# An astrobiological study of an alkaline-saline hydrothermal environment, relevant to understanding the habitability of Mars

A thesis submitted for the Degree of Doctor of Philosophy

By

**Lottie Elizabeth Davis**

Department of Earth Sciences

University College London

March 2012

I, Lottie Elizabeth Davis confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

## Abstract

The on going exploration of planets such as Mars is producing a wealth of data which is being used to shape a better understanding of potentially habitable environments beyond the Earth. On Mars, the relatively recent identification of minerals which indicate the presence of neutral/alkaline aqueous activity has increased the number of potentially habitable environments which require characterisation and exploration. The study of terrestrial analogue environments enables us to develop a better understanding of where life can exist, what types of organisms can exist and what evidence of that life may be preserved.

The study of analogue environments is necessary not only in relation to the possibility of identifying extinct/extant indigenous life on Mars, but also for understanding the potential for contamination. As well as gaining an insight into the habitability of an environment, it is also essential to understand how to identify such environments using the instruments available to missions to Mars. It is important to be aware of instrument limitations to ensure that evidence of a particular environment is not overlooked.

This work focuses upon studying the bacterial and archaeal diversity of Lake Magadi, a hypersaline and alkaline soda lake, and its associated hydrothermal springs. Culture dependent and culture independent analytical methods have been used and have led to the identification of potentially novel isolates. In addition, the effects of simulated Martian conditions such as desiccation and UVC radiation upon single strains isolated from this environment, and the effects upon a complex soil community have been assessed. Results have also provided an indication of what issues may arise with identifying minerals associated with these environments using the equipment such as the ExoMars PanCam. It is hoped that this work has contributed to our understanding of the possible habitability of Mars.

## Acknowledgements

There are many people I want to thank for their help, advice and guidance over the past three and a half years. Firstly want to thank my supervisors Dr. Ian Crawford and Prof. John Ward for their constant enthusiasm, guidance and support throughout, and Dr. Adrian Jones and Dr Graham Shields-Zhou for their assistance and help during my PhD. I cannot thank Dr. Claire Cousins enough for all her advice and patience right from the start, and for sitting with me in the middle of nowhere in the cold, rain and hail to teach me how to sample in the field. I must also thank Dr. Stephanie Hunter, Dr. Markus Gershater and Dr. Lewis Dartnell, for their invaluable guidance and assistance in the laboratory.

This work is almost entirely centred upon samples collected from Kenya, therefore I also want to thank Dr. Abigail Church, the Kenyan field work team, Ian Patmore and Professor Eric Odada, without whom the field work in Kenya could not have been possible. I also want to thank Dr. Andrew Griffiths who allowed me to test the PanCam instrument, Matt Gunn for his help with analytical instruments, and the stranger in the raman laboratory who brought me a cup of tea when everything seemed to be going wrong. I must also thank Dr. Manish Patel for allowing me to use the Mars chamber facilities and Dr Terry McGenity and Mr tony Osbourne for their help with water analyses.

I want to give a big thank you to my two offices, firstly to Ceri, Claire, Josh, Shoshana, Pete, Dom, Helen and everyone in the planetary office (at some point) for numerous entertaining and often somewhat bizarre lunchtime conversations and for the all-important tea breaks and cake time. I also want to say a massive thank you to the biology office, to Oriana, Steph, Pedro, John (Mr), Markus, Lewis, Julio & Vishal for making the office and laboratory such an entertaining, friendly and fun environment, and for the much needed light relief during tea breaks and evenings out. I also want to thank the support staff at UCL, whose upbeat good mornings made the early starts in the laboratory more bearable. Also thank want to thank Esme, for your help and advice, and all the Biggin Hill Billies (and their partners) for listening to me gabble on about my work. I am also so grateful to Laura, for being there throughout it all, field work, transfer reports, and thesis writing, you have been fantastic friend and for that I thank you.

I must thank my family, Mum, Dad, George, Gran, Grandad, Peter, Sheila, Jane, Malcolm and Lily and all the Davis', the Wynne-Jones', Howses and Hobdays, for the help and confidence that they have always had in me. Without your support, this would not have been possible.

Finally, I must thank my boys, Bertie and my wonderful Mikey. Mike, I could not have done this without you, your belief in me, your patience and support, your dinners and the constant supply of yet more teas. I cannot thank you enough for putting up with my stress and endless yabbering on. I just hope I can be as much of a rock for you, as you have been for me.

# Contents

# List of abbreviations

| | |
|---|---|
| **ANOVA** | Analysis of Variance |
| **AP+** | Africa Peptone Medium (microbial growth medium) |
| **APS** | Ammonium Persulphate |
| **BLAST** | Basic Local Alignment Search Tool |
| **BSA** | Bovine Serum Albumin |
| **MEGA** | Molecular Evolutionary Genetics Analysis |
| **CCA** | Canonical Correspondence Analysis |
| **CCD** | Charge Coupled Device |
| **CFU** | Colony Forming Units |
| **CRISM** | Compact Reconnaissance Imaging Spectrometer for Mars |
| **DGGE** | Denaturing Gel Gradient Electrophoresis |
| **DMSO** | Dimethylsulphoxide |
| **DNA** | Deoxyribonucleic Acid |
| **EDS** | Energy Dispersive X-Ray Spectrometer |
| **ESA** | European Space Agency |
| **EPS** | Extracellular polysacchride |
| **FWHM** | Full Width Half Maximum |
| **GR** | Green rock |
| **HRC** | High Resolution Camera |
| **IMP** | Imager for Mars Pathfinder |
| **IPTG** | Isopropyl β-D-1-thiogalactopyranoside |
| **IR** | Ionizing Radiation |
| **LCTF** | Liquid Crystal Tunable Filters |
| **LED** | Light Emitting Diode |
| **LIBS** | Laser Induced Breakdown Spectroscopy |
| **MastCam** | Mast Camera |
| **MER** | Mars Exploration Rover |
| **MGS** | Magadi Soda Company |
| **MIMA** | Mars Infrared Mapper |
| **MSL** | Mars Science Laboratory |
| **MVSP** | Multivariate Statistical Package |
| **NASA** | National Aeronautics and Space Administration |
| **NIR** | Near Infrared |
| **NB** | Nutrient Broth (microbial growth medium) |
| **NBAF** | NERC Biomolecular Analysis Facility |
| **NCBI** | National Centre for Biotechnology Information |
| **NERC** | Natural Environment Research Council |
| **OMEGA** | Observatoire pour la Minéralogie, l'Eau, les Glaces et l'Activité |
| **PanCam** | Panoramic Camera |
| **PBS** | Phosphate Buffered Saline |
| **PCA** | Principle Components Analysis |
| **PCR** | Polymerase Chain Reaction |
| **Prop.** | Proportion |
| **rDNA** | Ribosomal DNA |
| **RDP** | Ribosomal Database Project |
| **ROS** | Reactive Oxygen Species |
| **SEM** | Scanning Electron Microscopy |
| **sp.** | Species |
| **SPSS** | Statistical Package for the Social Sciences |
| **SR** | Soft rock |
| **TAE** | Tris base, Acetic acid and EDTA buffer |
| **TEMED** | N,N,N',N'-Tetramethylethylene diamine |
| **TGY** | Tryptone-Glucose-Yeast Extract (microbial growth medium) |
| **USGS** | United States Geological Survey |
| **UV** | Ultraviolet |
| **VIS** | Visible |
| **WAC** | Wide Angle Camera |

# List of Figures

## Chapter 1- Introduction

## Chapter 2- Material and methods



## Chapter 3- The isolation and analysis of bacterial and archaeal communites from Lake Magadi

### Chapter 4- Characterisation of novel bacterial isolates from Lake Magadi





Figures

Figures

## Chapter 5- Chapter Tolerance of isolates from Lake Magadi to desiccation and UV-C irradiation





Figures

## Chapter 6- Response of a single isolate, and a complex soil community to simulated Martian conditions





Figures

## Chapter 7- Testing PanCam multispectral filter sets on geological samples typical of alkaline environments

## Chapter 8-Discussion

# List of Tables

## Chapter 1- Introduction



### Chapter 2- Materials and methods



## Chapter 3- The isolation and analysis of bacterial and archaeal communites from Lake Magadi

## Chapter 4-Characterisation of novel bacterial isolates from Lake Magadi

## Chapter 5-Tolerance of isolates from Lake Magadi to desiccation and UV-C irradiation



## Chapter 6- Response of a single isolate, and a complex soil community to simulated Martian conditions



## Chapter 7-Testing PanCam multispectral filter sets on geological samples typical of alkaline environments

# Chapter 1

## A study of alkaline-saline environments relevant to understanding the habitability of Mars

This thesis focuses upon the microbiology of extreme saline, alkaline soda lake environments. Studying the reactions of the communities to environmental stresses provides a wealth of information on the ability of communities to survive or adapt to unfavourable conditions. This in turn provides greater insight into the sorts of organisms which could inhabit extraterrestrial environments such as Mars. Investigations into characterising novel isolates may lead to the identification of organisms which can survive conditions beyond what has been documented before. Finally, critical to the whole process in terms of the search for life beyond the Earth is that in order to identify organisms which occupy a particular environment, either past or present, it must be possible to identify that environment using the instrumentation available to space missions. These are all areas which this thesis will examine in relation to Lake Magadi, an African soda lake.

Astrobiology is a field of science which encapsulates the study of the origin, evolution and distribution of life in the universe. The study of the geology and biology of terrestrial analogues, be these environmental or biological analogues, is the first step towards answering whether there has been or is life beyond the Earth. The identification of aqueous alteration on Mars and the potential for a subsurface liquid ocean on Europa (Carr, 1998: Kivelson, 2000) and Enceladus (Waite Jr et al., 2009: Postberg et al., 2011) provides evidence of liquid water at some time in the history of these planetary bodies. At present, water is regarded as a necessary condition for life as we know it and its identification (past and/or present) elsewhere in the solar system is necessary in the search for extraterrestrial life (Rothschild and Mancinelli, 2001: Hubbard et al., 2003: Marion et al., 2003). To date the identification of life, on any planetary body other than the Earth remains elusive. However, this could be an absence of evidence rather than evidence of absence.

Until recently Mars analogue studies in astrobiology focused markedly on acidic and arid environments (Amils et al., 2007: Fernandez Remolar and Knoll, 2008). As a result the study of near neutral to alkaline environments as Martian analogues has largely been overlooked in favour of acidic, sulphate forming ones (Ehlmann et al., 2010a: Ehlmann et al., 2011a). However, detailed observations of Mars have revealed that it has had a much more environmentally diverse geological history than previously thought (Ehlmann et al., 2008b). As a consequence astrobiological research requires the study of a variety of environments, not just acidic, but alkaline and saline terrestrial environments as well. Analogue research can focus upon the environmental and geological similarities between two environments, however this thesis will focuses upon the study of organisms which may have adaptations to conditions

analogous to those which do, or may exist on Mars. This work will also address the ability to recognise alkaline environments on Mars, so that the identification of an analogous environment to Lake Magadi might be possible, if such an environment were present on the surface of Mars.

### 1.1. Alkaline environments and the origin of Life

There are a number of hypotheses for the origin of life, including the protein first hypothesis, in which amino acids spontaneously join up to form proteins and therefore these were the first informational molecule (Mellersh and Smith, 2010). An alternative hypothesis is the RNA world, which states that the first genes and enzymes were RNA based. The proposed 'Alkaline World' provides possible answers for an energy source, the introduction of substrates, dynamic flow and removal of waste for early life (Mellersh and Smith, 2010).

The conditions on the early Earth have been hypothesised to include areas where cracks in the crust allowed the seepage of cold acidic waters. These waters would have been involved in the process of serpentinisation, or the formation of serpentinite ($Mg_3Si_2O_5$ $(OH)_4$), through the hydration and metamorphic transformation of ultrabasic rock in the Earth's mantle (Sleep et al., 2004). The waters would have become reducing, warmer, more alkaline and enriched in ions such as magnesium and silica. The mixing of these alkaline waters with the probably acidic Hadean Ocean waters (pH 5 - 6) could have caused the precipitation of minerals which could have formed semi-permeable, tube like structures, with a proton gradient to provide energy (Lane, 2010: Mellersh and Smith, 2010). The flow of waters through these tubes would allow the trapping and concentration of molecules, and those not trapped will flow out into the ocean (Martin, 2008: Mellersh and Smith, 2010: Lane, 2010).

Examples of this kind of environment exist on present-day Earth: the Lost City hydrothermal field in the North Atlantic on the Atlantic Massif is comprised of white smokers formed by the process of serpentinisation (Figure 1.1). The fluids in these areas are alkaline (pH9-11) and upon contact with the seawater precipitate carbonate for the chimney-shaped deposits (Martin, 2008). The temperatures are typically lower than the more well studied black smokers (>300°C (Pirajno, 2005)), with temperatures between 40 °C and 91 °C, and are found further away from the spreading ridges than the higher temperature vents (Martin, 2008). These alkaline hydrothermal vents support an ecosystem of predominantly anaerobic methanogen bacteria (Brazelton et al., 2006: Martin, 2008). The concept of life originating in an alkaline environment, such as these hydrothermal vents on the Earth, makes alkaline hydrothermal environments on Mars of great interest to astrobiology.

*(Figure removed for copyright reasons)*

Figure 1.1. Comparison of (A) alkaline hydrothermal vent at Lost City and (B) A black smoker hydrothermal vent. Scale is 1 metre. Taken from (Lane, 2010)

**1.2. Terrestrial saline alkaline environments**

Soda lakes are naturally occurring alkaline settings in which organisms must withstand extreme conditions. Examples exist on all continents: for example Mono Lake and Summer Lake in North America (Raymond and Sistrom, 1967), Lake Van in Turkey (Kempe and Kazmierczak, 2002), Lake Malyi Kasytui, Siberia (Bryantseva et al., 1999) and Lake Magadi in the Kenyan region of the East African Rift Valley (Baumgarte, 2003). Those lakes in the East African Rift Valley have been studied in terms of their geology, hydrology, chemistry and biology (Jenkin, 1932: Baumgarte, 2003: Duckworth et al., 1996: Jones et al., 1998: Rees, 2004: Grant, 2006: Grant, 1986: Tindall, 1980), but not in an astrobiological context.

Soda lakes form in a similar way to purely salt lakes, however in these systems the carbonate and carbonate complexes are the major anions in solution (Jones et al., 1994). A number of climatic and geological factors contribute to the formation of soda lakes. The climate of the area controls how much water enters and leaves the system and therefore, the concentration of the lake fluids. The geology of the surrounding area is also important as it is directly linked to the types of solutes which find their way into the lake waters as meteoric water passes over the sediments. The source of the carbonate in these types of lakes is from rocks comprised of high

levels of sodium carbonate that, is leached as ground water flows over (Baker, 1955). Soda lake environments characteristically contain low levels of calcium and magnesium ions, due to the absence of Ca-rich and Mg-rich source rocks in the surrounding area(Warren, 2006).

### 1.3. Saline-alkaline lakes of the East African Rift Valley

Lake Magadi (Figure 1.3.A) is in the East African Rift Valley, which runs from Ethiopia through Kenya to Tanzania. There are ~20 lakes in the East African rift all of which are sodic in nature except Lake Baringo and Lake Naivasha (Uku and Mavuti, 1994: Darling et al., 1996). These lakes are hydrologically closed, either due to being crater lakes, or due to their position in the rift in a natural depression (Warren, 2006). The salinity of the lakes in the rift valley varies from 5% in the more northern lakes (Bogoria, Nakuru, Sonachi, and Elementia) to approximately 30% (saturation) halite/trona saturation (Lake Magadi, Lake Natron). The pH of these lakes is generally around 9, reaching up to a pH of 11.5 in the more alkaline depressions. Lake Magadi is one of the smallest and most saline lakes in the East Rift (Warren, 2006).

*(Figure removed for copyright reasons)*

*Figure 1.2. Map of Lake Magadi. A. Satellite image of Lake Magadi, (Google-Earth, 2011) B. Map of Lake Magadi with spring locations and alkaline lagoons (Baker, 1955).*

Lake Magadi is at latitude 1°51' S and longitude 36°15' E with a surface area of around 90 $km^2$ and 604m above sea level (Baker, 1958: Warren, 2006). The climate is hot and dry, the average dry season air temperature reaches 40°C, the mudflat sediments around the lake edge which are dark in colour can reach between 66 °C and 68°C (Baker, 1958). The mean average precipitation is around 1, 000 mm/year, with an evaporation rate of 1, 750 mm/year and is an arid basin (Olaka et al., 2010). The rainy season occurs between March to May (Baker, 1958). For the majority of the year evaporation exceeds rainfall creating a negative hydrological balance (Warren, 2006).

The Lake Magadi area has an archean aged basement rock and trachyte, basaltic and alkaline lavas (Figure 1.3.B), deposited during the Pleistocene and Pliocene periods (Baker, 1955). The Nguruman escarpment rises around 2 km along the western side of the lake, the result of various periods of faulting in its geological history (Spink and Stevens, 1946). To the east Olorgesailie volcano produced a build-up of alkali lava deposits, with the rift floor experiencing numerous basalt flows(Spink and Stevens, 1946). They are overlain by a thin layer of lake beds (Oloronga beds) comprised of reworked volcanic dust and debris. The area in which the modern lake Magadi sits is the result of grid faulting and down faulting and has experienced deposition from lakes of various ages up until current Lake Magadi. The rift valley is a volcanically active region: in the Lake Magadi, Lake Natron area, the younger the volcano the more alkaline it is. The youngest volcano in the area, Ol Doinyo Lengai is a carbonatite volcano and spews lava comprised of a mixture of sodium and calcium carbonate salts (Warren, 2006).

*(Figure removed for copyright reasons)*

*Figure 1.3. Geological map of the Lake Magadi area*

**1.4. Hydrochemistry and geology of Lake Magadi**

The springs at Lake Magadi (Figure 1.2) are hot-alkaline springs, defined as waters whose principal cations are sodium or potassium and which have a concentration of dissolved solids exceeding 2000 parts per million (a salinity greater than 0. 2%) (Baker, 1955). In general the springs around the lake tend to be seepages that occur in the shingle around the lake shore. The spring temperatures vary from 33°C to 86°C, and groups of springs may vary in temperature within a range of 5°C (Baker, 1955). The spring waters are clear at the source but algae are present in pools and near spring seepage locations. The salinity of the spring waters is generally lower than lake waters (which can reach saturation ~35% (w/v) NaCl) into which they feed (Warren, 2006). These areas can be considered as a refuge during times of extended drought in the lake area (Warren, 2006: Duckworth et al., 1996).

Meteoric waters contain normal levels of calcium and magnesium ions, these waters flow over highly reactive volcanic rocks on their way to the lake depression. The calcium within the residual glass in the volcanic rocks is more tightly bound up than the sodium therefore the sodium is more readily released on contact with inflow (Eugster, 1986). The calcium and magnesium is deposited as pedogenic and phreatic carbonates creating path 1 brines, with a high Na/Ca+Mg and ($HCO^-_3$+$CO_2$)/ ($SO_4$/$Cl^-$) ratios (Warren, 2006) (Figure 1.4).

*(Figure removed for copyright reasons)*

*Figure 1.4. Flow diagram of water chemistry evolution from undersaturated inflow, to possible final brine chemistry with example lakes and associated saline mineral which are related to Lake Magadi geology. L = lake, V = valley. Adapted from Warren (2006).*

By the time these waters reach the lake they are characterised by a four-fold dominance of $HCO^-_3$ over the alkaline earths Mg and Ca (Warren, 2006). Sulphate, and therefore sulphate-

rich brines, such as those of Great Salt Lake are not formed (Eugster, 1986). The low levels of calcium and magnesium in the inflow water to the lake means that carbonates such as calcite and magnesite (Table 1.1) do not form in any significant amounts.

The Na-$CO_3$-Cl water chemistry at Lake Magadi produces the saline minerals; halite, nahcolite, natron, thermonatrite and trona (Figure 1.4). Saturated lake brines contain high levels of $Na^+$, $Cl^-$ and $HCO_3$, precipitation removes $HCO^-_3$and $CO_3^{2-}$resulting in a rise in pH (Eugster, 1986: Warren, 2006). The $HCO_3^-$ in the most abundant ion and will combine with the Na in the waters and produce evaporitic minerals mentioned above and a rise in pH (by removing $HCO^-_3$ (Eugster, 1986)), therefore an environment in which trona forms must be alkaline. The reduction in $HCO^-_3$ trona formation means an enrichment of chlorine relative to $HCO_3$, this leads to an increase in the salinity and results in the crystallisation of halite (Warren, 2006).

The evaporite series underlies the trona deposits in the centre of the lake. The trona above ground is massive, coarsely crystalline and porous. The liquor which is found within the holes in the porous trona is present 5cm to 2m below the trona surface, this liquor is depleted in $Na^+$ and is relatively enriched in chlorine due to the trona precipitation (Eugster, 1986) . The fluid is pink-brown in colour and can tinge the trona. This is due to the presence of red halophilic bacteria in the fluid (Tindall, 1980). Trona precipitation is halted by the depletion of $HCO_3^-$ and formation will only continue following the addition of $CO_2$ to the waters (Eugster, 1986). Mineral deposits such as trona and natron (Table 1.1) are not associated with the evaporation of waters with the composition of modern sea water.

*Table 1.1. Minerals associated with alkaline environments*

| Mineral Class | Mineral Subclass | Mineral Group | Mineral | Chemical Formula |
|---|---|---|---|---|
| Carbonate | Hydrated | - | Trona | $Na_2(CO_3)(HCO_3).2(H_2O)$ |
| | | | Thermonatrite | $Na_2CO_3.\ H_2O$ |
| | | | Natron | $Na_2CO_3.10H_2O$ |
| | Anhydrous | Calcite | Magnesite | $MgCO_3$ |
| | | | Siderite | $FeCO_3$ |
| | | | Calcite | $CaCO_3$ |
| Chloride | | | Halite | NaCl |
| Sulphate | Anhydrous | - | Anhydrate | $CaSO_4$ |
| | Hydroxide | - | Gypsum | $CaSO_4.2H_2O$ |
| | | - | Jarosite | $KFe_3S_2On.3H_2O$ |
| Silicate | Tectosilicates | Zeolites | Analcime | $NaAlSi_2O_6{\cdot}H_2O$ |
| | | | phillipsite | $(Ca,Na_2,K_2)_3Al_6Si_{10}O_{32}{\cdot}12H_2O$ |
| | Phyllosilicates | Clay | Montmorillonite | $(Na,Ca)_{0.33}(Al,Mg)_2(Si_4O_{10})(OH)_2{\cdot}nH_2O$ |

Sodium aluminosilicate minerals or zeolites (for example analcime Table 1.1)) minerals can be found in association with evaporite minerals, these are microporous crystalline solids containing alkaline earth metals (Warren, 2006). Zeolites form in saline alkaline lakes (Eugster and Jones, 1968: Eugster, 1980), soils and land surfaces (e. g. Dry Valleys of Antarctica (Poulet, 2010), deep-sea sediments, low-temperature open and closed tephra systems (Sheppard and Hay, 2001) and burial diagenesis and hydrothermal systems (Ruff, 2004a: Weisenberger, 2009). The pH of interstitial water is generally the most important control on zeolitization, and the waters will preferentially contain bicarbonate (Hall, 1998). The high pH and ion ratios needed to form zeolites are typical of closed hypersaline basins in areas such a continental rift zones or collision belts.

Zeolites have been recorded in the Lake Magadi area (Lagaly, 1975: Surdam, 1976: Eugster and Jones, 1968). In addition sodium-aluminium silicate gel deposits, up to 5cm thick have been identified at Lake Magadi and are associated with zeolite formation (Eugster, 1967). The texture of these gels ranges from a soft gelatine-like substance to rubbery and cement-like, incorporating biological detritus. It is thought that these gels form through the interaction of hot (over 60°C) alkaline fluids with alkali trachyte lava flows (Baker, 1955). Zeolites begin to crystallise once the pH of the environment rises to between 9 and 10(McHenry et al., 2009). The zeolite analcime can form from a precursor mineral such as phillipsite or erionite, but can feasibly form from a gel precursor (Surdam, 1976: Eugster and Jones, 1968) and will also form from sodium rich solutions (English, 2001). The soil chemistry in the Magadi area is controlled by the leaching and base exchange within the soils, these being dominated by insoluble carbonates and the clay minerals which form are predominately the aluminium silicate clay bentonite (Baker, 1955), which is comprised mostly of montmorillonite (Millot, 1970).

The anhydrite and gypsum (Table 1.1) are not compatible with an alkaline environment. This is because the dominance of $HCO^{-}_{3}$ in the inflow water means that calcium precipitates as calcite before the calcium concentration is high enough for these minerals to form (Eugster, 1986).

### 1.5. Identification of alkaline-saline deposits in the geological record

Saline alkaline lake deposits are found throughout the geological record (Eugster, 1986). It is important to study modern saline alkaline lakes and the deposits they form to assist the identification of such areas in the past even after the alteration of evaporitic products. When looking for alkaline, saline lakes in the geological record minerals such as zeolites and cherts are particularly relevant as these are less soluble than trona, and so maybe better preserved (Eugster, 1986).

The Green River Formation of Lake Gosiute in Wyoming is a 52-44Ma depositional environment. Sedimentary beds up to 3km thick of trona and halite, as well as Magadi-type cherts (Eugster, 1986), analcime and other zeolites (Surdam and Parker, 1972) are present. The trona beds indicate a high pH geochemistry prevailing, as does the high silica content and

alkaline zeolites. The hydrochemistry of this lake differs to that of Lake Magadi by having a different Ca to Na ratio, this resulted in more deposition of calcite around spring outflows than is seen at Lake Magadi. Once the calcium was removed from the waters by precipitation, trona was deposited (Eugster, 1986).

Searles lake in California is an endoheric (closed – no outflow) dry drainage basin with evaporite deposits laid down during the Pleistocene (Warren, 2006). These deposits have high levels of bicarbonate (although not as high as at Lake Magadi), indicating a path I brine origin and a non-marine chemistry. Again phillipsite, analcime and fluorite are present indicating a high pH hydrochemistry (Eugster, 1986).

Analyses of the paleolake deposits of Olduvai Gorge in northern Tanzania showed zeolites including phillipsite and authigenic potassium feldspars indicating that these sediments are of an alkaline-saline lake origin. However, jarosite- a hydrated potassium sulphate- has also been identified (McHenry et al., 2009). The jarosite in this location is found in the altered tephra in association with phillipsite, in the alkaline-saline lake deposits and the alkaline nature of the lake waters is undisputable (McHenry et al., 2009). Jarosite is typically indicative of an acidic environment, forming in locations with a pH below 4 (Bigham et al., 1996). The jarosite maybe modern, otherwise it would have been expected to have been destroyed in a non-acidic environment, or it might be that the mineral was stable under the very extreme alkaline-saline lake conditions present in the area (McHenry et al., 2009). This discovery may affect how deposits that contain jarosite are interpreted.

## 1.6. Microbiology of alkaline and saline environments

### 1.6.1. Environmental microbiology

The microbiological study of environmental samples can be via non-culture based techniques, culture based techniques or both. Culture based methods provide a wealth of information on individuals of a sampled community, including details on morphology, growth limits and growth optima. Culture based studies can involve the isolation of organisms on media, which can then be identified using the 16s rRNA sequence. The 16s rRNA sequence has an alternating pattern of conserved and hyper-variable regions which vary between species (Neefs et al., 1990). Comparison of the 16S rDNA sequence of an isolate with a database such as the National Centre for Biotechnology Information (NCBI) database allows the identification of the organism, and its comparison relative to other strains already identified. A drawback of culture based methods is that typically less than 1% of a community can be cultured (Rhodes et al., 1996).

Another culture based analytical method useful for environmental studies is the Biolog$^{TM}$ Ecoplate, microbial community analysis assay, Biolog$^{TM}$ Ecoplate plates have been designed to characterise the metabolic activity of a single strain or community (Garland and Mills, 1991:

Biolog, 2010). The plate wells contain a carbon source (31 individual carbon sources plus a water control, in triplicate) and the tetrazolium redox dye; 4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide. This is a yellow tetrazole which is reduced to a violet formazan, a reaction catalysed by mitochondrial dehydrogenases. These enzymes are only active in living cells and so give a measure of cell viability (Luttmann et al., 2006). The inoculation of a Biolog$^{TM}$ GN (gram negative) or Biolog$^{TM}$ GP (gram positive) with a strain or culture will produce a finger-print of carbon usage which is specific to a particular type of organism.

The pattern of well colour change can be compared to a database of known well colour change patterns for various strains. This method can therefore be used for identification of the strain when comparing the carbon usage fingerprint to known organisms, or for the characterisation of carbon usage by a strain (Smalla et al., 1998: Fynan et al., 2009). This method is can also be applied to soil communities to provide a carbon usage profile for a particular soil sample to distinguish between the communities of different soil types (Choi and Dobbs, 1999) (Figure 1.5) or for examining soil response after a particular treatment, for example after exposure to acid mine drainage (Gomez et al., 2006: Weber et al., 2008).

*(Figure removed for copyright reasons)*

*Figure 1.5. Example of Biolog$^{TM}$ Ecoplate response indicating colour change in wells where the carbon source is utilised and respiration has occurred (dark wells) and where no carbon metabolism has occurred (light wells). Taken from (Fynan et al., 2009).*

Culture-independent methods can provide a more complete picture of a community present in a sample. Denaturing Gradient Gel Electrophoresis (DGGE) is a non-culture based analytical technique in which DNA extracted from a sample is amplified through PCR using universal primers with a GC clamp on one end. The GC clamp is 40-60 nucleotides of guanine and cytosine which results in a high denaturing temperature (Roelfsema et al., 2005). The amplified DNA is run through an acrylamide gel which is made up with a gradient of the denaturing agents urea and formamide. The strands migrate through the gel until the proportions of the denaturing agent mix is sufficient to denature the DNA fragment except for the GC clamp section. The strand forms a Y shape which sticks in the gel at the position the fragment was

denatured(Roelfsema et al., 2005). It produces a 'fingerprint' of DNA bands which vary depending upon the organisms present (Figure 1. 6. A).

The number of bands present gives an indication of the diversity of a sample (Muyzer, 1993). The relative intensity of each band also provides an indication of the relative abundance of a particular species in a community (Muyzer, 1993: Muyzer, 1998). Clustering algorithms can also be used to compare the diversity pattern for different samples (Figure 1. 6. B). Finally the bands from a gel can be extracted, cloned and sequenced in order to identify which organisms contribute to a particular band.

*(Figure removed for copyright reasons)*

*Figure 1.6. Example DGGE gel. A. DGGE of 9 samples (wells A1-5 and B1-4) and reference lanes (R), demonstrating separation of bands and their varying intensity. B. Clustering analysis of each lane by their band distribution, indicating the samples produce two distinct groups. Taken from (Vanhoutte et al., 2005).*

DGGE complements the sequencing of isolated organisms and a reference ladder of isolated organisms can be used to identify which bands may be attributed to that species. However, the drawback of any technique which utilises PCR amplification is the introduction of PCR bias due to differences in template efficiency (Acinas et al., 2005).

### 1.6.2. Extremophile and extreme-tolerant organisms

Biology can exploit a variety of environments including those with a high pH, high salinity and extremes of temperature. Extremophile organisms are those which grow optimally in conditions which we would consider as being harsh and unfavourable for life (Cavicchioli, 2002). Organisms that need extreme conditions such as high pH or salinity to live are termed obligate extremophiles. These differ to tolerant organisms which can grow under extreme conditions but grow optimally at less extreme conditions (Cavicchioli, 2002). Polyextremophilic or polytolerant bacteria and archaea are able to live in the presence of a combination of extreme conditions, for example, at high pH, high salinity and high temperature. Possible definitions used to describe different environmental factors and examples of bacteria that can live in such environments are outline in Table 1. 2.

*Table 1.2 Examples of definitions of environmental parameters and examples of organisms which can live in their presence*

| Environmental parameter | Type | Conditions | Examples |
|---|---|---|---|
| Salinity | Halotolerant | 0. 2M-0. 5M NaCl (0.6% - 3% w/v), but can tolerate up to 2. 5M NaCl (14. 6% w/v NaCl)(Mohapatra 2008) | Halomonas subglaciescola (Franzmann et al., 1987) |
| | Halophile | Optimal growth in at least 1. 5M NaCl (9% NaCl) (Mohapatra 2008) | *Haloquadratum walsbyi* (Burns et al., 2007) *Natronobacterium* sp. (Tindall et al., 1984) |
| | Hyperhalophile | Optimal growth at 2. 5M -. 5M NaCl (14. 6%- 29% NaCl) (Mohapatra 2008) | *Salinibacterruber* (Mongodin et al., 2005) |
| pH | Alkaliphile | Optimal growth pH> 8.5, cannot grow below pH 7. 5 (Mohapatra 2008) | *Halomona scampisalis* (Boltyanskaya et al., 2004) |
| | Facultative alkaliphile | Optimal growth pH >8. 5, can grow at pH <7. 5 (Mohapatra 2008) | Clostridium thermoalcaliphilum (Li et al., 1994) |
| | Acidophile | pH0-4(Seckbach, 2000) | *Thiobacillus sp.* (Harrison, 1984) |
| Temperature | Hyperthermophile | Optimal growth 80°C - 85 °C(Mohapatra 2008) | *Aquifexaeolicus* (Deckert et al., 1998) |
| | Thermophile | 50->60 °C(Mohapatra 2008) | *Thermusaquaticus* (Brock and Freeze, 1969) |
| | Mesophile/ Thermotolerant | 25-45°C, Optimal growth <50 °C (Mohapatra 2008) | *Clostridium sp.* (Warnick et al., 2002) |
| | Psychrophile | -20-<15°C(Mohapatra 2008) | *Psychrobacterimmobilis* (Juni and Hwym, 1986) |
| Hydrostatic pressure | Barophiles | Growth is enhanced by pressures greater than 1 atmosphere (Nogi and Kato, 1999) | *Moritellayayanosii. (Kato et al., 1998: Nogi and Kato, 1999), Marinitogapiezophila* (Alain et al., 2002) |
| Radiation | | Radiation tolerant | *Deinococcus radiodurans* (Anderson et al., 1956) |
| Desiccation | Xerophiles | Organisms can withstand desiccation and enter ananhydrobioticstate showing little damage after rehydration (de Castro et al., 2000) | *Mycococcusruber (Imshenetsky et al., 1973)* |
| | Xerotolerant | Can withstand high osmotic pressures and low water activity (Speck, 1976) | |

### 1.6.3. Adaptation to extreme environments and stress factors

#### 1.6.3.1. High NaCl concentration

Microorganisms in highly saline environments require adaptations which are energetically costly. These organisms possess several adaptations to maintain their cytoplasm in an iso-osmotic state with the environment (Oren, 2001). The two main strategies employed are a salt-out/compatibility solute strategy or a salt-in strategy. Generally only one is used by an organism, with the exception of methanogenic archaea which utilise both (Siddiqui and Thomas, 2008). The salt-out method maintains the osmotic pressure by using organic compatibles such as betaine or glycerol; halophilic organisms can contain up to 1M betaine when grown in 20% NaCl (Siddiqui and Thomas, 2008). As the NaCl concentration of the environment decreases the levels of betaine are also reduced, this allows the organisms to grow over a wide range of NaCl concentrations (Siddiqui and Thomas, 2008).

The salt-in strategy involves the intracellular accumulation of salt to balance the osmotic pressure. Some organisms obtain equimolar volumes of KCl within the cells in relation to the extracellular NaCl concentration (Oren, 2002c: Siddiqui and Thomas, 2008) and the NaCl is actively excluded from the cell, which is particularly energetically costly to the organism (Oren, 2001). Those organisms which utilise a salt-in strategy need intracellular proteins which are adapted to high salt. These organisms then require high salt for protein activity and structural integrity, this need means that they are incapable of growing at lower salt concentrations and are obligate halophiles (Siddiqui and Thomas, 2008).

Several studies have shown that some bacteria and archaea strains can survive being encapsulated in the fluid inclusions in halite (NaCl) crystals (Norton and Grant, 1988: Mormile et al., 2003: Stan-Lotter et al., 2003: Schubert et al., 2009). An archaeon *Natronomonas sp*. has been isolated from 34kyr halite crystals (Schubert et al., 2010) and 6 strains of archaea have been isolated from 250 Myr halite crystals (Vreeland et al., 2000). Bacterial strains of *Bacillus* have reportedly been isolated from 121 Ma and 112 Ma crystals (Vreeland et al., 2007). In these studies the surfaces of crystals have been sterilised to ensure that the cultured strains were preserved inside the crystals and not from the crystal surface(Park et al., 2009). These are important findings in terms of astrobiology, if any biology has existed on Mars, then it may be possible that the evidence of life could be preserved in halite crystals. It will be halophilic, or halotolerant strains which are likely to be able to be persevered in halite fluid inclusions.

#### 1.6.3.2. Alkaline pH

The domains of bacteria, archaea and eukarya all contain examples of alkaliphiles. Alkaliphilic organisms have been identified in many alkaline environments, including the soda lakes and soda deserts, but also in environments with a pH near to neutral (Shirai, 2008: Yumoto et al., 2011). It is hypothesised that this is achieved by the organisms creating a localised pH through the induced production of extracellular alkaline substances (Chadwick, 1999: Horikoshi, 2006).

Alkaliphilic bacteria are capable of maintaining an internal environment less alkaline than their external environment, for example, the extreme alkaliphile *Bacillus pseudofirmus* maintains its cytoplasmic pH around 2 pH units lower than its external environment (Slonczewski et al., 2009). The cells of an alkaliphile can have barriers against external pH such as the presence of acidic polymers which may reduce the pH at the cell surface or they can utilise cell porters such as $Na^+/H^+$ antiporter system to maintain pH homeostasis (Horikoshi, 1999). The role of membranes and secondary wall polymers in the resistance of alkaliphiles requires further investigation (Slonczewski et al., 2009).

Alkaliphiles are commonly isolated using sodium carbonate buffer, regardless of the environmental chemistry they are isolated from, as most strains require at least some sodium (Horikoshi, 1999).

#### 1.6.3.3. Desiccation

Environments around Lake Magadi experience desiccation, as described in Chapter 1, section 1. 3. Highly saline environments are often accompanied by desiccating environments, as the high salinity can be the result of concentration of solutes by drying.

The dehydration of cells due to desiccation introduces damage to the organism's DNA, including single strand and double breaks, where the DNA strand is severed (Storz et al., 2000: Fredrickson, 2008: Kottemann et al., 2005a: Mattimore, 1996: Potts, 1994). Single strand breaks (SSB) are more common and the continued acquisition of SSB can eventually lead to double strand breaks (DSB). DSB are consider to be the more lethal type of DNA damage because no complimentary strand is available as a template for DNA repair (Daly, 2009: Kobayashi and Handa, 2001) and breaks in the DNA strand prevent DNA replication, transcription and protein synthesis (Kobayashi and Handa, 2001).

One way of limiting the effects of DSB is by providing a DNA template for repair through the presence of more than one set of chromosomes per cell (Daly, 2009: Minton and Daly, 1995). Studies have shown however, that isolate resistance is not simply the result of multiple chromosomes in the cell and in addition, resistance to desiccation is not simply a factor of the amount of damage to the genome (Daly et al., 2007: Fredrickson, 2008). Studies using *Deinococcus radiodurans,* a desiccation and radiation resistant bacterium, have shown that the numbers of DSB are comparable between resistant 'wild type' strains and sensitive 'mutants', a strain which has an inactivated uvrA gene, which is involved in DNA repair, for example (Udupa et al., 1994: Mattimore, 1996: Cox and Battista, 2005: Daly et al., 2007: Fredrickson, 2008: Slade, 2011). This suggests that direct DNA protection does not explain resistance.

Instead, resistance to desiccation/radiation identified in organisms such as *D. radiodurans* is actually the result of an ability to retain functional DNA repair mechanisms, namely the proteins

involved (Minton, 1996: Daly et al., 2004: Daly et al., 2007: Fredrickson, 2008: Daly, 2009). Cellular damage due to dehydration has been primarily attributed to the formation of reactive oxygen species (ROS), such as hydroxyl and peroxyl radicals (Nauser et al., 2005: Fredrickson, 2008: Kottemann et al., 2005a: Daly, 2011). The cellular proteins damaged during desiccation(Krisko and Radman, 2010)include those proteins involved in DNA repair(Fredrickson, 2008). Studies into protein damage related to oxidation suggest that the difference between resistant and non-resistant organisms is the amount of protein damage accumulated, with the amount of protein damage being lower in resistant organisms (Daly et al., 2007: Fredrickson, 2008: Krisko and Radman, 2010).

#### 1.6.3.4. Ultraviolet-245nm (UV-C) radiation

Ultraviolet radiation also causes direct damage to both DNA and proteins, in part due to oxidation (Daly, 2009: Chan et al., 2006). DNA damage due to UV-C radiation can include strand breaks (Slieman and Nicholson, 2000: Setlow and Setlow, 1996: Setlow, 2001) and the formation of photoproducts, such as pyrimidine dimers, which form between adjacent pyrimidine bases on the same DNA strand (Tyrrell, 1973: Maloy, 1994: Sancar, 1994: Cadet and Douki, 2010). These types of DNA damage interrupt the replication of DNA resulting in genome rearrangement as well as mistakes and/or the ceasing of DNA replication(Maloy, 1994).

The process of dimer formation in bacteria and archaea can be reversed by either photoreactivation or dark repair (Harm and Harm, 1968: Rastogi, 2006: Kowalski, 2009). Studies have shown a difference when comparing strains which have been exposed to UV-C and are then either incubated in the light (photoreactivation) or the dark (dark repair) for sufficient time to allow the cells time to recover, and DNA repair to occur. Those cells incubated without light can demonstrate up to an order of magnitude lower survival of cells compared to those incubated in the presence of light (Maloy, 1994).

Photoreactivation dimer repair firstly involves the formation of a complex between a photoreactivation enzyme (PRE), such as photolyase, and the dimer to be repaired (Maloy, 1994: Sancar, 1994: Rastogi, 2006). Photolyase will cleave the dimer in the presence of cofactor folic acid, upon exposure to visible light the folic acid absorbs the light, the enzyme breaks the dimer light and the PRE is released. This process is directly related to the quantity of visible light received (Maloy, 1994: Sancar, 1994: Rastogi, 2006).

Dark repair mechanisms (as the name suggest) do not require light and include; nucleotide excision repair (NER), post replication repair (PRR) /recombination repair and SOS repair. In *E. coli* NER involves the enzyme complex UvrABC endonuclease, proteins within this complex identify distortions in the double helix (i.e. thymine dimers), excises the damage and then a template DNA strand is used with the enzyme, DNA polymerase I, to infill the gap and repair the DNA (Yeung et al., 1986: Sancar and Tang, 1993: Maloy, 1994: De Laat et al., 1999: Cadet and Douki, 2010). PRR occurs when NER cannot function, for example, due to the absence of

template DNA for synthesis across the damage. In this instance, the section of damaged DNA can be replaced by intact segment of DNA from a different, but homologous duplex of DNA, this occurs post replication (Rastogi, 2006). The RecA protein is vital in this process, promotes single strand DNA (ssDNA) pairing between homologous, but not identical sequence from dsDNA (Maloy, 1994: Witkin, 1976). PRR initiates once the replicating DNA (Pol III in *E. coli* for example) attempts to replicate through a lesion, it will stall and then reinitiate polymerisation beyond the lesion, resulting in a gap in the newly synthesised daughter strand. A complementary section (to the gap in the daughter strand) on an undamaged parent strand is removed and ligated into the gap in the daughter strand. The gap in the parent strand is then in-filled by DNA Pol I (Trun and Trempy, 2004).

SOS repair a mechanism induced by DNA damage, and is a bypass system which allows DNA chain growth to occur over damaged segments of DNA. The SOS system involves the relaxation of the DNA editing system and allows DNA polymerisation across the damage (rather than removing it) (Lu et al., 1986: Maloy, 1994: Rastogi, 2006). In this process, the RecA protein is involved in suppressing this proofreading function, therefore, SOS repair results in polymerised DNA of the damage, which is prone to error (Maloy, 1994: Vandewiele et al., 1998). This mechanism is unlike photoreactivation, NER and PRR, which result in faithful DNA repair (Maloy, 1994).

#### 1.6.3.5. Ionizing radiation (IR)

There is an apparent relationship existing between desiccation and IR tolerance (and UV-C tolerance), DSB breaks and the production of ROS are produced by both forms of stress (DiRuggiero et al., 1999: Fredrickson, 2008: Mattimore, 1996). The evolution of IR resistance is debated; the radiation adaptation hypothesis suggests that desiccation resistance is a secondary result of evolved IR resistance (Sghaier et al., 2005: Sghaier et al., 2007). One theory is that extreme radiation resistance could have evolved through accumulated background radiation inducing DNA damage in organisms in permafrost/semi-frozen environments (Richmond et al., 1999).

In contrast, IR resistance could instead be a secondary benefit acquired due to the evolution of resistance to desiccation and/or UV radiation (Arrage et al., 1993a: Mattimore, 1996: Fredrickson, 2008). The 'desiccation adaptation hypothesis' suggests that similar types of cellular damage occur due to desiccation in arid environments and through IR radiation, therefore IR resistance is a secondary benefit of the cellular strategies evolved to cope with desiccation (Mattimore, 1996: Slade, 2011). This hypothesis works with the fact that organisms are resistant to IR in spite of the limited advantages of IR resistance on Earth (Mattimore, 1996). Studies related to IR resistance have shown that the actual repair proteins involved in DNA repair are themselves, unremarkable. For example, the replacement of the DNA Polymerase 1 gene (involved in DNA replication) in *D. radiodurans* with the DNA Polymerase 1 gene from *E. coli* still results in an IR resistant *D. radiodurans* mutant strain (Gutman et al., 1994: Daly et al.,

2007). This supports the concept that the actual protection of the proteins is the key to cellular resistance against oxidative stress caused by IR radiation.

#### 1.6.3.6. Resistance to Desiccation, UV-C and Ionizing radiation

Resistance to DNA damage and oxidative stress has been attributed to a variety of methods employed by different bacteria and archaea. These methods include; the presence of molecules such as sucrose and trehalose, which replace water hydrogen bonds and stabilise the cytoplasm, reducing the release of free radicals/ Reactive oxygen species (ROS) and therefore reducing protein oxidation (Slade, 2011: Welsh and Herbert, 1999). Other strains use an accumulation of KCl, which has been shown to reduce the effects of oxidative free radicals; KCl reacts with hydroxyl radicals to produce less reactive chloride radicals (Kottemann et al., 2005a: Shahmohammadi et al., 1998). In strains such as the cyanobacterium *Nostoc* sp., extracellular polysaccharides (EPS) can provide protection against desiccation, where it can absorb and retain water to slow down the drying process, in addition, it can protect against UV radiation (Potts, 1994: Ophir and Gutnick, 1994: Hill et al., 1994: Ehling-Schulz et al., 1997). Another mechanism to provide cellular protection includes carotenoids, which have also been attributed with protection from oxidation by their capacity to scavenge ROS (Krinsky, 1979: Tian et al., 2009: Kottemann et al., 2005a: Carbonneau et al., 1989).

Recent work on desiccation and IR resistance has indicated that there may be a correlation between increased resistance, and a high Mn/Fe ratio in the cells of *D. radiodurans* (Gérard et al., 2001: Daly et al., 2007: Fredrickson, 2008: Anjem et al., 2009: Daly, 2009). The Mn/Fe ratio has an effect on the level of protein oxidation, with the continued presence of DSB in the DNA helix indicating that the does not directly affect the DNA damage itself (Gérard et al., 2001: Daly et al., 2004). Redox cycling within the cell between $Mn^{2+}$ to $Mn^{3+}$ prevents the proliferation of Fe-dependent ROS and plays a role in reducing the number of Fe dependent ROS being formed (Daly, 2009). X-ray fluorescence microspectroscopy of *D. radiodurans* cells demonstrated that manganese is distributed throughout the cell, with iron being restricted to certain areas (Daly, 2009). The protection of intracellular proteins results in the continued function of proteins required to repair DNA damage, therefore the survival of the organism.

## 1.7. Alkaline-saline environments as extraterrestrial analogues

The study of analogue environments is a vital area of research to increase our understanding of environments elsewhere in the solar system. The study of analogue environments provides the opportunity not only to study the minerals present in a particular environment but also to understand the depositional environment under which they formed. These analogue studies also provide an indication of the types of life which can survive in a certain environment, and if nothing can live there, we can analyse why. All this information can then contribute to an understanding of the interactions between the environment and the biological community, the limits of life and the presence of any evidence for life in that environment. One of the most important aspects of studying analogue environments is to

identify of whether or not it is possible to distinguish between different depositional environments and to detect signs of life using instruments available to planetary orbiters, landers and rovers.

The missions searching for evidence of life require some sort of direction. This applies to the geological as well as the biological studies of Mars and it is necessary that these explorations have an idea of what they are looking for. The main assumption that most astrobiological research has to make is that extraterrestrial life in our solar system will be carbon based with structures, metabolic pathways and replication systems which are very similar to life as we know it on Earth. We are also assuming that water is necessary for life to exist.

The study of alkaline-saline environments such as Lake Magadi has relevance to locations such as Mars, Europa and Enceladus in our solar system. All these planetary bodies are known to have at some point in their history alkaline or alkaline-saline environments. The relevance of Lake Magadi in relation to Europa and Enceladus will be touched upon, however the focus of this work is Mars. There have been a variety of Martian analogue sites which have been examined, these have focussed upon areas including acidic rivers (Amils et al., 2007: Fernandez Remolar and Knoll, 2008), hydrothermal systems (Hagerty and Newsom, 2003: Bishop et al., 2004) and saline environments (Forsythe and Zimbelman, 1995: McDonald et al., 1999: Grasby and Londry, 2007) and more specifically: Antarctic dry valleys (Wentworth et al., 2005: MacClune et al., 2003), Svalbard (Steele et al., 2004: Steele et al., 2007) and the Atacama Desert (Wettergreen et al., 2005: Skelley et al., 2005). Following the discovery of neutral to alkaline environments on Mars, studies into analogues of such environments are now being conducted, e. g. (Ehlmann et al., 2011a: Dyar et al., 2012). The study of Lake Magadi its geology and biology will contribute to the Mars analogue studies of alkaline-saline environments.

## 1.8. Relevance of alkaline-saline environments to planetary bodies within our solar system

### 1.8.1. Europa

Europa is the smallest of the Galilean satellites and is ~1,300 km in diameter and therefore smaller than the Earth's Moon (Chela-Flores, 2010) and is thought to have an ocean (Kivelson, 2000: Chela-Flores, 2010). The cracked surface of this moon (Figure 1. 7) is thought to indicate the presence of tectonic processes, the result of warmer material existing underneath a cold crust (Harada, 2006). In addition, the lack of impact craters on the surface would imply active geology (Greenberg, 2002). The tidal processes on Europa are believed to be the dominant interior heat source, which resulted in liquid water on the moon (Greenberg, 2010) and the implication of hydrothermal vents on the ocean floor of Europa due to this internal heating could play a part in sustaining life on the moon (Chela Flores, 2010).

The infrared spectroscopy by the Galileo spacecraft has indicated the possible presence of hydrated salts, with mixtures of: epsomite ($MgSO_4.7H_2O$), bloedite ($MgSO_4.Na_2SO_4.4H_2O$), mirabilite ($Na_2SO_4.10H_2O$) and natron ($Na_2CO_3.10H_2O$), providing good spectroscopic matches (Zolotov and Shock, 2001). However, a deficiency of Na in the ocean, modelled by Zolotov et al. (2001), would argue against the hypothesis of a soda ocean with a pH 11-12 which has been suggested by others (Sleep, 2001: Kempe and Kazmierczak, 2002). Alternatively, the theorised aqueous weathering of ultrabasic rocks would lead to an alkaline solution with a pH 10-12, which may then be diluted to a pH 8-9, a pH which could be representative of both primordial and present Europan ocean (Zolotov, 2004). The ocean of Europa may also be salty, and could contain NaCl (Kargel et al., 2000: Hand and Chyba, 2007: Prieto-Ballesteros et al., 2011).

*(Figure removed for copyright reasons)*

*Figure 1.7 Surface ridging on Europa, Image is 200 km across(Carr, 1998)*

The presence of a briny/alkaline ocean on Europa provides another possible habitat for life beyond the Earth(Marion et al., 2003). The launch of missions to Europa will provide more data regarding the composition of its ocean. The Europa Jupiter System Mission EJSM planned to be launched around 2020 is a joint mission between ESA and NASA which will orbit the moons of Ganymede (ESA) and Europa (NASA).

### 1.8.2. Enceladus

The Cassini orbiter's Visual and Infrared Mapping Spectrometer revealed a surface, predominantly of water ice, with simple organics and $CO_2$in association with fractures in the south pole (Brown, 2006). Cassini Composite Infrared Spectrometer identified that the southern polar region is anomalously warm (Spencer, 2006). Cassini's Ultra-Violet Imaging Spectrograph confirmed the existence of water vapour plumes in the south polar region (Hansen, 2006), with the Imaging Science Subsystem actually captured the eruption of icy plumes from 'tiger stripes on the surface(Porco, 2006) (Figure 1. 8). These plumes, and the tiger striped trenches in the

southern hemisphere (which have a higher temperature than the surrounding surface), suggest that the moon is geologically active and warm(Porco, 2006).

*(Figure removed for copyright reasons)*

*Figure 1.8 (A) Image of Tiger stripes on the surface of Enceladus. (B) Image of plume erupting from surface of Enceladus (Porco, 2006).*

Cassini mission data has suggested that the plumes from Enceladus may replenish material in the E-ring of Saturn(Spahn, 2006). Analysis of the E-rings grains has identified sodium chloride and possibly sodium carbonate minerals, with the source of the plumes suggested to have pH 8-11 and contain 0.2% and 2% (w/v) NaCl and $Na_2CO_3$ 0.02-0.1 M (Zolotov, 2007: Postberg et al., 2009). The Cassini's Cosmic Dust Analyser produced mass spectra for a plume from Enceladus allowed the identified of 0. 5–2% by mass of sodium and potassium salts, which resemble the theorised composition of the Enceladian ocean (Postberg, 2011).

As with Europa, the presence of a liquid ocean on Enceladus provides another possible habitat for life. Competition between the EJSM and the Titan Saturn System Mission (TSSM) has resulted in priority being given to the EJSM, with TSSM being continued for potential launch at a later date (Talevi and Brown, 2009). These missions will increase our understanding of these systems and develop our understanding of habitable environments in the universe (Rampelotto 2011).

### 1.8.3. Mars

#### 1.8.3.1. Exploring the surface of Mars

Mars is a key target for planetary research and an astrobiological aspect to this work is becoming increasingly prominent. Exploring Mars has involved the acquisition of data using both orbital and rover missions over the years (Klein et al., 1976: Golombek, 1997: Farrand, 2008: Ehlmann et al., 2009: Hecht et al., 2009: Arvidson et al., 2011).

Each mission has been equipped with a variety of instruments in order to characterise the surface of Mars (Table 1.3). The Alpha Proton X-Ray Spectrometer, Miniature Thermal

Emission Spectrometer/Infrared-Mapper, Raman Spectrometer, Laser Induced Breakdown Spectrometer (LIBS), Mass spectrometer and X-ray Diffractometer instruments are all designed to analyse the mineralogy and/or the elemental chemistry of the planet. The Mössbauer spectrometer allows the analysis of iron-bearing minerals. Optical microscopes provide images of small scale features, with atomic-force microscopes imaging on nm scale. Wet chemistry analysis of pH, conductivity and the analysis of the redox potential of a soil can be conducted using instruments such as the Conductivity Analyzer (MECA), aboard the Phoenix lander. The addition of the Life Marker chip, an antibody assay-based life detection instrument on the ExoMars rover would enable a search for specific molecules indicative of life (Martins, 2011). Finally, camera instruments have been part of the payload of several missions (Bell et al., 2003: Griffiths, 2005: Malin et al., 2005: Griffiths et al., 2006). The camera systems can capture high resolution images, panoramic images and are capable of making multispectral observations (Griffiths et al., 2006).

The first astrobiology-focused mission was the Viking program, with its associated orbiter and Viking lander missions Viking 1 and Viking 2 in the 1970's, landing in Chryse Planitia and Utopia Planitia respectively (Mutch and Mutch, 1976a: Mutch and Mutch, 1976b) (Figure 1.9). The aims of the Viking Program were to take high resolution images of the surface, identify the main composition of the atmosphere and look for past signs of life (Radmer, 1971: Soffen and Snyder, 1976). The imaging provided detail pictures of regions of the surface of Mars but the biological experiments conducted provided no convincing evidence of life (Klein et al., 1976: Klein, 1978).

*Table 1.3. Missions to Mars, past and future detailing their instrument payloads*

| Mission and launch date | | Instrument payload |
|---|---|---|
| Viking 1 and Viking 2 1975 | Orbiter/ lander | • Gas Chromatograph/Mass spectrometer<br>• X-ray florescence spectrometer<br>• Stereo colour cameras<br>• Biology instrument |
| Mars Observer 1992 | Orbiter | • Gamma ray spectrometer (GRS)<br>• Mars Observer camera (MOC)<br>• Mars Observer laser altimeter (MOLA)<br>• Thermal emission spectrometer (TES)<br>• Magnetometer/electron reflectometer (MAG/ER)<br>• Pressure modulator IR radiometer (PMIRR) |
| Mars global surveyor 1997 | Orbiter | • Magnetometer/electron reflectometer (MAG/ER)<br>• Mars Observer laser altimeter (MOLA)<br>• Thermal emission spectrometer (TES)<br>• Mars Observer camera (MOC) |
| Mars Odyssey 2001 | Orbiter | • Thermal Emission Imaging System (THEMIS)<br>• Gamma Ray Spectrometer (GRS)<br>• Mars Radiation Environment Experiment (MARIE) |
| Mars Express 2003 | Orbiter | • High Resolution Stereo Camera (HRSC)<br>• Energetic Neutral Atoms Analyser (ASPERA)<br>• Planetary Fourier Spectrometer (PFS)<br>• Visible &Infra Red Mineralogical Mapping Spectrometer |

| | | |
|---|---|---|
| | | (OMEGA)<br>• Sub-Surface Sounding Radar Altimeter (MARSIS)<br>• Mars Radio Science Experiment (MaRS)<br>• Ultraviolet and Infrared Atmospheric Spectrometer |
| | Lander- Beagle 2 | • Gas Analysis Package (GAP)<br>• **Panoramic camera (PanCam)**<br>• Mössbauer spectrometer<br>• X-ray detector<br>• Ultra violet sensor<br>• Pressure sensor<br>• Horizontal wind gauge<br>• Dust impact detector |
| Mars Exploration rovers 2003 | Twin rovers- Opportunity and Spirit | • **Panoramic camera (PanCam)**<br>• Microscope Imager (MI)<br>• Miniature Thermal Emission Spectrometer<br>• Mössbauer Spectrometer<br>• Alpha particle X-ray spectrometer (APXS)<br>• Magnet Array |
| Mars reconnaissance orbiter 2005 | Orbiter | • High resolution imaging science experiment (HIRISE)- visible stereo imaging camera<br>• Context camera (CTX)<br>• Mars Color Imager (MARCI)<br>• Compact Reconnaissance Imaging Spectrometer for Mars (CRISM)- visible/near infrared spectrometer<br>• Mars Climate sounder (MCS)<br>• Shallow Radar (SHARAD) |
| Phoenix 2007 | Lander | • Robotic arm and camera<br>• Microscopy, Electrochemistry & conductivity analyser (MECA)<br>• Thermal and evolved gas analyzer (TEGA)<br>• Mars Descent Imager (MARDI)<br>• Meteorological station (MET)<br>• Atomic force microscope |
| Mars Science Laboratory 2011 | Rover | • **Mast Camera (MastCam)**<br>• Mars Hand lens imager (MAHLI)<br>• Mars Descent Imager (MARDI)<br>• Alpha particle X-ray spectrometer (APXS)<br>• Chemistry and camera (ChemCam)<br>• Chemistry and mineralogy diffraction/X-ray fluorescence instrument (Chemin)<br>• Sample Analysis at Mars (SAM Instrument suite<br>• Radiation assessment detector (RAD)<br>• Rover environmental monitoring station<br>• Mars science laboratory entry descent and landing instrument |
| ExoMars/ MaxC 2018 | Rover | • **Panoramic camera (PanCam)**<br>• Water Ice and Subsurface deposit information on Mars (WISDOM)<br>• Miniaturised Mössbauer spectrometer (MIMOS-II)<br>• Mars multispectral imager for subsurface studies (MA_MISS)<br>• The Raman- Laser Induced Breakdown Spectrometer (Raman-LIBS)<br>• MircoOmega- Visible/IR microscope<br>• Mars X-ray Diffractometer (Mars- XRD)<br>• Mars Organic Molecule Analyser (MOMA)<br>• Life Marker Chip (LMC) |

The ill-fated Mars Observer (MO) spacecraft was intended to analyse the global topography and gravitational fields of Mars, as well as determining the elemental and mineralogical character of surface materials (Albee, 1992). The loss of this orbiter led to the design and launch of the Mars Global Surveyor Mission (MGS), which utilised many spare components and instruments from MO, and followed many of the same science objectives (Dallas, 1997: Albee et al., 1998). This mission imaged relatively young landforms on Mars, thought to suggest geologically young aqueous activity (Malin and Edgett, 2000: Heldmann et al., 2010: Lanza et al., 2010).

*(Figure removed for copyright reasons)*

*Figure 1.9. Elevation map of Mars with landing sites (http://martianchronicles.wordpress.com)*

In the last 10 years 3orbiters have been sent to Mars, these being the ESA-led Mars Express and the NASA-led Mars Odyssey and Mars Reconnaissance Orbiter (MRO). The Mars Express orbiter and its associated Beagle 2 lander was intended to identify any signs of life and water, however contact with the lander was lost once it was separated from Mars Express (Griffiths, 2005).

The scientific objectives of Mars Odyssey, launched in 2001, included elemental mapping of the surface, ascertain subsurface hydrogen abundance, imaging of surface mineralogy and to provide details on surface morphology (Saunders et al., 2004). Odyssey identified subsurface water ice, pole ward of ± 60º latitude, through hydrogen mapping beneath the surface (Feldman et al., 2002). Mars Odyssey has also played a crucial role in communication relay for subsequent ground based missions (Edwards et al., 2004).

MRO was launched in 2005. The concept of following the water led to the definition of four main goals for this mission. The first objective was to search for any past or present life on Mars, secondly, to characterise the climate on Mars, thirdly, to characterise the geology of mars and fourthly, to prepare for human exploration (Zurek, 2007). The overall aim of the mission was to increase the understanding of how Mars has evolved, as well as considering the presence and extent of water, as well as its role in evolving Mars' climate, over its history (Zurek, 2007).

The Mars Exploration Rover (MER) mission was launched in 2003, with twin rovers Opportunity (Meridiani Planum) and Spirit (Gusev Crater). MER had several aims: to identify and characterise rocks with a hydrated history, to characterise the rocks present at the landing sites; to determine the geological processes involved in shaping the surface of Mars; and to try and answer whether or not Mars has had suitable environments for life (Roncoli and Ludwinski, 2002: Squyres, 2005). In addition, the mission was intended to provide calibration and validation for orbital measurements(Roncoli and Ludwinski, 2002: Rogers, 2008).

The Phoenix mission launched in 2007, its primary objectives were to look for water ice, and study the habitability of Mars at a high latitude (Smith et al., 2008). This mission was able to confirm the presence of water ice (Smith et al., 2009b), which had been detected by Mars Odyssey(Feldman et al., 2002), it also identified perchlorate chemistry and a moderately alkaline pH of 7. 7 ±0.5 (Hecht et al., 2009).

The next phase in exploration involves a number of rovers due to land on the planet later this decade. The Mars Science Laboratory is a rover (Curiosity) mission aimed for launch in late 2011, which will assess the past habitability of Mars, landing in Gale Crater (Mahaffy et al., 2008). Its aims include identifying organic compounds and determine their nature, take an inventory of carbon, hydrogen, nitrogen, oxygen, phosphorus and sulphur, the key 'building blocks of life' and to identify features which may be the result of biological processes (Mahaffy et al., 2008).

Following this, there is planned to be a joint European Space Agency/NASA mission ExoMars and Mars Astrobiology Exploration Cache (MaxC) rover planned for launch in 2018. The ExoMars rover is intended to search for signs of past and present life on Mars and to characterise the water and geochemical environment of the shallow subsurface of the planet. The ExoMars PanCam instrument has been designed with collaborators from institutions including the Mullard Space Science Laboratory (MSSL), University College London (UCL) and Aberystwyth University. The filter set for the PanCam instrument is being designed with the mission's astrobiological focus in mind.

#### 1.8.3.1.1. ExoMars PanCam instrument

The PanCam instrument is made up of two wide-angle stereo cameras (WAC) and a high resolution camera (HRC) (Figure 1.7). Its purpose is to gather data to provide details on the mineralogy of the rover's surroundings to allow the characterisation of the environments. The PanCam will provide high resolution colour imaging and enable multispectral observations of the environment.

The wide angle cameras will enable the acquisition of colour imaging of the surrounding environment (Griffiths et al., 2006). In addition the presence of a filter wheel will enable the rover to capture multispectral images of the environment. The filter wheel contains a set of glass

filters which are each made to a specific wavelength and bandwidth. Images taken through each filter will allow reflectance values to be obtained for the field of view. The combination of 12 filters, for the ExoMars PanCam, produces a spectral profile for each pixel within the field of view (Griffiths et al., 2006: Cousins et al., 2010).

*(Figure removed for copyright reasons)*

*Figure1.10. ExoMars PanCam optical bench indicating the position of the Wide angle stereo cameras and high resolution camera (Credit: MSSL/UCL).*

The PanCam filter set has been developed from the Beagle 2 filter set which was in turn based on the Pathfinder filter set. The Pathfinder filter set was designed with the aims of identifying ferric oxides and oxyhydroxides and to determine silicate mineralogy present on Mars (Smith et al., 1997). The ExoMars mission has a slightly different aim: although the PanCam instrument cannot directly identify the presence of life, it can identify the existence of hydrated minerals and the presence of pigments present in the rocks. This has led to the redesign of the filter using sulphate, phyllosilicates, opaline silicate and calcium carbonate (Cousins et al., 2010). The limited use of minerals from alkaline/saline environments for the design of the filter sets may limit the capability of the filter sets to identify such minerals on Mars. This is an important point and will be addressed in Chapter 6 of this thesis.

All filter sets designed for the ExoMars PanCam have incorporated 12 filters with wavelengths between 440 nm and 1000 nm. This wavelength range limits the PanCam systems ability to identify rock types, because the majority of diagnostic spectral features occur beyond 1000 nm, further into the near-infrared (Cousins et al., 2010). Nevertheless there are features within the 440 nm- 1000 nm region which can be used to identify certain minerals, and with the combined analysis by other instruments aboard the rover a clear understanding of the mineralogy present can be obtained.

#### 1.8.3.2. Environments on present day Mars

Mars has become a prime target in the search for life beyond the Earth due to the evidence for liquid water on the surface during its history and the identification of contemporary subsurface ice (Fassett and Head, 2005: Fassett and Head, 2008: Smith et al., 2009b). This, coupled with the increasing information on the tolerances of life to extreme conditions makes the possibility of finding evidence of life, past or present, more promising. The habitability of Mars can be considered both in terms of its present and past environments.

Mars has an atmosphere which is predominantly carbon dioxide ~95.3%, with 2.7% nitrogen, 1. 6% argon and trace amounts of oxygen 0.13% water 0.02% and carbon monoxide 0.07% (Hansen et al., 2009). Pressure was measured at the two Viking lander sites as varying between 6.7mbar and 9.9mbar (Tillman et al., 1993) and temperature varies between -123 °C and +25 °C with diurnal and seasonal fluctuations (Horneck, 2000). In addition, the surface is exposed to solar UV radiation and ionizing radiation (IR) of solar energetic protons and galactic cosmic rays (Kminek and Bada, 2006: Dartnell et al., 2007b). The effects of radiation, pressure, desiccation etc. on organisms and amino acids is a strong argument for the most likely place to look for evidence of life being the subsurface of the planet (Dartnell et al., 2007a: Kminek and Bada, 2006). However, it is important that we understand the effects of these parameters on Life and the limits to which life can be exposed in order to gain a greater understanding of the possible habitability of Mars.

Water in the form of ice is present on the surface and is stored at the poles as ground ice (Squyres, 1984: Feldman et al., 2002: Smith et al., 2009b). The presence of contemporary water as ice on Mars is important, but the existence of liquid water on Mars today would be more exciting for the search for life on Mars. Data from the Phoenix mission has led to the hypothesis that with the presence of deliquescent salts, liquid saline water could be present on Mars. Freeze-thaw cycles can lead to the formation of saline solutions with a freezing point below the summer ground temperatures recorded at the Phoenix lander site (Renno et al., 2009) and the existence of briny, liquid water has also been suggested by the interpretation of darkened soil streaks on equator facing slopes (McEwen et al., 2011). Spectral evidence for liquid saline water has also been reported at the polar region (Renno and Mehta, 2011). The possibility that brine fluids are present on Mars means the study of organisms from salty, desiccating environments is very relevant.

#### 1.8.3.3. Environments on past Mars

Even if there is no extant life on Mars, it is still possible that life may have existed early on in its history. The chronology of Mars is defined by a crater count timescale and a mineral alteration timescale; both are split into three geological periods (Figure 1. 8). These periods are generally characterised by different depositional environments, starting with a predominantly clay depositing environment during the Noachian/Phyllosian. This is followed by a sulphate dominated depositional environment in the early Hesperian/Theiikian and finally an anhydrous

ferric oxide dominating environment during the late Hesperian-Amazonian/Siderikian period, which extends until the present.

*(Figure removed for copyright reasons)*

*Figure 1.11. Periods of Martian history divided by crater density timescale- Noachian, Hesperian and Amazonian and mineral alteration timescale-Phyllosian, Theiikian and Siderikian, both compared to the equivalent geological eras on the Earth. Martian periods are accompanied by the dominant depositional environments at that time. Adapted from (Bibring et al., 2006).*

Noachian Mars, its earliest geological period (Figure 1.8), is hypothesised to have been environmentally similar to the early Earth and more hospitable for life than modern Mars (Kempe, 1997: Westall, 2005: Carr, 2006). It is possible that evidence of life in the Noachian, if life were present, may be preserved and identified by surface missions. The possibility that Mars was more habitable in its past is supported by the strong evidence of past liquid water on its surface: with morphological features including outflow channel and basin like structures (Baker, 1979: Baker, 2001) (Figure 1.9). In the past it is thought that water could have existed as standing bodies as lakes or as an ocean (Baker, 1979).

*(Figure removed for copyright reasons)*

*Figure 1.12. (A) High resolution Mars Orbiter Camera image of a fluvial channel, south of Cerberus Rupes, Image is 4km across and (B) Small gullies in Hale Crater. Scale bar 200m. Taken from (Baker, 2001: Kottemann et al., 2005a)*

There are a large number of craters Noachian in age which may have contained standing bodies of water (Fassett and Head, 2008) and it is thought that both surface runoff and groundwater flow were important input factors to these 'lakes', such as that in the Eridania Basin (Fassett and Head, 2008) and the Jezero Crater palaeolake (Ehlmann et al., 2008a: Fassett and Head, 2005). These Noachian lakes are thought to then have disappeared through the Hesperian (3.7-3.0 Gya Figure 1.8) as the surface water would have gradually frozen and sublimed. This would have concentrated salts and other solutes, producing highly saline, brine pockets(Mancinelli et al., 2004: Mancinelli, 2005b). With a higher salt content the freezing point of the water would have been lowered, resulting in the water remaining liquid for longer on the surface and potentially providing a last refuge for life (Mancinelli et al., 2004: Mancinelli, 2005b: Grasby and Londry, 2007). If the dominant salts to form were halide then the most likely organisms to live in these environments would be halophilic (Mancinelli, 2005b).

The presence of minerals which are the product of rock, ash and/or soil and water interactions provide more evidence for past water on Mars. These minerals can either be hydrated, or anhydrous, but require water to form. Hydrated minerals usually have a hydroxyl group (OH) in

their structure or may adsorb water to their surfaces (Bishop, 2005). To remove the water from minerals where it is integral to their structure would result in a change to the composition of that mineral, whilst water adsorbed to the mineral's surface is more readily removed without altering the mineral structure.

Hydrated minerals have been identified remotely using visible-infrared spectrometers (such as the Compact Reconnaissance Imaging Spectrometer for Mars or CRISM): water and OH produce indicative bands in the spectrum at 1.4 and 1.9 μm (Gendrin et al., 2005). Hydrated minerals were deposited through the Noachian to the Hesperian period although differences in the types of minerals are apparent. The oldest deposits are Mg, Fe and Al-rich phyllosilicate clays, identified as Noachian in aged in the Mwarth region (Loizeau et al., 2007: Wray et al., 2008) and in the Nili Fossae region (Ehlmann et al., 2008c). Analcime (a zeolite mineral) has also been identified in the Nili Fossae region (Ehlmann et al., 2009). These minerals all require waters at an alkaline or neutral pH to form (Millot, 1970), which is in contrast the Hesperian-aged deposits of sulphate minerals such as gypsum which were deposited later in the stratigraphic record, and which indicate a more acidic environment (Gendrin et al., 2005).

Results from the CRISM instrument imply that the Hesperian period had a variety of aqueous environments, including those with a neutral to alkaline pH. This suggests that the Hesperian was not a period when the aqueous activity of the planet was solely acidic and sulphate-forming in nature (Ehlmann et al., 2008b). The depositional environments of the sorts of minerals identified, or hypothesised to occur on Mars are discussed in the subsequent sections.

#### 1.8.3.4. Hydrated minerals on Mars

##### 1.8.3.4.1. Phyllosilicates

Phyllosilicates are sheet silicate minerals which are formed from parallel sheets of silica tetrahedral (Weaver, 1989) and contain water in their structure. Smectite clays are a group of phyllosilicates which contains the minerals nontronite ($Ca_{.5}$ ($Si_7Al_{.8}Fe_{.2}$) ($Fe_{3.5}Al_{.4}Mg_{.1}$)$O_{20}$ $(OH)_4$) and montmorillonite ($(Na, Ca)_{0.33}$ $(Al, Mg)_2$ ($Si_4O_{10}$) $(OH)_2 \cdot nH_2O$) are aluminium-rich silicates and are the first weathering product of basic, igneous rocks(Weaver, 1989). On the Earth these clays form in the equatorial, temperate and in polar regions (Kloprogge et al., 1999: Poulet, 2005). The formation of aluminium-silicate clays is controlled by factors such as the composition of the source bedrock and the topology of the area, as well as factors such as temperature and the availability of liquid water (Millot, 1970). Smectite clays form under neutral to alkaline conditions and degrade rapidly in the presence of a low pH (Bibring et al., 2006: Ehlmann et al., 2008a: McKeown et al., 2009). The clay deposits on Mars could potentially preserve evidence of life, therefore making them exciting astrobiological targets (Ehlmann et al., 2008a).

Spectra acquired by the CRISM instrument have indicated the presence of minerals with water in their structure, suggested by absorption features at 1900 nm and 1400 nm (Mustard, 2008).

These deposits are predominantly Fe/Mg rich, with some Al rich deposits and been identified as smectite clays, including Nontronite and montmorillonite. Phyllosilicate clay deposits have been identified globally (Figure 1.10), although they tend to be restricted to older terrains (Mustard, 2008). These areas include; Eberswalde, Holden and Jezero Crater (Ehlmann, 2008), Mwarth Vallis, where magnesium rich clay and Na rich montmorillonite deposits form part of a large geological unit (Loizeau et al., 2007: Bishop et al., 2008), and Syrtis Major region, where deposit were confirmed as beds of clay, rather than a coating of material (Poulet, 2005: Bibring et al., 2006: McKeown et al., 2009). Deposits of iron-magnesium smectite bearing rocks have been identified in several lake basins and associated deltas, suggesting the presence of large scale aqueous reservoirs of alkaline waters, due to the non-acidic pH required for clay formation (Bibring et al., 2006).

*(Figure removed for copyright reasons)*

*Figure 1.13. CRISM targeted images surveyed for clay minerals, these have been group by depositional environment; crustal clays (b), sedimentary clays (c) and clay in stratigraphy (d). Open symbols represent areas where no clays were identified. Taken from (Ehlmann et al., 2011b)*

Possible environments in which the phyllosilicates could have formed include subsurface hydrothermal activity and impact cratering, with subsurface water mixing with impacted minerals (Poulet, 2005: Bibring et al., 2006: Fairen et al., 2010: Ehlmann et al., 2011b). A review on the clay deposits suggested that they were formed through hydrothermal groundwater circulation, which would allow for a cool and dry Martian surface, with only transient surface water (Ehlmann et al., 2011b). These materials do not require a prolonged period of liquid water on the planet's surface.

#### 1.8.3.4.2. Carbonates

Carbonate minerals are important for astrobiology because they generally require water to form (Bishop, 2005). Evidence from Martian meteorites containing carbonates(McKay et al., 1996), geochemical models for Mars predicting carbonate formation (Ehlmann et al, 2008b), and the identification of carbonate in the Martian dust (Bandfield, 2002)implied that it was only a matter of time before carbonates were identified in outcrops.

The CRISM instrument aboard MRO was the first to identify carbonate deposits on Mars, in an area within the Nili Fossae region (Ehlmann et al., 2008b). The spectra obtained for these carbonates best fit to the spectra for the magnesium carbonate magnesite (Ehlmann et al., 2008b). These deposits are associated with Fe-Mg smectite-bearing formations and olivine units, which were deposited during the Noachian to early Hesperian (Ehlmann et al., 2008b). It is believed that these Martian carbonates were formed in either hydrothermal fluids or near surface waters which were neutral to alkaline (Ehlmann et al., 2008b). An alternative hypothesis is that olivine rich rocks were weathered at the surface at ambient temperatures, during fluid activity in the Nili Fossae region, or that ephemeral lakes rich in $Mg^{2+}$ produced these carbonate deposits on the surface (Ehlmann et al., 2008b).

Subsequently, the Spirit rover identified a concentration of magnesium and iron-rich carbonates in situ, in outcrops in the Columbia Hills of Gusev Crater (Morris, 2010), which are similar to the carbonates present in the meteorite ALH84001 (Chassefiere and Leblanc, 2011). These deposits are thought to have formed by hydrothermal fluids, at a near neutral pH(Squyres et al., 2006). This is a contrast to the acidic ground waters believed to have existed in the Meridiani Planum area explored by the Opportunity rover (Squyres et al., 2006).

Following this, the TEGA and MECA instruments aboard the Phoenix lander identified around 3-5 wt% calcium carbonate in the soil (Boynton et al., 2009). This carbonate could have formed through the precipitation of calcium carbonate from saturated films of water in the subsurface, alternatively, they could have formed from hydrothermal aqueous alteration (volcanic or impact) (Boynton et al., 2009).

#### 1.8.3.4.3. Chloride minerals

Chloride salts are a group of evaporite minerals and include the NaCl mineral halite. Evaporites are water soluble deposits which, on Earth, precipitate from a concentrated fluid due to evaporation and are described as either marine, or non-marine (Jackson and Bates, 1997: Warren, 2006). On Earth, chloride deposits form in saline lakes, in hydrothermal brines, as volcanic sublimates, or by the direct crystallization of salts onto sediment grains (efflorescence) (Osterloo et al., 2008). Water is necessary for the formation of evaporites and the composition of the minerals which form is dependent upon the composition of the water at the point when evaporation occurs (Osterloo et al., 2008: Warren, 2006). Terrestrial chlorides, such as the sodium chloride mineral halite, generally precipitate last in alkaline environments, following the deposition of carbonates, sulphates and silica (Osterloo et al., 2008). On Mars, loss of ground water to the atmosphere by evaporation/sublimation would lead to saline conditions, which could occur in many of the crater basins (Forsythe and Zimbelman, 1995).

Halite has been identified in the Nakhla meteorite in association with siderite and anhydrite (Bridges and Grady, 1999). Halite rinds and rock coatings have been identified at Meridiani Planum, which are believed to be the result of a fluid evaporating to dryness (Tosca et al.,

2008). Chloride salt deposits of mid Noachian and Hesperian age have been identified in the southern highlands of Mars, by the Mars Odyssey orbiter, and are thought to have formed from brines (Osterloo et al., 2008).

Halite is potentially a very important mineral in relation to astrobiology. Firstly its occurrence indicates that liquid water has been present in that locality, in addition it is a hygroscopic mineral, meaning that it has the ability to attract water molecules from the surrounding environment (Vitek et al., 2010). Some hygroscopic minerals attract water so readily that they dissolve in the water they attract and are termed deliquescent; halite is one of these minerals (Vitek et al., 2010). As well as providing a layer of liquid water around halite, fluid inclusions of liquid water occur within the mineral during its formation. It is within fluid inclusions that organic compounds have been preserved for long periods and contentious results show that bacterial spores have been extracted and revived (Vreeland et al., 2007: Vreeland et al., 2000: Mormile et al., 2003: Fish et al., 2002: Satterfield et al., 2005). Halite can provide protection for organisms through scattering of UV (Fendrihan et al., 2009) whilst being transparent enough for photosynthesis(Cockell and Raven, 2004) and studies have shown that organisms can still metabolise whilst contained within halite(Rothschild et al., 1994).

#### 1.8.3.4.4. Tectosilicates - Zeolites

Zeolites are microporous and crystalline, aluminosilicate minerals, generally forming where volcanic rocks and ash layers react with alkaline ground waters (Eugster and Jones, 1968: Ehlmann et al., 2009: Poulet, 2010). The microporous nature means that they can contain $H_2O$ molecules in the interlayers (Bish, 2003).

Terrestrial analogue studies (in Antarctica) of possible Martian weathering processes have indicated that zeolites may predominate over clay minerals when the weathering is in a cold and dry environment (Fialips, 2005). Zeolites having been recognised in the dust of the Martian surface (Ruff, 2004a), as well as in deposits, identified as analcime, in the central peaks and walls of craters in Nili Fossae (Ehlmann et al., 2008a: Ehlmann et al., 2009). Those identified in impact craters are possibly related to impact hydrothermal fluids, but this does not rule out the potential of finding alteration products associated with alkaline, non-impact related fluids (Ehlmann et al., 2008c). OMEGA spectral data around the Phoenix landing site showed the presence of minerals with distinct hydration features which are consistent with zeolites, for example chabazite (Poulet, 2010). Identifying the extent of zeolites on Mars is problematic, this is due to difficulties in distinguishing between zeolites and polyhydrated sulphates minerals using the CRISM instrument (Wray et al., 2009).

It is thought that the zeolites may contain significant amounts of $H_2O$ (Bish, 2003). The potential for saline brine ground water on Mars, along with the hydrolysis of volcanic glass which would lead to elevated pH levels, are perfect conditions for zeolite formation (Ruff, 2004a). These environmental conditions would be potentially habitable for life on Mars (Ruff, 2004a).

#### 1.8.3.4.5. Other hydrated minerals on Mars

Jarosite is a hydrated potassium sulphate which on Earth predominantly forms in acidic environments, it is rarely identified at a pH>4 (Dutrizac and Jambor, 2000: Elwood Madden et al., 2004). In contrast to these conditions, usually required for its formation, a small quantity of Jarosite has been identified in association with soda lake deposits in Oldovai Gorge, Tanzania (McHenry et al., 2009). This jarosite is found in association with zeolites, predominately phillipsite, Mg/Fe smectites which indicates a saline, high pH (pH>9) environment (McHenry et al., 2011). Jarosite has also been identified in a neutral, freshwater spring environment and in an acidic microenvironment in a highly buffered carbonate environment (Ashley et al., 2004: Leveille, 2007: Darmody et al., 2007).

Iron and magnesium sulphate minerals such as gypsum and jarosite have been identified in areas including the Terra Meridiani (Squyres, 2004), Valles Marineris (Bibring et al., 2006), Meridiani Planum (Andrews Hanna et al., 2007) and around the northern polar cap in layered deposits (Bibring et al., 2005: Bibring et al., 2006). Evaporative sulphate deposits would have formed during the arid and acidic conditions which occurred in the later Noachian and Hesperian (Andrews Hanna et al., 2007). The Opportunity rover has identified jarosite in the Meridiani Planum landing site on the ground, although it has not been identified in this region by orbital instruments (Elwood Madden et al., 2004).

Although there are in many areas where there is spatial separation between the phyllosilicates and the sulphates, this is not necessarily the case for all deposits (Poulet, 2005: Farrand et al., 2009). These close associations between minerals forming in acidic and alkaline environments on Mars suggests a variation in pH on a local scale, with phyllosilicates possibly forming in the subsurface and sulphates on the surface (Baldridge et al., 2009a). This would signify that the presence of one mineral should not be used as an indicator of the pH of an area as a whole (Baldridge et al., 2009a). It is possible that palaeolake deposits, such as those at Columbus Crater, Terra Sirenum or Terra Meridiani(Wray et al., 2009: Poulet et al., 2008) could have complex depositional histories, similar to that identified at Oldovai(McHenry et al., 2011).

## 1.9. Thesis summary

### 1.9.1. Motivation for this work

The study of analogue environments on Earth is an integral part of understanding the possibility for extraterrestrial life. Acidic environments have received the highest proportion of attention in Martian analogue studies thus far, due to the idea that Mars has been a solely acidic environment. However, the increasing amount of information on the geology of Mars suggests it is actually a geologically heterogeneous planet, with a complex depositional history. In addition, there is a possibility that alkaline/saline environments exist on Europa and Enceladus. For these reasons, the study of alkaline/saline aqueous environments needs to be a part of future analogue and astrobiological studies, with this thesis contributing to this area of research. It is

important to understand the limits of life from these environments; this includes understanding community structure of bacteria and archaea which live there and understanding the resistance of isolates to conditions which would be experienced on Mars. The need to identify deposits associated with a neutral-alkaline pH and high salinity on Mars means that it is necessary to test the resolution and capabilities of equipment. This testing must be done using minerals from alkaline/saline environments, in addition to those minerals which form in more acidic environments. This testing can ensure that both types of environmental condition can be identified by surface equipment, so that alkaline environments could be identified and studied on the surface of Mars.

### 1.9.2. Summary of chapters

**Chapter 2**, the materials and methods chapter describes the materials which were sampled from Lake Magadi, as well as outlining methods and protocols which are widely used in environmental microbiology and/or astrobiological research.

**Chapter 3** involves the design of a novel growth medium and subsequent isolation of strains from various samples from Lake Magadi. This is complemented by the culture independent DGGE analysis of several of the samples. Finally, three novel isolates from this study are chosen for more in depth description and analysis of their phylogenetic relationships.

**Chapter 4** details the characterisation of three novel strains from Lake Magadi which were isolated in Chapter 3. It focuses upon characterising their pH, temperature and NaCl concentration limits and optima, as well as determining their antibiotic resistance, enzyme production and ability to utilise different carbon sources.

**Chapter 5** examines the response of Lake Magadi isolates when exposed to desiccation and UV-C irradiation, using culture based techniques to assess the short term and longer term (where possible) effects upon the survival fraction of each isolate, isolated in Chapter 3, with several isolates also characterised in Chapter 4.

**Chapter 6** assess the effects of simulated Martian conditions on the novel isolate *Planococccus sp.* LMLD02, isolated in Chapter 3 and characterised in Chapters 4 and 5. It also focuses on the effects of simulated Martian conditions on a complex soil community, whose culturable community and diversity were assessed in Chapter 3. A variety of techniques are implemented to assess effects of simulated Martian conditions on the community including: measuring the effects upon the number of colony forming units calculated from the colonies present on 0.7%, 6.8% and 15% NaCl media, assessing changes in metabolic activity through the use of Biolog™ Ecoplates and finally the identification of organisms capable of surviving Mars chamber exposure through the sequencing of isolates cultured after the Mars chamber incubation.

**Chapter 7** tests the filter sets designed for the ExoMars, Pathfinder/Beagle 2, MSL and MER missions to Mars in terms of their ability to reproduce the spectra for a variety of minerals which form in alkaline/saline environments. This included sample collected from Lake Magadi, samples from the Geological Collections-UCL which are known to form at Lake Magadi but were not sampled and carbonate minerals which form from alkaline environments but with a different hydrochemistry. In addition, in order to demonstrate the sorts of biosignatures produced by the microbial communities present in alkaline/saline environments the filter sets were tested using spectra obtained from biological mats which were studied in Chapter 3.

# Chapter 2

# Materials and Methods

Astrobiology is an area of research which necessitates the use of a variety of different techniques. Its multidisciplinary nature requires the use of analytical methods drawn from the fields of biology, chemistry and geology. This chapter describes the sampling localities, laboratory methods and analytical techniques used throughout this thesis.

### 2.1. Sampling environment

The sampling locations were chosen on the basis of ease of access and in order to include samples from a variety of environmental conditions. African Rift valley contains around 30 active volcanoes. Ol Doinyo Lengai is a unique volcano, being the only active carbonatite volcano in the world and is 97.8 km to the southwest of Lake Magadi. Volcanism began in the southern part of the rift between 16 to 20 Ma. The region is arid (Chapter 1, Section 1.3), the average temperature and rainfall in the area are outlined in table 2.1.

*Table 2.1. Mean temperature and rainfall in the Lake Magadi area. Taken from World Meteorological Organization (WMO)*

| | Mean Temperature °C | | Mean Total Rainfall (mm) |
|---|---|---|---|
| | Daily minimum | Daily maximum | |
| January | 11.5 | 24.5 | 64.1 |
| February | 11.6 | 25.6 | 56.5 |
| March | 13.1 | 25.6 | 92.8 |
| April | 14.0 | 24.1 | 219.4 |
| May | 13.2 | 22.6 | 176.6 |
| June | 11.0 | 21.5 | 35.0 |
| July | 10.1 | 20.6 | 17.5 |
| August | 10.2 | 21.4 | 23.5 |
| September | 10.5 | 23.7 | 28.3 |
| October | 12.5 | 24.7 | 55.3 |
| November | 13.1 | 23.1 | 154.2 |
| December | 12.6 | 23.4 | 101.0 |

#### 2.1.1. Biological sampling sites

Samples were collected from seven sites around Lake Magadi in the May of 2009. Sites were located in the surrounding flood plain area, the hydrothermal springs and lagoons. Two sample sites were located to the south west of the lake and five sites were located around the lake, in areas of hydrothermal spring emergence (Figure 2.1.A).

*(Figure removed for copyright reasons)*

*Figure 2.1 Map of Lake Magadi **(A)** Google Earth satellite image, marking sampling sites (Table 2.2), **(i)** Northern end of Little Magadi, **(ii)** Northern end of Lake Magadi, **(iii)** Western Lagoon, **(iv)** Southern end of Lake Magadi. **(B)** Map indicating springs, lagoons and trona platform (Baker, 1958).*

The spring locations for sampling were chosen on using data presented by Baker (1958), which mapped the hydrothermal springs, and provided measurements of temperatures at each location (Figure 2.1.B). The data demonstrated a temperature gradient, with those the most northerly springs reaching temperatures of ~85 °C and those in the south ~40 °C (Baker, 1958).

The sites were chosen to provide samples for biological analysis from a range of temperature environments and due to the potential for different water chemistries. On this basis, samples were collected from three broad localities: the lower temperature springs and lagoon samples to the south, <44 °C (Figure 2.1 and Figure 2.2.C-H), the springs to the north of Lake Magadi, ~67 °C (Figure 2.1) and to the north of Little Magadi, >81 °C (Figure 2.1 and Figure 2.2.I-K).

The sample localities and samples collected are listed in Table 2.1. The samples collected from the springs and lagoons have been the focus of the biological analyses described in Chapter 3, with the isolated strains from these samples being the focus of the work detailed in Chapters 4, 5 and 6.

The sampling areas 2, 3, 4 and 7, to the south of Lake Magadi (Figure 2.1.A) had no significant salt crusting or trona deposits and were easily accessible. The springs bubbled up into pools which then drained into the lagoon. The springs and pool waters were very clear.

The main pool at site 13 (area 3) (Figure 2.2.C.ii) was surrounded by smaller 'puddles' which probably separated from larger pools as it evaporated. In some areas biomats were present on the ground, and were partially submerged in shallow water, these were dark green or pink in colour (Figure 2.2.D and Table 2.2). Other springs had a covering of yellow biomass of which the spring water flowed (Figure 2.2.E and G). Springs to the east (area 7) emerged into a sandy lagoon bed where the water could be seen bubbling up. The waters were again clear but some green material with bubbles was present on the bottom and the surface of the lagoon.

Site 4 was the Western lagoon; there were no springs at this site (Figure 2.2). The waters were murkier than at the spring pools and lagoon bed sediment was unconsolidated and brown. There were no biomats present with the exception of a small, bright pink coloured material on the lagoon bank (Table 2.2).

Site 5, to the North of Lake Magadi differs to all other sites by the presence of a generally thin <1cm white mineral deposits over the ground (Figure 2.2.I & J) and was difficult to access. The springs flowed as narrow streams towards the north of Lake Magadi. Biological samples were very dark green or dark red with no biomats comparable to the yellow and pink thick biomats seen at the south.

Finally, area 6 to the north of Little Magadi was the highest temperature site, there was a strong smell of sulphur. This area also lacked the extensive yellow and pink/bright green biomats which we found at the south but unlike site 5, there was no extensive white precipitate on the soil surface (Figure 2.2.K). The springs, as at the north of Lake Magadi, immediately flowed south into Little Magadi as channels. Along the stream there were small pools where some biomass material was present. There were some slightly raised areas which had small 0.5 m diameter pools within the main stream, containing orange and red particulate biological material (Table 2.2).

Of all the samples collected only a percentage of these samples could be analysed in this study (those in black in table 2.2). Samples were chosen based upon similarities between sample types between sample areas and simply based upon a desire to analyse a soil sample, water sample and biological sample, the latter being chosen, in part, due to the presence of bright pigmentation in the sample.

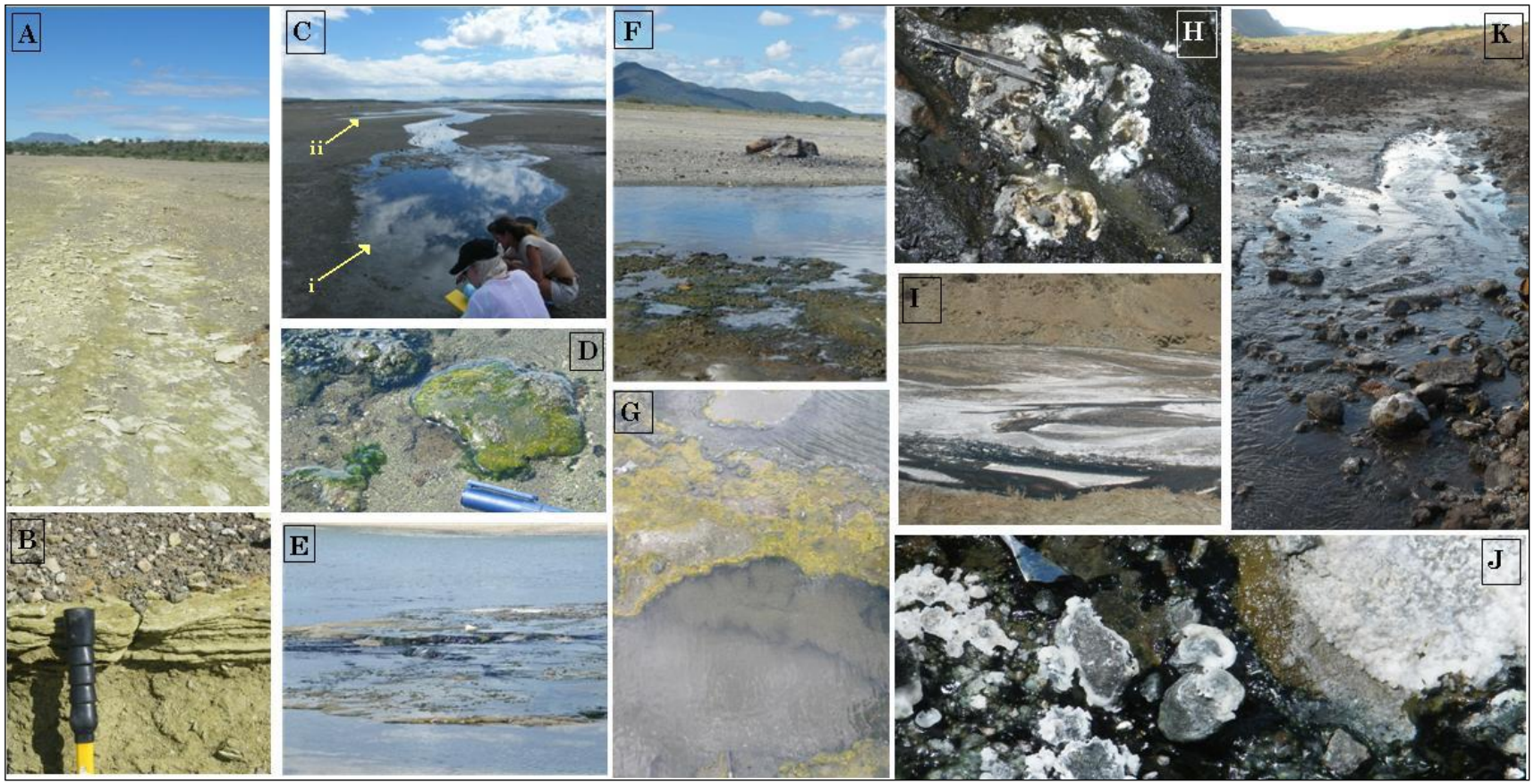



*Figure 2.2.* ***(A)*** *Area 2, Site 11* ***(B)*** *Sample 48 (GR1) (Table 2.3),* ***(C)*** *Area, 3* ***(i)*** *Site 12* ***(ii)*** *Site 13* ***(D)*** *Sample 61* ***(E)*** *Area 4, Site 18* ***(F)*** *Area 3, Site 13* ***(G)*** *Sample 76* ***(H)*** *Sample 133* ***(I)*** *Area 5* ***(J)*** *Area 5, Site 20* ***(K)*** *Area 6, Site 25* (Table 2.1).

*Table 2.2. Biological, sterile sampled samples collected from each site during May 2009 from various localities around Lake Magadi and its vicinity. N/R- not recorded. Those in black have been analysed in this study.*

| Region | Site | GPS | Temperature | pH | Sample | Comment |
|---|---|---|---|---|---|---|
| Area 1 | 10 | | N/R | N/R | 44 | Salt encrustation from soil surface |
| Area 3 | 12 | 02/00/157 36/12/887 | 45°C | 10.01 | 52a | Water sample from smallest pool-biological sample |
| | | | | | 52b | Water sample from smallest pool-cation and anion samples |
| | | | | | 53 | Red/pink and green covering of soft biological material covering rock surface |
| | | | | | 54 | Sample of bedrock- fine grained, light brown |
| | | | | | 55 | Thin layers of green biological material, particulate, rises and sinks to bottom of pool |
| | | | | | 56 | Bright green material on surface of water, bubbly appearance |
| | | | | | 57 | Gravel from bottom of pool with layer of green material |
| | 13 | 02/00/150 36/13/688 | 39.5°C | 9.97 | 58 | Bright pink soft material associated with yellow soft material |
| | | | | | 59 | Blocks from spring flow, encrusted with green material on the surface |
| | | | | | 60 | Orange, soft material on surface of bedrock |
| | | | | | 61 | Soft, green material, oval in shape and around 1.5 cm thick. Located in shallow water |
| | 14 | 02/00/127 36/13/889 | 44.4°C | 9.96 | 63a | Water sample- biological sample |
| | | | | | 63b | Water sample- cation and anion sample |
| | | | | | 64 | Encrusted block with dark green material, some patches have a pink colouration |
| | | | | | 65 | Biological material sampled from the bottom of the pool. Soft and yellow/pink coloured |
| | | | 31.3°C | 9.97 | 66 | Pink/red material – 'seaweed like' in appearance in small pool 0.5m from sample 65 pool |
| Area 4 | 16 | N/R | N/R | N/R | 70 | Samples from northern area of south western lagoon. Bright pink, wet material on mud |
| | | N/R | 35.7°C | 10.0 | 71a | Water sample-biological sample |
| | | | | | 71b | Water sample- cation and anion sample |
| | | | | | 72.1 | Brown/pink material, of a particulate nature. Floats on the surface of the water in the |
| | | | | | 72.2 | Mud and water scoop sampled from bottom of lagoon (near edge) |

*Table 2.2 continued.*

| Region | Site | GPS | Temperature | pH | Sample | Comment |
|---|---|---|---|---|---|---|
| | 17 | N/R | N/R | N/R | 73 | Vesicular blocks brown and black from edge of the lagoon |
| | 18 | 02/00/067 36/13/916 | 44.6°C | 9.97 | 74a | Water sample- biological sample |
| | | | | | 74b | Water sample- cation and anion sample |
| | | | | | 75 | Pink material floating in current, under surface is green in colouration |
| | | | | | 76 | Slightly soft, yellow material attached to bedrock surface |
| | | | | | 77 | Black and pink material submerged in water, covering bedrock surface |
| Area 5 | 20 | 01/45/273 36/18/267 | 64.4°C | 9.07 | 80a | Water sample- for biological analysis |
| | | | | | 80b | Water sample- cation and anion sample |
| | | | | | 81 | Pink, soft material, particulate and breaks up when sampled |
| | | | | | 82 | 'Stringy' material, dark green in colour |
| | | | | | 83 | Dark green soft material on the floor of the spring |
| | 21 | 01/45/354 06/18/289 | 64.3°C | 9.29 | 85a | Water sample- biological sample |
| | | | | | 85b | Water sample- cation and anion sample |
| | | | | | 86 | Scoop sample of mud- pink and white fine grained material within it |
| | 22 | 01/45/367 36/18/287 | 66°C | 9.38 | 87 | Pink and orange material scoop sampled near edge of pool |
| | | | | | 89 | Dark red/orange material attached to dark green material which is at the surface of the |
| | | | | | 90 | Salt crust of a pinky/orange colouration on mud at edge of spring |
| | 23 | 01/45/363 36/18/298 | 57.1°C | 9.31 | 91 | Orangey/brown, bubbly looking material, very shallow water |
| | 24 | 01/45/220 36/18/242 | 63.1°C | 9.31 | 94 | Dark red material in a pool. Dark green floating at surface attached to red material below |
| Area 6 | 25 | 01/43/143 36/13/267 | 59.1°C | 9.49 | 95 | Water sample at top of spring area |

*Table 2.2 continued*

| Region | Site | GPS | Temperature | pH | Sample | Comment |
|---|---|---|---|---|---|---|
| | 26 | 01/43/147<br>06/16/266 | 82.7°C | 9.48 | 102 | Block with precipitate on surface |
| | | | | | 103a | Water sample- biological sample |
| | | | | | 103b | Water sample- cation and anion sample |
| | | | | | 104 | Sediment sample |
| | | | | | 105 | Vesicular block with precipitant on surface |
| | 27 | N/R | 82.4°C | 9.51 | 106a | Water sample- biological sample |
| | | | | | 106b | Water sample- cation and anion sample |
| | | | | | 110 | Grey material precipitate on blocks in flow |
| | | | | | 113 | Scoop sample of sediment and water |
| | | | N/R | N/R | 114 | Non submerged rock and white precipitate on surface |
| | | | 38°C | N/R | 107 | Raised area of pooling, biological material |
| | | | 44°C | 9.51 | 108 | Red material in raised area |
| | | | | | 109 | Orange particulate matter in same pool as sample 108, on surface |
| | | | | | 110 | Grey material |
| | 28 | N/R | N/R | N/R | 115 | Pinky, bubbly material on surface of water |
| Area 7 | 29 | 01/59/883<br>36/15/442 | 36.6°C | 9.97 | 116a | Water sample- biological sample |
| | | | | | 116b | Water sample- cation-anion sample |
| | | | | | 117 | Green material attached to wood at bottom of spring |
| | | | 36.6°C | 9.97 | 133 | Pinky/ white material in blocks on sediment surface |
| | 30 | 01/59/878<br>36/15/444 | 40.2°C | 9.97 | 121a | Water sample- biological sample |
| | | | | | 121b | Water sample- cation and anion sample |
| | | | | | 123 | Green material floating on water surface, bubbly appearance |
| | | | | | 124 | Crumbly unconsolidated white material |

*Table 2.2 continued*

| Region | Site | GPS | Temperature | pH | Sample | Comment |
|---|---|---|---|---|---|---|
| | 31 | 01/59/870 36/15/450 | N/R | N/R | 125 | Dark sediment sample |
| | | | N/R | N/R | 126 | Scoop sample of mud and white crust |
| | | | 40.3°C | 9.98 | 127 | Green material from bottom of spring, slimy nature |
| | | 01/59/872 36/15/451 | 39.3°C | 9.97 | 129 | Bright green material from bottom of pool |
| Area 8 | 32 | N/R | 32.9°C | 9.78 | LME-1 | Soil sample from lake edge-0.5 m |
| | | N/R | 32.9°C | 9.78 | LME-2 | Soil sample from edge of lake- 3.5 m |
| | | N/R | 32.9°C | 9.78 | LME-3 | Whiteish, opaque/translucent geological sample with green colouration around edge of sample |

### 2.1.2. Geological sampling sites

In addition to the biological samples geological samples were collected from different sites around the lake. Samples of prominent rock outcrops were collected from site 2 (Figure 2.1A), including sample 48-GR1 and sample SR1 from site 6 (Table 2.3). Samples of the trona platform were obtained, courtesy of from the Magadi Soda Company (Table 2.3). It was not possible to gain access to the trona platform by foot at either Little Magadi or Lake Magadi. The samples from Magadi Soda Company were broken up pieces of the trona platform which had been dropped on the way to the trona processing, therefore were untreated. These geological samples have been the focus of the alkaline mineral, multispectral instrument testing in Chapter 7.

## 2.2. Sample collection

### 2.2.1. Sediment water and biomat samples for culturing

Sampling equipment was sterilised using 70% ethanol and flamed. Samples were collected in sterile, autoclaved containers, pre-sterilised falcon tubes and Whirlpak$^{TM}$ sterile bags. Samples were stored in cool boxes on ice. The samples were then stored at 4°C upon arrival in the UK.

### 2.2.2. Water samples for anion/cation analysis

Water samples for compositional analysis were collected and filtered using a syringe and stored in 15 ml containers. 50% nitric acid solution was added to stabilise the cation samples. All samples were filtered through a 0.45 µm filter and stored in cool boxes with ice. Samples were collected for carbonate titration; the containers were filled to the very top to limit air contact with the sample and again stored in cool boxes.

The temperature and pH was taken in situ at every site using a separate thermometer and pH meter. Conductivity and dissolved oxygen readings could not be taken as the equipment failed in the field. Water samples were analysed by the Wolfson Laboratory for Environmental Geochemistry using Horoboa JY Ultima 2C CP-AES for anion analysis and Dionex Ion Chromatograph for cation analysis

*Table 2.3 Geological samples collected from each site during May 2009 from various localities around Lake Magadi and its vicinity. N/R- not recorded. Samples labelled MGS were obtained from the Lake Magadi Soda Company which dredges the Lake. Those in bold have been analysed in this study*

| Region | Site | GPS | Sample | Comment |
|---|---|---|---|---|
| Area 1 | 10 | 02/04/622 36/04/222 | 42 | Broken up salt encrusted blocks, dark brown (fine grained) with black specks within |
| | | | 45 | Broken open sediment nodule with zoning of dark areas within |
| | | | 46 | Salt encrustation from sample 45, thinner layer than sample 43 or 44 |
| Area 2 | 11 | 02/01/086 36/13/939 | GR1 (48) | Chalky, light material green/yellow in colour, sampled from a dried up channel area. Fine grained |
| | | | 50 | Dark green, fine grained, chert like material |
| | | | 51 | Rock sample of white fine grained material, cracks between with grey fine grained matrix |
| Area 3 | 15 | 02/00/054 36/13/821 | 67 | Dark black sediment, fine grained |
| | | | 68 | Salt crust 1-2mm, light brown, fine grained sediment below. |
| Area 5 | 20 | 01/45/273 36/18/267 | 93 | Sediment sample |
| Collected on the way back from | - | N/R | SR1 | Sample of light brown, and lightweight sediment/rock |
| Collected from MGS | | N/R | MGS 1 | Slightly crumbly sample with transparent sharp pieces ~1cm in length & 2mm depth |
| | | N/R | MGS 2 | Black and green semi translucent sample |
| | | N/R | MGS 3 | Black semi translucent sample with cream/white layers within |
| | | N/R | MGS 4 | A white/grey coloured sample with patches of red on the surface |
| | | N/R | MGS 5 | Opaque/semi translucent greenish material with a covering of grey/white material. |
| | | N/R | MGS 6 | Dark brown material with thin layer of white powder |
| | | N/R | MGS 7 | Grey green material with layering present visible on surface |
| | | N/R | MGS 8 | Cream/beige conglomerate material with clasts of approximately 1 cm in diameter within a white grey fine grained matrix. One surface is black |
| | | N/R | MGS 9 | White opaque sample |
| | | N/R | MGS 10 | Cream beige sample with layer visible on surface |
| | | N/R | MGS 11 | White opaque sample |
| | | N/R | MGS 12, 13,15, 17 | Crystalline samples yellow/cream coloured and semi transparent |

## 2.3. Analytical techniques in biology

### 2.3.1. Isolation of strains

#### 2.3.1.1. Sterilization

All reagents, culture media and lab ware to be sterilized were autoclaved at 121°C for 20 minutes.

#### 2.3.1.2. Growth media

The application of each growth medium is described in the appropriate chapter but the composition of the widely used growth media is outline below.

##### 2.3.1.2.1. Tryptone Glucose Yeast (TGY) medium

TGY agar contains 0.5% Bacto-tryptone, 0.3% yeast extract, 0.1% glucose, and 1.5% agar. TGY broth contains 1% Bacto-tryptone, 0.6% yeast extract and 0.2% glucose (Battista, 1997).

##### 2.3.1.2.2. Nutrient broth 2 (NB2) medium

NB2 medium contained 1.5% Sigma Nutrient Broth 2 (4.3 g $l^{-1}$ casein peptone, 4.3 g $l^{-1}$ meat peptone, 6.4 g $l^{-1}$ of NaCl) and 1% agar. NB2 broth contained just 1.5% Sigma Nutrient Broth 2.

##### 2.3.1.2.3. Culture media for Lake Magadi isolates

Developed and described in Chapter 3, section 3.4.2.

### 2.3.2. pH calibration

pH of the basic AP+ medium was altered using $Na_2CO_3$/$NaHCO_3$ buffer, the pH of the medium was measured using a Mettler Toledo Seven Easy pH meter and a probe with a range of pH 0-14. A pH of 9.6 was obtained for the growth medium and was calibrated at 37 °C or 50 °C.

### 2.3.3. Growth conditions

AP+ plates were incubated at 37 °C for period between 1 day and 3 days, and at 50 °C for two weeks. From each batch of agar, control plate was also incubated to ensure no contamination of the agar from other microorganisms. Pure cultures were obtained by sub culturing from 1 to 4 times onto fresh media.

### 2.3.4. Isolation from soil and biomass samples

Samples of soil (~100µl) were suspended in 900µl of sterile phosphate buffered saline and vortexed. Samples were diluted depending upon the organic content of the sample, 100-150 µl of the sample was plated onto solid media.

### 2.3.5. Isolation from water samples

150 µl of water sample was plated directly onto solid media.

### 2.3.6. Isolation from soil and biomass samples

Isolates were streaked onto fresh media using either a flame sterilised loop or disposable inoculation loop.

### 2.3.7. Storage of pure cultures

Pure cultures from plates were suspended in a ≥35% glycerol solution and frozen at -20 °C or -80 °C.

## 2.4. DNA extraction

DNA was extracted from samples using E.Z.N.A Soil DNA Kit (D5625) using 0.5g of sample. The soil DNA kit protocol was followed; for step one the samples were taped horizontally to a vortex and vortexed for five minutes to lyse the cells

## 2.5. Polymerase chain reaction (PCR)

### 2.5.1. Sequencing primers

The primers BF1 and UN1492R (MWG Operon) were used for sequencing bacterial samples in the forward and reverse direction respectively. Arch 21F and UN1492R (MWG Operon) were used for sequencing archaea isolates. For DGGE analysis, the primers 16S_341FGC and 16S_518R (MWG Operon) were used with the primers 16S_341F and 16S_518R being used to sequence the bands extracted and cloned into *E. coli* described below in section 2.3.5.4.

*Table 2.3 Primer pairs used for PCR amplification for Bacteria and archaea*

| Use | Primer pair | Sequence | Target | Reference |
|---|---|---|---|---|
| Colony PCR | BF1<br>UN1492R | TGAATAACGAGGGGCAATATAAC<br>GGTACCTTGTTACGACTT | Bacteria | (DeLong, 1992: Lehmann et al., 2008) |
| Colony PCR & extracted DNA PCR | Arch 21F<br>UN1492R | TTCCGGTTGATCCYGCCGGA<br>GGTACCTTGTTACGACTT | Archaea | (DeLong, 1992) |
| Extracted DNA PCR | 16S_341FGC<br>16S_518R | CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG<br>ATTACCGCGGCTGCTGG | Bacteria | (Muyzer, 1993) |
| Extracted DNA PCR | 16S_341F<br>16S_518R | CCTACGGGAGGCAGCAG<br>ATTACCGCGGCTGCTGG | Archaea / Bacteria | (Muyzer, 1993) |

### 2.5.2. Colony PCR

For amplification of individual colony DNA a single colony was picked from solid media using a sterile pipette tip and suspended in 450 µl of PBS (Sigma). This was used as a template. The total reaction volume of 50 µl was comprised of: 1µl template DNA, 0.5 µl 10x BSA (NEB), 1 µl of 100% DMSO (Sigma), 5 µl 10x polymerase buffer with $MgCl_2$ (Sigma), 0.3 µl BF1 forward primer for bacterial amplification and Arch21F for archaea amplification, 0.3 µl UN1492R reverse primer for both bacterial and archaea amplification (Table 2.4), 0.5 µl Taq DNA polymerase (Sigma), 0.05 µl Pfu DNA polymerase (Sigma) and 41.35 µl of molecular grade water (Sigma). The PCR reaction was run on either a PCR Sprint Thermohybaid or Techne TC-512 Thermal Cycler. PCR products were run on a 1% agarose gel. Hyperladder 1 was used to estimate fragment size.

*Table 2.4 Touchdown PCR program to reduce the effects of non-specific binding*

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Denaturation | 96 °C | 5 minutes | 1 |
| Annealing step 1-5 | 94 °C | 1 minute | 1 cycle for each annealing temperature |
| | 60.5 °C, 59.5 °C, 58.5 °C, 57.5 °C, 56.5 °C | 30 seconds | |
| | 72 °C | 2 minute | |
| Annealing step 6 | 94 °C | 1 minute | 20 |
| | 55.5 °C | 30 seconds | |
| | 72 °C | 2 minute | |
| Extension | 72 °C | 10 minutes | 1 |
| Holding | 10 °C | - | - |

**2.5.3. Extracted DNA PCR**

For bacterial amplification, extracted DNA (Section 2.4.2) was used as a template. The amplification of archaea strains for DGGE analysis required the initial amplification of the extracted DNA (Chapter 2, section 2.3.2) using the Arch21F and UN1492R primers and PCR program 1, outlined in Table 2.3. The PCR product was then diluted 1:25 µl with molecular grade water. This was then used as template DNA for nested PCR using the reaction mix outlined below.

The total reaction volume of 50 µl was comprised of 1µl template DNA, 0.5 µl BSA,5 µl Polymerase buffer, 0.5 µl 16S_341FGC forward primer, 0.5 µl 16S_518R reverse primer (Table 2.3), 0.5 µl Taq DNA polymerase, 0.05 µl Pfu DNA polymerase and 41.95 µl of molecular grade $H_2O$ (reagents sourced as above). The second round of nested archaea PCR amplification used PCR program 2 in table 2.5, which was also used for the PCR amplification of the bacterial DNA extracted.

*Table 2.5 PCR programs for amplifying DNA extracted with E.Z.N.A kit*

1. *PCR program for 1st round of nested PCR for the extracted archaea DNA (Arch21F forward primer and UN1492R reverse primer)*

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Denaturation | 96 °C | 5 minutes | 1 |
| Annealing step | 94 °C<br>58 °C<br>72°C | 1 minute<br>30 seconds<br>2 minute | 30 |
| Extension | 72 °C | 10 minutes | 1 |
| Holding | 10 °C | - | - |

2. *PCR program for extracted bacterial DNA, and for 2nd round of nested PCR for the extracted archaea DNA (16S_341FGC forward primer and 16S_518R reverse primer)*

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Denaturation | 96 °C | 5 minutes | 1 |
| Annealing step | 94 °C<br>57 °C<br>72°C | 1 minute<br>30 seconds<br>2 minute | 30 |
| Extension | 72 °C | 10 minutes | 1 |
| Holding | 10 °C | - | - |

### 2.5.4. DNA concentration quantification

The concentration DNA following PCR amplification was ascertained using Nanodrop ND-100 Spectrophotometer.

## 2.6. Agarose gel electrophoresis

1% agarose gel was used with 5µl ethidium bromide per 50ml Tris borate EDTA buffer (Sigma). 1 µl of loading buffer (0.4% (w/v) bromophenol blue, 0.4% (w/v) xylene cyanal, 50% (v/v) glycerol) was added to 5 µl of DNA. Hyperladder 1 (Sigma) was used to estimate fragment size. The gel was run in a horizontal gel tank (BioRad Laboratories Ltd., Herts, UK) for 45 minutes at 110 V and imaged under UV light using GeneGenius Bio Imaging System.

## 2.7. Denaturing gradient gel electrophoresis (DGGE)

A 0% and 100% denaturant solution were made up (Table 2.6); the 100% solution was heated to 50 ˚C in order to dissolve the urea crystals. Both solutions were then degassed for 3 minutes under vacuum (Table 2.6). The gel was made up from a low, 30% denaturation solution and a high 70% denaturation solution, comprised of proportions of 0% and 100% denaturant solutions, 10% ammonium persulphate (APS) and N,N,N',N'-Tetramethylethylene diamine (TEMED) (Table 2.6). A gradient mixer was used to mix the correct proportions of denaturant for an even gradient in the gel. The gradient mixer was placed onto a magnetic stirrer and a flea was placed into the downstream chamber (Figure 2.3.A). The 70% denaturant mix was added to the upstream chamber, the valve was then opened to allow a

small amount of denaturant into the next chamber. The 30% denaturant was then placed into the downstream chamber, the valves between the two chambers and that leading to the gel were opened and the denaturant was pump into the gel frames (Figure 2.3.A).

The DNA samples were diluted to ensure each well contained a comparable concentration of DNA. Gels were run at 60 °C in a vertical, circulating tank (BioRad DCode Universal Mutation Detection System) (Figure 2.3.B) in 1 x Tris base, acetic acid and EDTA buffer (TAE) for 16 h at 63 V. To image the gels they were stain in 20 ml of 1 x SYBR Gold for 30 minutes, then de-stained in $dH_2O$ for 10 minutes.

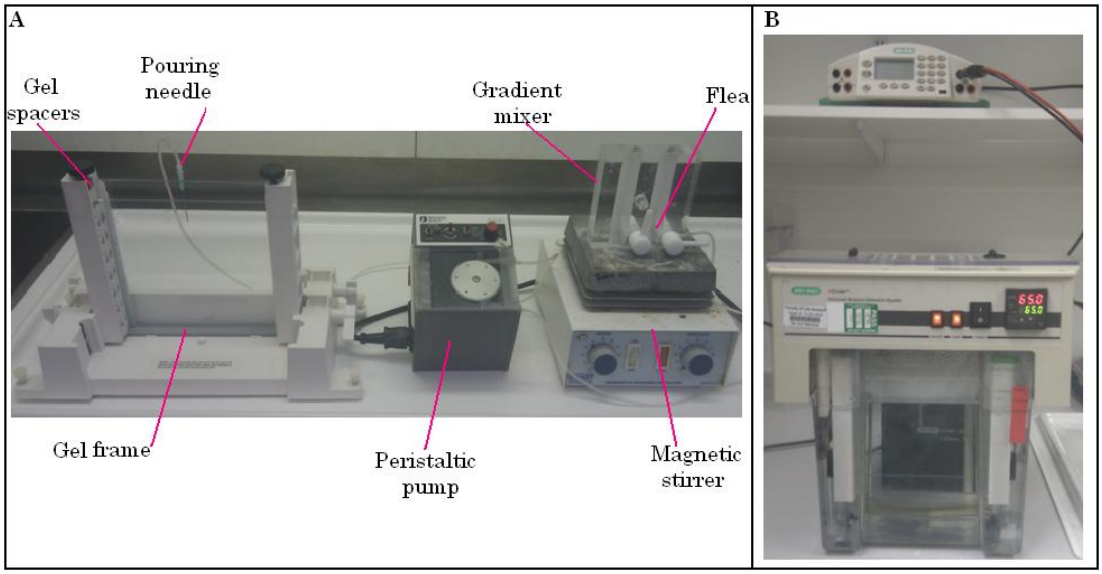



*Figure 2.3 DGGE gel set up.* ***(A)*** *Gel pouring equipment* ***(B)*** *Tank set up with gel*

*Table 2.6 DGGE denaturant solutions*

| Solution | Volume | Components | Volume |
|---|---|---|---|
| **0% denaturant** | 20 ml | 40% Acrylamide (Sigma) | 5 ml |
| | | 50x TAE (Sigma) | 0.4 ml |
| | | $dH_2O$ | 14.6 ml |
| • Degas for 3 mins under vacuum | | | |
| **100% denaturant** | 20 ml | 40% Acrylamide (Sigma) | 5 ml |
| | | 50x TAE (Sigma) | 0.4 ml |
| | | Deionized formamide (Sigma) | 8 ml |
| | | Urea (Sigma) | 8.4g |
| | | $dH_2O$ | 6.6 ml |
| • Heat to 50 ºC until urea crystals dissolve | | | |
| • Degas for 3 mins under vacuum | | | |

| Solution | Volume | Component | Volume |
|---|---|---|---|
| **Low Denaturant solution (30%)** | 16ml | 0 % denaturant solution | 11.2ml |
| | | 100 % denaturant solution | 4.8ml |
| | | 10 % APS (Sigma) | 150 µl |
| | | 10 % TEMED (Sigma) | 8.5 µl |
| **High Denaturant solution (70 %)** | 16ml | 0 % denaturant solution | 4.8ml |
| | | 100 % denaturant solution | 11.2ml |
| | | 10 % APS (Sigma) | 150 µl |
| | | 10 % TEMED (Sigma) | 8.5 µl |

### 2.7.1. Cultured ladder design for DGGE analysis

A ladder of known bacteria was compiled for comparison against the DGGE gel for each sample. An individual colony for 10 bacterial isolates and two archaea isolates were amplified in the method described for the PCR amplification of extracted DNA, section 2.4.3.3. A DGGE gel was run with each well containing a different strain and the position of the band for each isolate was noted on the ladder which was compiled of every strain.

### 2.7.2. DGGE profile analysis using Phoretix 1D Pro program

For each gel replicates of the homogenised samples which had been prepared separately after homogenisation were run for to assess intra-sample variability.

The DGGE gel image was analysed using the Phoretix 1D Pro program. Initially, the lane boundaries were created and correction factors were applied to eliminate error due to smiling of gel lanes as the DNA does not migrate evenly. The gel bands were then detected by the software and then manually checked for accuracy. Each lane was compared to a reference lane containing all the DGGE bands, each sample lane then scores 1 when a band is present and 0 when a band is absent. A dendrogram of the samples was then produced using neighbour joining clustering analysis.

### 2.7.3. Canonical components analysis of binary data

Canonical components analysis (CCA) was conducted on the binary DGGE data matrix of presence and absence of bands at each location, obtained using Phoretix 1D Pro, using MVSP 3.1.

### 2.7.4. DGGE band sequencing

Bands were excised from the gel and frozen at -20 ºC until needed. Before use the gel was left at 4 °C for 12 hours to allow the DNA to diffuse into the gel. 1 µl of the DNA/gel mix was used as a template for amplification and amplified using the 16S_341F forward primer, the 16S_518R reverse primer (Table 2.3) and the PCR programs in table 2.5. The PCR products were prepared for sequencing using Qiagen PCR cleanup kit.

### 2.7.5. DGGE band identification

Bands excised from gels were transformed into *E. coli,* the DNA insert was then digested and sequenced.

#### 2.7.5.1. Preparing chemically competent cells

*Escherichia coli* Top10 cells were streaked onto NB2 agar plates (Section 2.3.1.2.2) and incubated overnight at 37 °C. A 5ml NB2 broth (Section 2.3.1.2.2) with 100 µl of 1M $MgSO_4$ was inoculated with one colony and grown overnight at 37 °C at 200 rpm. 100ml of NB2 broth (Sigma) was pre-warmed in a 500ml flask and inoculated with 1 ml of the 5ml overnight culture, and incubate for 2 hours at 37 °C. The culture was transferred to two chilled, 50ml falcon tubes and incubated on ice for 10 minutes. The cultures were then centrifuge at 3300g for 5 minutes at room temperature. The supernatant was removed and the pellet was resuspended in 10 ml of ice cold 0.1M $CaCl_2$, with 15% glycerol, which was incubated on ice for 30 minutes. The cells were centrifuged again, as before, and the pellet was resuspended in 1ml of ice cold 0.1M $CaCl_2$ and 15% glycerol. The suspended cells were transferred to chilled Eppendorf tubes in 100 µl aliquots and stored at -80 °C until needed.

#### 2.7.5.2. Ligation

The PCR products were ligated into pUC19 plasmid (NEB), pre-cut with Sma1 restriction enzyme to provide blunt ends. A vector:insert ration of 1:1 was used and the reaction was comprised of: 1 µl ligase buffer (NEB), 0.5 µl T4 ligase (NEB), then appropriate proportions of vector and insert, to a total volume of 10 µl. Ligations were left over night at 4 °C.

#### 2.7.5.3. Transformation

5 µl of the ligation was added to 50 µl of competent *E. coli* Top10 cells and incubated on ice for 30 minutes. The cells were then heat shocked at 42 °C for 45 seconds and incubated on ice for 2 minutes. 250 µl of NB2 broth (Section 2.3.1.2.2) was added to the cells and this was incubated at 37 °C at 200 rpm for 1 hour and 30 minutes. Blue white bacterial screening selection was used to check for the presence of an insert. All the 250 µl culture was plated onto ampicillin (Sigma) (100 µg/ml), X-gal (Sigma) (80mg) and IPTG (Sigma) (0.5 mM) NB2 agar medium. This was incubated over night at 37 °C.

Up to 15 white colonies (which should contain the inserted DNA) were picked off and used to inoculate 2ml of NB2 broth containing ampicillin (100 µg/ml). These cultures were incubated overnight at 37 °C. The overnight culture was then spun down at 4 °C for 5 minutes at 3300 g and the pellet frozen until needed. The plasmid was purified from the pellet using a Qiaprep Minprep kit.

#### 2.7.5.4. Plasmid digestion

The presence of the insert was ascertained by digesting the plasmid. The digestion was comprised of: 7.9 µl of plasmid DNA, 0.5 µl EcoR1, 0.5 µl Pst1, 1.0 µl of buffer 3, 0.1 µl BSA for a total volume of 10 µl. The reactions were incubated at 37 °C for 1 hour. 10 µl of digestion reaction was run on an agarose gel (section 2.3.4) using Hyperladder 1 to estimate fragment size.

### 2.7.6. DNA sequencing

#### 2.7.6.1. Sequencing colony PCR products

The colony PCR products were cleaned up and sequenced at NBAF - The Gene Pool - NERC Biomolecular sequencing facility. Sequencing was in one direction, using the BF1 primer.

#### 2.7.6.2. Sequencing DGGE band clones

The DGGE band inserts into *E. coli* clones were sequenced at the Wolfson Institute for Biomedical Research sequencing facility, using the M13-40 and M13Rev primers to sequence in both directions.

#### 2.7.6.3. Aligning and molecular phylogenetic analysis of sequences

The DNA sequences were trimmed using BioEdit software (Hall, 1999). Sequences were then aligned and analysed using Molecular Evolutionary Genetic Analysis (MEGA 5.0) (Tamura et al., 2011). Alignment was conducted using the multiple sequence alignment computer program ClustalW, sequences were then trimmed to a uniform size and a molecular phylogeny analysis conducted using the Neighbour joining clustering method. Phylogeny test was conducted using interior branch test, 1000 replications. The archaea strain *Methanocaldococcus jannaschii* DSM 2661 was used as an out group for bacterial trees and *Deinococcus radiodurans* for archaea trees.

#### 2.7.6.4. Gram staining

Gram staining was carried out according to the Gram Staining Kit (Bacterial Staining Kit according to Gram) protocol.

#### 2.7.6.5. Schaeffer-Fulton stain

Carried out as described in Bartholomew, 1950 (Bartholomew, 1950).

#### 2.7.6.6. Microscopy

Prepared slides were analysed under an optical microscope with 40x magnification plus 10x eyepiece magnification.

#### 2.7.6.7. Shannon diversity

Shannon diversity index (H) was calculated using MVSP.

## 2.8. Geological techniques

### 2.8.1. Water activity

Water activity measured at 25 °C using a Novasina Aw Sprint TH 500.

### 2.8.2. Raman Spectroscopy

Samples were crushed and placed onto microscope slides, raman spectra were acquired using a Renishaw InVia Raman Spectrometer, with a Leica microscope attached. Excitation was achieved using a 514nm laser. A x50 microscope objective was used and the laser spot size was around 10 µm. 2-11 accumulations were used with a laser power from 1% - 50%, adjusted depending on the influence of fluorescence. Acquisition time was typically 10-40 seconds. Spectra were compared to reference spectra using the Crystal Sleuth software (Laetsch and Downs, 2006).

### 2.8.3. X-Ray Diffraction (XRD)

XRD analysis was conducted by the University of Aberystwyth, the samples were sent to Aberystwyth where they were crushed to a uniform size prior to analysis. The equipment used was a Bruker D8 Advance and was fitted with a Vantec Super speed detector. The spectra were compared to the spectral library on the XRD. The spectra were then confirmed where possible using Crystal Sleuth software (Laetsch and Downs, 2006).

### 2.8.4. Scanning Electron Microscopy/ Energy Dispersive Spectroscopy (SED/EDS)

Sample size for analysis was between 7mm-15mm in size was used for analysis. Samples were mounted on an SEM/EDS stub and gold coated, or mounted and impregnated with resin and analysed as a polished thin section. The precise sample preparation is described in the appropriate chapter.

## 2.9. Statistical tests

### 2.9.1. Student t-Test

In order to assess if the mean value of two data sets were statistically different from one another, a t-Test was conducted in Microsoft Excel. The assumptions of the specific t-Test are described in the appropriate chapter. A $p<0.05$ was considered statistically significant.

### 2.9.2. Analysis of variance (ANOVA)

Where the number of data sets was greater than 2, Analysis of Variance (ANOVA) statistical test in conjunction with Tukey Post-hoc analysis was used to identify differences between the means of all the groups, assessing if the differences are significant and identifying precisely which groups differ from one another. This analysis was conducted using SPSS 17.0, Statistical Analysis Software. A $p<0.05$ was considered statistically significant.

## Chapter 3

# The isolation and analysis of bacterial and archaeal communities from Lake Magadi

### 3.1. Soda lake environments

Soda Lakes such as those in the East African Rift Valley are the most stable alkaline naturally occurring environments on the Earth (Duckworth et al., 1996: Rees, 2004: Wani et al., 2006: Deshmukh et al., 2011) and often have high salinity (Lin et al., 2004: Warren, 2006). They generally form in closed basins which experience high evaporation rates (See Chapter 1). The hydrothermal springs associated with some soda lakes, such as those at Lake Magadi, may also have an alkaline pH due to the interaction of groundwater with silicate minerals present in the surrounding geology (Jones et al., 1998). Despite the highly alkaline pH and in some instances high salinity, these environments are described as one of the most productive aquatic environments in the world (Jones et al., 1994: Jones et al., 1998: Lefèvre et al., 2011), with a high bacterial and archaeal diversity (Mwatha, 1993: Rees, 2004: Mwirichia et al., 2010a: Mwirichia et al., 2010b).

### 3.2. Microbiology of soda lake environments

Previous studies conducted on the bacterial and archaeal diversity of soda lakes (including Lake Magadi) have utilised both culture dependent methods, using a variety of different growth media and sequencing isolates, and culture independent methods including denaturing gradient gel electrophoresis (DGGE) and rDNA clone libraries to study the diversity of a soda lake and compare them to other soda lake environments (Jones et al., 1994: Dubinin, 1995: Duckworth et al., 1996: Jones et al., 1998: Grant et al., 1999: Ma et al., 2004b: Rees, 2004: Wani et al., 2006: Joshi et al., 2008: Mwirichia et al., 2010b: Mwirichia et al., 2010a: Deshmukh et al., 2011).

The phylogenetic analysis of alkaliphiles has shown that representatives can be identified in every major group of bacteria and archaea (Rees, 2004: Grant, 2010), alkaliphiles from soda lake environments demonstrate a significant phylogenetic diversity (Wani et al., 2006). Halophiles are also a phylogenetically diverse group, a number of branches of the phylum Proteobacteria contain halophiles, many of which have close non halophilic relatives, as well having groups containing only halophilic bacteria (Oren, 2002b: Oren, 2008). Soda lakes with high pH and salinity (as well as high temperatures in some areas, and periods of desiccation) apply multiple stress factors to a community. Therefore, soda lakes only provide a habitable environment for those organisms able to withstand all the environmental stresses being applied (for example, salinity, pH and desiccation). These isolates are termed polyextremophiles (Bowers et al., 2004, Wiegel, 2010: Bowers and Wigel, 2011),

*Deniococcus radiodurans,* the radiation, oxidation and desiccation resistant strain has been described as one such polyextremophilic bacterium (Richmond et al., 1999a).

In spite of this overall diversity of alkaliphiles and halophiles, and the high diversity of soda lake environments identified through culture independent techniques, the list of isolated genera identified from soda lake environments remains fairly limited (Jones et al., 1998: Wani et al., 2006). Reflecting both the limitations of culture based studies, but also the potential for the isolation of novel (poly-extremophilic) strains with the use of different growth conditions.

The dominant genera identified in soda lakes (identified through either culture dependent or culture independent techniques) include *Bacillus* and *Alkalibacillus* as well as members of the phylum *Proteobacteria*, including the genera *Halomonas*, *Vibrio, Pseudomonas, Dietzia, Micrococcus* and *Idiomarina* (Rees, 2004: Wani et al., 2006: Mwirichia et al., 2010b: Deshmukh et al., 2011). In addition to studies focusing on the bacterial populations of soda environments, analyses of archaeal communities in soda lakes has identified a number of haloalkaliphilic isolates assigned to the genera *Natronococcus, Natrialba, Natronobacterium* and *Natronolimnobius* (Grant et al., 1999: Xin et al., 2001: Xu, 2001: Itoh et al., 2005: Mwirichia et al., 2010a). Interestingly the diversity of archaea in soda lake environments is generally lower than for the bacterial community (Wani et al., 2006).

### 3.3. The relevance of soda lake environments to astrobiology

In the context of astrobiology and considering the habitability of Mars in terms of indigenous life and the potential of contamination, organisms which live in environments where several detrimental factors exist (i.e. desiccation, UV, high pH and high salinity) may have survival characteristics which are similar to those required to survive on Mars. In addition, soda lake microbiology has been described as being potentially being inhabited by an ancient prokaryotic community and a “refuge for relict communities” (Jones et al., 1998: Zavarzin, 2000). If it were the case that soda lake communities harbour ancient lineages of prokaryotes, this could be an important point when considering the search for life beyond the Earth. Remembering that is through the characterisation and study of extreme terrestrial environments, that we can gain a better understanding of where to look for past or present extraterrestrial life (DasSarma, 2006).

The purpose of this chapter is to attempt to isolate novel strains of bacteria and archaea capable of growing at high salinity, temperature and pH. It is intended that through the development of a new growth medium supplemented with varying salt concentrations, alkaliphilic/alkalitolerant strains which can tolerate both high and low salt concentrations will be isolated, as well as those isolates which are only able to grow in the presence of extreme environmental conditions. Ultimately, isolates cultured in this chapter will be the focus of further characterisation and survival studies in subsequent chapters.

### 3.4. Aims of this chapter

1. To design a series of growth medium based on the water chemistry of Lake Magadi
2. To culture strains of archaea and bacteria from Lake Magadi using different temperatures
3. To culture strains of archaea and bacteria from Lake Magadi using varying salt concentrations
4. Assess the uncultured bacterial and archaeal diversity of different samples using DGGE analysis
5. Select three isolates for further characterisation

### 3.5. Materials and methods

#### 3.5.1. Samples from Lake Magadi

Twelve samples were selected for water analysis and twenty five samples, including twenty three samples from the 2009 field trip and two samples from 2010 field trip (Chapter 2, Table 2.2 and Table 2.3) were chosen for biological analysis.

The samples have been divided by their geographic location into samples from northern end Little Magadi, northern end Lake Magadi, southern end Lake Magadi and Western Lagoon, which contains samples collected from a lagoon along the western side of Lake Magadi, rather than from a spring (Chapter 2, section 2.1.1.1). For each division samples were selected so that one water and one sediment sample was analysed per geographic location. In addition, a selection of samples which included; biomass and possible mineral samples were chosen from each division (Table 3.2).

*Table 3.1. Samples selected for biological analysis, for each of the four divisions, outlining sampling locality, pH and temperature measurements.*

| Area | Site | Temperature | pH | Sample | Description |
|---|---|---|---|---|---|
| Division 1: Northern end Little Magadi | | | | | |
| 6 | 25 | 57.3 °C | 9.31 | 95 | Water sample |
| | | | | 99 | Water sample |
| | | 82.7 °C | 9.48 | 103 | Water sample |
| | 27 | 82.4 °C | 9.51 | 106 | Water sample |
| | | 44 °C | 9.51 | 108 | Red material in raised area |
| | 28 | 44 °C | 9.51 | 109 | Orange particulate material |
| | | 82.4 °C | 9.51 | 110 | Grey material |
| | | 82.4 °C | 9.51 | 113 | Scoop sample of sediment and water |
| | | - | - | 114 | Rock with white precipitate |
| | | 82.4 °C | 9.51 | 115 | Pinky, bubbly material on surface of water |
| Division 2: Northern end Lake Magadi | | | | | |
| 5 | 20 | 64.4 °C | 9.07 | 80 | Water sample |
| | | | | 81 | Pink, soft, particulate material |
| | 21 | | | 82 | 'Stringy' material, dark green in colour |
| | 24 | | | 83 | Dark green soft material on spring bed |
| | | 64.3 °C | 9.29 | 85 | Water sample |
| | | | | 86 | Scoop sample of sediment- pink & white fine grained material |
| | | 63.1 °C | 9.31 | 94 | Dark green floating at surface attached to red material below |
| Division 3: Western Lagoon | | | | | |
| 4 | 16 | N/R | N.R | 70 | Bright pink, wet material on mud flat |
| | | 35.7 °C | 10.01 | 71 | Water sample |
| | | | | 72.2 | Sediment and water scoop sampled from |
| Division 4: Southern end Lake Magadi | | | | | |
| 8 | - | 32.9 °C | 9.78 | 1 | Sediment sample from lake edge-0.5 m |
| | | 32.9 °C | 9.78 | 2 | Sediment sample from edge of lake- 3.5 m |
| 3 | 13 | 45.1 °C | 10.01 | 52 | Water sample |
| | | 39.5 °C | 9.97 | 58 | Bright pink soft material associated with |
| | 14 | | | 60 | Orange, soft material on surface of bedrock |
| | | | | 61 | Soft, green material, oval in shape and |
| | | 44.4 °C | 9.96 | 63 | Water sample |
| 4 | 17 | 44.6 °C | 9.97 | 74 | Water sample |
| | | | 9.97 | 76 | Soft, yellow material attached to bedrock |
| | | | | 116 | Water sample |
| 7 | 29 | 36.6 °C | 9.97 | 133 | Pinky/ white material in blocks on sediment |
| | | | | 121 | Water sample |
| | 30 | 40.2 °C | 9.97 | 123 | Green material floating on water surface, |
| | | | | 124 | Crumbly unconsolidated white material |
| | 31 | | | 125 | Dark sediment sample |
| | | | | | |

**3.6. Results**

### 3.6.1. Water analysis of samples from Lake Magadi

Temperature measurements showed a temperature gradient from north to south of Lake Magadi, with the springs at the northern end of the lake (sites 5 and 6) measuring between 82.7 °C and 57.3 °C, and those at the south (sites 3, 4 and 7) between 35.7 °C and 45 °C (see Table 3.1). The water activity measured varied between 0.977 $a_w$ and 0.984 $a_w$, a greater water activity level than the average water activity of sea water (0.97 $a_w$ (McGenity, Pers. Comm.)). The lowest $a_w$ was recorded for sample 71 from the Western Lagoon (Table 3.2).

Cation analysis of the water samples showed that the levels of calcium, iron and magnesium in the system were below the detection limit in all samples. Sodium levels were very high >3662 ppm and potassium levels were >64.10 ppm compared to sodium and potassium levels of 7.0 ppm and 2.3 ppm recorded for the Ewaso Ngiro river.

Anion analysis showed that phosphorus was only present in the springs in the south, and the lagoon (phosphate content was below the detection limit in all those samples from the north of the lake) where levels were between 0.56 ppm and 0.94 ppm, compared to 0.08 ppm for Ewaso Ngiro. Chlorine levels were high in all samples >5333.90 ppm compared to only 4.0 ppm in Ewaso Ngiro. Levels of silicon, fluorine and $SO_4$ were all much larger than levels recorded for the Ewaso Ngiro River.

*Table 3.2. Water chemistry analysis of samples from the springs and lagoon around Lake Magadi. * Signifies Lagoon sample. N/R measurement not taken. Ewaso Ngiro data taken from (Warren, 2006).*

| **Site and sample number** | | **pH** | **Temp. ( °C)** | **$a_w$** | **Ca (ppm)** | **Fe (ppm)** | **K (ppm)** | **Mg (ppm)** | **Na (ppm)** |
|---|---|---|---|---|---|---|---|---|---|
| 3 | 52 | 10.1 | 45.1°C | N/R | -0.82 | -0.07 | 71.20 | -0.52 | 4521.68 |
| 3 | 63 | 9.96 | 44°C | N/R | -0.81 | -0.07 | 79.82 | -0.52 | 5017.83 |
| 4 | 71* | 10.1 | 35.7°C | 0.977 | -0.78 | -0.06 | 146.28 | -0.52 | 7184.18 |
| 4 | 74 | 9.97 | 44.6°C | 0.983 | -0.83 | -0.07 | 75.22 | -0.52 | 4659.67 |
| 7 | 116 | 9.97 | 36.6°C | N/R | -0.83 | -0.07 | 69.32 | -0.52 | 4722.56 |
| 7 | 121 | 9.97 | 40.2°C | N/R | -0.82 | -0.07 | 64.10 | -0.52 | 4439.13 |
| 5 | 80 | 9.07 | 64.4°C | N/R | -0.82 | -0.07 | 83.10 | -0.52 | 3838.80 |
| 5 | 85 | 9.29 | 64.3°C | 0.983 | -0.81 | -0.07 | 96.73 | -0.52 | 4244.31 |
| 6 | 99 | 9.31 | 57.3°C | N/R | -0.83 | -0.07 | 95.98 | -0.52 | 3977.89 |
| 6 | 95 | 9.31 | 57.3°C | 0.984 | -0.82 | -0.07 | 92.36 | -0.52 | 4262.13 |
| 6 | 103 | 9.48 | 82.7°C | N/R | -0.83 | -0.07 | 85.00 | -0.52 | 3662.65 |
| 6 | 106 | 9.48 | 82.7°C | N/R | -0.83 | -0.07 | 130.54 | -0.52 | 4856.61 |
| Ewaso Ngiro | | 7 | N/R | N/R | 6.5 | N/R | 2.3 | 3.7 | 7.0 |

*Continued from Table 3.2 Water chemistry analysis of samples from the springs and lagoon around Lake Magadi. * Signifies Lagoon sample. N/R measurement not taken. Ewaso Ngiro data taken from (Warren, 2006).*

| Site and sample number | | P (ppm) | S (ppm) | Si (ppm) | Sr (ppm) | F- (ppm) | Cl- (ppm) | $NaNO_3$ (ppm) | $SO_4$ (ppm) |
|---|---|---|---|---|---|---|---|---|---|
| 3 | 52 | 0.68 | 25.82 | 7.29 | 0.17 | 121.12 | 7752.36 | 1118.48 | 165.12 |
| 3 | 63 | 0.71 | 27.52 | 8.14 | 0.14 | 202.90 | 7277.37 | 1342.11 | 517.00 |
| 4 | 71* | 0.94 | 41.52 | 13.50 | 0.17 | 179.78 | 11344.51 | 1469.73 | 322.31 |
| 4 | 74 | 0.85 | 25.75 | 7.38 | 0.15 | 90.23 | 6500.77 | 708.03 | 161.08 |
| 7 | 116 | 0.62 | 23.69 | 4.08 | 0.16 | 163.76 | 7257.23 | 1205.09 | 165.82 |
| 7 | 121 | 0.56 | 21.40 | 4.03 | 0.17 | 124.69 | 6938.17 | 1035.77 | 158.62 |
| 5 | 80 | -0.03 | 18.80 | 10.17 | 0.18 | 120.62 | 5689.12 | 1140.91 | 130.85 |
| 5 | 85 | -0.03 | 17.28 | 9.87 | 0.20 | 125.37 | 5615.67 | 966.51 | 148.25 |
| 6 | 99 | -0.02 | 19.48 | 9.70 | 0.18 | 128.33 | 5333.90 | 1035.14 | 114.79 |
| 6 | 95 | -0.01 | 16.98 | 9.53 | 0.18 | 133.04 | 6380.93 | 1269.86 | 131.09 |
| 6 | 103 | -0.03 | 17.97 | 8.94 | 0.18 | 136.36 | 5680.05 | 1012.35 | 127.26 |
| 6 | 106 | -0.01 | 24.04 | 12.15 | 0.18 | 180.99 | 6077.57 | 1039.45 | N/R |
| Ewaso Ngiro | | 0.08 | N/R | 20 | N/R | 0.2 | 4.0 | N/R | 2.4 |

Sample 71 from the lagoon at site 4 has the highest concentration of K, Na, S, Si, Cl and $NaNO_3$. The chlorine content is 46% greater in the lagoon sample than in any of the other samples from around the lake, other studies also show an increased chlorine content in the lagoons compared to the springs (Warren, 2006). This sample also has the highest concentration of phosphorus, one of the highest pH's measured.

### 3.6.2. Development of alkaline growth medium

Using the water analysis data from section 3.4.1 the AP+ growth medium was made up from 7 components, which were autoclaved separately (Table 3.3).

*Table 3.3 Working concentration of media components in each AP+ growth medium. Those components in a Sol. (solution) group were autoclaved together.*

| Sol. | Growth medium | | AP+ 0.7% | AP+ 6.8% | AP+ 15% |
|---|---|---|---|---|---|
| 1 | NaCl working concentration | | 0.12 M | 0.93 M | 2.0 M |
| | Oxoid peptone | | 1g/100ml | 1g/100ml | 1g/100ml |
| | Agar | | 1g/100ml | 1g/100ml | 1g/100ml |
| 2 | Solution A | $K_2SO_4$ | 5.4 mM | 5.4 mM | 5.4 mM |
| | | NaF | 16.8 mM | 16.8 mM | 16.8 mM |
| 3 | Solution B | $NaNO_3$ | 20 mM | 20 mM | 20 mM |
| | | $NaPO_4$ | 0.02mM | 0.02mM | 0.02mM |

*Table 3.3. Continued.*

| Sol. | Growth medium | AP+ 0.7% | AP+ 6.8% | AP+ 15% |
|---|---|---|---|---|
| 4 | $Na_2CO_3$ | 54 mM | 54 mM | 54 mM |
| 5 | $NaHCO_3$ | 46 mM | 46 mM | 46 mM |
| 6 | $MgSO_4$ | 16 mM | 16 mM | 16 mM |
| 7 | Trace element mix (Table 3.3) | 0.1ml/100ml | 0.1ml/100ml | 0.1ml/100ml |

The peptone, agar and NaCl were dissolved in 352ml of distilled water and autoclaved together. A 100x stock solution of $K_2SO_4$ and NaF (solution A) and a stock of $NaNO_3^-$ and $NaPO_4$ (solution B) were autoclaved separately and added to the molten agar. The medium was buffered to desired pH using $Na_2CO_3$ and $NaHCO_3$, which were also autoclaved separately and added to the molten agar. Finally $MgSO_4$ and the trace element mix (Table 3.4) were added to the molten agar.

*Table 3.4 Trace element mix components*

| Stock concentration | Compound |
|---|---|
| 10 mM | $FeCl_3.6H_2O$ |
| 0.1 mM | $ZnCl_2.4H_2O$ |
| 0.08 mM | $CoCl.6H_2O$ |
| 0.025 mM | $NaMoO_4.2H_2O$ |
| 0.07 mM | $CaCl_2.2H_2O$ |
| 4 mM | $CuCl_2.2H_2O$ |
| 0.08 mM | $H_3BO_3$ |
| | Concentrated HCl |

### 3.6.3. Culturable bacterial diversity of samples from Lake Magadi

Growth was achieved on solid media at 37 ºC, details of isolation, restreaking and storage are given in Chapter 2 (section 2.3). Attempts were made to culture on solid media at 50 °C, however no growth was achieved. A selection of isolates and their closest database match and closest cultured isolate match are given in Table 3.3, the full list of sequenced isolates are given in Appendices A-D.

#### 3.6.3.1. Culture based study of Division 1: Northern Little Magadi

##### 3.6.3.1.1. Sample 106

Water sample 106 had the lowest cultured colony density with only four colonies being isolated. Although these strains match to *Caldalkalibacillus uzonensis* and this strain was returned as one of the nearest neighbour by the Greengenes database (Table 3.5), theses strains are not closely related according to the neighbour-joining phylogenetic tree (Figure 3.1- purple arrow).

#### 3.6.3.1.2. Sample 108

The isolates from biological sample 108 were predominantly related to strains of *Halomonas campisalis* (bauxite deposits) and *H. campisalis* (Lonar Lake, India) (Figure 3.1 and Table 3.5). In addition, strains related to other species of *Halomonas* including *H. meridiana* (Sambhar salt lake India), *H. pantelleriensis* (Pantelleria island) and *H. variabilis* (glyphosate-polluted soil) were also isolated (Figure 3.1).

Isolates related to organisms of the Gamma Proteobacteria were isolated, with varying degrees of similarity to the closest species database matches of *Idiomarina baltica* and *Idiomarina seosinensis* (81% and 92% respectively) (Table 3.5).

Isolates related to members of the phylum Firmicute were isolated from this sample, being most closely related to strains of the genus *Bacillus, related to B. pseudofirmus, or B. okhensis, Alkalibacterium (*fermenting, salted food, mud volcanoes and a soda lakes) *related to A. psychrotolerans* or *A. pelagium* (Yumoto et al., 2004: Yumoto et al., 2008), or *Planococcus*, most closely related to *P. maritimus* (isolated from Thondi, India) (Table 3.5).

#### 3.6.3.1.3. Sample 109

Several isolates related to *Halomonas campisalis* (95-99% similarity) were isolated from the orangey biological sample 109. Along with isolates related to species of *Alkalibacterium* (98%-99% similarity), *A. pelagium and A. psychrotolerans* (92%-100% similarity). Two isolates was most closely related to *Idiomarina* from a solar saltern.

Strains related to *P. maritimus* (from the Andaman Sea) were also isolated from this sample, although the similarity was lower for these isolates than for the 6.8%_109GA isolate (76%).

#### 3.6.3.1.4. Sample 110

Once again isolates were most closely related to database strains of *Halomonas campisalis* and other species of *Halomonas* including *H. pantelleriensis* (mud volcano), *H. nitritophilus* (mud volcano) and *H. venusta* (Sambhar Salt Lake) (Table 3.5). Seven isolates were most closely related to species of *Idiomarina,* including *I. seosinensis* (hypersaline saltern and deep sea water column), *I. fontislapidosi* (hypersaline habitats in Spain) and *Idiomarina* salinarum (*Pseudoidiomarina salinarum*, from a marine solar saltern). Sample 110 was a grey rock covering material.

Six isolates were most closely related to database strains of the phylum Firmicute. Isolates related to strains of *Alkalibacterium* were also cultured from this sample, again related to strains of *A. psychrophilus* and *A. pelagium*. Two isolates were most closely related to species of the genus *Bacillus, B. aurantiacus* (Hungarian soda lake) and *B. agardhaerens* (sea shore).

#### 3.6.3.1.5. Sample 113

Five of the isolates were isolated from the water and sediment sample 113 and were most closely related to *Halomonas* (95-99% similarity), including *H. nitrophilus* (mud volcano) and *H. campisalis,* (alkaline saline lake-Soap Lake, bauxite). One strain most closely related to *Idiomarina zobellii* was isolated, with 91% similarity (Table 3.5).

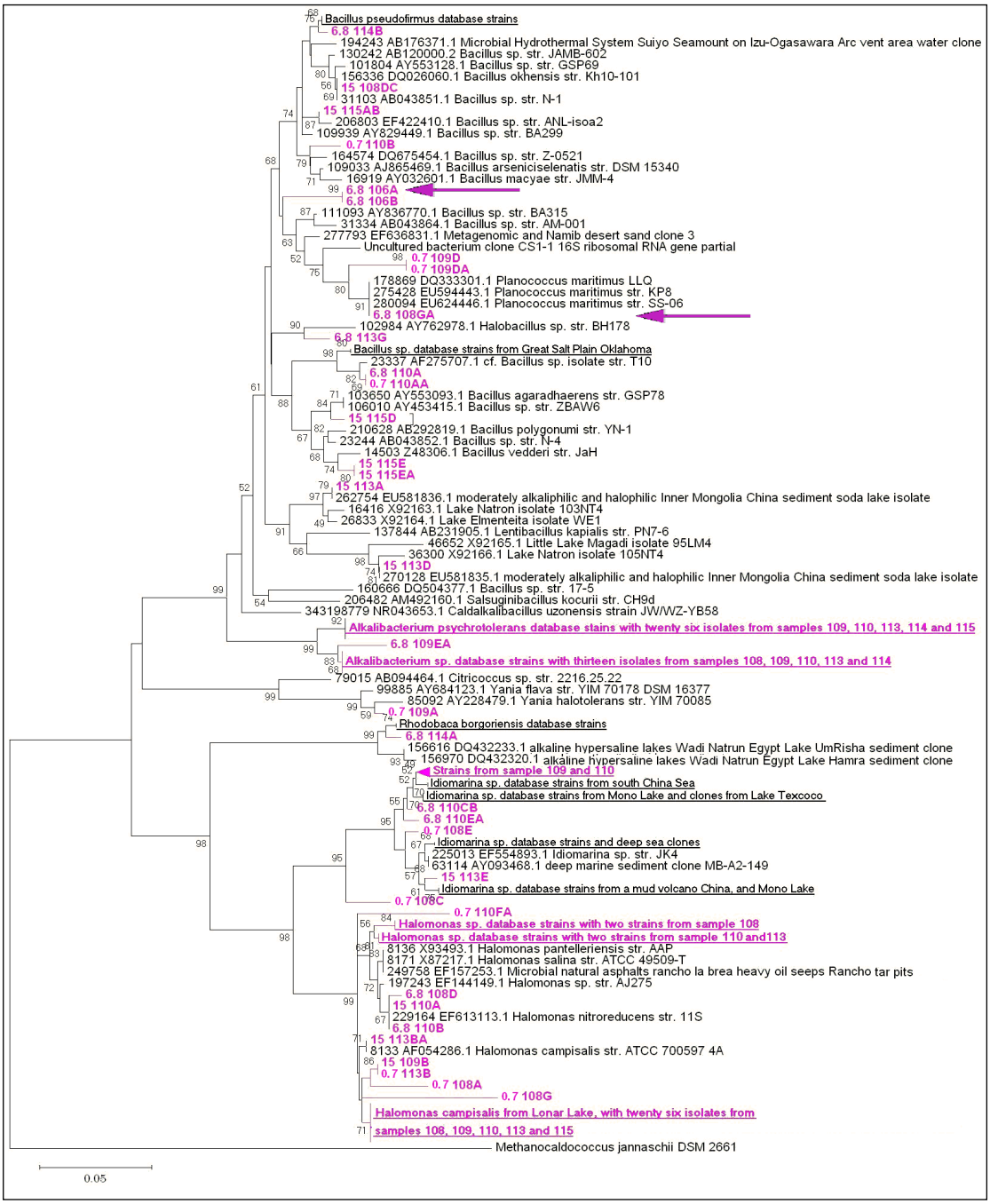



*Figure 3.1. Phylogenetic tree for the cultured isolates (pink) from samples 108, 109, 110, 113, 114, and 115 and near neighbour and nearest neighbour matches (black) using Greengenes alignment software (DeSantis et al., 2006). M. jannaschii DSM 2661 was used as an out group. Interior branch values, 1000 replications.*

Seven isolates were most closely related to strains of the phylum Firmicute, one strain isolated on 15% NaCl medium was most closely related to *Bacillus agardhaerens* (sea shore). In addition, an isolate was most closely related to *Salsuginibacillus halophilus* (Inner Mongolian soda lake) and 94% similar to a strain of the genera *Piscibacillus halophilus* (hypersaline Iranian lake). Finally, again a significant proportion, four isolates were most closely related to *Alkalibacterium* isolates, either *A. psychrotolerans* or *A. pelagium, o*ne isolate, 6.8%_113BC was only 88% similar to the database sequences (Figure 3.1).

**3.6.3.1.6. Sample 114**

Four isolates were successfully sequence from this rock and precipitate sample, two strains related to *Alkalibacterium* (Table 3.5) and *A. pelagium* and an isolate related to *Bacillus pseudofirmus* (Figure 3.1). 6.8%_114A was related to a strain of *Rhodobacter* (hypersaline Ekho Lake).

**3.6.3.1.7. Sample 115**

Two isolates were most closely related to *H. campisalis* with 98-99% similarity were isolated from this pink biological material. Five isolates were most closely related to *Alkalibacterium* (Table 3.5) and four isolates of *Bacillus*, *B. arseniciselenatis* (soda lake Khadyn) and *B. vedderi* (bauxite waste). *B. arseniciselenatis* is an arsenate respiring bacterium.

**3.6.3.2. Culture based study of Division 2: Northern Lake Magadi**

**3.6.3.2.1. Sample 81**

Fifteen isolates were most closely related to *H. campisalis* and were between 92% and 100% similarity, in addition, one strain, isolated on 15% NaCl was most closely related to *H. nitrophilus* (Table 3.5).

Three isolates were most closely related to *A. psychrotolerans* and one was most closely related to *A. pelagium* (98%-99%). Three strains isolated on 6.8% medium were most closely related to species of *Bacillus*, with 99% similarity to *B. polygoni* (indigo balls) and *B. clarkii* and 85% similarity to *B. cohnii* (Figure 3.2).

**3.6.3.2.2. Sample 82**

Most of the strains isolated and sequenced from this sample (eleven isolates) were most closely related again to *H. campisalis* (97%-100% similarity). In addition, strains related to other species of *Halomonas* were also isolated, including *H. kenyensis* (mixed sediments, soda lakes Kenya) (Table 3.5). One strain isolated on 6.8% medium was 92% related to *Idiomarina salinarum.*

Four strains were most closely related to isolates of the phylum Firmicute, two were related to A. psychrotolerans and three to the genus *Bacillus* (Figure 3.2). The *Bacillus* strains were

related to *B. vedderi* (97%), *B. hemicellulosilyticus* (96% similarity) and *B. pseudofirmus* (97% similarity).

#### 3.6.3.2.3. Sample 83

Isolates related to *Halomonas campisalis* and *A. psychrotolerans* and *A. pelagium* were cultured. One strain if *Idiomarina salinarum* was isolated with 92% similarity to the database strain. Three isolates were most closely related to *Bacillus,* being >97% similar to the species *B. alkalinitrilicus* (soda solonchak soil) or *B. pseudofirmus* (Figure 3.2). Isolate 6.8_83C was most closely related to *Halolactibacillus xiariensis* (Inner Mongolian soda lake) (Table 3.5).

#### 3.6.3.2.4. Sample 85

Only isolates related to strains of Firmicute were cultured and sequenced from this water sample. Two strains (6.8%_85D and 6.8%_85DB) were most closely related to *B. pseudofirmus* and isolate 0.7%_85A to *B. alkalinitrilicus* (Figure 3.2) (anodic solution of microbial fuel cell), along with an isolate related to a strain isolated from Lonar Lake and finally, a strain related to *Halolactibacillus xiariensis* (Inner Mongolian soda lake) (Table 3.5).

#### 3.6.3.2.5. Sample 86

Several strains were similar to strains of *H. campisalis* to varying degrees, 95%-99% similarity were isolated from the water and sediment sample. Isolates related to *A. psychrotolerans* were also cultured from this sample and on strain was 92% similar to *Idiomarina seosinensis*. Strains similar to *Bacillus* strains were also isolated from this sample, including *B. alkalinitrilicus, B. vedderi* and *B. cohnii* (western Kunlun mountains) (Figure 3.2 and Table 3.5).

#### 3.6.3.2.6. Sample 94

This sample was dark green biological material. Strains related to *H. campisalis* were isolated with 97%-99% similarity; in addition, an isolate related to *H. pantelleriensis* was also cultured (94% similarity). Strains related to *Idiomarina seosinensis* and *Idiomarina salinarum* were sequenced with isolates having a 92%-93% similarity to the database strains.

Seven isolates were cultured which were most similar to *Bacillus* strains, these were most similar to the species: *B. okhensis, B. vedderi, B. alkalinitrilicus* and *B. locisalis* (soda lakes Kenya and Tanzania) (Table 3.5). Finally, a single isolate most closely related to *Dietzia,* an Actinobacterium, was isolated on 0.7% NaCl (Figure 3.2 and Table 3.5).

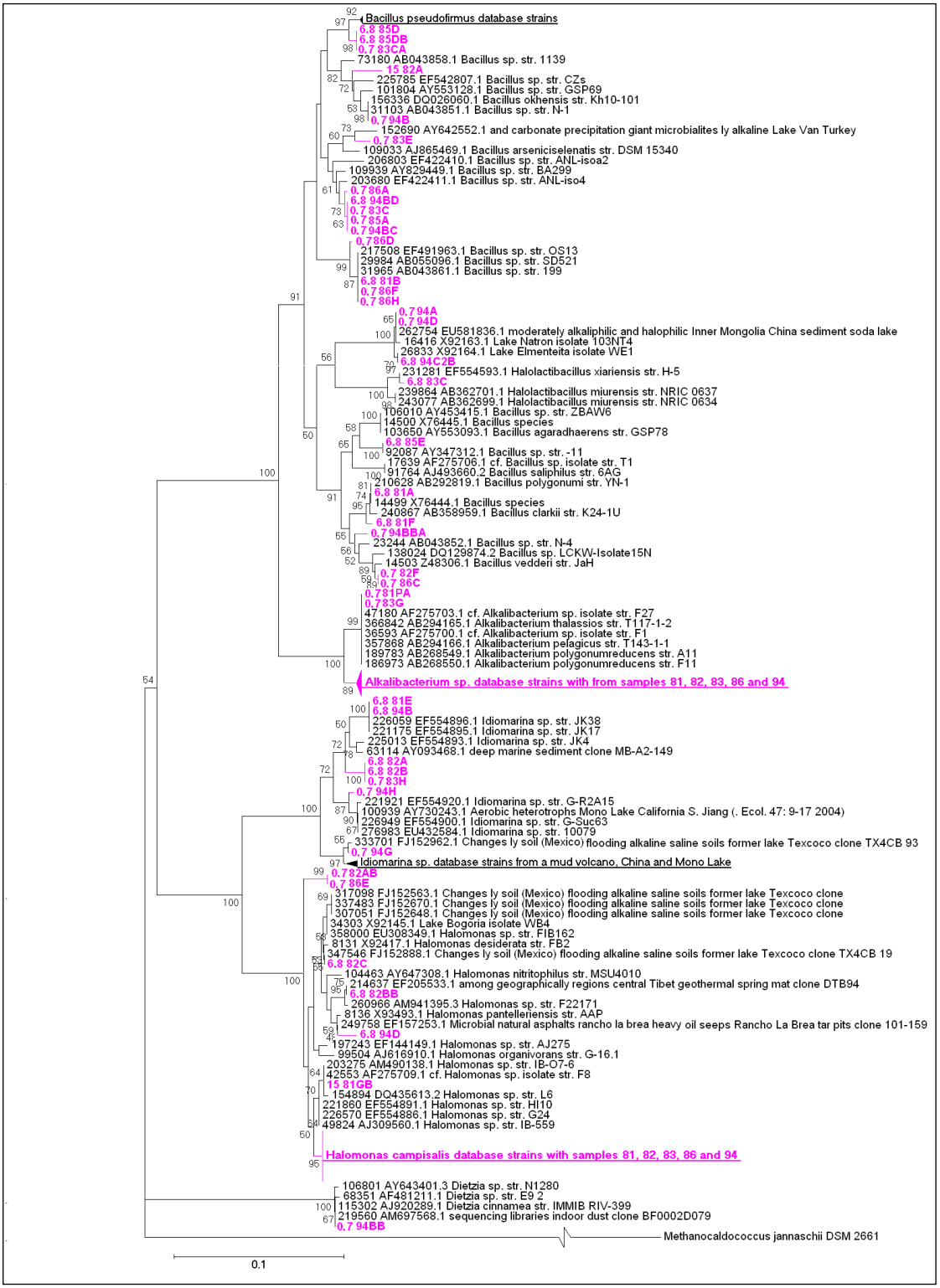



*Figure 3.2 Phylogenetic tree for the cultured isolates (pink) from samples 81, 82, 83, 85, 86 and 94 and near neighbour and nearest neighbour matches (black) using Greengenes alignment software (DeSantis et al., 2006). M. jannaschii DSM 2661 was used as an out group. Interior branch values, 1000 replications.*

### 3.6.3.3. Culture based study of Division 3: Western Lagoon

#### 3.6.3.3.1. Sample 70

This sample was a bright pink material from the lagoon beach. The common isolates of *H. campisalis*, *A. psychrotolerans* and *I. seosinensis* were isolated from this sample (Figure 3.3). In addition, one strain related to *Dietzia* was cultured on 0.7% medium, with 95% similarity (Table 3.5).

#### 3.6.3.3.2. Sample 72.2

Again, isolates related to *H. campisalis* and *H. pantelleriensis* strains were isolated and sequenced which range in similarity between 93% and 99% similarity. 0.7%_72.2E was most closely related to *Pseudoidiomarina* sp. (Table 3.5). A strain related to *Alkalibacterium* was also isolated from this sediment and water sample (Figure 3.3). One strain was isolated from this sample, which was most closely related to *Caldalkalibacillus uzonensis* (92% similarity).

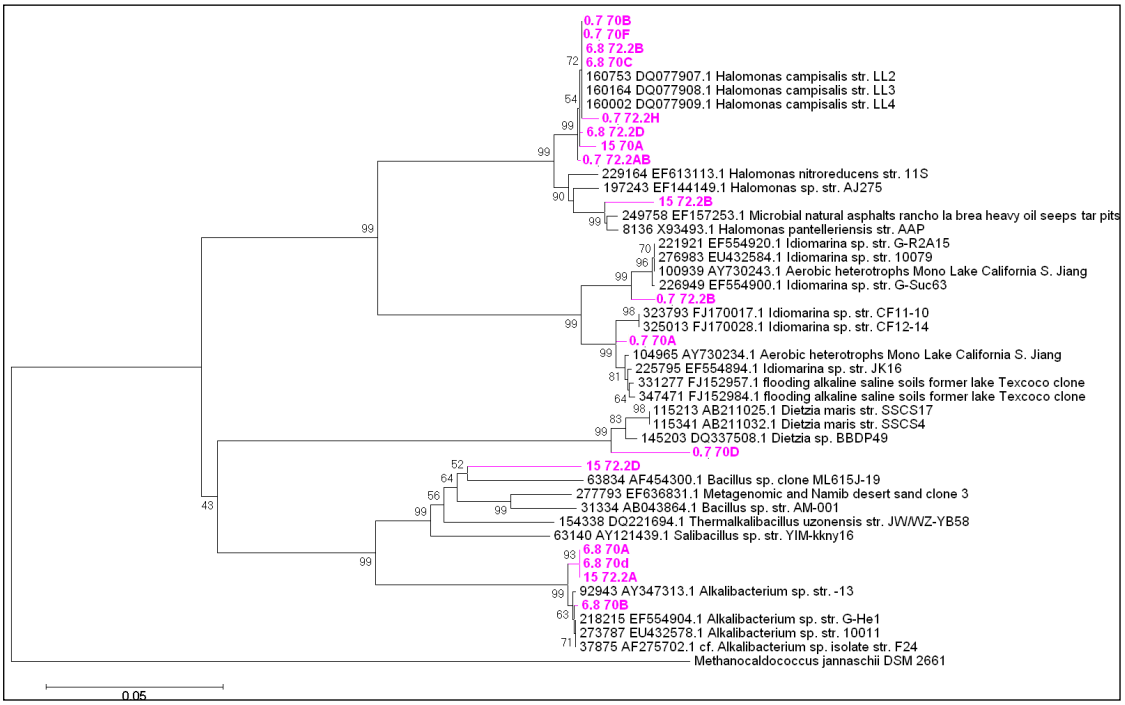



*Figure 3.3. Phylogenetic tree for the cultured isolates (pink) from samples 70 and 72.2 and near neighbour and nearest neighbour matches (black) using Greengenes alignment software (DeSantis et al., 2006). M. jannaschii DSM 2661 was used as an out group. Interior branch values, 1000 replications.*

### 3.6.3.4. Culture based study of Division 4: Southern Lake Magadi

#### 3.6.3.4.1. Sample 1

All isolates were most closely related to strains of the phyla Proteobacteria or Firmicute. The isolates related to *Bacillus* strains were slightly more varied in terms of their

closest species match, including *B. pseudofirmus, B. hemicellulosilyticus, B. clarkii, B. polygoni, B. vedderi, B. agardhaerens* and *Bacillus locisalis* (Kenyan soda lake) (Figure 3.4).

Three genera dominate (as with the previous samples) these being *Halmonas, Bacillus,* and to a lesser extent *Idiomarina (Pseudoidiomarina).* The closest *Halomonas* database matches strains were predominately *H. campisalis* with a single isolate (0.7%_1-4B) sequenced most closely relate to *H. kenyensis* (Kenyan lakes). Two isolates, on 0.7% NaCl and another on 15% NaCl were most closely related to *Alkalispirillium mobile*, a moderately halophilic, alkaliphilic bacterium (Rijkenberg et al., 2001) (Table 3.5).

**3.6.3.4.2. Sample 2**

As with sample 1, no isolates of *Alkalibacterium* were isolated from this soil sample from the edge of the lake. Nine isolates were most closely related to species of *Bacillus*, *B. alkalinitrilicus, B. arseniciselenatis, B. vedderi, B. selenitireducens* were isolated as well as *B. poygoni* and *B. vedderi*. Similarity was as low as 93% for some isolates (6.8%_2-3B).

Again *H. campisalis* was a dominant isolate with five isolates most closely related to the species. Finally isolate 0.7%_2-3C was most closely related to *I. seosinensis* was also identified (93% similarity) and isolate 0.7%_2-3K) has a similarity of only 94% to an *Idiomarina* (*Pseudoidiomarina*) sp. isolate (Figure 3.5).

**3.6.3.4.3. Sample 58**

This was a sample of bright pink and yellow biomat. Strains related to *B. vedderi*, *B. cohnii* and *B. clarkii* (Figure 3.4) were also isolated with a 94%-99% similarity. Isolates related to *H. campisalis* were again cultured, varying in similarity between 91% and 99%. In addition, two other strains cultured on 6.8% NaCl medium were related to *H. pantelleriensis* and *H. taeheungii* (mud volcano) (Figure 3.4). Finally, three isolates related to *Pseudoidiomarina* (*Idiomarina*) sp. with between 93% and 95% similarity were isolated (Figure 3.5 and Table 3.5).

**3.6.3.4.4. Sample 60**

The first isolate related to *Piscibacillus* was isolated from this sample, with 95% similarity to the isolate from a hypersaline Iranian lake (Figure 3.4 and Table 3.3). The majority of isolates sequenced successfully were again related to *A. psychrotolerans* and *H. campisalis*, with strains relating to the latter by between 95% and 99%. Strains related to *H. pantelleriensis* were again isolated with 94% similarity to the database strain (Figure 3.5). This sample was an orange biomat.

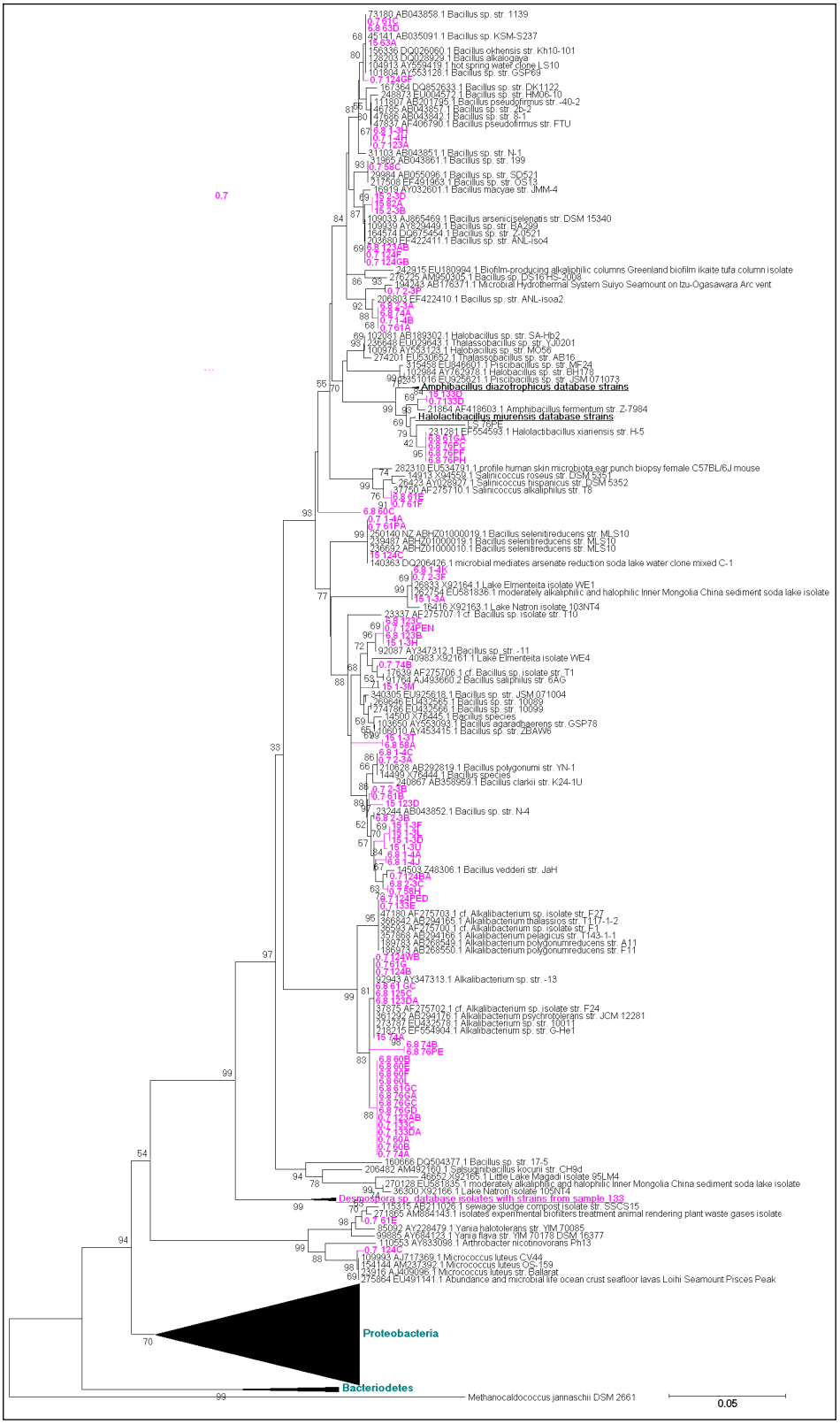



*Figure 3.4. Phylogenetic tree for the cultured isolates (pink) from samples 1, 2, 58, 60, 61, 63, 74, 76, 123, 124, 125, and 133 of the phyla Firmicute and Actinomycete and near neighbour and nearest neighbour matches (black) using Greengenes alignment software (DeSantis et al., 2006). M. jannaschii DSM 2661 was used as an out group. Interior branch values, 1000 replications.*

#### 3.6.3.4.5. Sample 61

Strains from this biomat sample were again predominantly related to *H. campisalis* and *A. psychrotolerans* with a similarity of >99%. Isolates 6.8%_61PB and 15%_61B were related to other strains of *Halomonas*, with 94% and 98% similarity to *H. pantelleriensis* respectively. Isolates related to *Pseudoidiomarina* were also identified with a similarity of 94% (Figure 3.5).

Isolates related to the genus *Bacillus were most closely related to a broader range of species including, B. akibai, B. vedderi, B. hemicellulosilyticus* and *B. selenitireducens.* Isolate 0.7%_61E was most closely related to an Actinomycetales bacterium, 98% similarity, although this strain has not been full described. 98% similarity was identified between isolate 6.8%_61GA and a *Halolactibacillus alkaliphilus*, a strain from an Inner Mongolian soda lake. Finally, two isolates on 0.7% and 6.8% NaCl were most closely related to *Salinicoccus alkaliphilus* (99% similarity), the database strain was also isolated from an Iranian hypersaline Lake (Figure 3.4 and Table 3.5).

#### 3.6.3.4.6. Sample 63

Strains related to *H. campisalis* were again isolated (97%-99% similarity), the database strains included an isolate from bauxite mining and from mud volcano from this water sample. *Bacillus* isolates related to *B. okhensis* and *B. akibai* were isolated (99% similarity). 0.7%_63AB was most closely related to *Alkalimonas amylolytica* (Lake Chahannor)(Ma et al., 2004a). Finally, isolate 6.8%_63AC was most similar to *Aquiflexum* sp. from deep sea sediment from Lonar Lake (Figure 3.5). Isolate 0.7%_63D was most closely related to *Alkalimonas delamerensis*, isolated from soda lake environment Kenya/China (Table 3.3).

#### 3.6.3.4.7. Sample 74

Isolates related to *Bacillus* were cultured, most closely related to the species *B. saliphilus* (99% similarity, mineral pool) and *B. hemicellulosilyticus* (94% similarity) were isolated from this water sample. Isolates from this water sample included *A. psychrotolerans* which varied in similarity between 87% and 99%. Another isolate related to *Aquiflexum* sp. was isolated (99% similarity). *Pseudoidiomarina (Idiomarina)* sp. isolates were also cultured (Figure 3.5).

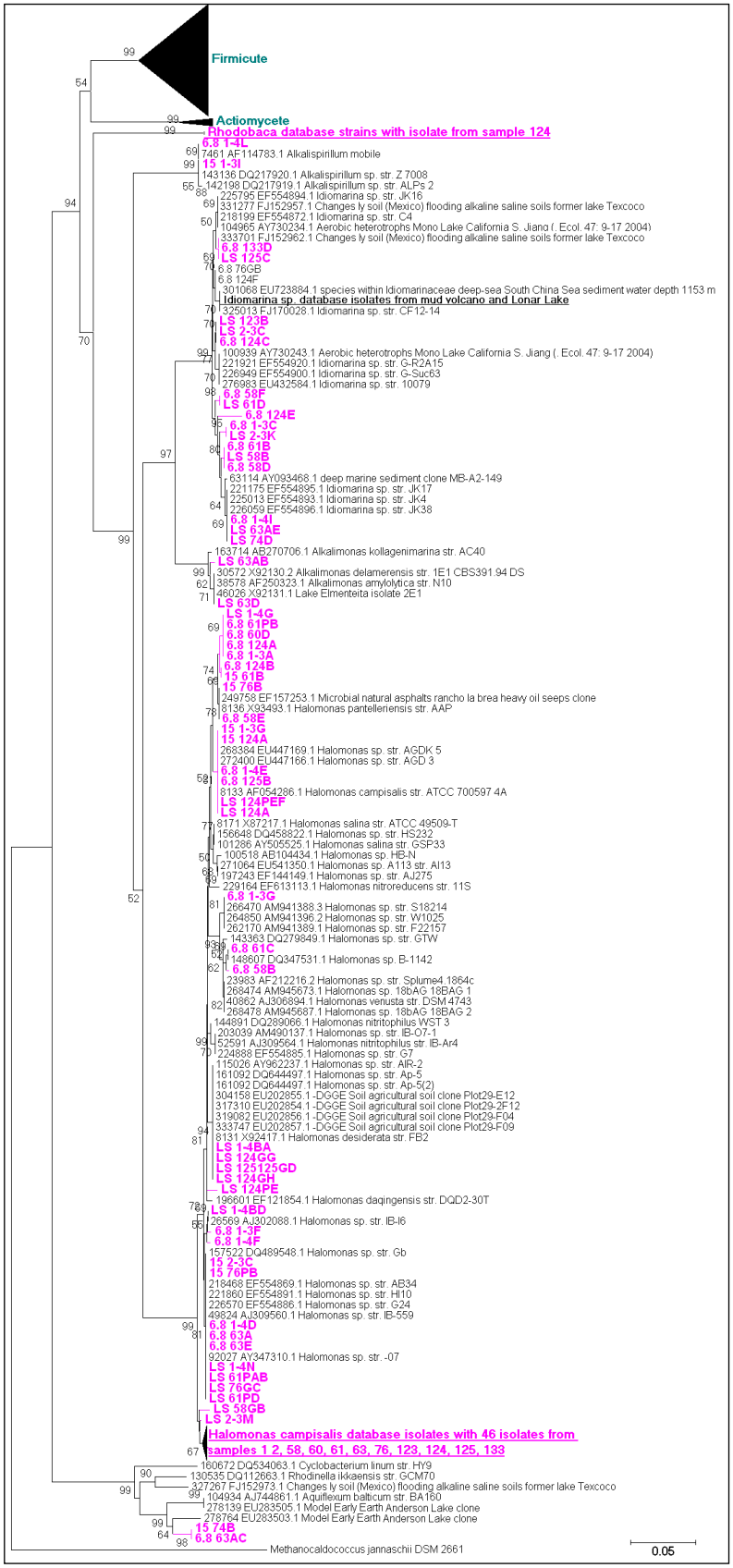



*Figure 3.5. Phylogenetic tree for the cultured isolates (pink) from samples 1, 2, 58, 60, 61, 63, 123, 124, 125, and 133 of the phyla Proteobacteria and Bacteroidetes and near neighbour and nearest neighbour matches (black) using Greengenes alignment software (DeSantis et*

*al., 2006). M. jannaschii DSM 2661 was used as an out group. Interior branch values, 1000 replications.*

**3.6.3.4.8. Sample 76**

Nine strain isolated and sequenced from this biomat sample which were related to *Halomonas* were most closely related to *H. campisalis* and *H. pantelleriensis* with strain 0.7%_76GC 99% similar to an unidentified species of Halomonas isolated from soda lake mud (Table 3.5). Three isolates cultured on 6.8% medium were related to *A. psychrotolerans* (Figure 3.5). In addition, four isolates on the 0.7% and 6.8% growth media were isolates and were most closely related to *Halolactibacillus alkaliphilus*. Isolate 0.7%_76PE demonstrated an 83% similarity, whilst those isolated on 6.8% medium were 99% similar to the database strain (Inner Mongolian soda lake- type strain).

**3.6.3.4.9. Sample 123**

Several isolates on 6.8% NaCl medium were most closely related to *Bacillus saliphilus* varying in similarity between 97% and 99%. Other *Bacillus* strains include 0.7%_123A- *B. pseudofirmus*, 6.8%_123AB- *B. macyae* (93% similarity) and 15%_123D (Table 3.5). Isolates related to *H. campisalis* and *A. psychrotolerans* were again cultured (Table 3.5). This sample was green material floating on the surface of the lagoon/spring.

**3.6.3.4.10. Sample 124**

This sample was a crumble white, unconsolidated material from the edge of the lake. Isolates related to *H. campisalis* (97%-99% similarity) were cultured, along with several isolates of *H. desiderata* (98%-99% similarity), *H. pantelleriensis* (94% similarity) and *H. nitriophilus* (96% similarity). Isolate 0.7%_124PED was most closely related to *Alkalibacterium pelagium* (marine), with 99% similarity. Six isolates were most closely related to species of *Bacillus*, related to *B. saliphilus* (99% similarity), *B. akibai* (94% similarity), *B. vedderi* (97% similarity), *B. selenitireducens* (98% similarity) and *B. alkalinitrilicus* (94% similarity) (soda solonchak soil) (Table 3.5).

Three isolates were most closely related to *Idiomarina seosinensis* demonstrating between 91% and 94% similarity. Isolate 0.7%_124C was most closely related to *Micrococcus luteus* (99%), 0.7%_124PENA to *Salsuginibacillus halophilus* (99%, Inner Mongolian lake) and finally, 0.7%_124D was most closely related to *Rhodobaca borgoriensis* (99% similarity).

**3.6.3.4.11. Sample 125**

This was a sample of soil, the isolates sequenced were all either 99% related to *H. campisalis, H. desiderata* or *A. psychrotolerans.* Isolate 0.7%_125C was most closely related to *I. seosinensis* (93% similarity) but 99% similar to an uncultured clone from Lake Texcoco.

*Table 3.5. Selected blast search results for different genera, species or percent similarity (%) for isolates. Full list see of isolates see Appendices A-D*

| Isolate name | Sequence length | Closest database match | % | Closest database strain match | % |
|---|---|---|---|---|---|
| 6.8%_106A | 784 | - | - | *Caldalkalibacillus uzonensis* JW/WZ-YB58 (NR_043653.1) | 93 |
| 0.7%_108A | 761 | *Halmonas* sp. 4AB3 (HM587243.1) | 88 | *Halmonas campisalis* strain HB10.1 (GU228480.1) | 88 |
| 0.7%_108C | 759 | Gamma proteobacterium E-113 (FJ764789.1) | 86 | *Idiomarina baltica* strain YCSD64 (GQ169047.1) | 81 |
| 0.7%_108E | 755 | Gamma proteobacterium E-034 (FJ764787.1) | 99 | *Idiomarina seosinensis* strain 2PR51-17 (EU440983.1) | 92 |
| 15%_108DB | 765 | *Alkalibacterium* sp. E-119 (FJ764767.1) | 99 | *Alkalibacterium pelagium* strain T143-1-1 (NR_041574.1) | 99 |
| 15%_108DC | 764 | *Bacillus* sp. T41 (AB111934.1) | 98 | *Bacillus okhensis* strain Kh10-101 (NR_043484.1) | 99 |
| 6.8%_108B2.A | 766 | Uncultured *Bacillus* sp. clone (EU676874.1) | 99 | *Bacillus pseudofirmus* strain NT10I3.2AB (GQ365198.1) | 99 |
| 6.8%_108C | 752 | *Halomonadaceae* bacterium GFAJ-1 (HQ449183.1) | 95 | *Halomonas meridiana strain* SL145 (JN645873.1) | 95 |
| 6.8%_108DA | 765 | *Alkalibacterium* sp. E-119 (FJ764767.1) | 99 | *Alkalibacterium pelagium* strain T143-1-1 (NR_041574.1) | 99 |
| 6.8%_108E | 749 | *Halmonas* sp. N1(2011) (JF937425.1) | 98 | *Halmonas variabilis* strain HTG7 (AY204638.1) | 97 |
| 6.8%_108GA | 719 | *Planococcus* sp. MTCC 8491 (JF775504.1) | 98 | *Planococcus maritimus* strain SS-06 (EU624446.1) | 98 |
| 0.7%_109A | 721 | *Yaniella* sp. G5 (FJ871122.1) | 99 | Actinomycetales bacterium SSCS15 (AB211026.1) | 97 |
| 15%_109D | 740 | *Alkalibacterium* sp. E-119 (FJ764767.1) | 99 | *Alkalibacterium pelagium* strain T143-1-1(NR_041574.1) | 99 |
| 0.7%_110AA | 772 | Unidentified Hailaer soda lake bacterium T10 (AF275797.1) | 99 | *Bacillus aurantiacus*, strain K1-10 (AJ605772.2) | 96 |
| 0.7%_110B | 778 | *Bacillus* sp. AMnr1 (FJ788526.1) | 97 | *Bacillus arseniciselenatis*, type DSM 15340T (AJ865469.1) | 97 |
| 0.7%_110FA | 743 | *Halmonas* sp. 10022 EU432574.1 | 84 | *Halmonas nitritophilus* strain 5-5-12 (GU113002.1) | 84 |
| 0.7%_110FC | 769 | Gamma proteobacterium E-410 (FJ764791.1) | 97 | *Idiomarina* fontislapidosi strain F23 (NR_029115.1) | 92 |
| 15%_110A | 709 | *Halmonas* sp. 15-7 (HM598402.1) | 95 | *Halmonas pantelleriensis* strain (GQ505338.1) | 95 |
| 6.8%_110AB | 761 | *Halmonas* sp. E-069 (FJ764763.1) | 98 | *Halmonas venusta* strain NBSL25 (FJ973522.1) | 98 |

*Table 3.3 continued from previous page*

| Isolate name | Sequence length | Closest database match | % | Closest database strain match | % |
|---|---|---|---|---|---|
| 6.8%_110B | 768 | Uncultured bacterium clone 101-159 (EF157253.1) | 96 | *Halmonas pantelleriensis* strain 4-5-9 (GQ505338.1) | 96 |
| 6.8%_110CB | 767 | Gamma proteobacterium E-410 (FJ764791.1) | 86 | Pseudidiomarina *salinarum* strain ISL-52 (NR_044246.1) | 81 |
| 0.7%_113B | 755 | *Halmonas* sp. AMP-12 (HM104378.1) | 95 | *Halmonas nitritophilus* strain 5-5-12 (GU113002.1) | 95 |
| 15%_113A | 773 | Bacillaceae bacterium halo-2 (EU581836.1) | 99 | *Bacillus agaradhaerens* strain Mi10-62 (GQ121032.1) | 93 |
| 15%_113BA | 747 | *Halmonas* sp. AGD 3 (EU447166.1) | 98 | *Halmonas campisalis* strain 4A (NR_028702.1) | 97 |
| 15%_113D | 777 | Bacterial sp. Lake Natron isolate 105NT4 (X92166.1) | 98 | *Salsuginibacillus halophilus* strain halo-1 (EU581835.1) | 98 |
| 15%_113E | 741 | Gamma proteobacterium E-410 (FJ764791.1) | 94 | *Idiomarina zobellii* strain M8B (GU397405.1) | 91 |
| 6.8%_113DB | 742 | Uncultured bacterium clone Luq_GN490_030 (HQ445815.1) | 99 | *Halmonas* sp. W1025 (AM941396.2) | 99 |
| 6.8%_113G | 772 | *Piscibacillus* sp. 401C1-1 (HM222702.1) | 94 | *Piscibacillus halophilus* HS224 (FM864227.1) | 94 |
| 15%_114B | 810 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | *Alkalibacterium* sp. 10011 (EU432578.1) | 98 |
| 6.8%_114A | 740 | Uncultured alpha proteobacterium clone WN-HSB-198 (DQ432305.1) | 98 | Rhodobacter sp. EL-50 strain EL-50 (NR_042212.1) | 97 |
| 15%_115A | 801 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | *Alkalibacterium* sp. 10011 (EU432578.1) | 98 |
| 15%_115AB | 786 | *Bacillus* sp. ANL-isoa2 (EF422410.1) | 97 | *Bacillus* arseniciselenatis type strain DSM 15340T (AJ865469.1) | 93 |
| 15%_115E | 804 | *Bacillus* cellulosilyticus DSM 2522 (CP002394.1) | 94 | *Bacillus* vedderi strain JaH NR_026526.1) | 94 |
| 6.8%_81PC | 778 | *Halmonas* sp. 4AB3 (HM587243.1) | 92 | *Halmonas campisalis* strain HB10.1 (GU228480.1) | 92 |
| 6.8%_81F | 801 | *Bacillus* sp. ISO_02_Chiprana (EU676882.1) | 99 | *Bacillus clarkii* strain DSM 8720 (NR_026141.1) | 99 |
| 6.8%_81B | 797 | *Bacillus* sp. B-3(2011) (N128828.1) | 85 | *Bacillus cohnii* strain D7055 (FJ161352.1) | 85 |

*Table 3.3 continued from previous page*

| Isolate name | Sequence length | Closest database match | % | Closest database strain match | % |
|---|---|---|---|---|---|
| 6.8%_81A | 800 | *Bacillus* sp. ISO_02_Chiprana (EU676882.1) | 99 | *Bacillus polygoni* strain YN-1 (NR_041571.1) | 99 |
| 15%_81GB | 785 | *Halmonas* sp. HI.br (GU228482.1) | 99 | *Halmonas nitritophilus* strain 5-5-12 (GU113002.1) | 99 |
| 6.8%_82C | 777 | Bacterial sp. (Lake Bogoria isolate WB4) (X92145.1) | 98 | *Halmonas kenyensis* strain AIR-2 (NR_043299.1) | 98 |
| 6.8%_82A | 770 | Uncultured bacterium clone MB-A2-149 (AY093468.1) | 96 | *Pseudidiomarina salinarum* strain ISL-52 (NR_044246.1) | 92 |
| 15%_82A | 767 | *Bacillus* sp. GSP69 (AY553128.1) | 96 | *Bacillus hemicellulosilyticus* strain C-11 (NR_040848.1) | 96 |
| 6.8%_83C | 755 | *Halolactibacillus miurensis* NRIC 0637 (AB362701.1) | 94 | *Halolactibacillus xiariensis* strain H-5 16S (NR_044282.1) | 96 |
| 0.7%_85A | 833 | *Bacillus* sp. ISO_06_Kulunda (EU676884.1) | 99 | *Bacillus alkalinitrilicus* strain ANL-iso4 (NR_044204.1) | 97 |
| 0.7%_86F | 797 | *Bacillus* sp. B-3 (2011) (JN128828.1) | 92 | *Bacillus cohnii* strain D7055 (FJ161352.1) | 92 |
| 0.7%_86E | 787 | *Halmonas* sp. HB.br (GU228481.1) | 97 | *Halmonas campisalis* strain 4-5-8 (GU112958.1) | 97 |
| 0.7%_94D | 809 | Bacillaceae bacterium halo-2 (EU581836.1) | 99 | *Bacillus locisalis* CG2 (FR714931.1) | 98 |
| 0.7%_94BB | 777 | Uncultured bacterium clone ncd2591f06c1 (JF227185.1) | 99 | *Dietzia cinnamea* strain :IMMIB RIV-399 (NR_042390.1) | 99 |
| 0.7%_94B | 804 | Uncultured bacterium clone ncd2190g02c1 (JF190672.1) | 99 | *Bacillus okhensis* strain Kh10-101 (NR_043484.1) | 100 |
| 0.7%_70D | 842 | Uncultured bacterium clone 46-SI5 (HQ116731.1) | 95 | *Dietzia maris* strain: SSCS4 (AB211032.1) | 95 |
| 0.7%_72.2B | 831 | Gamma proteobacterium E-034 (FJ764787.1) | 99 | *Pseudidiomarina* sp. (AK5 strain FN995239.1) | 93 |
| 6.8%_1-4L | 816 | *Alkalispirillum* sp. ACO1 (FJ976677.1) | 99 | *Alkalispirillum mobile* strain DSM 12769 (NR_024961.1) | 99 |
| 6.8%_1-4K | 809 | *Bacillus* sp. ISO_02_Chiprana (EU676882.1) | 97 | *Bacillus clarkii* strain DSM 8720 (NR_026141.1) | 97 |
| 6.8%_1-4J | 840 | *Bacillus* sp. ISO_02_Chiprana (EU676882.1) | 96 | *Bacillus polygoni* strain YN-1 (NR_041571.1) | 96 |
| 6.8%_1-4A | 839 | Bacillaceae bacterium halo-2 (EU581836.1) | 95 | *Bacillus* sp. CG2 16S rRNA gene, strain CG2 (FR714931.1) | 94 |

*Table 3.3 continued from previous page*

| Isolate name | Sequence length | Closest database match | % | Closest database strain match | % |
|---|---|---|---|---|---|
| 0.7%_1-4A | 819 | *Bacillus selenitireducens* MLS10 (CP001791.1) | 99 | *Bacillus selenitireducens* strain M1S4-7 (GU112950.1) | 97 |
| 15%_1-3M | 838 | Uncultured bacterium clone nbw287f02c1 (GQ086265.1) | 95 | *Bacillus agaradhaerens* strain Mi10-62 (GQ121032.1) | 95 |
| 15%_1-3H | 811 | *Bacillus* sp. X10-7 (HM598405.1) | 99 | *Bacillus saliphilus* strain 6AG (NR_025554.1) | 96 |
| 15%_1-3A | 808 | Bacillaceae bacterium halo-2 (EU581836.1) | 99 | Bacterial sp. (Lake Elementaita isolate WE1) (X92164.1) | 99 |
| 0.7%_58B | 839 | *Pseudidiomarina* sp. QA8 )GQ202579.1 | 99 | *Pseudidiomarina* sp. (AK5 FN995239.1) | 95 |
| 6.8%_58B | 829 | Halomonadaceae bacterium GFAJ-1 (HQ449183.1) | 89 | *Halmonas taeheungii* strain M112P1-7 (GU112966.1) | 89 |
| 6.8%_60C | 839 | *Piscibacillus* sp. W9B(2010) (HQ433445.2) | 95 | *Piscibacillus* sp. strain HS224 (FM864227.1) | 95 |
| 0.7%_61F | 780 | - | - | *Salinicoccus alkaliphilus* strain X2B (GU397409.2 | 99 |
| 0.7%_61E | 768 | *Yaniella* sp. G5 (FJ871122.1) | 99 | Actinomycetales bacterium SSCS15 (AB211026.1) | 98 |
| 0.7%_61C | 786 | *Bacillus* sp. NBRC 101221 (AB681407.1) | 99 | *Bacillus alibai* strain 1139 (NR_028620.1) | 99 |
| 6.8%_61GA | 792 | - | - | *Halolactibacillus alkaliphilus* NBRC 103919 (AB682143.1) | 98 |
| 6.8%_61C | 767 | Halomonadaceae bacterium GFAJ-1 (HQ449183.1) | 99 | *Halmonas* sp. strain ECS-str.1 (HE586889.1) | 98 |
| 0.7%_63D | 837 | Bacterial sp. (Lake Elementaita isolate 2E1) (X92131.1) | 98 | *Alkalimonas delamerensis* strain :1E1 (NR_044879.1) | 98 |
| 6.8%_63AC | 842 | Aquiflexum sp. DL6 (JF812063.1) | 96 | *Fontibacter* sp. AK8, strain (FR687204.1) | 95 |
| 6.8%_63A | 810 | *Halmonas* sp. HB.br 16S (GU228481.1) | 99 | *Halmonas* sp. IB-G4 (AM490139.1) | 99 |
| 0.7%_76GC | 817 | *Halmonas* sp. HB.br 16S (GU228481.1) | 99 | *Halmonas* sp. IB-G4 (AM490139.1) | 99 |
| 6.8%_123AB | 841 | *Bacillus* sp. JAEA (AB437410.1) | 93 | *Bacillus macyae* strain JMM-4 (NR_025650.1) | 93 |
| 0.7%_124PENA | 830 | *Salsuginibacillus halophilus* gene (AB682229.1) | 99 | *Salsuginibacillus halophilus* strain halo-1 (EU581835.1) | 99 |

*Table 3.3 continued from previous page*

| Isolate name | Sequence length | Closest database match | % | Closest database strain match | % |
|---|---|---|---|---|---|
| 0.7%_124D | 841 | Uncultured bacterium clone x287 (GU083689.1) | 99 | *Rhodobaca bogoriensis* strain LBB1 (NR_025089.1) | 99 |
| 0.7%_124GD | 839 | Uncultured *Halmonas* sp. clone Plot22-2H06 (EU665122.1) | 85 | *Halmonas desiderata* strain FB2 (NR_026274.1) | 85 |
| 0.7%_124D | 841 | Uncultured bacterium clone x287 (GU083689.1) | 99 | Rhodobaca *bogoriensis* strain LBB1 (NR_025089.1) | 99 |
| 0.7%_124C | 818 | *Micrococcus* sp. OS5 clone F12 GU003860.1 | 99 | Micrococcus *luteus* strain PCSB6 (HM449702.1) | 99 |
| 0.7%_133D | 808 | Uncultured low G+C Gram-positive bacterium clone WN-HSB-264 (DQ432299.1) | 97 | Am*phibacillus fermentum* strain Z-7984 (NR_025193.1) | 96 |
| 0.7%_133D | 797 | *Alkalibacterium* sp. 10011 (EU432578.1) | 98 | *Alkalibacterium* psychrotolerans strain IDR2-2 (NR_024830.1) | 97 |
| 0.7%_133A | 840 | - | - | *Desmospora activa* strain : IMMIB L-1269 (NR_042692.1) | 96 |

### 3.6.3.4.12. Sample 133

The pink/peach consolidated sample (magadiite- see chapter 7) yielded strains again related to *H. campisalis, A. psychrotolerans* and *I. seosinensis*. 0.7%_133E was most closely related to *Alkalibacterium pelagium* and 0.7%_133D and 15%_133D were both most closest related to *Amphibacillus fermentum*, with 96%-97% similarity. Isolate 0.7%_133A was 96% similar to an isolate of *Desmonspora active* (Table 3.5).

## 3.6.4. Culturable archaeal diversity of samples from Lake Magadi

Touchdown PCR was used to amplify the 16s rDNA of the microbial isolates due to the continuous issue of non-specific binding. A selection of isolates and their closest database match and closest cultured isolate match are given in Table 3.6. Only five isolates were sequenced using the archaeal primers so these will be discussed collectively rather than by area. All sequenced archaea isolates are given in this chapter.

*Table 3.6 Archaeal isolates cultured and sequenced from all samples*

| Isolate | Sequence length | Closest database strain match | % |
|---|---|---|---|
| 15%_71A | 842 | *Natronococcus occultus* strain: JCM 8859 (AB477981.1) | 99 |
| 15%_115A | 841 | *Natronococcus occultus* strain: JCM 8859 (AB477981.1) | 98 |
| 15%_72.2C | 789 | *Natronococcus occultus* strain: JCM 8859 (AB477981.1) | 98 |
| 15%_125A | 800 | *Natronococcus occultus* strain: JCM 8859 (AB477981.1) | 99 |
| 15%_70A | 602 | *Natrialba magadii* ATCC 43099 (CP001932.1) | 99 |

Four of the isolates were most closely related to strain *Natronococcus occultus*, with 98%-99% similarity (East African soda lakes), one isolate from sample 15%_70A was most closely related to strain *Natrialba magadii* (Lake Magadi), this sequence did have a low query coverage (89%), compared to the other strains compared through BLAST. Subsequent attempts to amplify and sequence this strain again failed. All isolates were bright pink in colour.

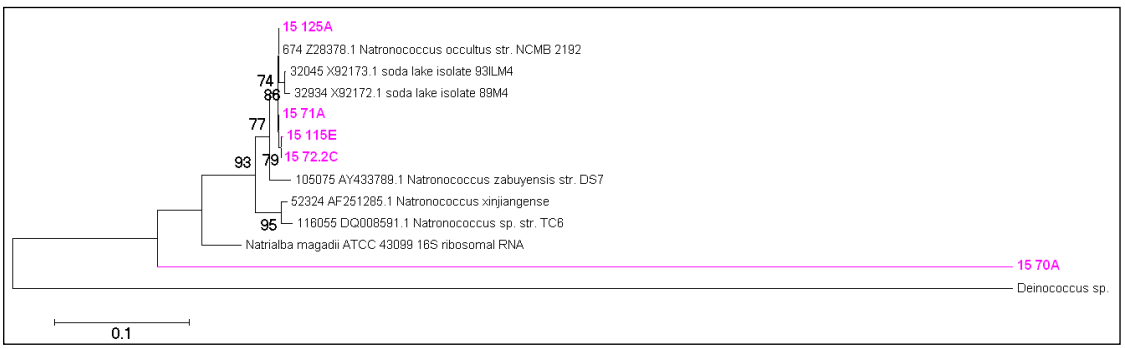


*Figure 3.6 Phylogenetic tree of archaeal isolates (pink) and near neighbour and nearest neighbour matches (black) using Greengenes alignment software (DeSantis et al., 2006). Deinococcus sp. was used as an out group. Interior branch values, 1000 replications*

### 3.6.5. Denaturing gradient gel electrophoresis analysis

DGGE analysis enables the assessment of the uncultured diversity of a sample, and look at similarities in community structure between different types of samples. The DGGE protocol is outlined in Chapter 2, section 2.2.2.6). The raw binary DGGE data is present in Appendix E.

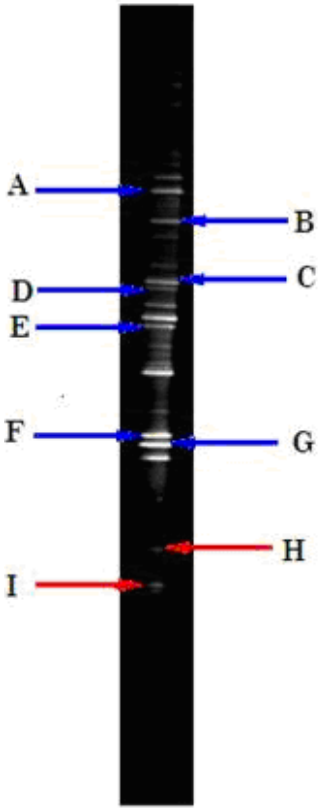
#### 3.6.5.1. DGGE ladder

A DGGE ladder was made by combining eleven isolates, nine isolates of bacteria and two isolates of archaea. The DGGE ladder was compiled by running 1 lane for each of the isolates, plus a lane which contained the combined DNA of the isolates. The presence of unassigned bands in the ladder is due to two reasons, firstly, the inclusion of Mars chamber isolates related to analyses in Chapter 5 (Section 6.3.3.5) and secondly due to multiple copies of 15S rRNA gene sequences in the same strain (Kang et al., 2010).

Strains included in the ladder were isolated in section 3.4.3 of this chapter, bacterial isolates include: 6.8%_114B, 6.8%_108GA, 15%_110A, 6.8%_76GB, 6.8%_106A, 6.8%_86C, 0.7%_58C, 0.7%_61F and 0.7%_110A, plus two archaea strains: 15%_72.2C and 15%_70A. In addition, one isolate from Chapter 6, isolated from the Mars chamber experiments was most closely related to *Halomonas campisalis*, this isolate was also included (Table 3.7).

The ladder profile of known isolates shows that in the instances of bands C and E, the resolution of the DGGE was not sufficient to distinguish between the 16 sRNA gene sequences of the different isolates.

*Table 3.7 Ladder key, DGGE ladder with bands assigned, blue arrows indicate bacterial bands, red arrows, archaea bands. Names assigned were the closest match from the NCBI database. In addition to the isolates from this chapter, one strain isolated from the Mars chamber in Chapter 6, which was most closely related to H. campisalis has been included (Band C- dark grey).*

| | Key | Closets uncultured database match | % | Isolate | Closest strain match | % |
|---|---|---|---|---|---|---|
|  | A | *Bacillus sp.* E-127 FJ76477.1 | 98 | 6.8%_114B | *Bacillus pseudofirmus* MC02 JN566125.1 | 96 |
| | B | *Planococcus* sp. JSM 076050. FJ477406.1 | 98 | 6.8%_108GA | *Planococcus maritimus strain* AMP-10. HM104376.1 | 98 |
| | C | *Halomonas* sp. *15-7*<br>*Halomonas sp.* 4AB3 HM587243.1 | 94<br>94 | 15%_110A<br>(6.8%_B3-1F) | *Halomonas pantelleriensis* 4-5-9. GQ505338.1<br>*Halomonas campisalis* strain HB10.1 GU228480.1 | 94<br>99 |
| | D | Gammaproteobacterium E-410. FJ764791 | 99 | 6.8%_76GB | *Idiomarina seosinensis* CL-SP19. NR_025826.1 | 93 |
| | E | *Bacillus* sp. 9-3AIA FN397519.1.<br>Gamma proteobacterium E-034. FJ764787.1<br>*Bacillus* sp. DV9-38 GQ407183.1 | 92<br>99<br>98 | 6.8%_106A<br>6.8%_86C<br>0.7%_58C | *Caldalkalibacillus uzonensis* NR_043653.1<br>*Idiomarina seosinensis* 2PR51-17. EU440983.1<br>*Bacillus cohnii* T-46 H202864.1 | 93<br>92<br>98 |
| | F | - | - | 0.7%_61F | *Salinicoccus alkaliphilus* X2B. GU397409.2 | 99 |
| | G | *Hailaer soda lake bacterium* T10 AF275707.1 | 99 | 0.7%_110A | *Bacillus aurantiacus* K1-10 AJ605772.2 | 97 |
| | H | *Natronococcus occultus* SP4. AB477981.1 | 99 | 15%_72.2C | *Natronococcus occultus* JCM 8859. NR_028255.1 | 99 |
| | I | *Natrialba magadii* ATCC 43099 CP001932.1 | 99 | 15%_70A | *Natrialba wudunaoensis strain Sua-E42* EU672838.1 | 99 |

### 3.6.5.2. DGGE profiling

Ten samples were chosen for analysis and are outlined in the Table 3.8, along with the environmental conditions associated with each sample (samples are described in detail in Section 3.3.1, Table 3.1). Samples 1A and 2A have a pH and temperature value associated, however a water analysis was not conducted on the waters associated with these samples. The samples include both biological material and soil samples which were also the focus of the culture based study, section 3.4.3. The samples 1A and 2A provide a comparison between samples from very close localities but which possibly different in soil conditions due to their different proximities to the lagoon edge.

*Table 3.8 Conditions associated with samples used in DGGE analysis.*

*Area codes relate to: 1-North Little Magadi, 2-North Lake Magadi, 3-Western Lagoon and 4-South Lake Magadi. Bio.= Biological samples, N/R denotes not recorded*

| Sample | **1A** | **2A** | **58** | **72.2** | **76** | **133** | **113** | **86** | **125** | **94** |
|---|---|---|---|---|---|---|---|---|---|---|
| Area | 4 | 4 | 4 | 3 | 4 | 4 | 1 | 2 | 4 | 2 |
| Sample type | Soil | Soil | Bio. | Soil | Bio. | Mineral | Soil | Soil | Soil | Bio. |
| Temp. (°C) | 32.9 | 32.9 | 39.5 | 35.7 | 44.6 | 36.6 | 82.4 | 64.3 | 40.3 | 57.3 |
| pH | 9.78 | 9.78 | 10.10 | 10.10 | 9.97 | 9.97 | 9.51 | 9.29 | 9.98 | 9.31 |
| Ca | N/R | N/R | -0.82 | -0.78 | -0.83 | -0.83 | -0.83 | -0.81 | -0.82 | -0.81 |
| Fe (ppm) | N/R | N/R | -0.07 | -0.06 | -0.07 | -0.07 | -0.07 | -0.07 | -0.07 | -0.07 |
| K (ppm) | N/R | N/R | 71.2 | 146.3 | 75.2 | 69.3 | 130.5 | 96.7 | 64.1 | 96.7 |
| Mg | N/R | N/R | -0.52 | -0.52 | -0.52 | -0.52 | -0.52 | -0.52 | -0.52 | -0.52 |
| Na | N/R | N/R | 4521. | 7184.2 | 4659.7 | 4722.6 | 4857 | 4244 | 4439 | 4244 |
| P (ppm) | N/R | N/R | 0.68 | 0.94 | 0.85 | 0.62 | -0.01 | -0.03 | 0.56 | -0.03 |
| S (ppm) | N/R | N/R | 25.82 | 41.52 | 25.75 | 23.69 | 24.04 | 17.28 | 21.4 | 17.28 |
| Si (ppm) | N/R | N/R | 7.29 | 13.5 | 7.38 | 4.08 | 12.15 | 9.87 | 4.03 | 9.87 |
| Sr (ppm) | N/R | N/R | 0.17 | 0.17 | 0.15 | 0.16 | 0.18 | 0.2 | 0.17 | 0.2 |
| F-(ppm) | N/R | N/R | 121.1 | 1 | 90.23 | 163.76 | 181 | 125.37 | 124.6 | 125.4 |
| Cl- | N/R | N/R | 7752 | 11345 | 6500.8 | 7257.2 | 6078 | 5615.7 | 6938. | 5615.7 |
| $NaNO_3$ | N/R | N/R | 1119 | 1470 | 708.03 | 1205.1 | 1040 | 966.51 | 1035. | 966.51 |
| $SO_{4(ppm)}$ | N/R | N/R | 165.1 | 322.31 | 161.08 | 165.82 | N/R | 148.25 | 158.6 | 148.25 |

### 3.6.5.3. Bacterial DGGE profile

The DGGE gel for the bacterial community of the ten samples (in triplicate- See Chapter 2, Section 2.3.5.2) is shown in Figure 3.7. The intensity of band provides an indication of the proportion of that species/ group of species, relative to others in the sample. The 'smiling' effect of the gel was corrected for during analysis (see Chapter 2, section 2.3.5.2). The dashed lines highlight bands which have migrated to the same point as a ladder band (Figure 3.7.A). Bands were then chosen to be excised from the gel and sequenced (Figure 3.7.B)

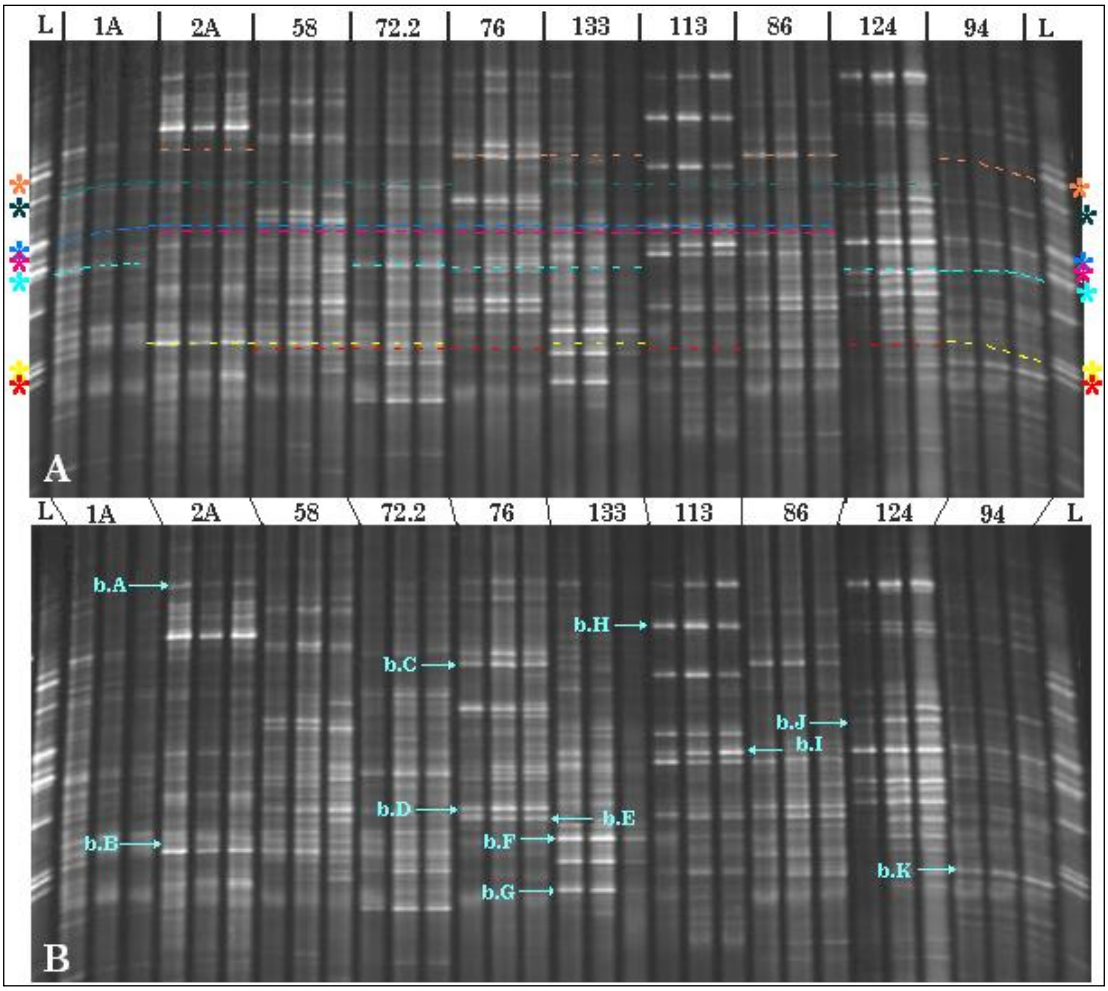



*Figure 3.7. DGGE gel of DNA amplified using bacterial primers. Each sample was run in triplicate (Chapter 2. Section 2.3.5.2)* ***(A)*** *the band in each lane which corresponds to a ladder band (Table 3.5) is highlighted. Orange dash line and* ✻ *denotes bands in corresponding location to the band for 6.8%_114B (Table 3.5 A). Dark green dash line and* ✻ *denotes bands in corresponding location to the band for 6.8%_108GA (Table 3.5 B). Dark blue dash line and* ✻ *denotes bands in corresponding location to the band for 15%_110A (Table 3.5 C). Pink dash line and* ✻ *denotes bands in corresponding location to the band for 6.8%_76GB (Table 3.5 D). Bright blue dash line and* ✻ *denotes bands in corresponding location to the band for 6.8%_106A, 6.8%_86C and LS_58C (Table 3.5E). Yellow dash line and* ✻ *denotes bands in corresponding location to the band for LS_61F (Table 3.5 F). Bright blue dash line and* ✻ *denotes bands in corresponding location to the band for* 0.7%_110A *(Table 3.5 G)*. L. denotes ladder lane. ***(B)*** *Indicates bands which were chosen to be excised and sequenced.*

All samples demonstrated a Shannon index of diversity (H) >3.13, which indicates all samples has a high diversity (Getachew et al., 2012) (Table 3.9).The high diversity of the samples indicates that number of different species and genera identified from samples when using

culture based technique under represents the diversity of the bacterial community, as would be expected. Despite the harsh conditions experienced at Lake Magadi including high pH, temperature and desiccation, the environment is still has a diverse community. The index value was then compared using analysis of variance statistical test which showed that there was no significant difference between the diversity of the samples analysed, suggesting that all samples were equally diverse despite the difference in sample type.

*Table 3.9 Shannon diversity score for each sample, calculated using MVSP. A value of ~1.5 indicates low diversity and a score of ~3.5 suggests a high diversity.*

| Sample | Average Shannon diversity index value (H) |
|---|---|
| 1A | 3.46 |
| 2A | 3.30 |
| 58 | 3.44 |
| 72.2 | 3.42 |
| 76 | 3.42 |
| 133 | 3.23 |
| 113 | 3.13 |
| 86 | 3.28 |
| 125 | 3.49 |
| 94 | 3.42 |

**3.6.5.3.1. Association of bands in bacteria DGGE profile with isolates**

Comparing the bands of the cultured ladder to the bands of the other lanes it is possible to pick out occurrence of particular bands across the samples. The band which includes 6.8%_114B, related *B. pseudofirmus* (Figure 3.7, orange line) was identified as present in S21, S94, S133, S86 and S76, with the band occurring most strongly in the latter two samples. This band is one of the most intense bands in sample S76.

The band corresponding to isolate 6.8%_108GA, most closely related to *P. maritimus* (Figure 3.7, dark green line) was only isolated from samples 108 and 109, but a band at the same position is present in samples 1A, 2A, 58, 72.2, 133, 113 and S125 it is strongest in sample 72.2 and relatively faint in the other samples. The band containing strain 15%_110A, related to *H. pantelleriensis* (Figure 3.7, dark blue line) was identified in samples 1A, 2A, 58, 72.2, 76, 113, 125 and 94. This band is absent in the lanes of sample 113 or 86.

A band which corresponds to a band for the isolate 6.8%_76GB, 93% similar to a strain of *Idiomarina seosinensis* (Figure 3.7, pink line) was identified in the profile of all samples except 2A, 125 and 94. Another strain related to *Idiomarina soesinensis,* isolate 6.8%_86C, was included in the ladder had a 92% similarity, this strain migrated further down the gel, indicating that relatively large separation does occur between strains which are most closely

related to a species of the same genus. This band into which the second *Idiomarina* species contributed also contained three other strains, but is only recorded as present in 1A, 72.2, 76, 133, 125 and 94. In sample 72.2 this is one of the most intense bands of this sample.

The band outlined by turquoise oval corresponds to the band for three of the cultured strains, isolate 6.8%_106A, related to *C. uzonensis*, isolate 0.7%_58C, related to *B. cohnii* and strain 6.8%_86C, related to *Idiomarina seosinensis*. The band can be identified in the triplicates of samples; 1A, 72.2, 76, 133, 125 and 94 (Figure 3.7, bright blue line).

The band which corresponds to the band for isolate 0.7%_61F, related to *Salinicoccus alkaliphilus* (Figure 3.7, yellow line), was present in samples 2A, 58, 72.2, 133 and 94. The corresponding band is also quite intense in sample 72.2 and 2A. A band corresponding to the ladder band for isolate 0.7%_110A, related to *B. aurantiacus* (Figure 3.8, red line) can be traced in samples 72.2 and 125 as well, but also in samples 58, 76 and 113.

**3.6.5.3.2. Sequencing of bacterial DGGE bands**

The sequencing of DGGE bands was conducted, through the cloning of DNA fragment into *E. coli* (described in Chapter 2, section 2.2.2.6.1), the sequencing of up to 5 clones per DNA band excised (Figure 3.7.B). The successful sequencing in both directions of 4 clones from 4 different bands of the bacterial DGGE was achieved. For the other excised bands cloning was unsuccessful, the amplified DNA from the gel failed to be cloned into the plasmid in the isolates picked and tested. The closest matches to these sequences are outline in Table 3.10. The strains were all most closely related to clones and strains from saline environments or saline alkaline environments.

*Table 310 The closest uncultured clone and isolated strain matches for clones from bacterial DGGE gel*

| Band | Closest uncultured | % | Nearest strain match | % |
|---|---|---|---|---|
| b.A5 | Uncultured bacterium clone ARDBACSS23. (EU869383.1) | 94 | *Selenihalanaerobacter shriftii* strain DSSE-1. (NR_028804.1) | 93 |
| b.B.1 | Halanaerobiaceae clone SLAb1-1. (EU855125.1) | 98 | *Halarsenatibacter silvermanii* strain SLAS-1. (AY965613.1) | 98 |
| b.D.1 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | *Alkalibacterium psychrotolerans strain: JCM 12281 (*AB294176.1) | 99 |
| b.E.5 | Uncultured bacterium clone MG18-H2-MF (HQ413944.1) | 100 | *Arsukibacterium ikkense* strain K1-118 (FR746050.1) | 97 |

Clone b.A5 was most closely related to clones from salt lake brine from Inner Mongolia, a hypersaline microbial mat clone from Mexico and a clone from saline soil, Sidi Ameur Salt

Lake in the Algerian Sahara. It is more closely related to these than it is to its closet database strain of *Selenihalanaerobacter shriftii*, an anaerobe from Dead Sea sediments which respires selenate. The similarity of the clone b.A5 to its nearest database match is only 93-94%, suggesting that this could be a novel species or genus.

Clone b.B1 was most closely related to a *Halanaerobiaceae* clone from Searles Lake, which is a salt saturated lake. This clone grouped with *Halarsenatibacter silvermanii*, an arsenate respire which was also identified from Searles Lake, USA and a clone from the sediments of the hypersaline lake, Lake Fazda, Egypt.

Clone b.D1 was most closely related to *Alkalibacterium psychrotolerans* JCM 12281 when run through the blast search, but the phylogenetic analysis places this strain basally to all the strains analysed. This database isolate differs to the closest database match of any of the strains isolated in section 3.4.3, of this Chapter.

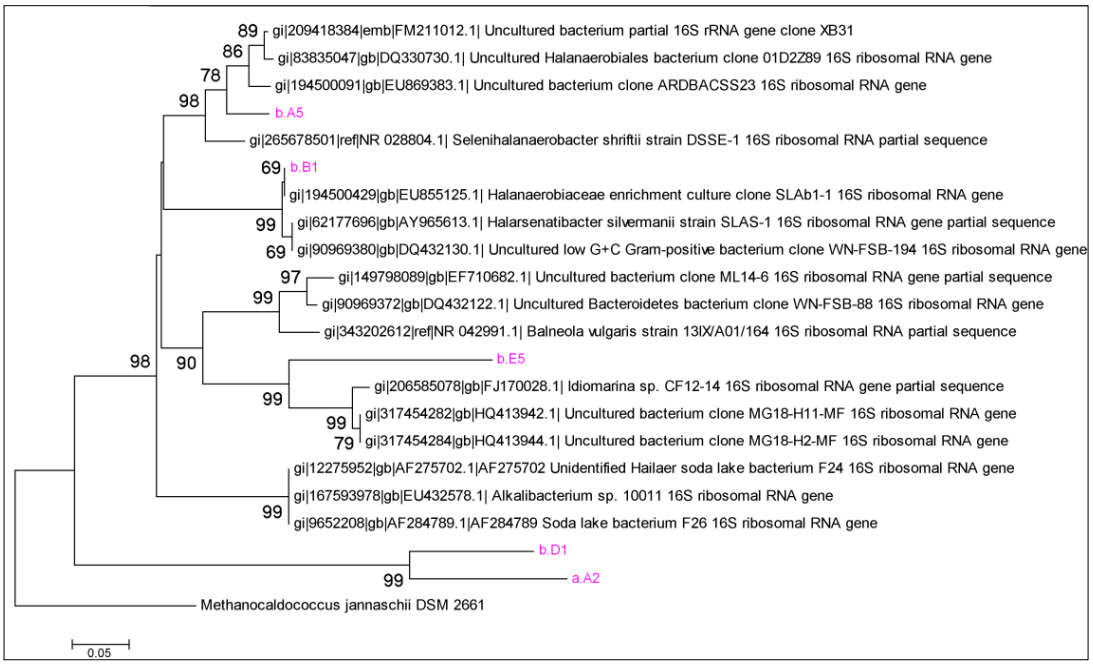



*Figure 3.8 Phylogenetic tree of bacterial clones (pink) and near neighbour and nearest neighbour matches (black) using Greengenes alignment software (DeSantis et al., 2006). Interior branch values, 1000 replications.*

Finally Clone b.E5 was most closely related to *Arsukibacterium ikkense* strain K1-118, a Proteobacteria from shallow soda ponds, with the *Idiomarina* sp. CF12-14 from the South China Sea, and two clones from the biological treatment of refinery spent caustics under halo-alkaline conditions.

### 3.6.5.3.3. Differences in community composition of bacteria in samples, detected by hierarchical cluster analysis

Neighbour joining cluster analysis of DGGE profile of different samples and replicates provides an indication of the relationship between sample replicates and between the replicates and the other samples analysed.

Clustering analysis of the bacterial DGGE lane profiles demonstrates that for every sample the triplicates are more closely related to each other than to any other triplicate (Figure 3.9). The samples generally form three clades, one containing samples 86, 113, 133, 76, 58 and 2A, a second containing just sample 125 and finally a clade containing 72.2, 94 and 1A. There seems to be no separation in relation to temperature or pH, with samples which have high temperature (sample temperatures and pH's group together), there is also no apparent separation due to sample type or geographical location.

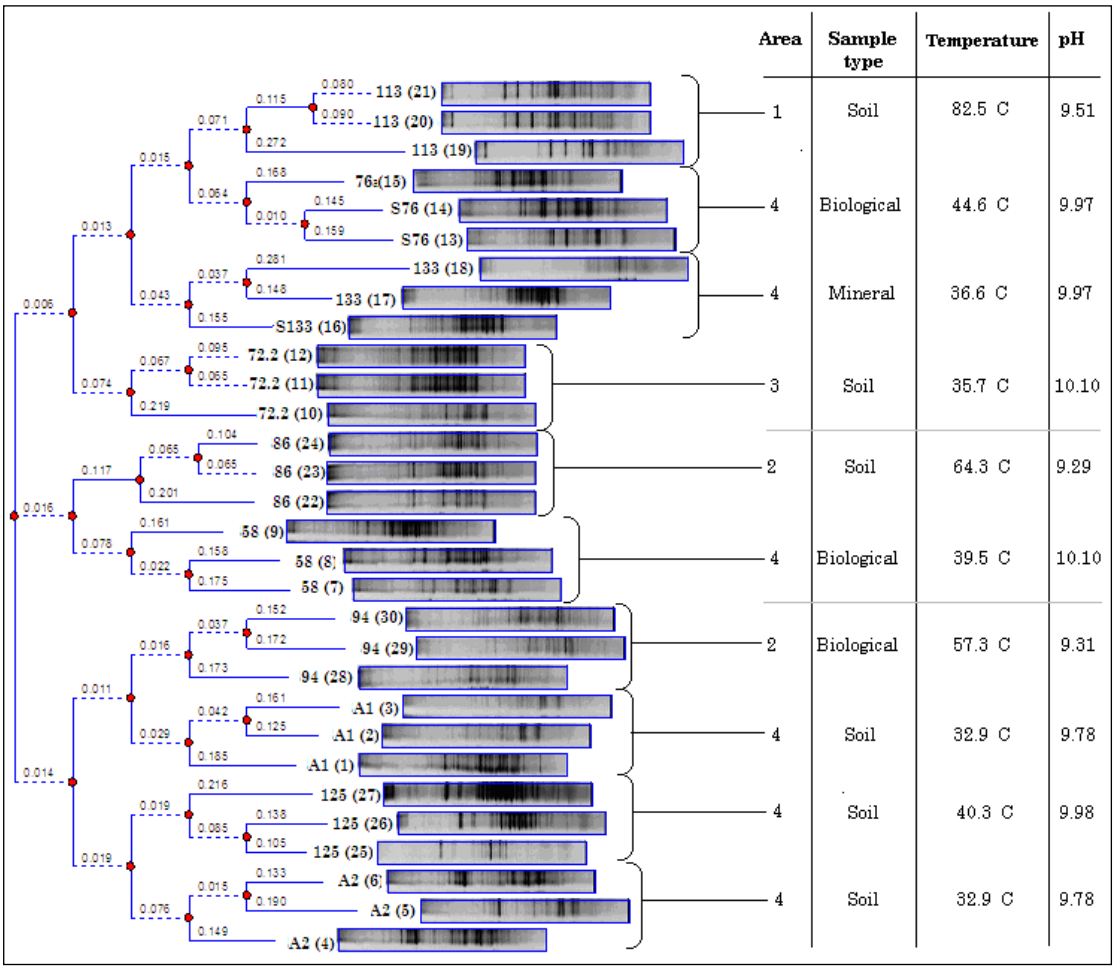



*Figure 3.9 Dendrogram of bacterial DGGE gel replicates for all samples using Neighbour joining clustering algorithm, the sampling area (key: 1-North Little Magadi, 2-North Lake*

*Magadi, 3-Western Lagoon and 4-South Lake Magadi), sample type, temperature and pH for each sample are also given.*

The two samples 1A and 2A cluster into the same clade but are not more closely related to each other than they are to other samples, demonstrating that there is a difference in the bacterial community in these two soils (Figure 3.9).

**3.6.5.3.4. Canonical correspondence analysis (CCA) of bacterial DGGE profile**

CCA is an ordination technique which relates the DGGE binary 'fingerprint' profile with environmental variables, including correlating species (band positions) to the environmental variables (pH and temperature) (Salles et al., 2004). This analysis is used to detect relationship between community composition and environmental factors (Zeng et al., 2009). In this study, the data analysed in MVSP was the binary data obtained during DGGE analysis (Appendix E) (Chapter 2, Section 2.3.5.2).

Due to the number of samples only six environmental variables could be compared, using canonical correspondence analysis. Samples 1A and 2A were excluded from the analysis due to an absence of water chemistry data available for these samples (Table 3.4).

The graph vector on a CCA plot corresponds to a single variable, the origin of the vector is an area reflecting the average score for that variable. The direction of the vector indicates the direction of most rapid change for that variable and the length of the vector is proportional to the rate of change in that direction. Those environmental variables which have a long vector (e.g. pH, Figure 3.9.) are more strongly correlated to the ordination axes than those with a short vector (e.g. phosphate, figure 3.11), and so are in turn are more closely related to the pattern of variability we see in the community on the plot (Ter Braak, 1987: Bossio and Scow, 1995).

The parameters were chosen by the presence of a value for samples 58 through to 94 and by their possible influence on the environment: temperature, pH, phosphorus, sulphur sodium nitrate and chlorine (chlorine levels can be used as a rough guide to NaCl content). Samples 1A and 2A were omitted from the CCA analysis due to the absence of associated water compositional data for these samples.

Temperature, phosphate and pH are the variables most correlated with the ordination axes, therefore they have the most effect on the variation seen in the bacterial DGGE profiles. The vectors for sodium nitrate, chlorine, phosphorus pH and sulphur show that these variables are negatively correlated to temperature.

The most distinct distributions on the bacterial DGGE profile CCA plot were samples 86 (Figure 3.10, blue squares) and 113 (Figure 3.10, black inverted triangles), which were sampled from the north of the lake and plot with a positive axis value, and sample 72.2 from the Western lagoon. Samples 86 and 113 plot towards the head of the temperature vector, indicating that these samples have a higher than average temperature, which is influencing their DGGE profile (and CCA plot distribution). The two samples from the North of Little Magadi do not plot together due to their separation along axis 2, due to sulphur, chlorine pH and/or phosphate content (or another factor not considered in this study). Their separation along axis 1 is in part due to a stronger negative correlation to sodium nitrate content for sample 113.

Sample 94, from the north of Lake Magadi plots around the origin of both axes and so is recorded as having an average level for all environmental variables relation to the other samples in the data set. Sample 94 plots close to sample 72.2. This sample has the highest recorded concentrations of anions and cations as well as pH. However, the sample plots with what seems to be a more average value for these variables than samples with a lower concentration of these cations/anions and pH. This distribution indicates that another variable must be acting upon the distribution of this sample other than the variables used in this study. The distribution of this samples replicates in this area maybe due to this sample being collected from the lagoon, rather than from the spring sites.

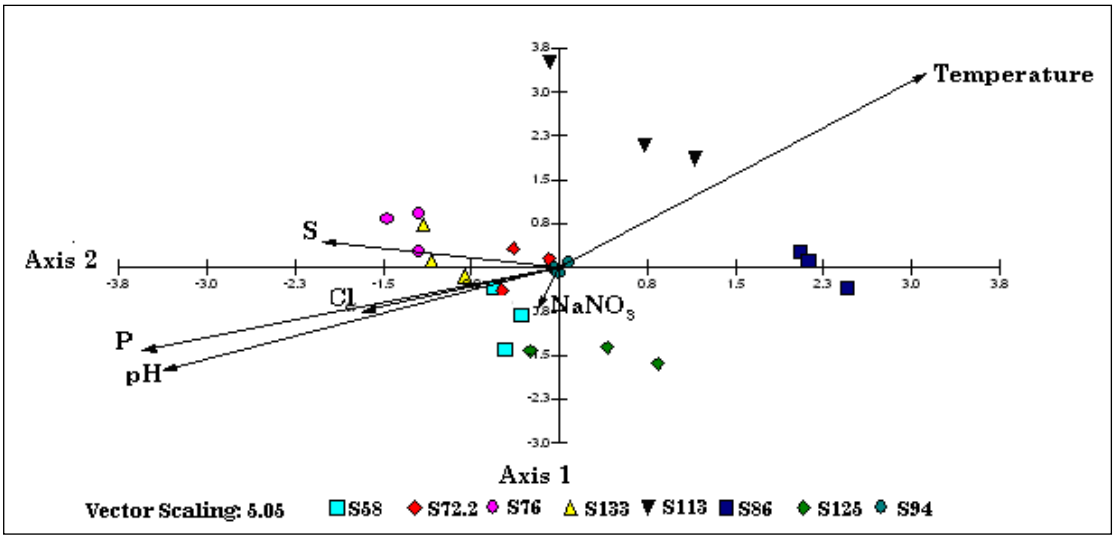



*Figure 3.10 CCA biplot relating bacterial DGGE binary data to environmental variables: temperature, P= phosphorus, pH, S=sulphur, Cl=chlorine and $NaNO_3$= nitrate. Direction of vector demonstrates direction of most rapid change and length of vector is proportional to the rate of change. Axis 1 represents 9.6% of variation and axis 2, 8.1% of variation.*

Samples 125, 133, 58 and 76 plot along a similar trajectory (Figure 3.11) with these samples all having similar water chemistry. These samples are separated however by a variable which plots samples within the positive axis 1, negative axis 2 quadrant. This variable, which has not been included in this study, could be related to the sample type.

#### 3.6.5.4. Archaea DGGE profile

The same samples (Table 3.4) and analytical procedure was conducted for the archaea DGGE analysis as for the bacterial DGGE analysis, however no archaeal DNA could be extracted from sample 94 so this was excluded from the analysis (Figure 3.12).

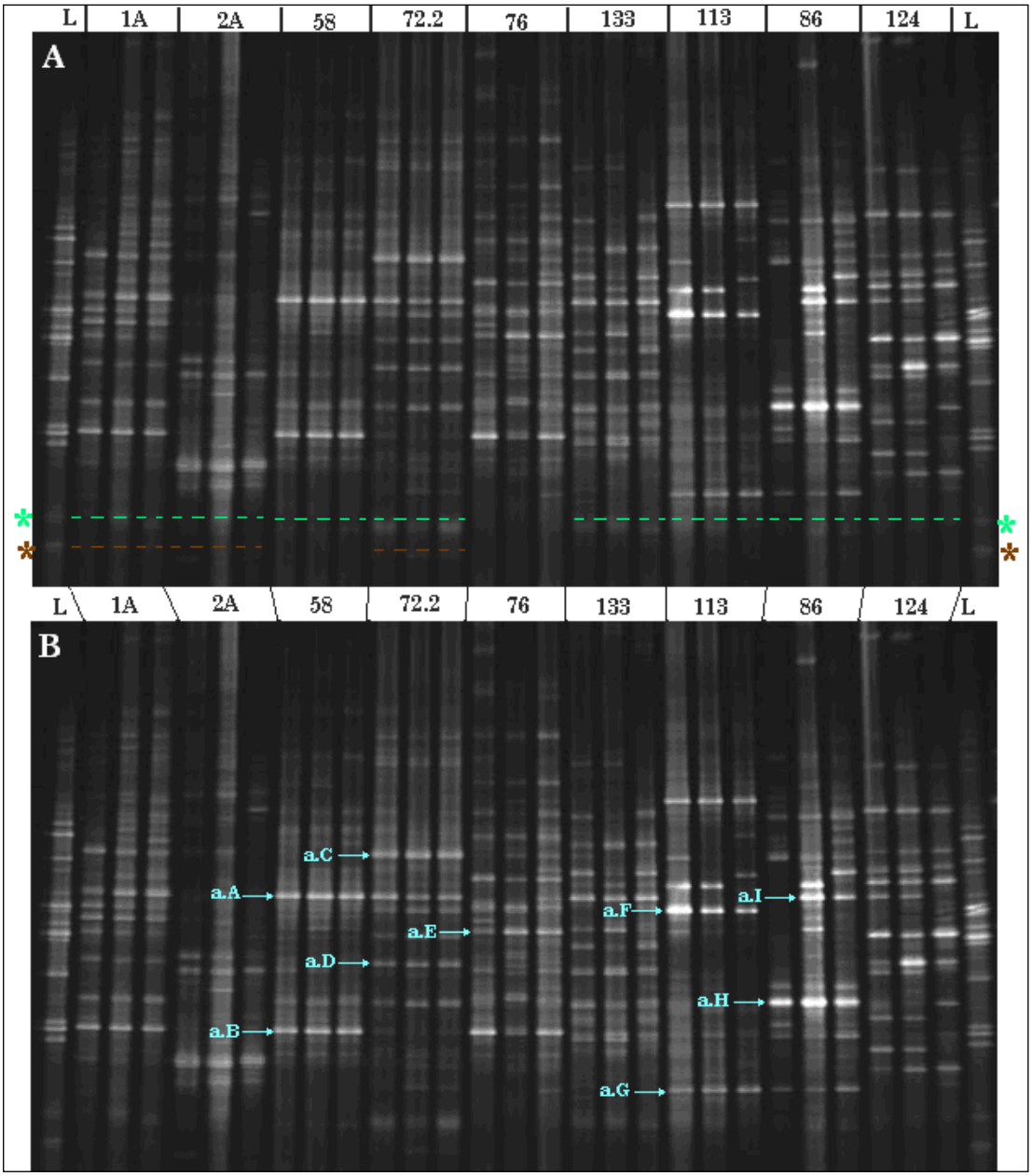



*Figure 3.11 DGGE gel of DNA amplified using archaeal primers. Each sample was run in triplicate (Chapter 2. Section 2.3.5.2)* ***(A)*** *the band in each lane which corresponds to a ladder band (Table 3.5) is highlighted. Mint green dash line and* ✻ *denotes bands in corresponding location to the band for 15%_72.2C (Table 3.5 H). Brown dash line and* ✻ *denotes bands in corresponding location to the band 15%_70A (Table 3.5 I).*L. denotes ladder lane. ***(B)*** *Indicates bands which were chosen to be excised and sequenced.*

The archaea DGGE also shows a large number of bands across most samples (Table 3.11). A high diversity in the samples was confirmed through the Shannon diversity index value for each sample (Table 3.11.). The ANOVA analysis of the index scores showed that the diversity

of sample 58 although still quite high (H=2.64), was significantly lower than the diversity of the other samples ($p$<0.012). This is important when considering the limited number of archaea isolated on solid media earlier in this chapter. A diverse archaea community (as demonstrated by high Shannon diversity score here) would be expected in this type of extreme environment, many halophilic thermophilic and alkaliphilic archaea have been identified in other environments and soda lake by other researchers (Benlloch et al., 2001: Mwirichia et al., 2010a).

*Table 3.11 Shannon diversity score for each sample, calculating using MVSP.* denotes significantly different from all other samples. Raw data see appendix F.*

| Sample | Average Shannon diversity index value (H) |
|---|---|
| 1A | 3.22 |
| 2A | 3.03 |
| 58 | 2.64* |
| 72.2 | 3.26 |
| 76 | 3.24 |
| 133 | 3.13 |
| 113 | 3.27 |
| 86 | 3.20 |
| 125 | 3.27 |

**3.6.5.4.1. Association of bands in archaeal DGGE profile with isolates**

All archaea strains isolated from the samples in section 3.4.5 of this chapter were either related to *Natronococcus occultus* or *Natrialba magadii* all having 98-99% similarity to either one or other of the species. A representative of each species were included in the DGGE ladder: 15%_72.2C (*Natronococcus occultus*) and 15%_70A (*Natrialba magadii)*.

Bands migrated to the same location as 15%_72.2C have been marked with mint green dashed line (Figure 3.11). A band migrates to the corresponding position as isolate 15%_72.2C in all samples except sample 76. The absence of its isolation from the samples where it appears to be present could indicate that this band is comprised of more than just strains of isolate 15%_72.2C, although its absence from isolation studies does not confirm its absence.

A band migrating to the same location as the band for 15%_70A (dark brown line, Figure 3.11) is less common, with a band only present in samples 1A, 2A and 72.2, all these samples were collected from lagoon area of the lake, suggesting that may be this isolate prefers higher pH and or the concentration of solutes such as NaCl.

Interestingly, samples 1A and 2A which were collected from the same site but from different distances away from the lake edge show a difference in their archaeal DGGE profiles. Strong bands can be seen in sample 1A at points 2, 3, 4, 5 and 6 which do not occur in sample 2A. The most intense bands in 1A also appear in samples 72.2, 76, 58, 113, 133 and 125. This differs to what was seen in the bacterial DGGE profile, where the two samples had more similar fingerprint pattern of bands.

#### 3.6.5.4.2. Sequencing of archaeal DGGE bands

Only two clones contained the inserted DGGE DNA and were successfully sequenced, these were clones containing DNA extracted from band a.A (Figure 3.11). One clone was identified as an archaeon, closely related to *Halalkalicoccus jeotgali* JCM 14584 and a clone from the soil around Lake Texcoco (Table 3.12).

*Table 3.12. The closest uncultured clone and isolated strain matches for clones from bacterial DGGE gel.*

| Band | Closest uncultured match | % | Nearest strain match | % |
|---|---|---|---|---|
| a.A.1 | Uncultured haloarchaea clone TX4CA_14. (EF690569.1) | 96 | *Halalkalicoccus jeotgali* JCM 14584. (AB477223.1) | 94 |
| a.A.2 | Uncultured Bacteroidetes bacterium clone WN-FSB-88. (DQ432122.1) | 93 | *Balneola vulgaris* strain 13IX/ A01/ 164. (NR_042991.1) | 88 |

The closest database strains are extremely halophilic, and haloalkaliphilic archaea (Roh et al., 2007). The phylogenetic analysis shows that the a.A1 clone was basal to all of the closest database matches.

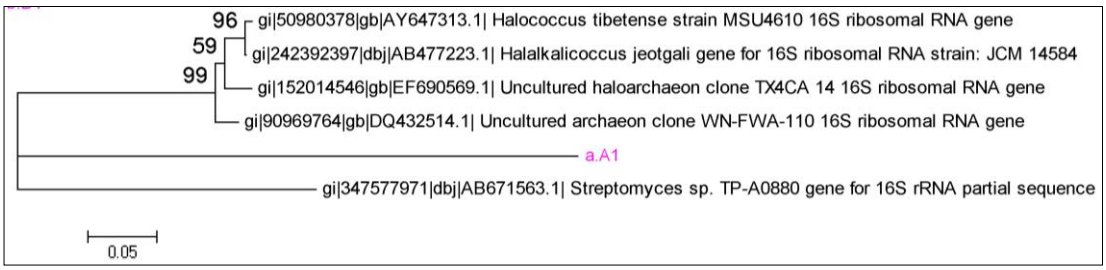



*Figure 3.12 Phylogenetic tree of archaeal clones (pink) and near neighbour and nearest neighbour matches (black) using Greengenes alignment software (DeSantis et al., 2006). Interior branch values, 1000 replications.*

The second clone successfully sequenced from band a.A was in fact a bacterial clone of the order *Sphingobacteriales* (Figure 3.13). The closest matches were to clones and strains from

saline and alkaline environments. The phylogenetic analysis places this clone most closely related to clone b.D1, related to *A. ikkense* (section 3.4.7.4). The presence of bacterial bands is possible due to the use of nested PCR culminating in the amplification with universal primers suitable for bacterial and archaeal amplification.

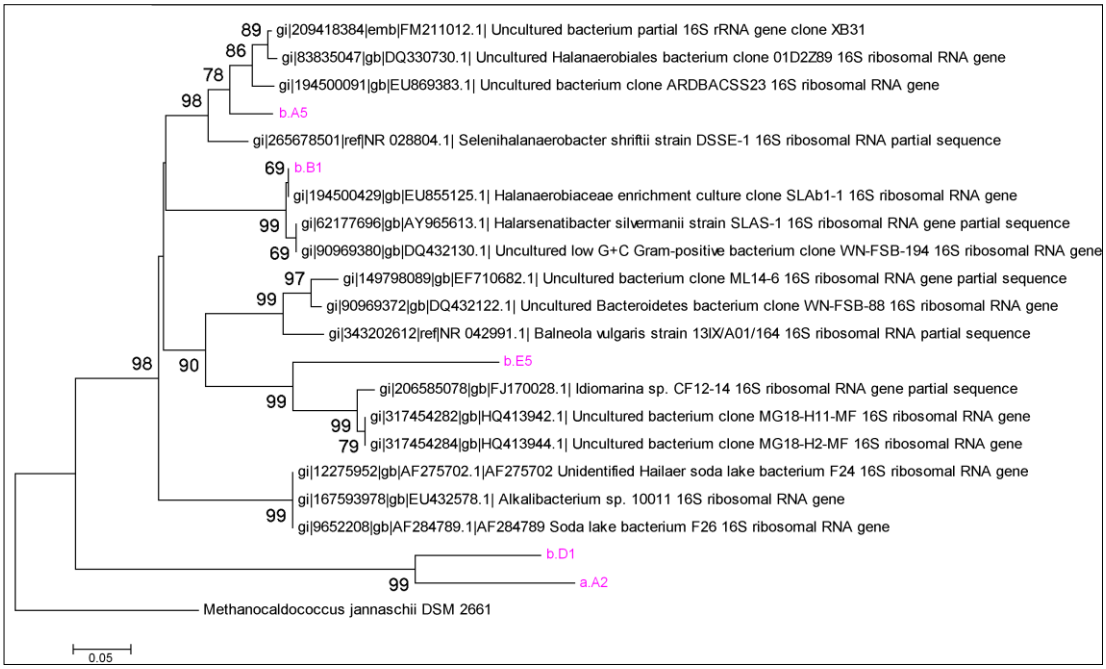



*Figure 3.13 Phylogenetic tree of bacterial clones (pink) from both bacterial and archaeal DGGE and near neighbour and nearest neighbour matches (black) using Greengenes alignment software (DeSantis et al., 2006). Interior branch values, 1000 replications.*

### 3.6.5.4.3. Differences in community composition of archaea in samples, detected by hierarchical cluster analysis

This relationship between the samples is also shown in the dendrogram which groups these samples as most closely related to each other than they are to the other samples (Figure 3.14). As before the triplicates for each sample are more closely related to one another than they are to any other sample.

As with the bacterial DGGE profiles there seems to be no grouping in relation to sample type or water chemistry. Interestingly sample 2A groups are more similar to sample 133 than to sample 1A. This difference may be due to the more desiccating nature of sampling area from which sample A2 was collected in comparison to 'wetter' sample 1A location, closer to the lake edge. The similarities in diversity between sample 133 and 2A may be a reflection upon the harsh sampling environment away from the lake edge and in relation to the mineral magadiite.

Sample 125 does not seem to group with any other sample, this sample was very dry, more so than any other sample. The archaeal community present in this sample therefore would have to be organisms which are capable of surviving these conditions, environmental conditions which were not affecting the communities in the other samples where water was more available.

The absence of separation sample 125 in the bacterial community DGGE profile may reflect the ability of some bacteria to protect themselves as spores in very dry environments and so can remain present in the sample until more favourable conditions return. Therefore the community representatives may not change drastically, as the organisms are still present, but other organisms may proliferate in such circumstances.

This may also explain the differences in archaeal DGGE profiles of samples 1A and 2A but absence of any very distinct difference in the bacterial profiles.

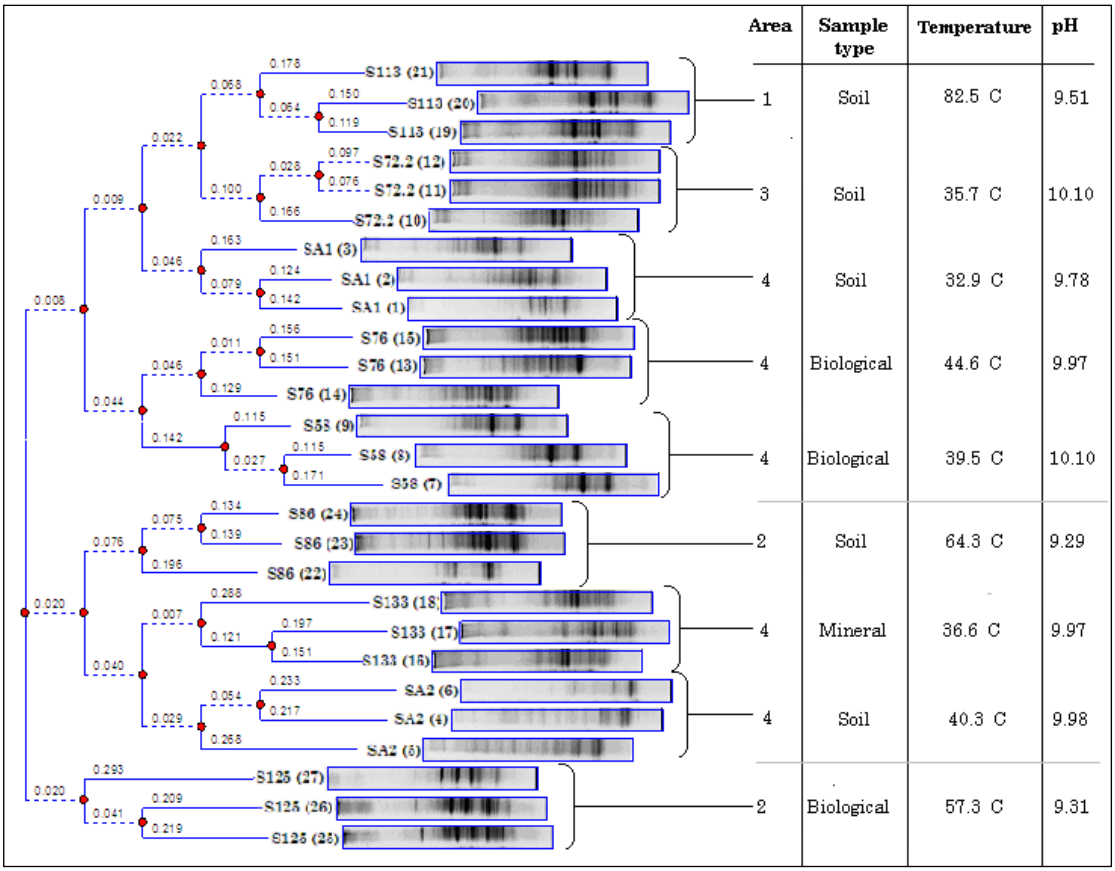



*Figure 3.14 Dendrogram of archaeal DGGE gel replicates for all samples using Neighbour joining clustering algorithm, the sampling area (key: 1-North Little Magadi, 2-North Lake*

*Magadi, 3-Western Lagoon and 4-South Lake Magadi), sample type, temperature and pH for each sample are also given.*

**3.6.5.4.4. Canonical correspondence analysis (CCA) of archaeal DGGE profile**

As with the bacterial DGGE, the analysis of fewer than 10 samples meant that only six environmental variables could be compared. Once again samples 1A and 2A were excluded from the analysis due to an absence of water chemistry data available for these samples. The direction of the vector arrow indicates the direction of most rapid change for that variable and the length of the vector is proportional to the rate of change in that direction.

The most influential variables on the archaea DGGE diversity of these samples are pH, temperature, nitrate and chlorine, compared to the dominant environmental factors: pH, temperature and phosphate and which influence bacterial DGGE diversity (section 3.4.6.3.4, this chapter). This suggests that phosphate content is an influencing factor on bacterial community structure at Lake Magadi, with chlorine content having less of an influence. In contrast chlorine (salinity) concentration has more of an influence on the archaeal community structure, microbial community at Lake Magadi than phosphate content. Both pH and temperature are significant environmental factors which affect the microbial community structure

Sample 72.2 plots with a more positive axis 1 value than any other sample, reflecting the possible influence of a higher than average chlorine, sulphur, pH, nitrate and phosphorus content in the sampling environment on the diversity pattern of this sample. The replicates for this sample plot separately from all other samples which all were sampled from environments with a lower concentration, temperature and pH measurements.

Sample 133 plots with a more positive axis 2 value than any other sample, and therefore plots distinctly from all other samples. The more negative plotting of the sample compared to other samples may be due to this sample being a mineral sample rather than soil or biological. It is possible that the variation in axis 2 is contributed to by sample type.

Unlike with the bacterial samples, there is no separation between those samples from the northern end of the lake and those from more southerly areas. However, the environmental variable of temperature does have an effect upon the sample diversity (long temperature vector) (Figure 3.13), and there is a correlation between temperature and latitude with the springs from the lower latitudes having a high temperature. This would suggest that another factor which has not been accounted for in this study is having an effect upon the archaeal diversity, although it is unclear what this might be.

Biological samples 58, 76 and 94 plot separately from one another along axis 2, but not along axis 1. This distribution is in part due to the lower sampling environment temperature and higher than average pH, Cl and $NaNO_3$ content for sample 58, compared to samples 58 and 94.

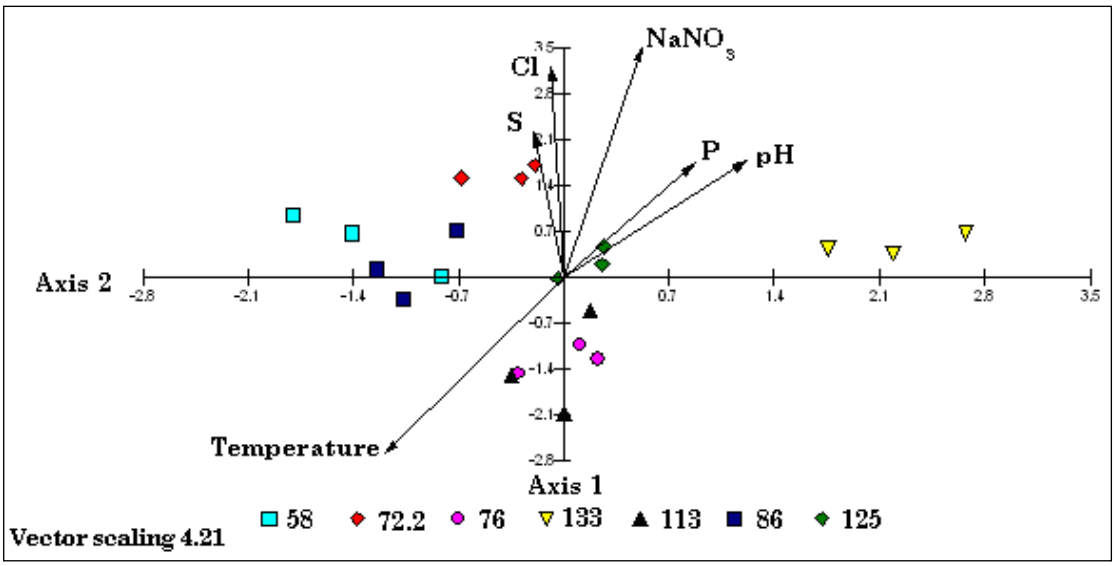



*Figure 3.15 CCA biplot relating archaeal DGGE binary data to environmental variables: temperature, P= phosphorus, pH, S=sulphur, Cl=chlorine and $NaNO_3$= nitrate. Direction of vector demonstrates direction of most rapid change and length of vector is proportional to the rate of change. Axis 1 represents 12.8% of variation and axis 2, 11.2% of variation*

### 3.6.6. Description and phylogeny of three novel isolates

A number of potentially novel isolates have been cultured during this study. As discussed previously, 16S rRNA gene sequencing has its limitations and cannot determine the phenotypic characteristics of the isolate. Therefore, three isolates have been chosen for further study and characterisation to compare the novel isolates from this chapter to related species and genera, this is the focus of Chapter 4. The morphology of the novel strains are described below.

#### 3.6.6.1. Isolate 6.8%_76GB

Isolate 6.8%_76GB will now on be referred to as *Idiomarina* sp. LMLD01 (figure 3.19.A). Plate growth occurred within 24 hours. Colonies appeared muccoidal, circular, cream in colour and 1-2 mm in diameter after 24 hours (Figure 3.19.B). Colonies developed a dark peach/brown colour in the middle of the colony after 3 days at 37 °C and when stored at 4 °C (Figure 4.4.C). Under a light microscope cells stain Gram negative and were rod shaped.

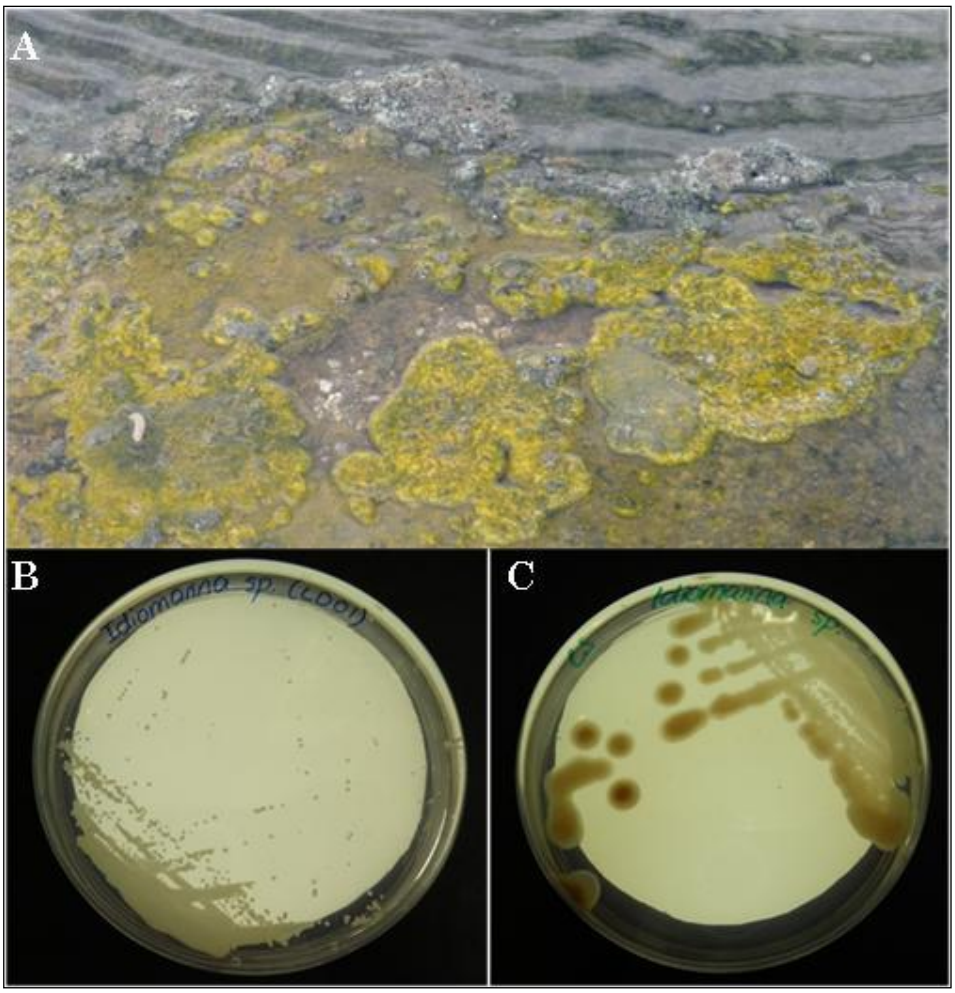



*Figure 3.16 Isolate Idiomarina* sp. LMLD01*. A. Sample in the field. B. Idiomarina* sp. LMLD01 *colonies after 24 hours incubation at 37 °C. C. Idiomarina* sp. LMLD01 *colonies after 1 days incubation at 37 ºC and 7 days storage at 4 °C.*

The NCBI blast search (Altschul et al., 1990) of isolate *Idiomarina* sp. LMLD01 returned a 99% match to a Gammaproteobacterium E-410 from lake Elementia, Kenya (Mwirichia et al., 2010b) and 98% match to an isolate *Idiomarina* sp. C4 (Table 3.3). The Ribosomal Database Project (RDP) (Maidak et al., 1994) matches the strain to the family *Idiomarina*ceae. Phylogenetic analysis which compared *Idiomarina sp*. LMLD01 to 10 species of that genus is shown in Figure 3.20.

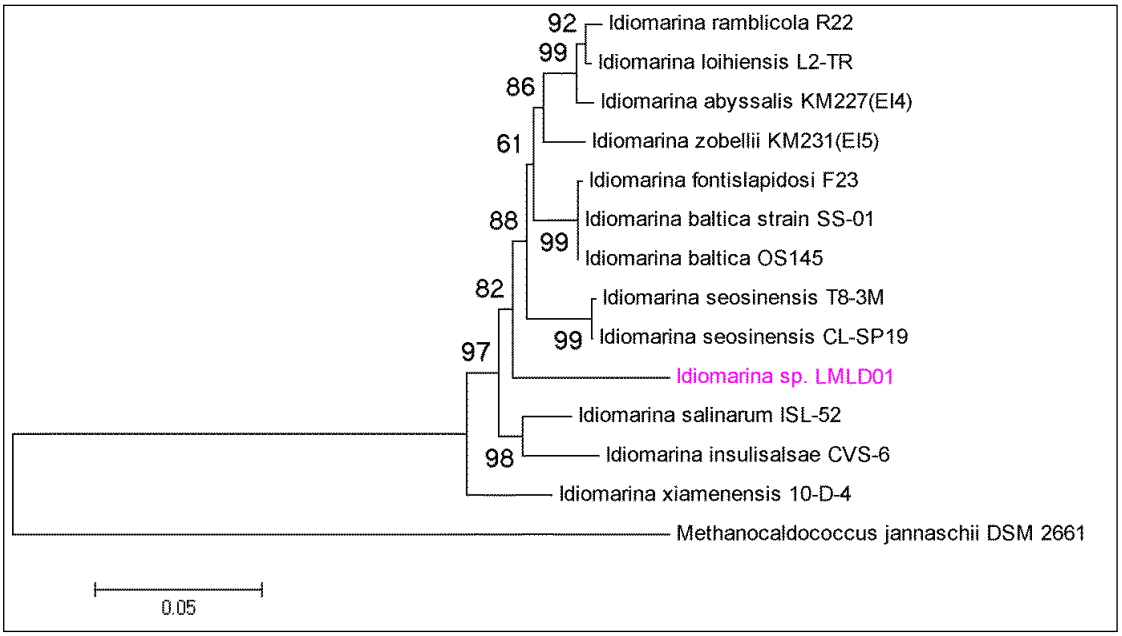



*Figure 3.17 Phylogenetic tree showing the relationship of isolate Idiomarina sp. LDLM01 (pink) to other members of the genus Idiomarina (black). The archaeon Methanocaldococcus jannaschii DSM 2661 has been used as an out-group. Interior branch values, 1000 replications.*

### 3.6.6.2. Isolate 6.8%_108GA

Strain 6.8%_108GA will from now on be referred to as *Planococcus sp.* LMLD02. Growth on 6.8% (w/v) NaCl agar medium at 37 °C occurs within 48 hours. Colonies are dark orange in colour, circular and around 1mm - 2 mm after 48 hours (Figure 3.21.A). Cells are Gram positive and coccoid (Figure 3.21.B).

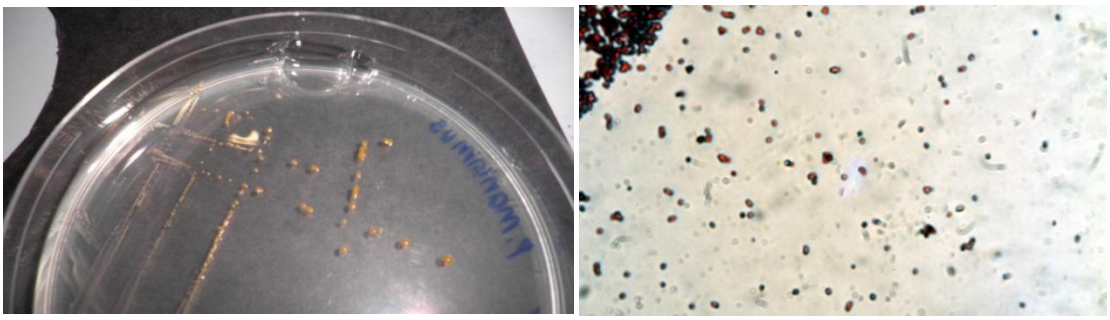


*Figure 3.18* ***(A)*** *Colonies of Planococcus sp. LMLD02 on AP+6.8% medium at 37 °C.* ***(B)*** *6.8%109GAA_LMLD02 cells under light microscope x100 magnification, field of view measure 150 µm across. Cells have been Gram stained.*

The isolate *Planococcus sp. LMLD02* has a 98% match in the NCBI database (Altschul et al., 1990) to *Planococcus maritimus* (Table.3.3). The RDP database (Maidak et al., 1994) placed

the strain in the genus *Planococcus.* The phylogenetic tree for *Planococcus sp.* LMLD02 shows the isolate is more closely related to *P. maritimus* AMP-10 (isolated from dyeing waste sludge) than to any other species of *Planococcus* (Figure 3.22).

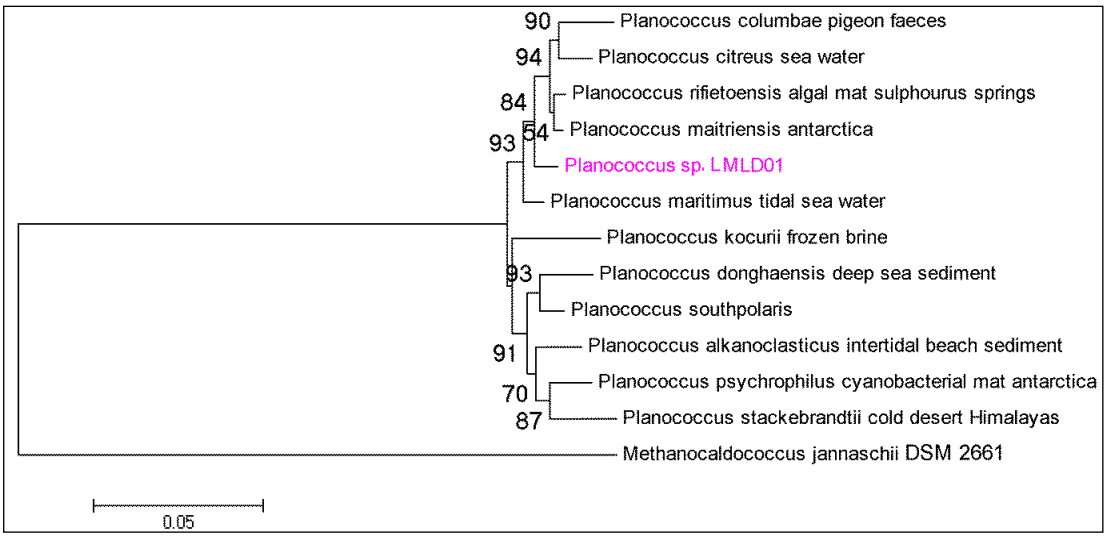



*Figure 3.19 Phylogenetic tree showing the relationship of isolate Planococcus sp. LMLD02 (pink) to other species of the genus Planococcus (black). The archaeon M. jannaschii DSM 2661 has been used as an out-group. Interior branch values, 1000 replications.*

### 3.6.6.3. Isolate 6.8%_106A

Finally isolate 6.8%_106A will now be referred to as *Caldalkalibacillus* sp. LMLD03. On a 6.8% (w/v) NaCl plate incubated at 37 °C, colonies are round translucent, slightly convex in shape and around 1 mm in diameter (Figure 3.23.A) after 3 days incubation at 37 °C. The colony surface has a slightly rough looking texture (Figure 3.23.B) are very difficult to scrape off the surface of the agar. Some colonies appeared to form a ‘fluffy’ material on the surface and about 1 mm around some colonies.

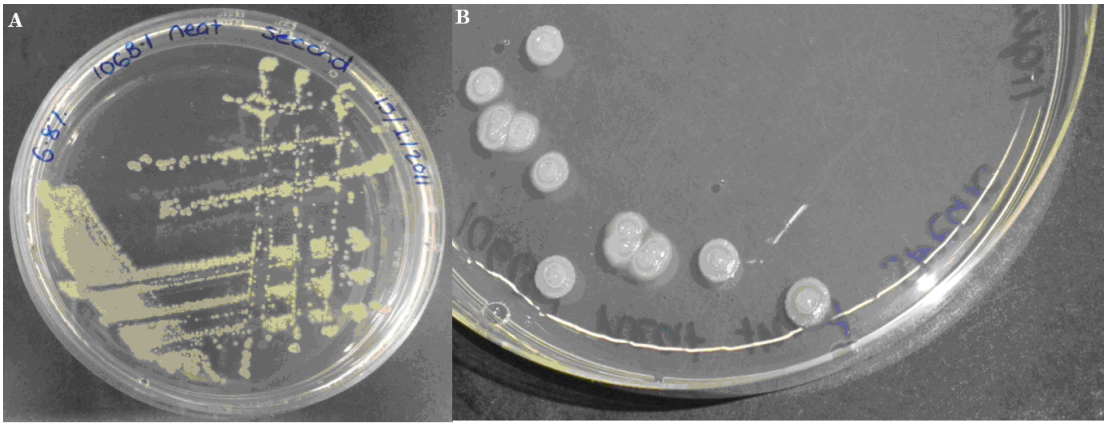



*Figure 3.20.* ***(A)*** *Caldalkalibacillus* sp. LMLD03 *colonies on AP+6.8% medium incubated at 37 °C.* ***(B)*** *Close up image of Caldalkalibacillus* sp. LMLD03 *colonies on same agar as for A. Showing limited growth when streaked on from a loop inoculated by 24 hour liquid culture.*

Analysis under a microscope of both liquid cultures and cells from agar plates showed the presence of both round structures and thin strands. Use of the Schaeffer-Fulton stain on the microscope slide showed the presence of vegetative rod shaped cells in pink, and round spores in dark green (Figure 3.33). Spores were observed in both the cell dispersion from the plates and in the liquid culture.

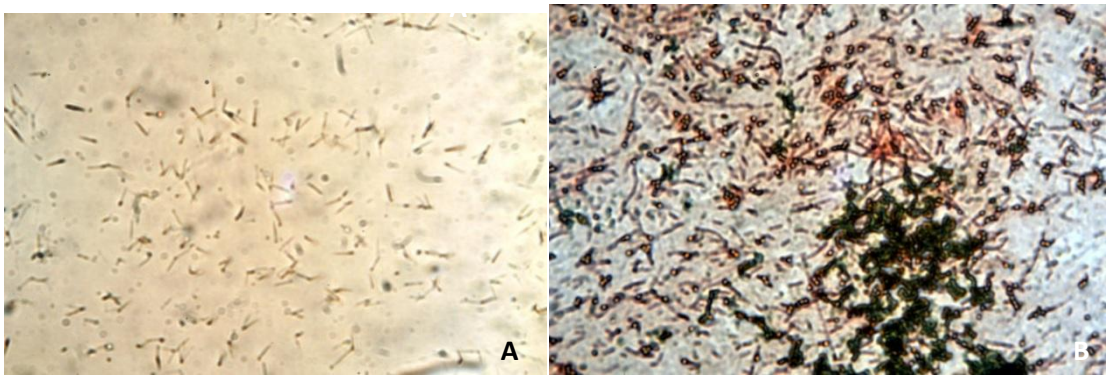



*Figure 3.21.* ***(A)*** *Caldalkalibacillus* sp. LMLD03 *cells, field of view is 150µm across.* ***(B)*** *Caldalkalibacillus* sp. LMLD03 *cells stained with Schaffer-Fulton stain, vegetative cells are red and spores are green. Field of view is 150µm across. 100x magnification.*

Isolate as *Caldalkalibacillus* sp. LMLD03 was most closely matched with *Caldalkalibacillus uzonensis* JW/WZ-YB58 (93%). Phylogenetic analysis places it at the root of the clade of the genus *Caldalkalibacillus* (Figure 3.24). The RDP database (Maidak et al., 1994) places the strain in the family Bacillaceae, but possibly within the genus *Bacillus, Caldalkalibacillus Piscibacillus, Halobaillus* or *Filobacillus*.

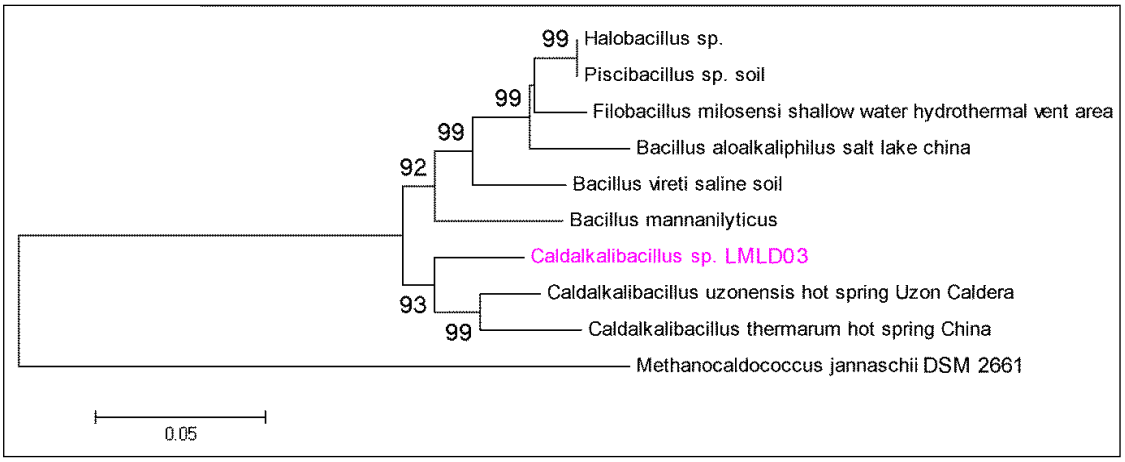



*Figure 3.22 Phylogenetic tree showing the relationship of isolate Caldalkalibacillus sp. LMLD03 (pink) the closest matches in the database and other representative taxa of the family Bacillaceae (black). The archaeon M. jannaschii DSM 2661 has been used as an out-group. Interior branch values, 1000 replications.*

### 3.7. Discussion

This work has involved the analysis of the bacterial and archaeal diversity of samples from Lake Magadi, using culture based, and the culture independent analysis method of denaturing gradient gel electrophoresis (which has not been conducted on Lake Magadi samples previously). The purpose was to identify novel isolates from this environment and assess the diversity of the samples from a variety of environments around Lake Magadi. Lake Magadi has been studied in terms of bacterial and archaeal diversity (section 3.1.1), however, the use of different culture media and the differing treatments of samples can potentially result in the identification of novel strains.

#### 3.7.1. Characterisation of environmental conditions in sampling locations

The chemical analysis of the different sampling divisions showed that calcium and magnesium content in all springs analysed were below the detection limit as would be expected in this type of soda lake environment. The data did demonstrate a difference with latitude, with the northern end of the lake characterised by higher temperature springs and phosphorus levels below the detection limit. The two most northerly areas were much more remote than the sites to the south, which are visited by humans and animals, and have a large amount of biomaterial associated with them. Phosphate is passed into the water cycle through erosion, animal excretion and the decomposition of animal and plant remains. The lagoons are also breeding grounds for thousands of flamingo which would contribute to the phosphorus levels in the area, therefore a high phosphorus content in the lagoon and more southerly sites would be expected.

The water chemistry of the Western Lagoon sample showed elevated levels of all solutes compared to the springs, in particular chlorine, most likely reflected an elevated NaCl concentration in the lagoon (also reflected in the $a_w$ measurements). This would be expected because of the desiccating and evaporating nature of the environment concentrating solutes in the lagoon. The organisms which would inhabit this environment would be able to tolerate a pH greater than 10 and high salinity. The ordination analyses of DGGE gels indicated that the biggest factors which were affecting the diversity pattern for samples were temperature, pH, phosphorus for bacterial diversity and temperature, pH, nitrate and chlorine, for archaeal diversity.

The choice of growth medium is an important part of the studying the microbiology of an extreme system, such as Lake Magadi. One possible culturing method could have involved using lake water instead of distilled water, to which carbon sources and agar can be added. This was not logistically possible in this study due to the volume of water which would be required; the alternative method was to produce a growth medium which is designed based on the average water chemistry of the springs.

Interestingly, other culture based studies of soda lakes, such as Lake Elementia (Mwirichia et al., 2010b), resulted in the isolation of similar organisms to those isolated in this study, although their conclusions were that varying growth media composition can result in the isolation of novel strains from soda lake environments. In this study, generally it was not possible to identify variation in the types of species and genera of bacteria isolated on the different salinities used, although 15% NaCl did yield several archaea which was not the case on the lower salinity growth media. This suggests that there was insufficient variation in salinity concentration, a broader range of salinities (0.7%, 15% and 25% NaCl for example) may have resulted in the isolation of a greater diversity of species and genera. Considering the influence of pH temperature and phosphorus on bacterial community according to the CCA analysis, varying these factors would potentially result in a broader isolation of novel strains. In addition, the variation of carbon source from special peptone in the AP+ growth medium may result in the isolation of more novel isolates. An important consideration however with the altering of growth media carbon source is the effects of yeast extract on growth at elevated salinity, which will be discussed in more detail in Chapter 4, section 4.4.1.

### 3.7.2. Bacterial diversity of Lake Magadi samples

A total of 410 isolates were successfully sequenced and demonstrated a dominance of isolates related to strains of the phyla *Proteobacteria* and *Firmicute,* with a few isolates related to strains of *Actinobacteria* and *Bacteroidetes*. Studies of the biology of Lake Magadi and other soda lakes in the Rift Valley, such as Lake Elementia, have also identified a

dominance of organisms with a relationship to *Proteobacteria* and *Firmicute* (Rees, 2004: Mwirichia et al., 2010b) as well as the soda lakes: Baer Lake, Inner Mongolia and Lonar Lake India (Ma et al., 2004b: Joshi et al., 2008: Deshmukh et al., 2011).

The DGGE analysis of samples showed a high bacterial (and archaeal) diversity across all samples. Studies of other soda lakes in Kenya have also shown a high diversity, however it has been noted that a high community diversity results in a low DGGE resolution (Rees, 2004), an issue which was also encountered in this study. This demonstrates the limitations of DGGE analysis, however, this technique does still provide some indication of diversity and allows for the identification of bands which can be traced across different samples. This study demonstrated that particular bands, including bands associated with cultured isolates can be traced in a number of samples from all around the lake. This suggests that organisms are not restricted at Lake Magadi by pH or temperature, for example and possibly have a broad growth range which could be beneficial in a changing environment. One issue with DGGE analysis is the presence of multiple 16S rRNA gene sequences in a single strain, which results in multiple DGGE bands for one strain. This causes problems with assessing the diversity of a profile as each band cannot be assumed as representing on organism. In addition, it cannot be assumed that each band only represents one strain. To counteract this, the sequencing of bands can confirm what the band is comprised of, although these efforts were hampered in this study. The use of blunt end vectors and inserts could be part of the problem, which limited the number of clones with the correct insert, resulting in the ligation of multiple inserts into the vector.

The high diversity of samples from these extreme conditions demonstrates that a variety of organisms can withstand these unfavourable conditions. If a large community is able to live in highly saline, alkaline and desiccating environments then this would demonstrate that at least these conditions would not limit the habitability of a similar environment on Mars. Therefore there are potentially a wide variety of organisms which could be studied in relation to understanding the habitability of Mars, limited by the ability to culture these strains.

Those isolates related to *Firmicute* database strains were generally related to members of the family Bacillaceae, which have been isolated from other soda lake environments (Rees, 2004: Wani et al., 2006: Deshmukh et al., 2011: Ma et al., 2004b). The Bacillaceae family contains genera such as *Bacillus* and *Caldalkalibacillus* (isolated in this study) which contain many species which can produce endospores under stressful environmental conditions, allowing them to survive for prolonged periods and the re-animate once conditions become more favourable again. Therefore the isolation of strains related to spore forming organisms would be expected, enabling these organisms to inhabit extreme environments, becoming a spore

for protection during periods of particularly extreme conditions. These types of bacteria are potentially of great importance to astrobiology, the protective nature of endospore could provide protection against detrimental factors which would be experienced on Mars.

Members of the phylum Proteobacteria are commonly isolated from soda lake environments, including organisms related to *Halmonas, Deleya, Pseudomonas, Idiomarina* and *Vibrio* (Rees, 2004: Wani et al., 2006: Mwirichia et al., 2010b: Deshmukh et al., 2011). The most common closest database strains of isolates cultured in this study were *Halomonas*, in particular *H. campisalis* and *Alkalibacterium*, in particular *A. psychrotolerans*. The predominant isolation of strains like *H. campisalis* and *A. psychrotolerans* suggests that the growth medium was particularly suited to these organisms, and potentially proliferated on the solid medium at the expense of slower growing organism. To counteract this issue, the use of a carbon source which these strains cannot grow upon would exclude these organisms and so could then allow slower growing organisms to grow. In addition, the isolation of genera such as *Halomonas, Alkalibacterium* and *Idiomarina* on both 0.7% NaCl and 15% NaCl suggests that the highest salt concentration used in this study was not high enough to exclude these organisms which may have outcompeted any slower growing organisms. Several halophilic archaea were isolated but may have been outgrown again by the faster growing strains as the salinity may be a little low for some halophilic bacteria and archaea.

In spite of this dominance, a variety of potentially novel isolates, and isolates which have not been described from this environment previously were identified. This includes isolates related to *Idiomarina* and *Planococcus* which had not been described from Lake Magadi. In addition, the genus *Alkalibacterium,* a common isolate in this study has not been described from Lake Magadi previously. These strains have been described from other soda lake environments, However their identification in this study extend our understanding of their geographic distribution (Joshi et al., 2007: Deshmukh et al., 2011). It is also true that here may be differences in phenotypic characteristics due to adaptations to a particular location, therefore a focused growth characterisation of these novel isolates could identify growth ranges not previously seen in a particular species or genera. This will be the focus of Chapter 4 of this thesis.

Studying the effects of simulated Martian conditions on common isolates from soda lakes (*Idiomarina* and *Alkalibacterium),* and those isolates from soda lakes which have a broad environmental and geographic distribution (*Planococcus* and *Idiomarina)* would contribute to our understanding of the potential habitability of Mars. The effect of simulated Martian conditions on soda lake isolates is the focus of Chapters 5 and 6 of this thesis. In addition assessing the biosignatures associated with these strains would enable us to understand

what evidence of life could be present in relation to saline, alkaline, desiccating environments on Mars.

### 3.7.3. Selection of bacterial isolates for further characterisation

Three strains related to *Idiomarina, Caldalkalibacillus* and *Planococcus* have been chosen for further study and characterisation, the focus of Chapter 4. These were all fast growing isolates, able to grow in liquid medium, with either a low database match (Table 3.5 *Caldalkalibacillus* sp. LMLD03 and *Idiomarina* sp. LMLD01) and/or had not previously been characterised from this sampling locality (*Idiomarina* sp. LMLD01 and *Planococcus* sp. LMLD02). Of particular interest is *Planococcus* sp. LMLD02*,* a bright orange strain which may have resistance to desiccation and UV irradiation due to the presence of pigments, an area which will be explored in Chapter 5 and 6.

### 3.7.4. Archaeal diversity of Lake Magadi samples

Saline soda lakes are known to support a variety of halophilic archaeal, including members of the order *Halobacteriales: Natronobacterium, Natronococcus, Natronomonas, Natrialba, Natronorubrum* and *Halorubrum* (Mwirichia et al., 2010a). The archaea isolated during this study were both pink pigmented, and were closely related to isolates previously cultured from Lake Magadi*, Natrialba* sp. and *Natronococcus* sp. (Tindall et al., 1984: Kanai and Kanai, 1995: Kamekura et al., 1997). Evidence of the presence of members of the order *Halobacteriales* has been obtained through culture independent studies of environments including the Dead Sea, solar salterns, Antarctic hypersaline lakes, alkaline African hypersaline lakes, and Solar Lake in Sinai (Benlloch et al., 2001: Cytryn et al., 2007: Mwirichia et al., 2010b). This identification of haloarchaea from a number of different environments demonstrates an ability of these organisms to occupy a variety of niches. Strains of halophilic archaea have been shown to be particularly resistant to conditions such as extreme desiccation and UV irradiation, which makes them particularly interesting organisms in terms of astrobiology.

The density of DGGE bands in the archaeal profile suggests that there is a high diversity of archaea present in the environment although only a limited number could be cultured and sequenced. One issue is the identification of a bacterial isolate from the cloning of the DGGE band DNA. The use of universal DGGE primers does mean that there is a risk of amplifying bacterial DNA even during nested PCR starting with amplification using archaeal primers. This could be addressed by using an alternative, more specific primer pair for archaea.

One limitation for this study is that the highest NaCl concentration used (15%, 2.6 M, NaCl) was still quite low compared to the growth optima of many halophilic archaea, although it is

well above the 1M concentration below which halophilic archaea can lyse, this concentration could limit the types of extremely halophilic organisms isolated. This is because of the lack of a rigid cell wall in most haloarchaea which instead rely upon salt concentration for structural stability; an exception to this is the genus *Natronococcus* which uses a different cell wall structure comprised of poly-(y-L-glutamine) (Niemetz et al., 1997: Mancinelli, 2005a).

The similarity of many of the isolates in this study to organisms from a variety of environments (soda lakes, deep sea and shallow marine environments) indicates the ability of these types of organisms to occupy a variety of niches. For example, unlike Lake Magadi, the inflow into Mono Lake and Lake Van contains sufficient calcium to allow the precipitation calcium carbonate in the form of tufa columns, at Lake Magadi most of the calcium is deposited as pedogenic and phreatic carbonates (Warren, 2006). This ability to occupy a variety of environmental niches is highly beneficial because these organisms are less restricted, than those which are limited to only growing in the presence of high pH or high salinity for example. This could be beneficial when considering the habitability of planets such as Mars, a broader range of habitable environments for an organism would mean a wider range of potentially habited locations for those types of organisms.

### 3.8. Conclusions

This study has contributed to the understanding of soda lake environments, and has increased the number of genera/ species that have been identified at Lake Magadi. Potentially novel isolates and isolates which have been previously described from Lake Magadi and other soda lakes were cultured and sequenced, suggesting that culture based studies are still and important part of environmental microbiology.

The majority of isolates were most closely related to genera of the phyla *Proteobacteria* and *Firmicute*, including *Halomonas, Alkalibacterium, Idiomarina, Alkalimonas, Bacillus*, *Planococcus, Caldalkalibacillus*, *Amphibacillus, Rhodobaca, Halolactibacillus and Salinicoccus* with a few isolates related to genera of the phylum *Actinobacteria* including *Dietzia* and *Yaniella* and *Aquiflexum* of the phylum *Bacteroidetes*. The identification of these genera correlates with the genera which have been isolated from other soda lake environments, however a number of isolates have a <96% similarity to database sequences, which may suggest the identification of novel species or even novel genera. Demonstrating the contributions which culture based studies can make to the understanding of community structure.

DGGE analysis of samples from Lake Magadi demonstrated a high diversity of microorganisms in this environment across all sampling localities which varied in terms of pH temperature and solute concentration. The identification of more than one isolate contributing

to a band indicating that the resolution of the DGGE gel was not sufficient to fully resolve the diversity of the samples. This does emphasise however how diverse soda lake environments are in term of bacterial and archaeal community despite the extreme environmental conditions present.

Haloarchaea isolates were cultured from a number of different samples although the diversity was low. The archaeal DGGE gel suggests that the diversity of archaeal community may be greater than the cultural community suggests. The potential importance of archaeal isolates, particularly haloarchaeal isolates to astrobiology would suggest that further work on the isolation, genotypic and phenotypical characteristics of these organisms is necessary.

### 3.9. Further work

The influence of temperature, pH and chlorine (salinity) on bacterial and archaeal diversity (DGGE profile), would indicate that varying these factors would potentially allow the isolation of a wider range of novel isolates. Although the salinity of the growth media designed in this study was varied, this was still a fairly low concentration compared to the growth ranges of some archaea and bacteria. In addition, only one carbon sources was used for culturing in this study, therefore by increasing the salinity above 15% NaCl and using less complex carbon source it may be possible to identify more novel species for growth characterisation and resistance studies. Finally, the isolates identified were related to either haloalkalitolerant or haloalkaliphilic bacteria, attempts were made to culture at higher temperatures to isolate polyextremophiles. Further study into why this failed in order to design a suitable growth medium at higher temperatures would allow the isolation of polyextremophiles from Lake Magadi.

The drawbacks of culture dependent and culture independent techniques have been discussed in section 3.1.2, however, the combined use of different techniques is necessary for an understanding of the community structure in extreme environments. Future work on the samples should include a more in-depth culture independent analysis such as using pyrosequencing to obtain an unbiased sample of all members of the sampled community which could then be compared to the culture community identified in this study.

The time constraints and lack of success with ligation and/or transformation of the DGGE DNA meant that only a limited number of DGGE bands could be successfully sequenced. The optimisation of the ligation protocol, and the use of alternative vectors may improve the success of insertion and subsequent successful transformation and sequencing of the DNA extracted from the DGGE gel. This would potentially mean that more of the bands could be sequenced and identified.

Finally, DGGE analysis has been used in this study to compare the diversity of samples, however it can also be applied to assessing the after effects of a treatment, for example, the prolonged exposure of a sample to extreme cold for example may instigate changes in community structure which could be recorded through DGGE analysis before and after treatment. This would result in the identification of what environmental factors may affect the community structure of samples from soda lake environments, with the sequencing of bands allowing the identification of organisms capable of surviving those conditions. However, it must be considered that under environmental conditions which can preserve DNA (constant low pressure/temperature for example), DGGE analysis cannot distinguish between the living and dead cells in a sample.

## Chapter 4

# Characterisation of novel bacterial isolates from Lake Magadi

This chapter focuses on characterising three prokaryotes isolated from Lake Magadi in terms of the growth conditions which permit growth and more specifically optimal growth. The high pH and salinity of Lake Magadi would mean that we may expect these isolates to be able to grow at high levels of NaCl and in the presence of an alkaline high pH, possibly with their optimum NaCl and pH at high levels. In addition, those isolates sampled from a higher temperature region of the lake would be expected to have a higher temperature optimum or at least a higher temperature growth range.

Chapter 3 described the isolation and identification of 410 isolates from Lake Magadi. One isolate of the phylum *Proteobacterium*, within the family *Idiomarinaceae* (*Idiomarina* sp. LMLD01) and two isolates related to members of the phylum *Firmicute*, within the families *Planococcaceae* (*Planococcus* sp. LMLD02) and *Bacillaceae* (*Caldalkalibacillus* sp. LMLD03) were chosen for further study and characterisation.

### 4.1. Characterisation of novel strains

The ability to isolate strains in a culture is considered as a limiting factor of studying bacteria and archaea (Jones et al., 1998: Janssen, 2006: Caesar-TonThat et al., 2008)) it is important to combine culture independent studies with culture based studies (DeLong, 2009). The isolation of strains from samples can potentially result in the identification of novel bacteria or archaea, which may be of novel species or genera (Chapter 3). Despite the biases and limitations associated with culture based studies, they are still indispensable methods for understanding the physiology, ecology, community relationships and metabolism of microorganisms (DeLong, 2009: Gontang et al., 2007: Mwirichia et al., 2010a: Mwirichia et al., 2010b: Palleroni, 1997: Vaz-Moreira et al., 2011).

Intraspecies variation and the adaptations of organisms to different environmental niches means that the growth limits identified for an isolate of a species from one environment, may differ to a similar species isolate from another. Genomic variation within a species related to protein synthesis or cell membrane structure for example, may potentially result in the ability of a strain to exploit different ecological niches. The primary drawback of 16S rRNA gene sequence analysis is that it cannot be assumed that just because two isolates share the same 16s rRNA gene sequence that they will have exactly the same phenotypes (Amann et al., 1990: Kisand and Wikner, 2003). The acquisition or alteration of a few genes may allow growth under different conditions whilst still retaining the same 16s rRNA sequence (Clarridge, 2004). It has been shown that organisms which have sufficient 16S rRNA gene

sequence similarity to be classed as the same species, in fact have enough genotypic and phenotypic variability to be classed as different species, if other regions of their genomes are studied (Fox, 1992). In addition, variability within the 16S rRNA gene can exist within the same strain. some archaea for example can demonstrate up to 5% dissimilarity between 16S rRNA gene sequence within the same cell (Amann et al., 1990).

Another important factor is the ability of many organisms to adapt to a particular environment through the 'borrowing' of genes from other organisms (Lorenz and Wackernagel, 1994: Garcia-Vallvé et al., 2000: Jain et al., 2003: Keeling and Palmer, 2008). Horizontal gene transfer (HGT) is a process by which an organisms can acquire foreign genes from unrelated organisms in response to changing environmental conditions, enabling them to adapt with the environment (Jain et al., 2003). This is a common occurrence in prokaryotes in particular, and can potentially result in phenotypic differences in genotypically (according to their 16S rRNA gene sequence) very similar strains (Jain et al., 2003: Keeling and Palmer, 2008). It is for these reasons culture based studies are still an important part of environmental microbiology and are an important complementary technique to culture independent analyses (Ma et al., 2004b). It is by characterising different organisms from extreme environments, and then assessing their response to certain stresses that we may gain a better understanding of the limits of life.

### 4.2. *Idiomarina*ceae

One isolate to be studied in more detail was identified as being related to *Idiomarinaceae* (of the class *Gammaproteobacteria)* was most closely related to the genus *Idiomarina* (Chapter 3). The family *Idiomarinaceae* was first proposed by Ivanova et al. (Ivanova, 2000) to describe a group of *Proteobacteria* which do not form spores (Taborda, 2009). The type strains for the species of this genus have been characterised from a range of environments, including sea surface waters, hypersaline dry ravines, hypersaline wetlands and hydrothermal vents (Table 4.1).

The genus *Idiomarina* is differentiated from other marine bacteria due to their broad tolerances of temperature, pH and NaCl concentration (Martinez-Canovas, 2004). Several of the type strains for the species are described as moderately halophilic (Donachie et al., 2003: Choi, 2005) and depending upon the definition of alkaliphilic and alkalitolerant, members of this genus could be defined as at least as alkalitolerant. Species of *Idiomarina* are generally cream or pale yellow, with no strong pigmentation. Some species can produce mucus and extracellular polysaccharides (EPS) (Martinez-Canovas, 2004). Studies into the production of EPS have shown that they play an important role in desiccation and freezing tolerance some bacteria (Roberson and Firestone, 1992: Tamaru et al., 2005: Knowles and Castenholz, 2008) (See Chapter 1, section 1.6.3.6). If *Idiomarina* sp. LMLD01 is shown to be able to

produce EPS then this may mean this strain could have an advantage against detrimental conditions, such as desiccation, which could be experienced on Mars.

*Table 4.1. Growth characteristics for 8 species of the genus Idiomarina. 1. I. baltica OS145 (Brettar et al., 2003), 2. I. ramblicola (Martinez-Canovas, 2004), 3. I. fontislapidasi (Martinez-Canovas, 2004), 4. I. liohiensis (Donachie et al., 2003), 5. I. abyssalis (Ivanova, 2000), 6. I. seosinensis CL-SP19 (Choi, 2005), 7. I. salinarum (Pseudoidiomarina salinarum) (Yoon et al., 2007: Jean, 2009). 8. I. xiamenensis (Wang et al., 2011). N/R denotes data not reported.*

| | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* |
|---|---|---|---|---|---|---|---|---|
| Colony colour | Yellowy | Cream | Cream | N/R | N/R | Yellowish | Pale yellow | Pale yellow |
| Amylase | N/R | N/R | N/R | N/R | N/R | - | N/R | - |
| Protease | N/R | N/R | N/R | N/R | N/R | N/R | N/R | N/R |
| Streptomycin resistance | N/R | N/R | N/R | N/R | N/R | N/R | N/R | N/R |
| Ampicillin resistance | N/R | N/R | N/R | N/R | N/R | N/R | N/R | N/R |
| NaCl range (% w/v) | 1%-10% | 0.5%-15% | 0.5%-25% | 0.5%-20% | 0.6%-15% | 1%-20% | NaCl needed Growth up to 15% | 0% - 15% |
| NaCl optimum | 3%-6% (w/v) | 3%-5% (w/v) | 3%-5% (w/v) | 7.5%-10% (w/v) | 3%-6% (w/v) | 5% - 10%(w/v) | N/R | 1% - 3% |
| TemP. Range | 8 °C-46 °C | 15°C-40 °C | 4 °C-45 °C | 4 °C-46 °C | 4 °C-30 °C | 4 °C- 40 °C | 4 °C-42 °C | 4 °C- 42 °C |
| Temp optimum | 30 °C-40 °C | 32 °C | 32 °C | - | 20 °C-22 °C | 30 °C-35 °C | 37 °C | 25 °C |
| pH Range | N/R | 5.0-10.0 | 5.0-10.0 | 0.5-10 | 5.5-9.5 | 6.0-10.0 | From 6.0 | 6 – 10 |
| pH Optimum | N/R | 7.0-8.0 | 7.0-8.0 | N/R | 7.8-8.0 | N/R | 7.0 – 8.0 | 7.0-8.0 |
| Isolation location | Baltic sea surface waters | Hyper-saline rambla Spain | Inland hyper-saline wet-land soil, Spain | Hydrothermal vents on the Lō'ihi Seamount, Hawai'i | North-western Pacific Ocean at a depth of 4000-5000m | Hypersaline water of solar saltern Korea | Marine solar saltern Korea | Surface seawater Xiamen Island |

### 4.3. Planococcaceae

The first novel isolate to be characterised of the phylum *Firmicute* is most closely related to a strain of the genus *Planococcus* (Chapter 3). *Planococcus* is within the family *Planococcaceae,* are cocci in shape and do not form spores (Suresh 2007). This genus has been isolated from variety of environments, including cold deserts in the Himilayas, Antarctica, sea water, fish curing brine and soda lakes (Lonar Lake) (Table 4.2.) although the genus *Planococcus* has not previously been reported by studies of Lake Magadi. Species of *Planococcus* are described as halotolerant as well as haloalkaliphilic (Reddy et al., 2002: Romano et al., 2003).

Pigments play an important role in cellular defence against oxidative stress (Wu et al., 1983: Carbonneau et al., 1989: Tian et al., 2009: Tian and Hua, 2010) and all species of *Planococcus* are brightly pigmented orange (Table 4.2). Research conducted on the pigmentation of species of *Planococcus* has demonstrated that changes in the biosynthesis of

carotenoids can be induced through varying the salt concentration in the growth medium. In addition, the pigmentation also varies depending upon the age of the culture (Thirkell and Summerfield, 1980). *Planococcus maritimus* produces a novel antioxidative glyco-$C_{30}$-carotenoic acid, which has been shown to have a more potent antioxidative activity than the carotenoids astaxanthin or β-carotene (Shindo, 2008). This may make this strain particularly interesting in terms of its ability to withstand the types of oxidative stress which might be experienced on Mars associated with radiation and desiccation.

*Table 4.2. Growth characteristics for 9 species of the genus Planococcus. 1. P. columbae (Suresh et al., 2007), 2. P. rifietensis (Romano et al., 2003: Sadfi Zouaoui et al., 2007), 3. P. maitriensis (Alam et al., 2003), 4. P. maritimus (Yoon, 2003), 5. P. kocurii (Engelhardt et al., 2001), 6. P. donghaensis (Choi et al., 2007), 7. P. southpolaris/P. antarcticus (Reddy et al., 2002), 8. P. psychrophilus (Reddy et al., 2002), 9. P. stackebrandtii (Mayilraj et al., 2005). N/R denotes data not reported.*

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Colony colour | Orange | Orange | Orange | Orange | Orange | Orange | Orange | Orange | Orange |
| Amylase | - | + | N/R | + | N/R | N/R | N/R | N/R | N/R |
| Protease | - | + | N/R | + | N/R | N/R | N/R | N/R | N/R |
| Streptomycin resistance | - | N/R | N/R | N/R | N/R | N/R | N/R | - | N/R |
| Ampicillin resistance | N/R | N/R | N/R | N/R | N/R | N/R | N/R | - | N/R |
| NaCl range (% w/v) | 0%-14% | Up to 15% | 0.1-12.5% | 0%-17% | 0%-10% | Growth occurs at 12% | up to 12% | up to 12% | up to7% |
| NaCl optimum (% w/v) | 4%-5% | - | - | 2% | - | 2% | - | - | - |
| Temp. Range | 8 °C – 42 °C | 5 °C – 42 °C | 0 °C - 30 °C | 4 °C – 41 °C | 15 °C – 37 °C | 4 °C – 37 °C | 2 °C – 30 °C | 2 °C – 30 °C | 15 °C – 30 °C |
| Temp. optimum | 30 °C | 37 °C | 25 °C | 30 °C | - | 25 °C – 30 °C | 20 °C | 22 °C | 25 °C |
| pH Range | 6.0-11.0 | 6.0-10.5 | 6.0-12.0 | Growth occurs at pH 5 | - | No growth at pH 6.0 but does occur at pH 7.0 | 6.0-12.0 | 6.0-12.0 | 5.6-11.0 |
| pH Optimum | 7.0-8.0 | - | - | 6.0-8.0 | - | 7.5-8.0 | - | 7 | - |
| Isolation location | From pigeon faeces | Algal mat from sulphurous springs in Campania | Cyanobacterial mat, Schirmacher Oasis, Antarctica | Sea water of a tidal flat in Korea | Fish, frozen foods, and fish curing brine. | East Sea, South Korea | Cyanobacterial mat samples collected from ponds in Antarctica | Cyanobacterial mat samples collected from ponds in Antarctica | Cold desert of the Himalayas, India |

### 4.4. Bacillaceae

The final isolate to be characterised in more detail is most closely related to *Caldalkalibacillus uzonensis* according to phylogenetic analysis (Chapter 2). The genus *Caldalkalibacillus* is also a member of the phylum *Firmicute*, within the family *Bacillaceae*. Members of the genus *Caldalkalibacillus* has been isolated from hot and alkaline environments (Russia and China), with the type strains for the genus being a thermophilic and

alkaliphilic bacterium (Xue et al., 2006). All species belonging to this genus which have been so far characterised have been able to form spores (Table 4.3).

*Table 4.3 Growth characteristics for 2 species of the genus Caldalkalibacillus. 1. C. uzonensis. (Zhao et al., 2008), 2. C. thermarum (Xue et al., 2006) N/R= data not reported*

| | *1* | *2* |
|---|---|---|
| Colony colour | Cream, translucent | Yellow white, translucent |
| Amylase | N/R | N/R |
| Protease | N/R | N/R |
| Streptomycin resistance | N/R | - |
| Ampicillin resistance | N/R | - |
| NaCl range (% w/v) | 0%-6% | 0% - 6% |
| NaCl optimum (% w/v) | - | 1.5% |
| Temperature range | 42 °C - 64 °C | 45 °C- 65 °C |
| Temperature optimum | 50 °C - 52 °C | 60 °C |
| pH Range | 6.4-9.7 | 7.5-10.0 |
| pH Optimum | 8.2-8.4 | 8.5 |
| Isolation location | Hot Spring , Uzon Caldera, Russia | Hot spring, China |

The initial study of the novel strain *Caldalkalibacillus* sp. LMLD03 in Chapter 3 identified the presence of endospores. Many *Bacillaceae* species are known to be endospore forming organisms (Clifton 1958), which makes them resistant to extreme stress including radiation and temperature (Setlow, 2006). The resistance of an organism to an environmental stress through the formation of spores ability of a microorganism to demonstrate a level of survival after exposure to an extreme stress requires their subsequent germination, trigger by the presence of particular nutrients, for example (Paidhungat and Setlow, 2000). The ability of the novel isolate *Caldalkalibacillus* sp. LMLD03 to form spores, followed by its successful germination, may demonstrate a resistance of *Caldalkalibacillus* sp. LMLD03 to some of the stress which may be experienced on Mars.

Details on the growth and tolerance of novel microorganisms from extreme environments will contribute to a fuller understanding of the limits of life Extraterrestrial locations where the environmental parameters are within the limits of life would be a higher priority target location in the search for life beyond the Earth. When studying environments on Mars in terms of astrobiology, it is important to understand firstly which environments could be habitable and which could not. The characterisation of novel isolates, such as *Idiomarina* sp. LMLD01, *Planococcus* sp. LMLD02 and *Caldalkalibacillus* sp. LMLD03, could provide more details on the limits of life (as we know it) in relation to pH temperature and salinity.

### 4.5. Aims of the chapter

1. To investigate for each strain;
    a. their carbon source utilisation

b. extracellular enzyme production
c. resistance to antibiotic

2. To investigate for each strain the range and optimal growth conditions for;
    a. Temperature
    b. pH
    c. NaCl concentration

## 4.6. Materials and methods

### 4.6.1. Bacterial strains

Strains were isolated as described in Chapter 2. *Idiomarina* sp. LMLD01 was maintained on 0.7% NaCl agar and *Planococcus* sp. LMLD02 and *Caldalkalibacillus sp. LMLD03* were maintained on 6.8% salt agar with a pH of 9.6 and all incubated at 37 °C in the dark, as described in chapter 3.

All strains were stored on agar plates at 4 °C, with stocks stored in 35-70% glycerol at -20 °C or -80 °C ready for use.

### 4.6.2. Carbon and nitrogen source studies

The AP+ 6.8% NaCl growth medium was altered by removing the peptone and replacing it with the alternative carbon source. The carbon sources tested were; L-glycine, L-alanine, Aspartic acid, L-asparagine, L-alginine and L-serine at a concentration of 0.4% (w/v). Glycerol, maltose, Na acetate and Na citrate were tested both with and without the addition of ammonium citrate as a reduced nitrate source. Peptone was used as a control for growth; these were all used at a concentration of 1% (w/v). An OD reading was taken using a WPA Biowave CO800 Cell Density Meter, a value of >0.1 was considered a positive reading.

### 4.6.3. Enzyme activity assay

The AP+ 6.8% NaCl agar medium described in Chapter 2 was supplemented with 0.5% (w/v) starch to test for digestion by amylase; 0.5% (w/v) skimmed milk was added to test for protease production. Each isolate was streaked onto the prepared plates and incubated for up to 72 hours.

In addition, the cultures were spun down at 4 °C at 3000 g for 10 minutes, the supernatant was removed, filter sterilised and pipetted into holes bored in the agar.

Enzyme activity was confirmed through the presence of zones of clearing on starch and on skimmed milk plates.

#### 4.6.4. Antibiotic resistance assay

Resistance to the antibiotics ampicillin and streptomycin was tested. The AP+ 7% NaCl agar medium described in Chapter 2 was supplemented with 100µg/ml of either antibiotic. Each isolate was streaked onto either of the antibiotics and incubated at 37 °C for 24 hours for *Idiomarina* sp. LMLD01 and *Planococcus* sp. LMLD02, and up to 72 hours for *Caldalkalibacillus sp. LMLD03*.

#### 4.6.5. Preliminary growth studies

Initial studies for the variables of pH, temperature and salt concentration were conducted to ascertain limits of growth. 15 ml culture in 50 ml Falcon tubes were inoculated with a single colony and incubated for seven days at 37 °C and 200 rpm.

#### 4.6.6. Focussed growth studies

Growth was studied in triplicate; three 10 ml starter cultures were inoculated with a single colony and were incubated at 37 °C for *Idiomarina* sp. LMLD01 and *Planococcus* sp. LDLM02 and 45 °C for *Caldalkalibacillus sp. LMLD03* at 200 rpm overnight.

The starter culture for NaCl studies were all grown in AP+ 6.8% NaCl, the pH and temperature studies used a starter culture grown in AP+ 0.7% NaCl for *Idiomarina sp. LMLD01* and *Planococcus sp. LMLD02*.

1 ml of overnight starter cultures were added to 50 ml of the appropriate broth medium in 500 ml flasks incubated at the appropriate temperature for the study at 180 rpm. A sample was taken from each flask for an OD reading at 1-2 h intervals from 0 hours to 24 hours.

When the full range of conditions could not be studied at the same time, starter cultures were diluted with sterile medium where necessary to a similar OD.

##### 4.6.6.1. NaCl concentrations

Growth was tested at 0% of added NaCl (but actually containing 0.034g/100ml of NaCl from the peptone) up to 16% NaCl initially, and then between 0.07% and 10% NaCl for the focussed studies.

##### 4.6.6.2. Temperature

Growth was first tested at 10 °C, 25 °C, 30 °C, 37 °C and 45 °C. The subsequent growth curve studies conducted for each isolate focused upon a range of 20 °C up to 50 °C at pH ~9.6.

##### 4.6.6.3. pH

The pH meter used was a Mettler Toledo Seven Easy and a probe with a range of pH 0-14 and up to 80 °C. The pH was calibrated for each temperature. pH was maintained at

~9.6 for each temperature tested. The pHs for growth were tested from 6 to 11 and conducted at either 37 °C or 45 °C. A pH of 6 to 7.5 were attained using a $NaH_2PO_4$/ $Na_2HPO_4$ buffer (Yumoto et al., 2004) at 0.1 M final concentration. The pH's 9 to 10.3 were achieved using the $Na_2CO_3$/$NaHCO_3$ buffer system. Finally pH 10.4 and pH 10.6 were achieved using $NaCO_3$/NaOH buffer system (Yumoto et al., 2004), An overlap between the different buffering systems was not possible.

The pH after growth was tested for each flask. 2 ml of culture was spun down at 37 °C at 11000 rpm for 10 minutes and the pellet discarded. The supernatant was maintained at 37 °C or 45 °C and the pH measured for each of the triplicates. The pH of the negative control for each medium was measured three times after 24 hours to ensure no change in pH in the blank control broth.

### 4.6.7. Data analysis

#### 4.6.7.1. Calculation of growth rates

The growth rate for each strain under each variable was calculated by plotting the log of the OD against time for at least three points during the exponential phase of growth in Microsoft Excel. An exponential trend line was fitted to the data and the factor of *X* was taken as the growth rate in units of $h^{-1}$. Error bars were fitted to each data point as 1 *x* the standard error. Values of 0.00 were plotted as 0.01 on the logarithmic scale. The experiments were conducted using three biological replicates.

#### 4.6.7.2. Statistical tests

Students t-Test was conducted as described in Chapter 2. The T-test was paired, and conducted assuming 2-tailed distribution for analysing the pH change after incubation. A *p* value < 0.05 was considered statistically significant. ANOVA statistical test and Tukey Post-hoc analysis were conducted as described in Chapter 2.

## 4.7. Results

### 4.7.1. Carbon source utilisation

The ability to use 10 different carbon sources was tested for each strain (Table 4.4). Neither *Idiomarina* sp. LMLD01 nor *Planococcus* sp. LMLD02 were able to utilise any of the carbon sources tested except for the peptone control. In contrast to this *Caldalkalibacillus* sp. LMLD03 was able to utilise L-alanine, L-arginine and Na citrate as sole carbon source, with the medium also containing $NaNO_3$.

*Table 4.4 Carbon utilisation over 7 day period at 37 °C in 6.8% NaCl. The presence of growth is indicated by + and an absence of growth by -.* N/R= datum not recorded.

| Carbon sources | *Idiomarina sp. LMLD01* | *Planococcus sp. LMLD02* | *Caldalkalibacillus sp. LMLD03* |
|---|---|---|---|
| Aspartic acid | - | - | - |
| Glycerol | - | - | - |
| Glycerol & $NH_4Cl$ | - | - | N/R |
| L Alanine | - | - | + |
| L- Asparagine | - | - | - |
| L-Arginine | - | - | + |
| L-glycine | - | - | - |
| L-Serine | - | - | - |
| Maltose | - | - | - |
| Maltose & $NH_4Cl$ | - | - | N/R |
| Na acetate | - | - | - |
| Na acetate & $NH_4Cl$ | - | - | N/R |
| Na citrate | - | - | + |
| Na citrate & $NH_4Cl$ | - | - | N/R |
| Peptone (control) | + | + | + |

### 4.7.2. Extracellular enzyme secretion

The production of both amylase and protease was confirmed for *Idiomarina sp. LMLD01* (Table 4.5). A small amount of clearing could be seen around the colonies for *Planococcus* sp. LMLD02 on skimmed milk as well as a small amount around the wells indicating protease activity. No clearing was seen around the colonies or supernatant incubated on the starch plates.

The presence of amylase and protease enzyme production was confirmed with zones of clearing around both supernatant wells and the colonies for *Caldalkalibacillus sp. LMLD03*.

*Table 4.5 Extracellular enzyme secretion on 6.8%? Agar, supplemented with either starch or skimmed milk powder. Presence of clearing around colonies is indicated by + and an absence of clearing by -*

| | *Idiomarina sp. LMLD01* | | *Planococcus sp. LMLD02* | | *Caldalkalibacillus sp. LMLD03* | |
|---|---|---|---|---|---|---|
| Enzyme | Colony | Well | Colony | Well | Colony | Well |
| Protease | + | + | + | + | + | + |
| Amylase | + | + | - | - | + | + |

### 4.7.3. Antibiotic resistance

No growth of *Idiomarina* sp. LMLD01 occurred on streptomycin antibiotic plates after 24 hours, however growth did occur on the ampicillin antibiotic plates. Growth of *Planococcus*

sp. LMLD02 occurred on both types of antibiotic plates within 24 hours. *Caldalkalibacillus* sp. LMLD03 did not grow in the presence of either antibiotic (Table 4.6).

*Table 4.6 Antibiotic resistance 6.8% Agar, supplemented with 100µg/ml of either ampicillin or streptomycin. Presence of growth is indicated by + and an absence of clearing by -*

| Enzyme | *Idiomarina sp. LMLD01* | *Planococcus sp. LMLD02* | *Caldalkalibacillus sp. LMLD03* |
|---|---|---|---|
| Ampicillin | + | + | - |
| Streptomycin | - | + | - |

### 4.7.4. Preliminary growth studies for NaCl concentration, temperature and pH

#### 4.7.4.1. Preliminary NaCl growth studies

All strains were able to grow in the absence of NaCl (Table 4.7). The strains *Idiomarina* sp. LMLD01 and *Planococcus* sp. LMLD02 grew in the presence of up to 15% NaCl, over a seven day period. No growth was achieved at 16% NaCl for either isolates. *Caldalkalibacillus* sp. LMLD03 was capable of growing in a salt concentration of 0% NaCl up to 13%, but not at 15%.

*Table 4.7. Outlining the occurrence or absence of growth at different salt concentrations when grown at 37 °C. Presence of growth between 1 and 7 days is indicated by + and an absence of growth after 7 days by –.* N/R= datum not recorded.

| Salt content | *Idiomarina sp. LMLD01* | *Planococcus sp. LMLD02* | *Caldalkalibacillus sp. LMLD03* |
|---|---|---|---|
| 0% | + | + | + |
| 1% | + | + | + |
| 2% | + | + | + |
| 3% | + | + | + |
| 5% | + | + | + |
| 10% | + | + | + |
| 12% | + | + | + |
| 13% | N/R | N/R | + |
| 15% | + | + | - |
| 16% | - | - | N/R |

The growth characteristics of each isolate in NaCl concentrations between 0% and 10% NaCl will be analysed in the focussed study.

#### 4.7.4.2. Preliminary temperature growth studies

Growth for *Idiomarina* sp. LMLD02 was achieved at 10 °C and up to 45 °C, with no growth occurring at 50°C. Growth was achieved at 10 °C with *Planococcus sp. LMLD02* and up to 40 °C but not at 45 °C (Table 4.8).

*Table 4.8 Outlining the occurrence or absence of growth at different temperatures when grown in a 1% salt concentration and pH 9.6. + denotes growth occurred between 1 and 7 days, - denotes no growth after 7 days. N/R denotes not recorded*

| Temperature | *Idiomarina* sp. LMLD02 | *Planococcus* sp. | *Caldalkalibacillus sp.* |
|---|---|---|---|
| 10 °C | + | + | N/R |
| 20 °C | + | + | - |
| 30 °C | + | + | - |
| 37 °C | + | + | + |
| 40 °C | + | + | + |
| 45 °C | + | - | + |
| 50 °C | - | - | + |
| 55 °C | N/R | N/R | + |
| 60 °C | N/R | N/R | + |
| 65 °C | N/R | N/R | - |

No growth occurred for *Caldalkalibacillus sp. LMLD03* at 30 °C, but it did grow at 37 °C. Growth occurred up to 60 °C but not at 65 °C. *Caldalkalibacillus* sp. LMLD03 was found to grow very poorly in a liquid medium at 37 °C, compared to the growth achieved at 45 °C. In addition growth at 37 °C in 6.8% NaCl broth was uneven and final OD's were low and not uniform. For these reasons the starter cultures for the strain *Caldalkalibacillus* sp. LMLD03 was incubated at 45 °C.

With these data a focussed study will be conducted for the growth range of 20 °C to 45 °C for *Idiomarina* sp. LMLD01 and 20 °C to 40 °C for *Planococcus sp. LMLD02*. The range for the focussed study of *Caldalkalibacillus* sp. LMLD03 will be between 37 °C and 60 °C.

#### 4.7.4.3. Preliminary pH growth studies

*Idiomarina* sp. LMLD02 was able to grow between pH 7 and pH 10.6 at 37 °C. Growth did not occur at a pH of 12 (Table 4.9). *Planococcus* sp. LMLD02 grew at pH of 7 up to a pH of 10.3, growth was not achieved at pH 10.6. Growth of *Caldalkalibacillus* sp. LMLD03 was achieved at a pH of 7.6, and up to a pH of 10.3.

*Table 4.9 Outlining the occurrence or absence of growth at different pH's when grown at 37 °C or 45 °C. + denotes growth occurred between 1 and 7 days, - denotes no growth after 7 days. N/R denotes not recorded.*

| Buffer system | pH | *Idiomarina sp. LMLD01* (at 37 °C) | *Planococcus sp. LMLD02* (at 37 °C) | *Caldalkalibacillus* sp. LMLD03 (at 45 °C) |
|---|---|---|---|---|
| $Na_2HPO_4$/$NaH_2PO_4$ | 6 | + | + | - |
| | 7 | + | + | - |
| | 7.5 | + | + | + |
| $Na_2CO_3$/$NaHCO_3$ buffer | 9 | + | + | + |
| | 9.6 | + | + | + |
| | 10 | + | + | + |
| | 10.3 | + | + | + |
| $Na_2CO_3$/NaOH Buffer | 10.6 | + | - | - |
| | 12 | - | N/R | N/R |

With these data, and the interest in the upper limits of pH to which these strains can growth, the growth rate and final OD from pH 10.3 to 7 was studied for the isolates: *Planococcus* sp. LMLD02 and *Caldalkalibacillus sp. LMLD03*. An addition measurement at a pH of 10.6 was studied for *Idiomarina* sp. LMLD01 as the preliminary growth data indicated an ability of this strain to grow at this slightly higher pH.

### 4.7.5. Growth of isolates at a range of NaCl concentrations

#### 4.7.5.1. *Idiomarina* sp. LMLD01

The growth curves for each treatment; 0.07%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8% and 10% are shown in Figure 4.1. *Idiomarina* sp. LMLD01 reached the stationary phase by 12 hours in all salt concentrations.

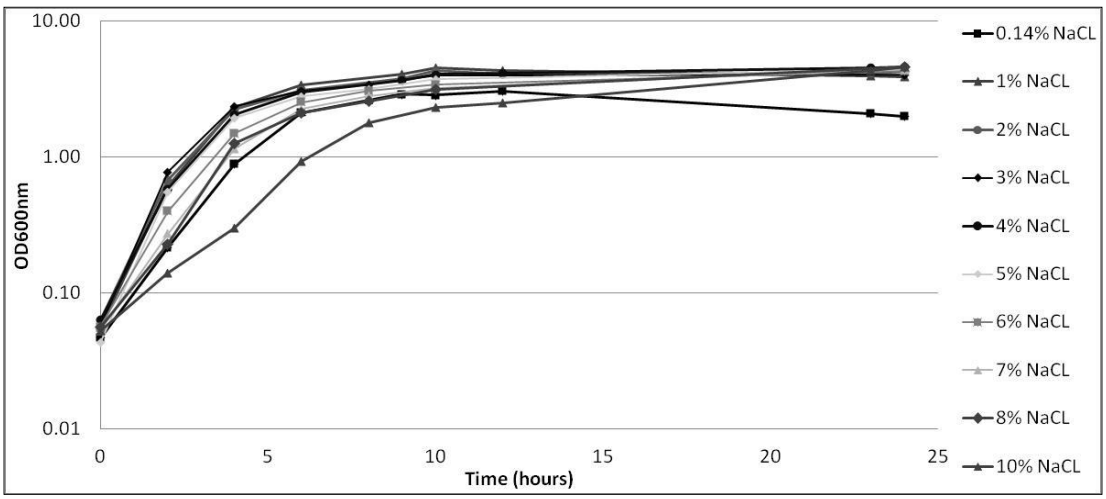



*Figure 4.1. Growth curve of Idiomarina* sp. *LMLD01 over a 24 hour period for medium with a NaCl concentration from 0% to 10%. Error bars are 1 x standard error.*

The highest growth rate was recorded in a NaCl concentration of 3% with a rate of 0.9336 $h^{-1}$ although this was not significantly different from the growth rate achieved in 1% - 5% NaCl ($p$>0.768). There was a trend of decreasing growth rate from 5% up to 8% NaCl for *Idiomarina* sp. LMLD01 (Figure 4.2). A sharp decreases in growth rate can be seen from 1% to 0.07% NaCl medium which was significantly lower than that at a concentration of 1% to 5% ($p$>0.002).

The lowest growth rate recorded was in a NaCl concentration of 10% with a rate of 0.4452 $h^{-1}$. The growth rate in 10% NaCl was significantly lower than that achieved in any salt concentration less than 10% ($p$=0.000). (See Appendix 1, table 1.1).

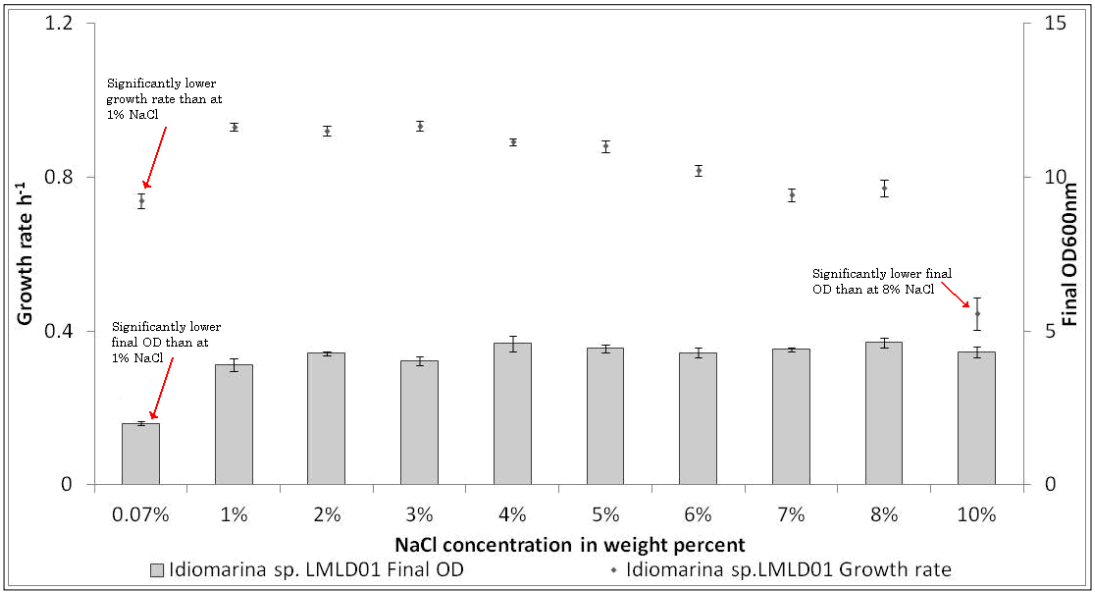



*Figure 4.2. Growth rates and final OD for Idiomarina sp. LMLD01 at increasing NaCl concentration (at 37 °C, pH 9.6). Biomass was measured using $OD^{600nm}$. Error bars are 1 x standard error. Statistical differences were assessed using ANOVA analysis of variance statistical test. Significance was attributed to values where p<0.05. Error bars are 1 x standard error.*

The final OD reading for each NaCl concentration shows that a similar final OD was achieved in a medium with a NaCl concentration between 1% and 10% NaCl. These final OD's are not significantly different from one another ($p$>0.057). The final OD measured for 0.07% NaCl was significantly lower than the OD achieved when incubated with a NaCl concentration between 1% and 10% ($p$=0.000) (See Appendix 2, table 1.2).

#### 4.7.5.2. *Planococcus* sp. LMLD02

*Planococcus* sp. LMLD02 reached the stationary phase by 12 hours in a NaCl concentration of 7% NaCl or less, and after 12 hours in a NaCl concentration of 8% or 10%

(Figure 4.3). The highest growth rate was recorded at 1% NaCl with a rate of .0247 $h^{-1}$. The lowest growth rate was recorded at 10% NaCl with a growth rate of 0.279 $h^{-1}$.

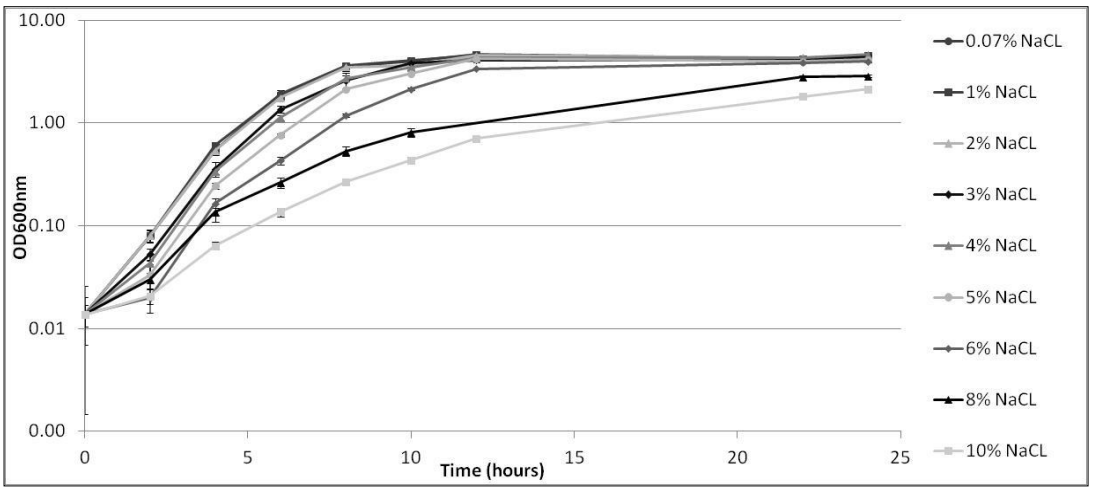



*Figure 4.3. Growth curves for Planococcus sp. LMLD02 over a 24h period for medium with NaCl concentration of 0.07% to 10%. Error bars are 1 x standard error.*

There was no significant change in the growth rate of *Planococcus* sp. LMLD02 when incubated in the presence of 1% NaCl compared to 0.07% ($p$=0.470) (Figure 4.4). In turn the growth rate at 1% was not signifcantly different from the growth rate at NaCl concentration of 2% to 5% NaCl ($p$>0.064). The growth rate at 8% and 10% NaCl were signficantly lower than for any other treatment tested ($p$=0.000), with the exception of 6% NaCl for which it was not significantly different ($p$=0.075).

The final OD recorded after 1%-6% treatment demonstrated no significant difference ($p$>0.05). However, there was a significant drop in the final OD reached when incubated in the presence of 8% and 10% NaCl, when compared to the final OD reached in the presence of any other NaCl concentration tested ($p$=0.000).

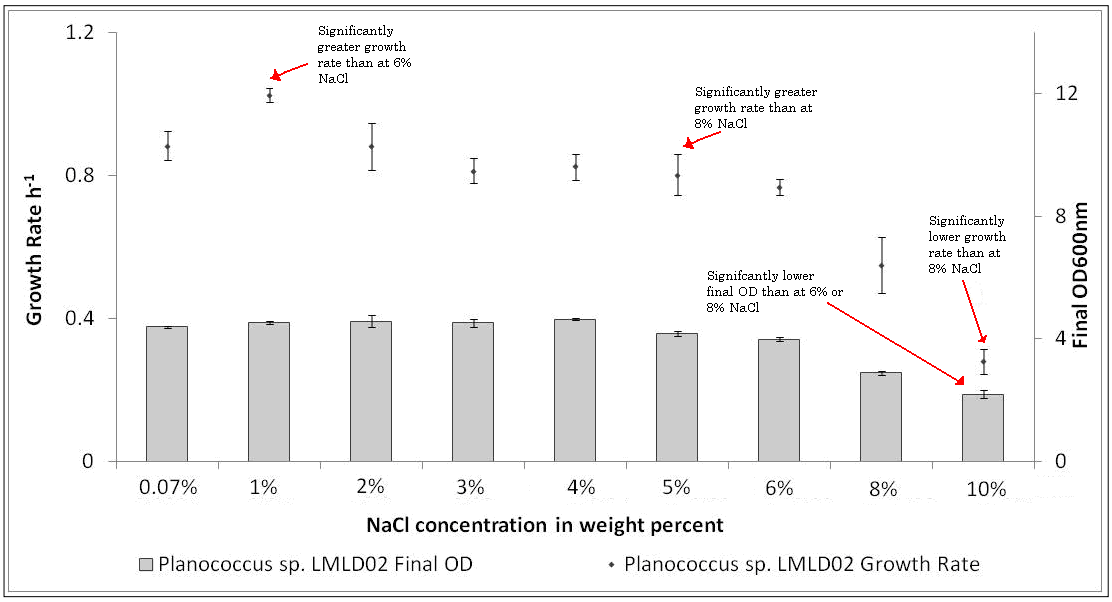



*Figure 4.4. Growth rates and final OD for Planococcus sp. LMLD02 at increasing NaCl concentration (at 37 °C, pH 9.6). Biomass was measured using $OD^{600nm}$. Error bars are 1 x standard error. Statistical differences were assessed using ANOVA analysis of variance statistical test. Significance was attributed to values where p<0.05. Error bars are 1 x standard error.*

#### 4.7.5.3. *Caldalkalibacillus* sp. LMLD03

*Caldalkalibacillus* sp. LMLD03 reached the stationary phase of growth by 12 hours at all NaCl concentrations, except for when grown in a concentration of 10% NaCl, where it did not enter the exponential phase until after 24 hours (Figure 4.6.). The highest growth rate recorded for *Caldalkalibacillus sp. LMLD03* was at 1% NaCl with a growth rate of 0.5640 $h^{-1}$ and the lowest growth rate was at 10% at 0.2927 $h^{-1}$.

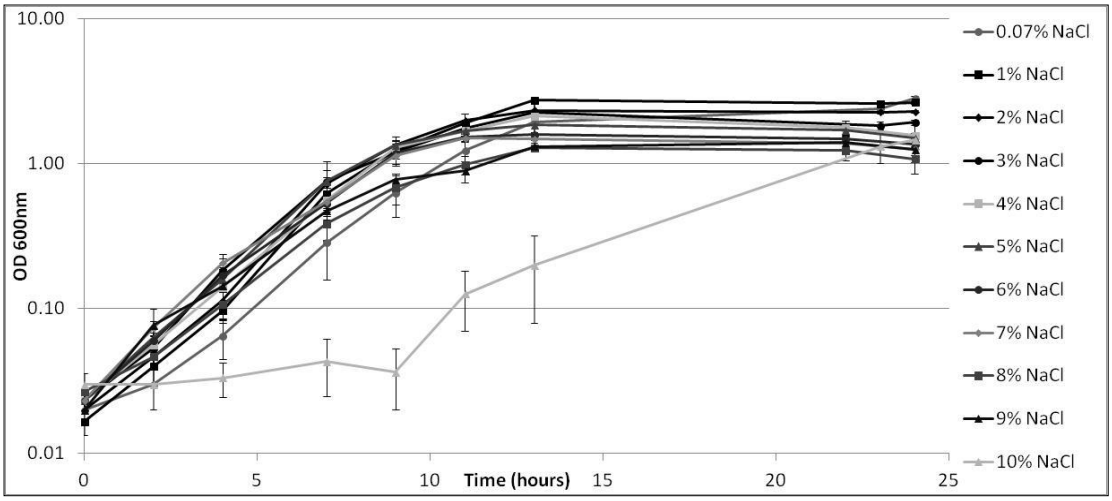



*Figure 4.6. Growth curve of Caldalkalibacillus sp. LMLD03 over a 24 hour period for medium with a NaCl concentration from 0.07% to 5%. Error bars are 1 x standard error.*

The growth rate at 1%, 2% and 3% were significantly greater than that recorded at 9% and 10% NaCl ($p$<0.036) (Figure 4.7.). There was no significant difference in the growth rate at 1%, 2% and 3% NaCl and that recorded at 4%, 5%, 6%, 7% and 8% NaCl ($p$<0.169).

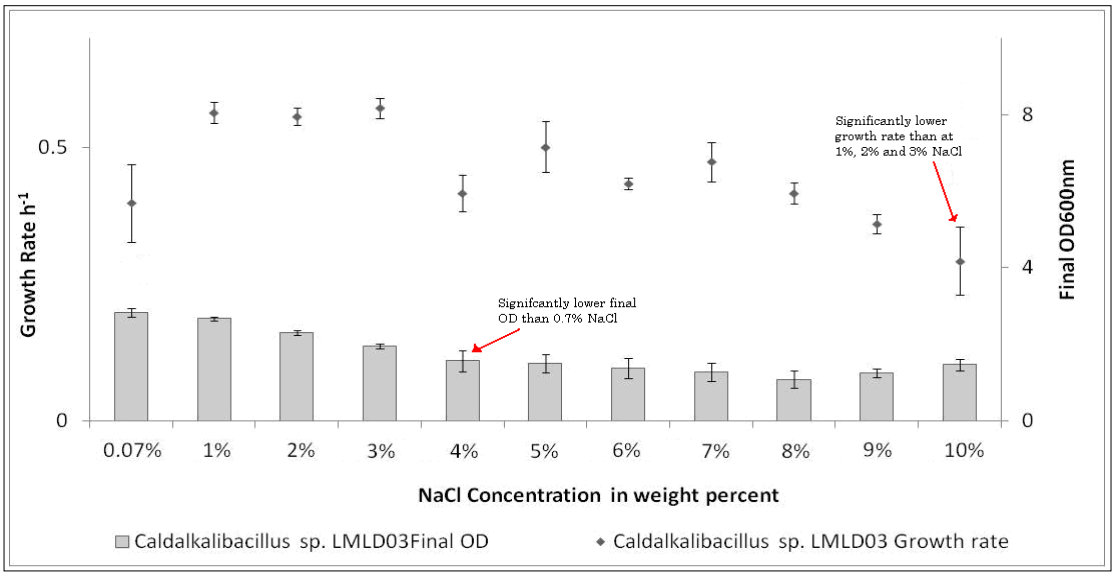



*Figure 4.7. Growth rates and final OD for Caldalkalibacillus sp. LMLD03 at increasing NaCl concentration (at 37 °C, pH 9.6). Biomass was measured using $OD^{600nm}$. Error bars are 1 x standard error. Statistical differences were assessed using ANOVA analysis of variance statistical test. Significance was attributed to values where p<0.05. Error bars are 1 x standard error.*

A trend of decreasing final OD can be seen with increasing NaCl concentration. The final OD achieved at 0.07% NaCl was not significantly different from that reached in a 1%-3% NaCl media with ($p$>0.639). The final OD in the 1% NaCl medium was significantly different to that achieved between 4% and 10% NaCl ($p$>0.014). The 9% and 10% NaCl medium produced a final OD which was significantly lower than recorded at 0.07% to 1% NaCl ($p$<0.006).

Some of the flask cultures for *Caldalkalibacillus* sp. LMLD03 contained particulate matter which developed (Figure 4.8.A.) in the broth, whilst the OD reading still remained very low. The material often collects on the flasks at the highest level (Figure 4.8.B). The uneven growth observed could be due to the mixture of spores and vegetative cells present on the plate or overnight culture from which the medium was inoculated. This isolate is known to form spores, see chapter 2. If the spores are then inoculated into a less favourable growth medium they may take longer to transform to vegetative cells or remain in spore form. This may result in an uneven growth rate, differing initiation time for the log phase of growth, or absence of growth in some cultures. This clumping behaviour may also be a factor of the presence of mycelium sticking the cells together.

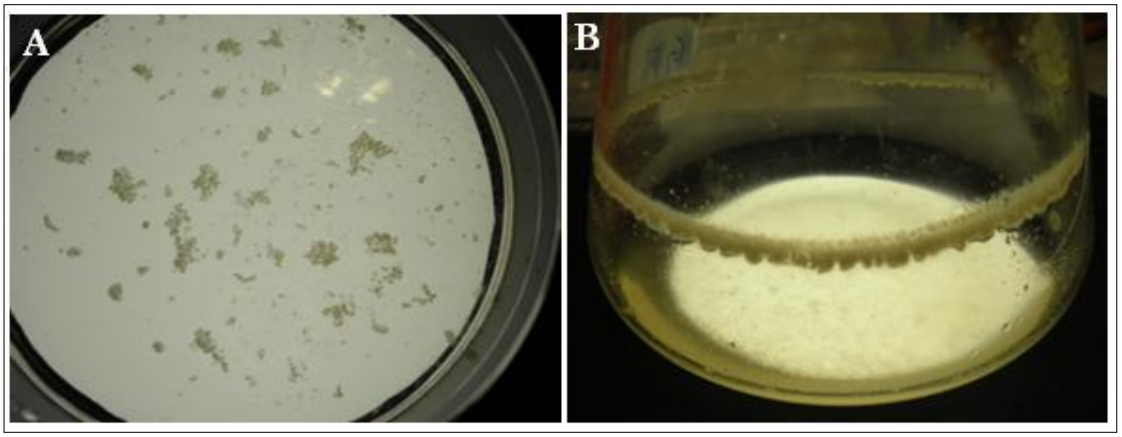



*Figure 4.8**. (A)** Particulate matter formed in the broth of Caldalkalibacillus sp. LMLD03, the material was allowed to settle to the bottom of the flask and the supernatant was removed. The material was then placed onto a petri dish, and viewed over a light box. **(B)** The tide mark inside the flask in which the Caldalkalibacillus sp. LMLD03 culture was grown.*

### 4.7.6. Growth of isolates at a range of temperatures

The salt concentration used for the pH and temperature studies was 1% as this was identified as a salt concentration which produced high growth rates for *Idiomarina* sp. LMLD01 and *Planococcus* sp. LMLD02. *Caldalkalibacillus* sp. LMLD03 was grown in AP+6.8% broth.

#### 4.7.6.1. *Idiomarina* sp. LMLD01

Growth was analysed at temperatures from 20 °C to 45 °C for *Idiomarina* sp. LMLD01, those treatments which resulted in growth over 24 hours all reach the stationary phase by 12 hours (Figure 4.9). No growth occurred at 45 °C over 24 hours. The highest growth rate of 0.888 $h^{-1}$ was achieved at 37 °C was statistically greater than that achieved at any other temperature ($p$=0.000). The lowest of 0.3648 $h^{-1}$ at 20 °C was statistically lower rate than at any other temperature ($p$<0.024).

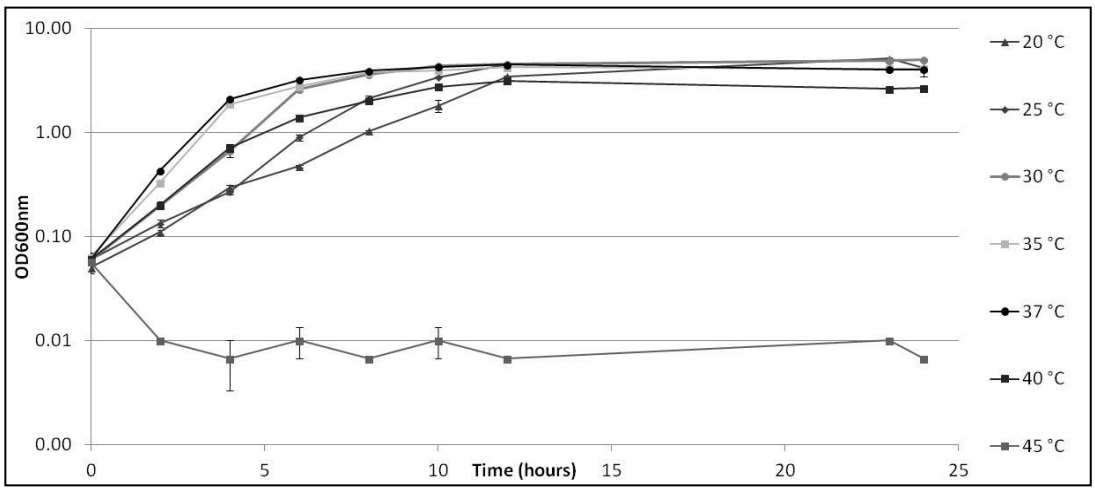


*Figure 4.9 Growth curve of Idiomarina sp. LMLD01 over a 24 hour period for a growth medium with a 1% NaCl concentration and pH 9.6 at temperatures from 20 °C to 45 °C. Error bars are 1 x standard error.*

The growth rate at 40 °C was significantly greater than the growth rate achieved at 25 °C and 20 °C ($p$<0.005), indicating that although *Idiomarina* sp. LMLD01 was near to its upper temperature limit at 40 °C, it grows faster at this temperature than at 30 °C or below (Figure 4.10). In contrast the final OD achieved for 40 °C was significantly lower however than for any temperature tested ($p$=0.000) (See Appendix 1 table 1.3 and 1.4).

The final OD decreased with increasing temperature, the OD reached at 20 °C, 25 °C and 30 °C was significantly greater compared to that reached at 35 °C, 37 °C & 40 °C ($p$>0.317).

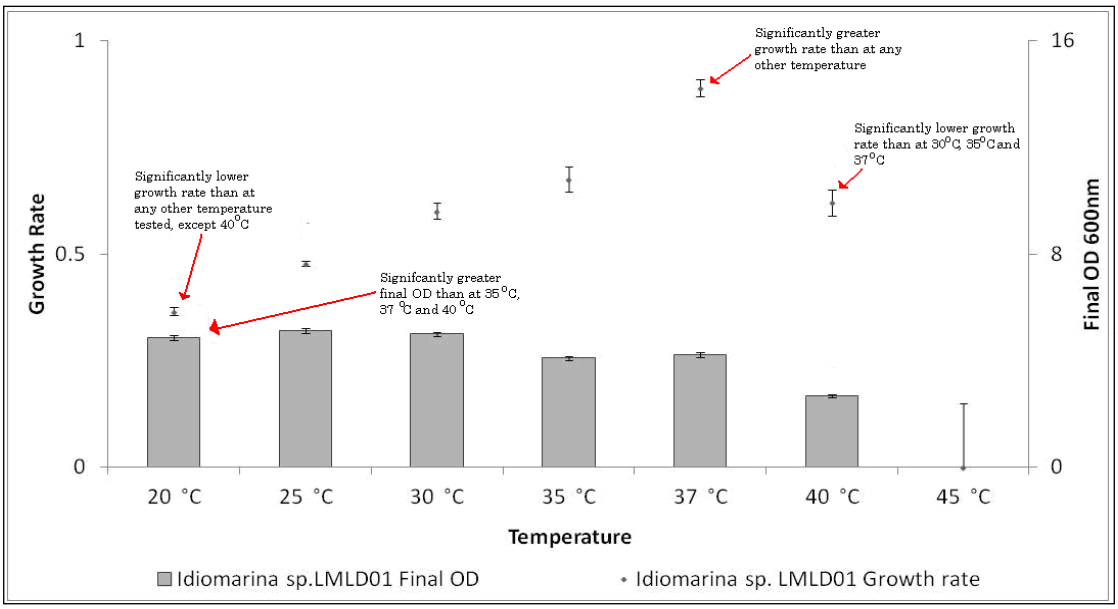



*Figure 4.10 Growth rates and final OD for Idiomarina sp. at increasing temperatures, in a growth medium containing 1% and pH 9.6. Biomass was measured using $OD^{600nm}$. Error bars are 1 x standard error. Statistical differences were assessed using ANOVA analysis of variance statistical test. Significance was attributed to values where p<0.05. Error bars are 1 x standard error.*

#### 4.7.6.2. *Planococcus* sp. LMLD02

*Planococcus* sp. LMLD02 reached the stationary phase by 12 hours when grown at temperatures >20 °C and <45 °C (Figure 4.11). This isolate reached the stationary phase of growth by 23 hours when grown at 20 °C, but did not grow over a 24 hour period at 45 °C. The highest growth rate recorded was 0.8297 $h^{-1}$ at 35 °C, the lowest growth rate was at 20 °C with a rate of 0.2546 $h^{-1}$ however the growth rate at 20 °C and 25 °C were not significantly different from one another ($p$=0.772).

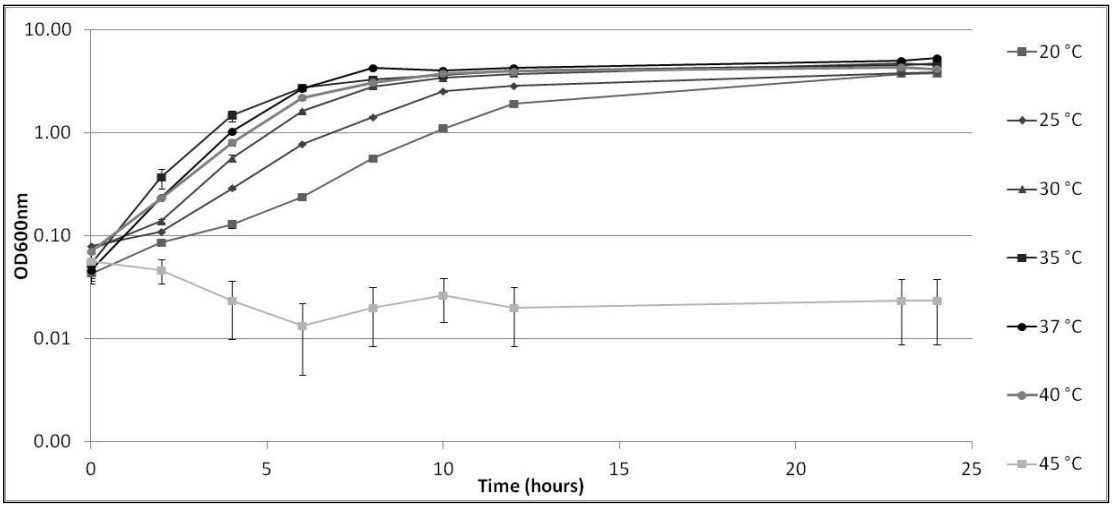



*Figure 4.11 Growth curves for Planococcus sp. LMLD02 over a 24 hour period for medium with NaCl concentration of 0.07% to 10%. Error bars are 1 x standard error.*

The growth rate at 35 °C was not significantly higher than the growth rate at 37°C ($p$=0.704), but both were significantly higher than the growth rate at 30 °C and 40 °C with ($p$<0.024). The Final OD for 25 °C to 37 °C were not significantly different from one another ($p$=0.083), but these are significantly greater than for all other treatments ($p$=0.038).

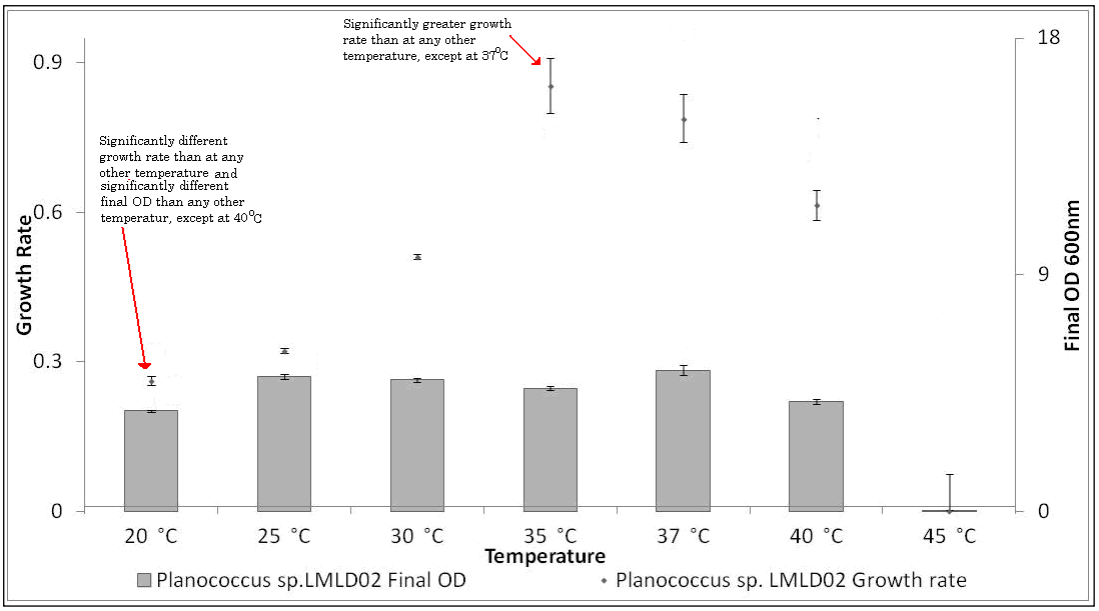



*Figure 4.12 Growth rates and final OD for Planococcus sp. LMLD02 at increasing temperatures, in a growth medium containing 1% NaCl and a pH of 9.6. Biomass was measured using $OD^{600nm}$. Error bars are 1 x standard error. Statistical differences were assessed using ANOVA analysis of variance statistical test. Significance was attributed to values where p<0.05. Error bars are 1 x standard error.*

#### 4.7.6.3. *Caldalkalibacillus* sp. LMLD03

All cultures grown at 45 °C and 50 °C reached the stationary phase of growth by 12 hours (Figure 4.13). When grown at 40 °C the isolate may have reached the stationary phase by 24, however it had not when grown at 37 °C.

Growth did not occur at 55 °C over a 24 hour period. Once again uneven growth occurred, particularly with growth at 37 °C and 50 °C (Figure 4.13). At 50 °C over 12 hour period only two of the replicates had an OD over 0.1, however after 24 hours all three replicates had an OD between 2.35 and 3.05. The triplicates for 37 °C still displayed an uneven OD reading even after 24 hours with the final OD reading being between 0.97 and 2.35.

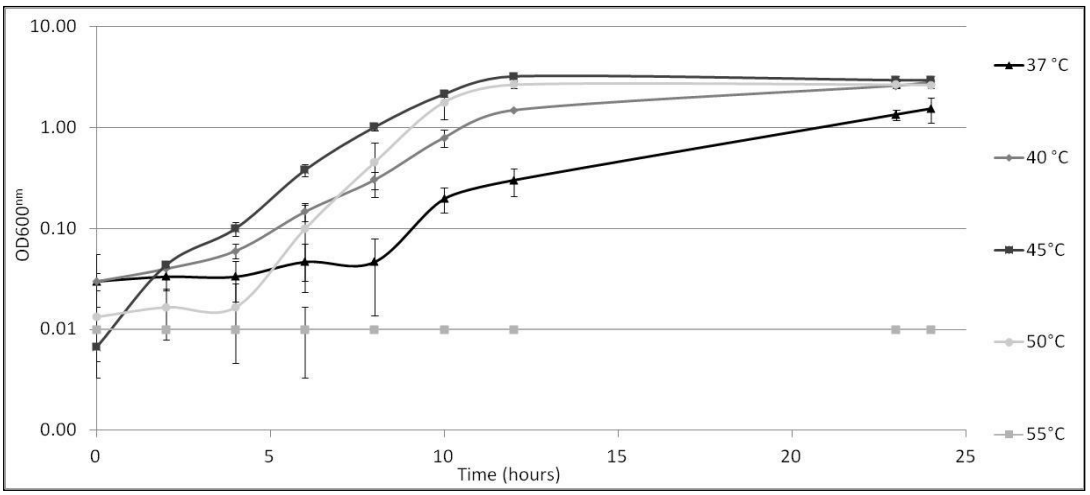



*Figure 4.13. Growth curve of Caldalkalibacillus sp. LMLD03 over a 24 hour period for increasing temperature in a growth medium with a NaCl concentration of 1% and pH of 9.6. Error bars are 1 x standard error.*

The highest growth rate was recorded at 50 °C, 0.8258 $h^{-1}$ , the lowest growth rate was recorded at 37 °C (0.4652 $h^{-1}$) (Figure 4.14), however the growth rates achieved were not statistically different from one another ($p$>0.129), (Appendix 3. Table 3.3).

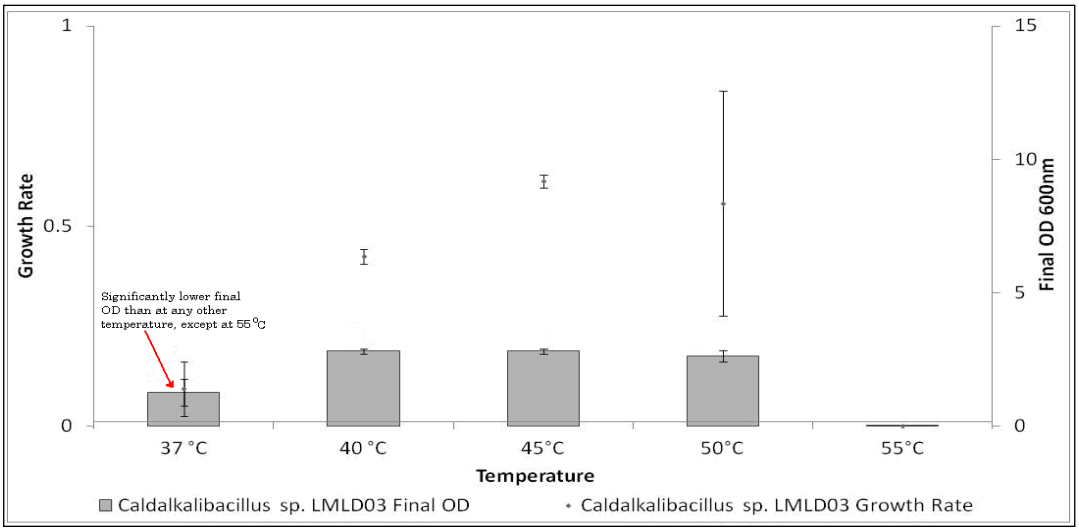



*Figure 4.14 $OD^{600}$ after 24 hours for Caldalkalibacillus sp. LMLD03 at increasing temperatures, in a growth medium containing 1% NaCl and pH 9.6. Statistical differences were assessed using ANOVA analysis of variance statistical test. Significance was attributed to values where $p<0.05$. Error bars are 1 x standard error.*

The final OD achieved by *Caldalkalibacillus sp. LMLD03* at 37 °C was significantly lower than the OD reached 40 °C to 50 °C, ($p$<0.035). The final OD achieved at 40 °C, 45 °C and 50 °C were not significantly different from one another.

When the values recorded for 50 °C were removed from the ANOVA test, (due to absence of growth in one triplicate until after 12 hours) the growth rate at 45 °C was significantly greater than that at 37 °C or 40 °C. In addition the final OD at 37 °C was significantly lower than that at 40 °C and 45 °C.

The absence of growth at 50 °C in one triplicate but the presence of a final OD comparable to the other two triplicates after 23 hours would imply that a long lag phase occurred for this triplicate. This was then followed by a log phase occurring within a 10 hour period, with the other triplicates completing the log phase of growth within an 8 hour period.

### 4.7.7. Growth of isolates at a range of pH

#### 4.7.7.1. *Idiomarina* sp. LMLD01

The strain reached the stationary phase by 10 hours when grown in a pH of at least 9 to pH 10.4 (Figure 4.15). At a pH of 10.6 (highest pH tested) the lag phase was recorded with the exponential phase initiating after 6 hour, but not reaching the stationary phase even after 24 hours. For a pH of 7, the lag phase was longer still and the exponential phase again was not observed within a 24 hour period.

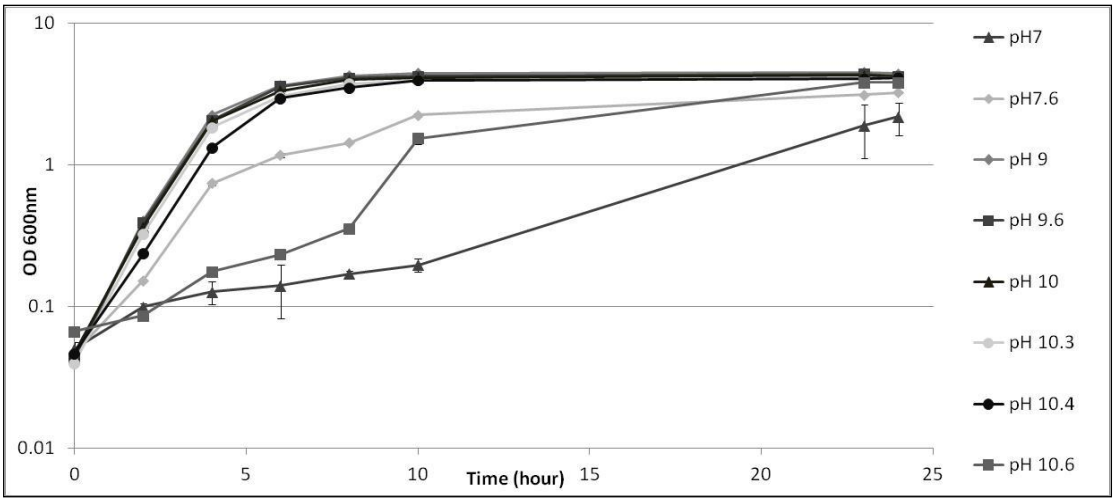



*Figure 4.15 Growth curve of Idiomarina sp. LMLD01 over a 24 hour period, in a medium with 1% NaCl and incubated at 37 °C. Error bars are 1 x standard error.*

The growth rate achieved by *Idiomarina* sp. LMLD01 increased with increasing pH up to a pH of 9, with a growth rate of 0.2324 $h^{-1}$, recorded at a pH of 7 and a growth rate of 1.0071 $h^{-1}$ at a pH of 9. The growth rate at a pH of 7 and 7.5 were significantly lower than growth rate at any other pH, except pH 10.6 ($p$=0.000) (Figure 4.16). However, there was no significant difference between growth rate at a pH of 9, 9.6, 10 or 10.3 using the $Na_2CO_3$/$NaHCO_3$ buffer system ($p$>0.185). The growth rate at a pH of 10.3 when using the $Na_2CO_3$/$NaHCO_3$ buffer system, was significantly different to the growth rate at pH 10.4 on the $Na_2CO_3$/NaOH buffer ($p$=0.000). Finally the growth rate at a pH of 10.6 was significantly greater than the growth rate at any other pH, except at pH7 ($p$=0.000).

The final $OD_{600}$ indicated that the biomass produced was higher in a medium with a pH between 9 and 10.6, which was significant compared to the amount of biomass produced at pH of 7 (Figure 4.16) ($p$>0.003). The final $OD_{600}$ at a pH 7.5 was statistically lower than that produced for the pH 9 to pH 10 ($p$<0.043) (See Appendix 1, table 1.5 and 1.6).

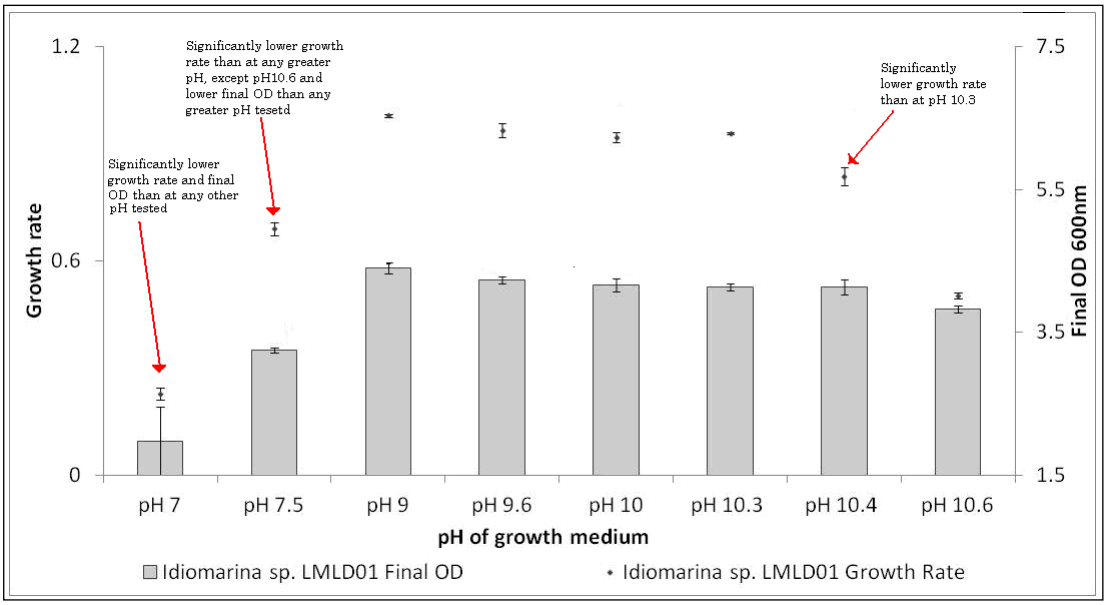



*Figure 4.16 Growth rates and final OD reading for Idiomarina sp. LMLD01 at different pH (at 37 °C, 1% NaCl). Growth rate is taken as the X value in figure 4.1 which is plotted to show differences in growth rate when incubated at increasing pH. Statistical differences were assessed using ANOVA analysis of variance statistical test. Significance was attributed to values where p<0.05. Error bars are 1 x standard error.*

#### 4.7.7.1.1. pH measurements for *Idiomarina* sp. LMLD01 growth medium

The starting pH of the pH 10.6 blank control dropped after 24 hours to pH 10.05, which indicated that the buffering capacity at this pH was not high enough. The pH remained constant for all other treatments (Table 4.10). The pH of all the inoculated medium significantly dropped compared to the pH of the negative control for treatments 9.6 to 10.05 (starting pH 10.6) and the pH significantly increases compared to the negative control for pH's of 7 and 7.5 ($p$>0.006). At a pH of 9 the change in pH was not significant ($p$=0.218).

*Table 4.10. pH for control pH blank after 24 hours compared to the average sample pH of isolate Idiomarina sp. LMLD01 medium after 24 hours incubation. Post incubation pH was recorded from triplicates. A 2-tailed, two sample t-test, assuming equal variance was used to identify differences in pH before and after incubation. Significance was attributed to values where p<0.05 **

| | Control pH blank | Sample pH after 24 incubation | p value |
|---|---|---|---|
| pH 7 | 7.06 ± 0.01 | 7.54 ± 0.04 | 0.006* |
| pH 7.5 | 7.42 ± 0.07 | 9.00 ± 0.01 | 0.002* |
| pH 9 | 9.10 ± 0.03 | 9.04 ± 0.03 | 0.218 |
| pH 9.6 | 9.70 ± 0.05 | 9.19 ± 0.04 | 0.002* |
| pH 10 | 10.11 ± 0.01 | 9.22 ± 0.03 | 0.000* |
| pH 10.3 | 10.32 ± 0.02 | 8.97 ± 0.03 | 0.001* |
| pH 10.4 | 10.42 ± 0.03 | 9.12 ± 0.03 | 0.001* |
| pH 10.6 | 10.05 ± 0.02 | 8.86 ± 0.01 | 0.002* |

#### 4.7.7.2. *Planococcus* sp. LMLD02

Limited growth occurred when incubated for 24 hours at a pH of 10.4 on $Na_2CO_3$/NaOH buffer (Figure 4.17). Growth at a pH of 7 to 9.6 reached the stationary phase after 12 hours; growth at a pH 10 and 10.3 had not reached the stationary phase by 24 hours.

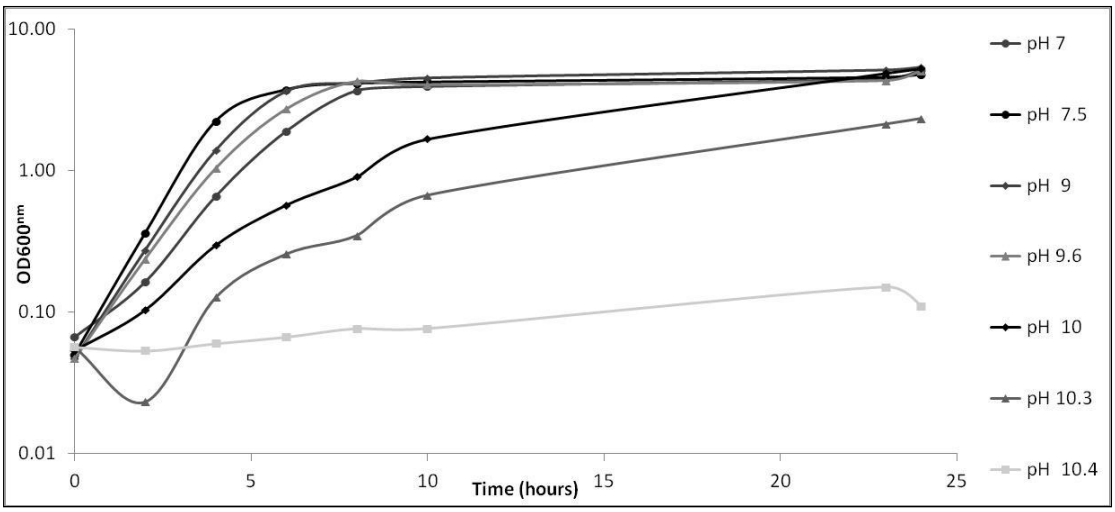



*Figure 4.17 Growth curve of Planococcus sp. LMLD02 over a 24 hour period, in a medium with 1% NaCl and incubated at 37 °C. Error bars are 1 x standard error.*

The highest growth rate of 0.952 $h^{-1}$ was recorded in a medium with a pH of 7.5. The lowest growth rates were 0.04897 $h^{-1}$ at pH 10.4 and 0.6032 $h^{-1}$ at pH 10.3. The growth rate at pH 7 was statistically lower than that achieved at a pH of 7.5 to pH 9.6 with ($p$<0.013). The growth rate at pH 7.5 was not statistically different to the growth rate at pH 9 ($p$=0.375) but was statistically greater than growth at all other pH's tested. The growth rate at pH 10 was not significantly different to that achieved in a pH of 7 with ($p$=0.346) or pH 10.3 ($Na_2CO_3$/$NaHCO_3$) ($p$=0.249).

The final OD for the pH 7 to pH 10 medium general appeared to be fairly constant, and were not statistically significantly different ($p$>0.497). The final OD reached at a pH of 10.3 was significantly lower than the OD reached for treatments pH 7 to pH10 ($p$=0.000).

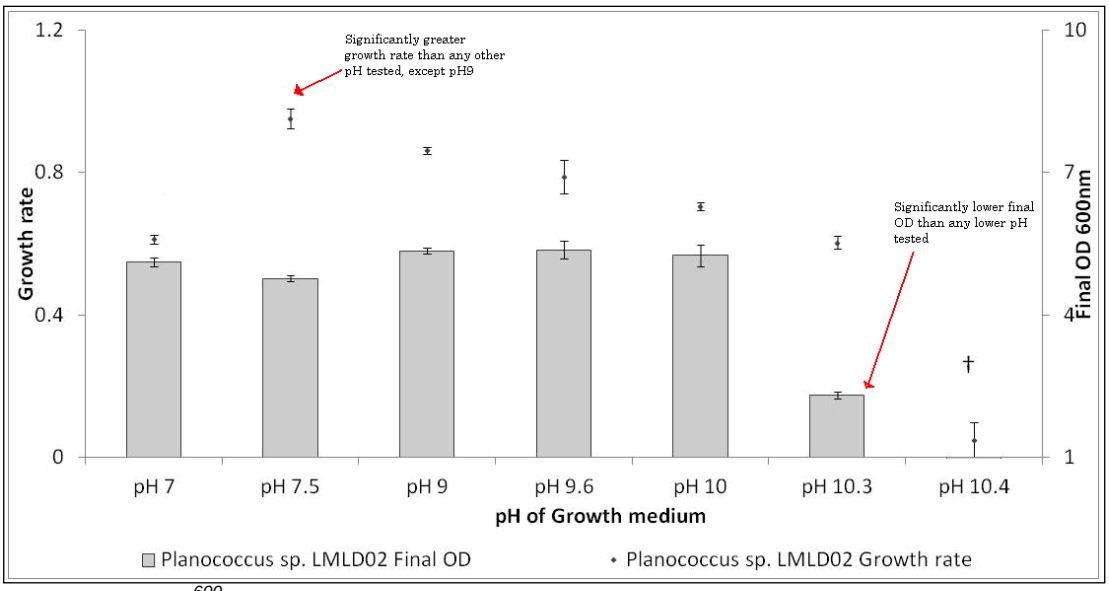



*Figure 4.18 $OD^{600}$ after 24h for Planococcus sp. LMLD02 at different pH (at 37 °C, 0.7% NaCl). Statistical differences were assessed using ANOVA analysis of variance statistical test. Significance was attributed to values where p<0.05. Error bars are 1 x standard error.*

#### 4.7.7.2.1. pH measurements for *Planococcus* sp. LMLD02 growth medium

The pH of the medium again changes after 24 hours incubation when inoculated with *Planococcus sp. LMLD02*. The media with a pH of 7 and 7.6 significantly rose to 7.53 and 8.24 respectively ($p$>0.009) (Table 4.11). The pH of the media which started at pH 9, 9.6, 10 and 10.3 all dropped, with the final pH being significantly different from the starting pH ($p$<0.0176). The decrease in pH for the 10.3 treatment was not as large as the decrease at the lower pHs, this may be due to the lower levels of biomass achieved after 24 h in this sample.

*Table 4.11. pH for control pH blank after 24 hours compared to the average sample pH of isolate Planococcus sp. LMLD02 medium after 24 hours incubation. Post incubation pH was recorded from triplicates. A 2-tailed, two sample t-test, assuming equal variance was used to identify differences in pH before and after incubation. Significance was attributed to values where p<0.05 **

| | Control pH blank | Sample pH after 24 incubation | p value |
|---|---|---|---|
| pH 7 | 7.06 ± 0.01 | 7.53 ± 0.01 | 0.0016* |
| pH 7.5 | 7.42 ± 0.07 | 8.24 ± 0.02 | 0.0088* |
| pH 9 | 9.09 ± 0.01 | 8.82 ± 0.02 | 0.0098* |
| pH 9.6 | 9.64 ± 0.02 | 9.33 ± 0.02 | 0.0176* |
| pH 10 | 10.12 ± 0.02 | 8.95 ± 0.02 | 0.0003* |
| pH 10.3 (Na2CO3/ NaHCO3) | 10.32 ± 0.02 | 9.37 ± 0.02 | 0.0170* |

#### 4.7.7.3. *Caldalkalibacillus* sp. LMLD03

Growth of *Caldalkalibacillus* sp. LMLD03 was tested on pHs from 7, 7.5, 9, 9.6, 10 and 10.3 on both buffer systems. Again problems were encountered with uniform growth and development of particulate material at some pH 's. Similar levels of growth were achieved for the triplicates grown at a pH of 9, 9.6 and 10, however beyond this the formation of particulate matter in the flasks again made accurate OD reading of the samples impossible as large amounts of particulate matter formed which just settled to the bottom of the cuvette.

The growth curves for the pH's where sufficient data could be gathered growth was sufficiently uniform are shown in figure 4.18. The growth curve for 106B pH 10.3 ($NaHCO_3$) which demonstrated very different rates of growth between the triplicates was also included to show quality of data obtained for the problematic pH tests.

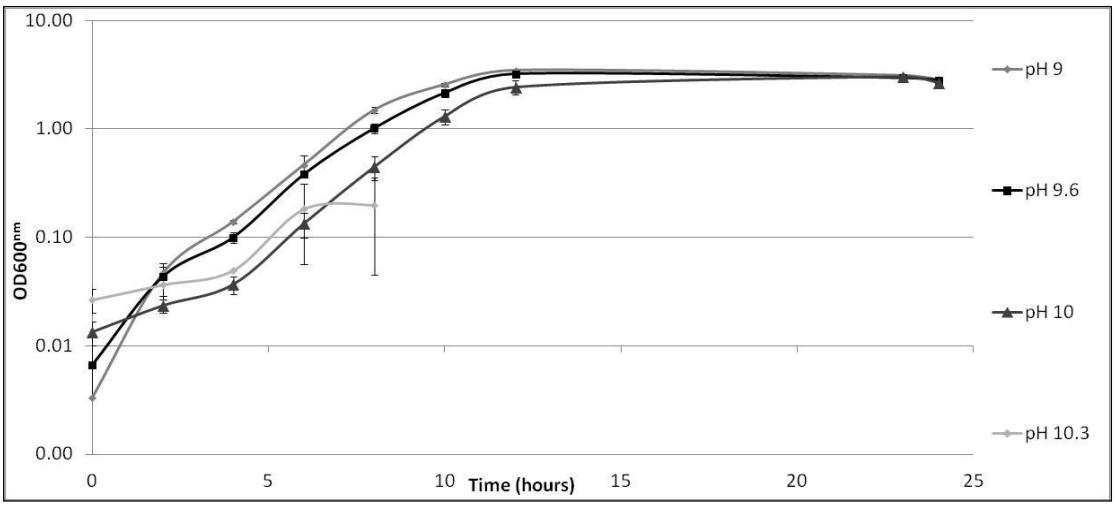



*Figure 4.19. Growth curve of Caldalkalibacillus sp. LMLD03 over a 24 hour period, in a medium with 1% NaCl and incubated at 37 °C. Error bars are 1 x standard error.*

The growth rate for *Caldalkalibacillus* sp. LMLD03 decreased as pH rises from pH 9 (Figure 4.20). The growth rate of $0.5756h^{-1}$ at pH 9 was significantly higher than the growth rate of $0.4357h^{-1}$ at pH 10 ($p$=0.032). However it was not significantly higher than the growth rate of $0.5428h^{-1}$ at pH 9.6 ($p$=0.489) (Appendix 3, Table 3.5). The growth rate of $0.5428h^{-1}$ at pH 9.6 was not significantly higher than that at pH 10 ($p$=0.143).

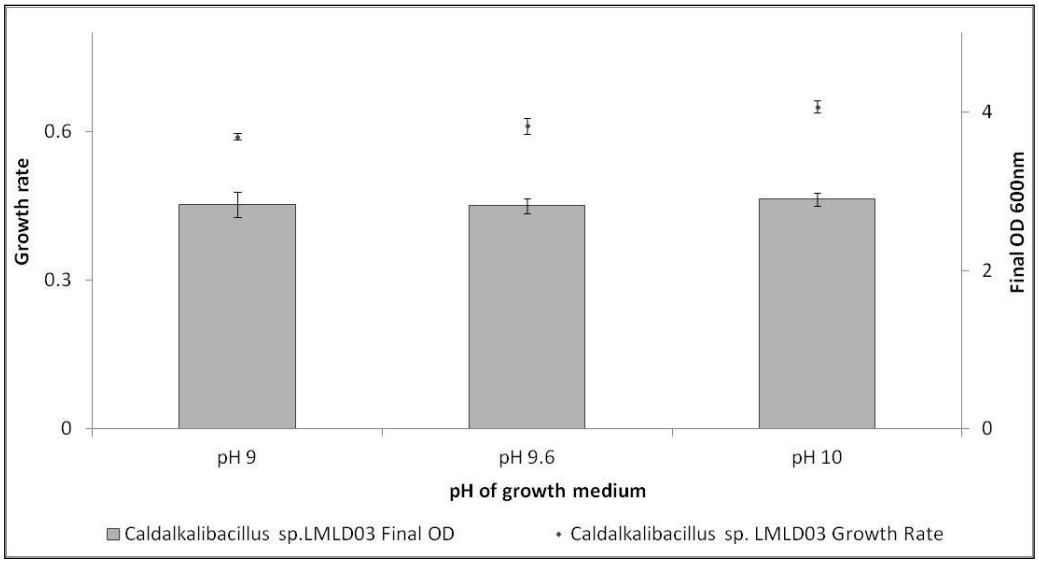



*Figure 4.20 Growth rate and final OD$^{600nm}$ after 24 hours for Caldalkalibacillus sp. LMLD03 at different pH (at 37 °C, 0.7% NaCl). Statistical differences were assessed using ANOVA analysis of variance statistical test. Significance was attributed to values where p<0.05. Error bars are 1 x standard error.*

The final OD reached by *Caldalkalibacillus* sp. LMLD03 was around 2.85 (Figure 4.20) for all treatment. There was no statistical difference between the final OD reached by each treatment, despite the differences in growth rate, ($p$ >0.878). (Appendix 3, Table 3.6).

#### 4.7.7.3.1. pH measurements for *Caldalkalibacillus* sp. LMLD03

The pH measured before and after 24 hours growth shows that the pH does not change when the starting pH 9 or 9.6. When the starting pH was at 10 however, the pH was lowered to around pH 9 (Table 4.12.).

*Table 4.12 pH for control pH blank after 24 hours compared to the average sample pH of isolate Caldalkalibacillus sp. LMLD03 medium after 24 hours incubation. Post incubation pH was recorded from triplicates. A 2-tailed, two sample t-test, assuming equal variance was used to identify differences in pH before and after incubation. Significance was attributed to values where p<0.05 **

| | Control pH blank | Sample pH after 24 incubation | P-value (T-test) |
|---|---|---|---|
| pH 9 | 8.97 ± 0.02 | 9.08 ± 0.05 | 0.236 |
| pH 9.6 | 9.61 ± 0.01 | 9.62 ± 0.03 | 0.776 |
| pH 10 | 9.94 ± 0.05 | 9.08 ± 0.05 | 0.01* |

### 4.8. Discussion

This chapter focusses upon the characterisation of three novel strains isolated from Lake Magadi, assessing their ability to utilise different carbon sources, as well as determining their ability to produce certain enzymes and their antibiotic resistance. Following this, the NaCl concentration, temperature and pH tolerances and optima for each isolate were examined. The analysis of a range of different growth characteristics can provide information

on phenotypic differences and similarities between strains with similar 16s rRNA gene sequences but isolated from different environments. Variability between strains, which are very similar in terms of their 16s rDNA sequence, but show marked difference at the DNA level and in their physiological characteristics, highlights why it is important to conduct growth studies on novel isolates (Fox, 1992). There is always the potential to extend the growth range of groups of organisms by identifying and characterising novel isolates.

### 4.8.1. Carbon source usage

*Caldalkalibacillus* sp. LMLD03 was able to use three different types of carbon source, with the type strain of *C. uzonensis* also able to use a range of carbon sources. In contrast, *Idiomarina* sp. LMLD01 and *Planococcus* sp. LMLD02 were unable to use any of the carbon sources tested. Some strains of *Idiomarina* are also shown to have limited carbon utilisation ability (Ivanova, 2000: Donachie et al., 2003), although studies of species of *Planococcus* generally show an ability to utilise a wider range of carbon sources (Romano et al., 2003: Suresh et al., 2007). Studies conducted with members of the *Halomonadaceae* have shown that the use of complex growth media can extend the salt tolerance (>3% NaCl), with the type of carbon source, in particular yeast extract, influencing the proportion of intercellular solutes such as betaine and ectoine, with different organisms displaying different solute patterns (Wohlfarth et al., 1990: del Moral et al., 1994). This could be a factor affecting other *Proteobacteria*, and possible could affect strains with the *Firmicutes*. Therefore it is possible that the absence of yeast extract (Chapter 2) which is present in special peptone is restricting the growth of *Idiomarina* sp. LMLD01 and *Planococcus* sp. LMLD02 on a single carbon source, in the presence of 6.8% NaCl. Interestingly, (Ivanova, 2000) tested the growth of *I. abyssalis* and *I. zobellii* on single carbon sources in the presence of 2.2% NaCl and recorded growth on a number of single carbon sources. In contrast, (Martinez-Canovas, 2004) recorded no growth for *I. ramblicola* and *I. fontislapidasi* on any of the single carbon sources they were testing; the growth media being used contained 5% NaCl. The absence of growth could simply be because those *I. ramblicola* and *I. fontislapidasi*, unlike *I. abyssalis* and *I. zobellii* are far more restricted in terms of the carbon sources they can utilise. However it could be that the high salt concentration used there was having an influence on the growth of the isolates. In order to clarify this point the growth of each strain in the presence of lower NaCl concentrations for each carbon source would be needed.

### 4.8.2. Enzyme production

The results showed that all strains produce protease and that all strains except *Planococcus* sp. LMLD02 produced amylase. This is in contrast to the type strain of *P. maritimus,* which produces both amylase and protease (Romano et al., 2003: Yoon, 2003: Suresh et al., 2007) (Table 4.10). Neither of the type strains for the genus *Caldalkalibacillus* have been analysed in terms of their enzyme production (Xue et al., 2006: Zhao et al., 2008). When comparing *Idiomarina* sp. LMLD01 to strains for the genus *Idiomarina*, where enzyme

activity have been analysed, no species of *Idiomarina* produce either amylase of protease, contrasting to the findings in this study (Choi, 2005: Wang et al., 2011). This indicates physiological difference between the Lake Magadi *Planococcus* and *Idiomarina* strains and other known strains of the same genus.

### 4.8.3. Antibiotic resistance

*Idiomarina* sp. LMLD01 was susceptible to streptomycin (inhibits protein synthesis) but not ampicillin (damages cell walls), this cannot be compared to other strains as these resistance of these antibiotics has not been tested. *Caldalkalibacillus* sp. LMLD03 was susceptible to both antibiotics tested, which is line with the antibiotic resistance of *C. thermarum* (Xue et al., 2006). *Planococcus* sp. LMLD02 was resistant to both streptomycin and ampicillin, which differs to the other species of *Planococcus* which have been tested, and are not resistant to either antibiotic, again demonstrating differences between these novel isolate and its nearest database match.

*Table 4.13 Description of novel isolates in grey (1, 3 and 5) and their nearest database match in white (2, 4 and 6 respectively). 1. Idiomarina sp. LMLD01, 2. Idiomarina seosinensis (Choi, 2005), 3. Planococcus sp. LMLD02, 4. Planococcus maritimus (Yoon, 2003), 5. Caldalkalibacillus sp. LMLD03, Caldalkalibacillus uzonensis (Zhao et al., 2008). N/R not recorded, N/D not determined*

| Genus | *Idiomarina* | | *Planococcus* | | *Caldalkalibacillus* | |
|---|---|---|---|---|---|---|
| Isolate | 1 | 2 | 3 | 4 | 5 | 6 |
| Colony description | Cream, mucoid, circular & convex. | Circular, smooth. Slightly yellowish. | Circular, convex and orange 1mm - 2mm | Circular, orange, smooth and convex | Transparent and circular | Circular and transparent |
| Cell description | Rod shaped | Slightly curved rods | Cocci | Motile cocci | Rod shaped | Straight rods |
| Gram staining | Gram negative | Gram negative | Gram positive | Gram positive | Gram positive | Gram positive |
| Spores | No spores observed | N/R | No spores observed | No spores | Spores | spores |
| Amylase | + | N/R | - | - | + | N/R |
| Protease | + | N/R | + | - | + | N/R |
| Ampicillin | + | N/R | + | N/R | - | N/R |
| Streptomycin | - | N/R | + | N/R | - | N/R |
| NaCl range | 0%–15% | 1%-10% | 0%–15% | 0%–17% | 0%–13%, not 15% | 0%–6% |
| NaCl optimum | 1%–5% | 3%–6% | 0%–6% | 2% | 1%–8% | N/R |
| Temp. Range | 10 °C – 40 °C, not at 45 °C | 8 °C–46 °C | 10 °C – 40 °C, not at 45 °C | 4 °C – 41 °C | 37°C – 60 °C, not at 65 °C | 42 °C – 64 °C |
| Temp. optimum | 35 °C–37 °C | 30 °C–40 °C | 35 °C – 37 °C | 30 °C | N/D | 50 °C - 52 °C |
| pH Range $^{37°C}$ | At least 6-10.6 | N/R | At least 6-10.3 | 6.0-11.0 | 7.5-10.3 | 6.4-9.7 |
| pH $_{37°C}$ Optimum | At least 9.0-10.3 | N/R | 7.6-9.6 | at least pH 5 | N/D | 8.2-8.4 |
| Isolation location | Lake Magadi | Baltic sea surface waters | Lake Magadi | Tidal flat, Korea | Lake Magadi | East Thermal Field, Uzon Caldera |

### 4.8.4. Response of *Idiomarina* sp. LMLD01 to NaCl concentration, temperature and pH

The 16S rRNA gene sequence for this isolate was most closely related (92% similar) to the species *I. seosinensis* (Chapter 3, section 4.4.1.1) The NaCl concentration growth range of *Idiomarina* sp. LMLD01 was comparable with that recorded for the other species of the genus *Idiomarina* (*I. ramblicola, I. xiamenensis and I. salinarium*) isolated from hypersaline environments (Section 4.1.2), but narrower than recorded for *I. seosinensis* (Choi, 2005). *Idiomarina* sp. LMLD01 can grow at NaCl levels as low as 0.035% NaCl, a lower NaCl concentration than recorded for *I. seosinensis* (Choi, 2005) .

The optimal range for *Idiomarina* sp. LMLD01 was broader than recorded for other species of the genus. The wider range of NaCl optimum and high levels of biomass reached at higher salinities may reflect adaptation or suitability of this strain to the variable nature of the Lake Magadi environment, which can vary drastically in salinity (Chapter 1). A broad growth range is beneficial as it will mean the organisms is less constrained in terms of habitable environments compared to those organisms which are restricted to a narrower growth range.

Growth rate of *Idiomarina* sp. LMLD01 decreases with NaCl content (above 1% NaCl), however, the organism was still capable of reaching comparable levels of biomass to that achieved at <1% NaCl which demonstrated a faster growth rate. Dispite the growth rate at 10% NaCl being lower than that at 0.07% NaCl concentration, *Idiomarina* sp. LMLD01 produces statistically more biomass at 10% NaCl. This would indicate that although growth was slower at a higher NaCl concentrations it does not have a detrimental effect on the final amount of biomass produced. The temperature upper limit for the novel isolate *Idiomarina* sp. LMLD01 *Idiomarina* was within the range of other species of the genus *Idiomarina* (40 °C - 46 °C) (Brettar et al., 2003: Martinez-Canovas, 2004: Donachie et al., 2003). Growth occurred at least as low as 10 °C but some members of the genus *Idiomarina* can grow down to a low of 4 °C, which could not be tested (Martinez-Canovas, 2004).

*Idiomarina* sp. LMLD01 growth data shows that despite the elevated growth rate with rising temperature up to 37 °C, the amount of biomass produced decreases. This would indicate that at a higher temperature the organism can grow rapidly but this rate of growth cannot be sustained to produce an equivalent amount of biomass. The temperature optimum of 37 °C recorded for *Idiomarina sp. LMLD01* was higher than for the other type species of between 20 °C- 35 °C (Engelhardt et al., 2001: Choi et al., 2007), except for that recorded for *I. salinarum* (Yoon et al., 2007).

The pH range for the *Idiomarina* type strain species is generally down between 5 and 6, and up to a high of 9.5 to 10 (Choi, 2005: Ivanova, 2000). This is a slightly narrower range than recorded for the isolate *Idiomarina* sp. LMLD01, which can grow between at a pH of at least pH 6 and up to a pH of at least 10.6. This higher upper limit might be due to the adaptation of

this particular strain to a highly alkaline environment with a particularly high pH and carbonate content compared to the isolation environments of the other species of the genus.

The analysis of the medium pH after incubation indicates that *Idiomarina* sp. LMLD01 preferentially grows at a pH of $9^{37\ °C}$ and seems to be able to adjust the pH of the growth medium to around this level either through the acidification or alkalisation of the extracellular environment. The final pH for the sample at a pH of $7^{37\ °C}$ was lower than the other final pH which may indicate that the organism was less capable of raising the pH from $7^{37\ °C}$ than it was from $7.5^{37\ °C}$. This might be because the pH was too low at pH $7^{37\ °C}$ for the organism to be able to raise the pH over a 24 hour period.

The optimum pH for the genus *Idiomarina* is standard across all species between a pH of 7 and 8, which is lower than recorded for the isolate in this study pH $9^{37\ °C}$ – $10.3^{37\ °C}$ (Ivanova, 2000). The preferred pH of $9^{37\ °C}$ indicated by the alteration of medium pH would reinforce the suggestion that the optimal pH of the Lake Magadi strain isolated here was greater than recorded for other species of *Idiomarina*.

Given the growth characteristics defined in this study *Idiomarina* sp. LMLD01 could be described as a halotolerant or slight halophilic, facultative alkaliphilic mesophile.

### 4.8.5. Response of *Planococcus* sp. LMLD02 to NaCl concentration, temperature and pH

The maximum NaCl concentration at which growth was recorded for *Planococcus* sp. LMLD02 was 15%, falling short of the 17% maximum NaCl concentration recorded for the type species *Planococcus maritimus* (Yoon, 2003). The growth characteristics of *Planococcus* sp. LMLD02 shows a general correlation between decreasing growth rate and final OD with increasing NaCl concentration. At 0.07% NaCl, dispite the lower growth rate, *Planococcus sp. LMLD02* still achieved a final OD comparible to that reached at 1% NaCl. The optimal range of 1% - 5% NaCl recorded for *Planococcus* sp. LMLD02 was wider than for those type strains where the optimum has been ascertained. This may again indicate a need for a wider NaCl optimum in a variable environment. The closest database match to *Planococcus* sp. LMLD02, *P. maritimus*, has quite a restricted NaCl optima of 1% NaCl, possibly demonstrating an adaptation of this particular isolate to the hypersaline nature of soda lake environments.

*Planococcus* sp. LMLD02 was capable of growing between 10 °C to 40 °C with the temperature growth range observed for members of the genus so far is between 0 °C and 42 °C. Growth at temperatures between 40 °C and 45 °C were not tested in this study and therefore it is unclear if this strain can grow at 42 °C. Both *Idiomarina* sp. LMLD01 and *Planococcus* sp. LMLD02 were isolated from waters around 45 °C which was at the upper limit or beyond the upper limit of growth for these strains. Once again there was variation

between the closest type strains optimal temperature and that recorded for the novel *Planococcus* isolate (Table 4.10).

The optimum temperature for members of the genus *Planococcus* occurs between 20 °C and 37 °C, with the optimum recorded for *Planococcus* sp. LMLD02 being between 35 °C and 37 °C, closest to the optimum recorded for *P. rifientensis* isolated from hot springs (Romano et al., 2003). The strain was isolated from an environment at about 45 °C with the surrounding water up to 82 °C, this strain was isolated from an environment with a temperature at the upper limits of its tolerance (no growth observed in this study at 45 °C).

The lower pH limit for members the genus *Planococcus* so far is around pH 5, and up to a maximum pH of 12. The strain *Planococcus* sp. LMLD02 could not be tested below a pH of $6^{37\,^{\circ}C}$ in this study due to experimental constraints of suitable pH buffer. The upper limit of pH 12 is higher than recorded for this strain with the closest similar upper pH limit of 10.5 recorded for *P. rifietensis* (Romano et al., 2003). The ability of *Planococcus* sp. LMLD02 to grow over a broad pH range means the strain has the advantage of potentially being able to occupy both alkaline and neutral environments and can withstand fluctuations and changes in its environment.

The optimum pH range of the members of the genus is generally between 6 and 8, a range which is lower than that recorded for *Planococcus* sp. LMLD02. *Planococcus* sp. LMLD02 also appears to alter the medium pH to between $8^{37\,^{\circ}C}$ and $9^{37\,^{\circ}C}$ during incubation, either by increasing or decreasing pH. Once again this provides an example of an organism which could occupy a micro-environmental niche within what may be considered an unfavourable environment given the organism's growth range.

*Planococcus* sp. LMLD02 could also be described as a halotolerant or slight halophilic, facultative alkaliphilic mesophile, given the growth characteristics defined in this study.

### 4.8.6. Response of *Caldalkalibacillus* sp. LMLD03 to NaCl concentration, temperature and pH

*Caldalkalibacillus* sp. LMLD03 was capable of growing in a range of 0.035% to 13% NaCl, a higher upper limit than that recorded for other members of the genus (0% to 6% NaCl (Xue et al., 2006: Zhao et al., 2008)), isolated from less saline environments. The optimum salinity recorded for other species of the genus was 1.5% NaCl compared to a range of around 1%-8% for the strain studied here. This difference in growth range between the isolates reflects the adaptation of this strain to grow in the presence of both low and high NaCl concentrations present in the isolation enviornment of *Caldalkibacillus* sp. LMLD03, compared to the NaCl levels in the isolation environments of *C. thermarum*.

The temperature growth range for *Caldalkalibacillus* sp. LMLD03 was somewhere between 37 °C and 60 °C differing to the temperature range for other members of the genus, of 42 °C to 65 °C (Xue et al., 2006: Zhao et al., 2008).

The variation of temperature appears to have a significant effect on the growth of *Caldalkalibacillus* sp. LMLD03, with the uneven growth and development of particulate matter and spores at higher and lower temperatures. This would indicate that this organism has a relatively limited temperature growth range and the application of temperatures beyond 45 ˚C could initiate spore formation. These spores would then require the presence of more favourable conditions to return to a vegetative state. The optimal growth temperature for *Caldalkalibacillus* sp. LMLD03 most likely occurs between 45 °C and 50 °C, lower than the type strain optima, although uneven growth made this difficult to determine.

The growth limit for *Caldalkalibacillus* sp. LMLD03 between pH $7.5^{45\ ^{\circ}C}$ and $10.3^{45\ ^{\circ}C}$ was greater than recorded for the other members of genus *Caldalkalibacillus* of between pH 6.4 – 10 (Xue et al., 2006: Zhao et al., 2008). Again this may indicate an adaptation of this strain to a highly alkaline environment in contrast to the isolation environment of other species of *Caldalkalibacillus*. The optimal pH for *Caldalkalibacillus* sp. LMLD03 appeared to be between at least pH $9^{45\ ^{\circ}C}$ and pH $10^{45\ ^{\circ}C,}$ cultures with at a pH either side of these values produced such uneven growth indicating that a pH $>10^{45\ ^{\circ}C}$ or at $7.5^{45\ ^{\circ}C}$ was detrimental to the growth and may induce the production of spores, this may suggest that this an alkaliphilic strain, requiring a high pH. *Caldalkalibacillus* sp. LMLD03 also altered the medium pH when starting pH was $>9.6^{45\ ^{\circ}C}$ to pH $\sim 9^{45\ ^{\circ}C}$. This would indicate a preferred pH of between 9 - $9.6^{45\ ^{\circ}C}$, in line with the environmental pH of 9 from which it was sampled.

Considering the similar growth range of *Caldalkalibacillus* to the type strains of the genus and the growth data recorded in this study, *Caldalkalibacillus* sp. LMLD03 could be defined as a halotolerant, moderately halophilic, facultative alkaliphilic moderate thermophile.

### 4.8.7. Response of novel bacterial isolates to environmental pH

The identification of the alteration of medium pH by the organisms was very interesting and shows that the organism may have been capable of creating a more suitable pH when that present was unfavourable. Many alkaliphilic organisms can occupy environments of a neutral to slightly acidic pH (Arora, 1998: Seckbach, 1999: Yumoto et al., 2010) and change the surrounding pH, through alkalisation or acidification of their local environment. This process produces more optimal conditions for those particular organisms and extends the range of environmental niches they can occupy. The ability to grow in a broad range of conditions is highly beneficial providing these organisms with an advantage over those with a more restricted growth range. Of the isolates studied *Idiomarina* sp. LMLD01 and *Planococcus* sp. LMLD02 would be interesting strains for further survivability

studies for the reasons discussed in section 4.1.1-3. *Caldalkalibacillus* sp. LMLD03 appears to respond negatively to changes in salinity, pH and temperature. However, its tolerance to desiccation is still of great interest providing it can be reanimated from the spore form.

In astrobiology understanding the growth ranges of 'tolerant' organisms is highly relevant, these organisms are equipped to cope with changes environment conditions across a broader range of environmental conditions. For example, work on archaeal halophiles shows that they can lyse in the presence of low salinities, and so cannot survive even exposure to a salinity <1M NaCl. However these novel Lake Magadi isolates, can grow in salinities up to 15%, as well as at lower salinities. This would suggest that they would be more suited to surviving the sorts of changeable environments we may have existed on Mars. If survival studies on temperature/NaCl/pH tolerant strains indicates an ability to survive prolonged exposure to stressors such as UV irradiation and desiccation, then the study of the physiology of these strains would be of even more important in respect to astrobiology. The tolerance of these strains, plus four other novel isolates from Lake Magadi from Chapter 3 will be studied in terms of their desiccation and UV tolerance in Chapter 5.

The comparisons of growth characteristics between novel strains and the database type strains would suggest that these strains are novel species, requiring further investigation.

### 4.9. Conclusions

The isolates *Idiomarina* sp. LMLD01, *Planococcus* sp. LMLD02 and *Caldalkalibacillus* sp. LMDL03 demonstrate differences in their growth characteristics in comparison to other species of the closest genus match. This suggests that *Planococcus* sp. LMLD02 maybe a novel isolate of *Planococcus* in spite of its similar 16S rRNA gene sequence to *Planococcus maritimus*.

The ecological ranges of the genera *Idiomarina, Planococcus* and *Caldalkalibacillus* have been extended to include soda lake environments, and specifically the hydrothermal system associated with Lake Magadi. These isolates generally demonstrate broader and higher growth range and optimum in terms of NaCl concentration, pH and/or temperature than previously demonstrated by strains of their respective genera.

The novel isolates *Idiomarina* sp. LMLD01*, Planococcus* sp. LMLD02 and *Caldalkalibacillus* sp. LMLD03 are capable of altering the pH of their extracellular environment, either by raising or lowering the pH, although the mechanisms by which this is achieved is unclear.

Having studied the tolerance of these strains to; temperature, pH and salinity, the tolerance of these isolates to other environmental parameters has then been conducted in Chapter 5.

When considering the potential for life, past or present, on planets such as Mars, the tolerance of life to desiccation and UV-C irradiation are important areas of research.

### 4.10. Further work

In addition to the characterisation methods employed in this chapter, an assessment of: the DNA–DNA similarity between the novel strains and members of the closest genus match through DNA–DNA hybridization, GC content and cellular fatty acid profiles would be beneficial. This would provide more details on the genotypic variation between the different strains.

The specific pigments present in *Planococcus* sp. LMLD02 could be extracted and defined. Does *Planococcus* sp. LMLD02 contain the same types of antioxidant pigments as *Planococcus maritimus* or does it contain other pigments which assist with its ability to inhabit a soda lake environment.

Further work on the characterisation of *Caldalkalibacillus* sp. LMLD03 is required in order to understand the initiation of spore formation in this isolate and how to reanimation the strain to a vegetative state. This capability to form spores could provide the strain with an ability to withstand factors such as desiccation.

This work focused upon maintaining two environmental factors (pH and temperature) whilst varying another (salinity), however it has not examined the interaction between these factors. For example, does pH tolerance increase as salinity and or temperature decreases. It is necessary to understand the interaction between different environmental conditions and how these affect the growth range of an organism.

## Chapter 5

# Tolerance of isolates from Lake Magadi to desiccation and UV-C irradiation

This chapter focuses on the response of seven novel strains isolated in Chapter 3, to desiccation and UV-C (245nm wavelength) irradiation. Lake Magadi experiences periods of desiccation and high light intensities due its equatorial location and therefore may yield organisms with adaptations which provide them with desiccation and UV-C tolerance/resistance. Studies on the desiccation and UV-C resistance in bacteria and archaea have been conducted by other researchers on thermophiles and hyperthermophiles (Gérard et al., 2001: Beblo et al., 2009: Beblo et al., 2011), methanogens (Fetzer et al., 1993: Kendrick and Kral, 2006: Morozova and Wagner, 2007: Morozova et al., 2007), isolates from Antarctica (Dartnell et al., 2010) and halophiles (Mancinelli et al., 2004: Kottemann et al., 2005a: Baxter et al., 2007a), however, the response of alkalitolerant or alkaliphilic strains appears to have been overlooked.

### 5.1. Conditions on Mars

Organisms on present day Mars nearer the subsurface would encounter an environment which is desiccating, with high UVC flux and high levels of Ionizing radiation (IR). Desiccation resistance is an important factor to study in relation to the habitability of Mars for two reasons, firstly, the surface of Mars at present, and during its history has experienced desiccation, possibly including desiccation in association with hyper saline environments (Mancinelli, 2005b). The second factor is the correlation between IR and desiccation due their relationship to oxidative stress (Mattimore, 1996: Makarova et al., 2001: Fredrickson, 2008). The response of isolates to IR could not be assessed in this chapter, but inferences regarding an organism's potential resistance can be made, identifying isolates which may be of particular interest for future IR resistance studies. Although UV-C radiation is not as significant a detrimental factor as IR, it is still a noteworthy issue causing extensive cellular damage. UVC flux is up to three times higher on Mars than on Earth (Schuerger et al., 2006) and should be considered when addressing the potential for life on Mars (Patel et al., 2002), although its direct effects are mostly limited to the upper surface of the planet (Osman et al., 2008).

### 5.2. Effects of desiccation and UVC radiation

Exposure to desiccation and radiation induces double strand breaks (DSB) in the DNA helix, however, as discussed in Chapter 1, section 1.5.3.3-4, the ability of an organism to survive desiccation or irradiation is not simply a factor of the amount of damage to its genome (Daly et al., 2007). The subsequent survival of the organism is dependent upon its

ability to repair the damaged DNA, through the presence of functional repair proteins (Daly et al., 2007).

It has been proposed that ionising radiation resistance could be a secondary effect of resistance mechanisms to other forms of oxidative stress such as desiccation and UV radiation (Mattimore, 1996; Fredrickson, 2008). Evidence supporting the concept of a relationship between desiccation and radiation resistance includes the isolation of more radiation resistant organisms from arid soils compared to non-arid soils (Fredrickson, 2008; Slade, 2011), plus the observations that desiccation sensitivity also occurs in IR-sensitive D. radiodurans mutants (Mattimore, 1996). This is a simplistic idea however, and resistance is clearly a complex phenomenon. The repeated exposure of E. coli to IR can develop a radiation resistance, but it remains sensitive to desiccation, it also true that not all desiccation resistant organisms are also resistant to radiation (Slade, 2011).

The survival of bacteria and archaea on the surface of Mars would rely, in part, to their resistance to desiccation and UV-C radiation. Mars chamber studies provide details of the effects of a combination of factors, such as desiccation, UV-C irradiation and temperature, but the specific relationship between a particular environmental stress and the subsequent response cannot be easily untangled. Culture-based studies on single isolates which allow the assessment of the effects of stressful conditions separately (desiccation, then UV-C radiation), which can lead onto combined studies in Mars Chambers (Chapter 6) is an important part of increasing the understanding of the potential habitability of Mars.

### 5.3. Aims of this chapter

1. To test desiccation resistance of 7 Lake Magadi isolates over a short period (3 days) and over an extended period of up to 28 days
2. To test the UV-C resistance of 7 Lake Magadi isolates
3. To identify any correlation between UV-C and desiccation resistance in alkalitolerant/alkaliphilic, halotolerant/halophilic strains from Lake Magadi
4. To test if any of the novel isolates from Lake Magadi make suitable model organisms for studying the potential for identifying alkali/halophilic organisms on Mars.

### 5.4. Methodology

#### 5.4.1. Growth media

Due to the different growth requirements of the control strains and the novel isolates different growth media have been used, each suitable for that particular strain. Tryptone Glucose Yeast (TGY) medium was chosen for the culturing and maintenance of *D. radiodurans* (Battista, 1997), Nutrient broth 2 (NB2) medium was used to culture *E. coli* and the AP+ growth medium designed in Chapter 3 (section 3.4.2) was used for the novel

isolates. In addition, an AP+ 20% medium which has not been described before but utilises the same basic medium with the addition of 3.4M NaCl.

### 5.4.2. Lake Magadi isolates and control strains

Seven isolates (isolated in Chapter 3), five bacteria: *Idiomarina* sp. LMLD01, *Planococcus* sp. LMLD02*, Caldalkalibacillus* sp. LMLD03 (characterised and described in Chapter 4), *Bacillus* sp. LMLD04 and *Salinicoccus* sp. LMLD05, and two archaea: *Natronococcus* sp. LMLD06 and *Natrialba* sp. LMLD07 (Isolated in Chapter 3) were grown over varying periods of time and in different culture media (Table 5.1).

*Table 5.1. Growth medium and incubation time for 10 isolates for desiccation and UV-C tolerance studies. Isolates marked with * denotes archaea. † denotes control strains.*

| Isolate | Growth medium | Incubation time for starter culture | Closest database strain match | % match |
|---|---|---|---|---|
| *Idiomarina* sp.LMLD01 | AP LS | Over night | *Idiomarina seosinensis* strain CL-SP19. | 93 |
| *Planococcus* sp. LMLD02 | AP-6.8% | Over night | *Planococcus maritimus* strain SS-06 | 98 |
| *Caldalkalibacillus* sp. LMLD03 | AP-6.8% | Over night | *Caldalkalibacillus uzonensis* NR_043653.1 | 93 |
| *Salinococcus* sp.LMLD04 | AP LS | Over night | *Salinicoccus alkaliphilus* X2B. GU397409.2 | 99 |
| *Bacillus* sp.LMLD05 | AP-6.8% | Over night | *Bacillus aurantiacus* K1-10 AJ605772.2 | 97 |
| *Natronococcus* sp. LMLD06* | AP-15% | 7 days | *Natronococcus occultus* JCM 8859. NR_028255.1 | 99 |
| *Natrialba* sp.LMLD07* | AP-15% | 6 days | *Natrialba wudunaoensis strain Sua-E42* | 99 |
| *Escherichia coli* DH1 (Rec-)† | NB2 | Over night | N/A | N/A |
| *Escherichia coli* W3110 (Rec+)† | NB2 | Over night | N/A | N/A |
| *Deinococcus radiodurans*† | TGY | 5 days | N/A | N/A |

All strains were maintained and starter cultures inoculated from plates incubated at 37 °C, except *D. radiodurans,* a desiccation, UV-C resistant control strain for which agar plates are

incubated at 30 °C. All isolate starter cultures were grown at 200 rpm and 37 °C, except *Caldalkalibacillus* sp.LMLD03 which was incubated at 45 °C and *D. radiodurans* which was incubated at 30 °C. *Caldalkalibacillus* sp.LMLD03 grew optimally at 45 °C and *D. radiodurans* grows optimally at 30 °C. Three control strains were used for comparison: two strains of *E. coli*, *E. coli* W3110 (Rec+) and *E. coli* DH1 (Rec-), and *D. radiodurans* as a radiation and desiccation tolerant strain. The *E. coli* strains differ by the presence (Rec+) or absence (Rec-) of the RecA gene and therefore provide interesting comparisons to the novel strains (refer to Chapter 1, section 1.6.3.4). *D. radiodurans* was included due to its known radiation and desiccation tolerance as discussed above and in Chapter 1, section 1.6.3.3.

### 5.4.3. Growth curve for novel isolates

The stationary phase of growth was ascertained for *Salinicoccus* sp. LMLD04, *Bacillus* sp. LMLD05, *Natronococcus* sp. LMLD06 and *Natrialba* sp. LMLD07 as these novel strains were not studied in Chapter 4. The protocol undertaken in this chapter was similar to that used in Chapter 4. Starter cultures were incubated in the media and for the length of time outlined in Table 5.1. These cultures were used to inoculate 50ml of the appropriate broth. The flasks were double foiled to reduce evaporation when incubated for long periods of time. An OD reading was taken every 2 hours over a 24 hour period, as described in Chapter 4 or every 6 to 24 hours if the growth study was conducted for longer than 24 hours. The OD reading was then plotted against time in Microsoft Excel, as in Chapter 4 in order to ascertain when the strain entered the stationary phase of growth.

### 5.4.4. Buffers

Phosphate buffered saline (PBS) which contained 1M $KH_2PO_4$, 1M $K_2HPO_4$ and 5M NaCl or 15% Halo Buffer which contained 0.1M $Na_2CO_3$, 0.1M $NaHCO_3$ and 2.6M NaCl were used to resuspend the cells.

### 5.4.5. Desiccation experiment setup

Starter cultures were incubated to the stationary phase of growth (Chen, 1973: Fredrickson, 2008: Chaibenjawong, 2011). Triplicates for each strain were spun down at 11000 rpm at 4 °C for 10 minutes. The supernatant was removed and the cells resuspended in 10 ml of PBS for all cultures, except *Natronococcus* sp. LMLD06 and *Natrialba* sp. LMLD07 which were resuspended in15%Halo buffer; a buffer suitable for haloalkaliphilic archaea which has a pH of 10 and a salinity of 15% (w/v) NaCl.

100 µl of the cell suspension was pipetted onto a 4 cm diameter tissue culture dish and shaken to disperse the cell suspension on the surface of the dish. The tissue culture dishes were then placed into sterile Nalgene containers, with 68g of $CaCl_2$ desiccant (Dartnell et al., 2010). The Nalgene container lid was tightly closed to prevent atmosphere interaction (Figure 5.1.). Data for seven time points was collected; 24 hours, 48 h, 72 h, 168 h (7 days), 336 h

(14 days), 504 h (21 days) and 672 h (28 days), three tissue culture dishes were removed for analysis.

At each data point 1 ml of appropriate buffer (PBS or 15%Halo buffer) was added to the culture dishes which were tilted on an adjustable tilt rocking platform for 10 minutes, at 10 tilts per minute. 1 ml of buffer was used for cell resuspension to ensure the cells which were desiccated to the culture dish surface, were fully resuspended. This procedure was the equivalent of diluting the original cell density by 10, therefore this was the lowest dilution which could be examined in this experiment. A dilution series was then produced for each triplicate to ascertain the proportion of cell survival at each time point for each strain.

Controls for contamination were run in parallel in each Nalgene container, testing for contaminants on the 3rd, 14th and 28th day. These controls were tissue culture dishes with 100 µl of sterile buffer (PBS or 15% Halo buffer). The controls were treated in exactly the same way as the samples, and plated onto the same type of growth medium as the strain being studied.

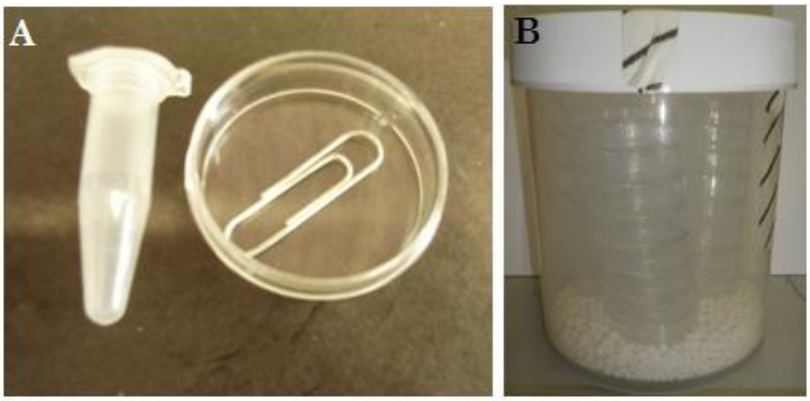



*Figure 5.1. Equipment used for desiccation and UV experiments. A. Tissue culture dish and paper clip 'flea', used to agitate sample in UV experiments, with Eppendorf tube for scale. B. Nalgene container containing $CaCl_2$ desiccant and the tissue culture dishes.*

**5.4.6. UV-C experiment setup**

The cultures were prepared as in paragraph 1, section 4.3.4.1. 1200 µl of the resuspended cells were added to tissue culture dish. A cell density of at most $10^6$ cells per ml was achieved by diluting the cell suspension with either PBS or 15% Halo Buffer. An autoclaved paperclip was used as a flea (Figure 5.1). The culture dish was placed in the centre of a magnetic stirrer to ensure the cells were dispersed within the sample.

The UV-C flux from the UV-C germicidal lamp (254nm) used was such that every minute of lamp exposure equated to 6.8 seconds of Mars surface flux (Patel pers. comm.). A sample was taken at the time points: 5 seconds, 10 seconds, 15 seconds, 30 seconds and 55 seconds irradiation, the radiation dose and Mars surface flux that these exposure times

equate to are outlined in Table 5.2. The samples and lamp were contained within a blackout card to eliminate visible light.

*Table 5.2. Sample irradiation time, resulting UV-C dose and equivalent Mars UV-C flux.*

| Time (seconds) | Irradiation dose ($Jm^{-2}$) | Mars surface flux (seconds) |
|---|---|---|
| 5 | 10 | 0.7 |
| 10 | 20 | 1.4 |
| 15 | 30 | 2.2 |
| 30 | 60 | 4.3 |
| 55 | 110 | 7.9 |

The sample was first irradiated for 5 seconds, the lamp was turned off and a sample removed. The lamp was then turned on again for a further 5 seconds, therefore totalling 10 seconds exposure, and another sample was removed. The lamp was turned on for a further 5 seconds, totalling 15 seconds exposure and a sample removed. Following this, the lamp was turned on for 15 seconds, equating to 30 seconds total exposure and finally 25 seconds, totalling 55 seconds total exposure. 100 µl of the sample was removed at each point and placed in an Eppendorf tube, and covered with foil to exclude the light for exactly hour to ensure sample continuity.

In order to ensure that only dark repair of any UV-C-induced dimers would occur, all light was excluded from the samples during the 1 hour cell recovery which was incubated at room temperature (~25 ºC). Photoreactivation of cells is related to the amount of visible light exposure (Maloy, 1994), there was no quantifiable light source available to ensure a unified light intensity across all samples. As a result, all samples were maintained in dark. A dilution series was then made at each time point, for each sample triplicate. 50 µl of the dilution was plated onto the appropriate agar (See table 5.1), the plates were then wrapped in foil and incubated at 37 °C or 30 °C.

### 5.4.7. Calculating cell survival fraction

Using Microsoft Excel the numbers of cells per ml were calculated for a blank control of untreated cell suspension. The numbers of cells per ml were then calculated after each treatment (either desiccation or UV-C) and divided by the blank control number of cells per ml, producing a survival fraction for the viable cells.

### 5.4.8. Calculating cell death gradient

The Survival fraction for each strain at each time point was plotted on a logarithmic scale in Microsoft Excel. For the desiccation study a trend line was fitted to the first four data points (days 0-3). An exponential trend line was fitted to all the data points for the UV-C study. The gradient of cell death, which equated to rate of cell death, was calculated in Microsoft

Excel fitting an exponential trend line to the data points. A high and therefore steep gradient represents a rapidly decreasing viable cell density.

## 5.5. Results

### 5.5.1. Growth curves for novel isolates

The growth characteristics of *Idiomarina* sp. LMLD01, *Planococcus* sp. LMLD02 and *Caldalkalibacillus* sp. LMLD03 have been studied in Chapter 4, a growth study was conducted for the novel strains not characterised in that Chapter. Both *Salinicoccus* sp.LMLD04 and *Bacillus* sp. LMLD05 reached the stationary phase by 12 hours (Figures 5.2.A.B.). The archaea strains took longer to reach the stationary phase, with *Natrialba* sp.LMLD07 reaching stationary phase by 37 hours, followed by *Natronococcus* sp.LMLD06, taking 73 hours (Figures 5.2.C.D).

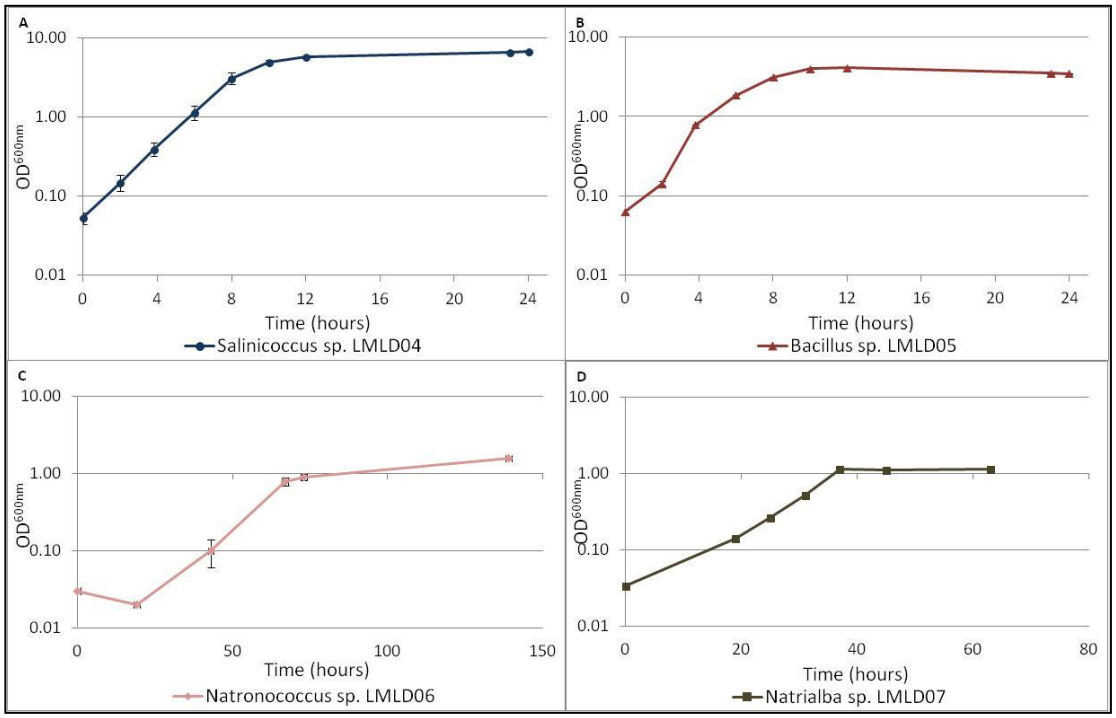



*Figure 5.2. Growth curves for Salinicoccus sp. LMLD04, Bacillus sp. LMLD05, Natronococcus sp. LMLD06 and Natrialba sp. LMLD07. Error bars are 1x standard error.*

### 5.5.2. Desiccation resistance of isolates

#### 5.5.2.1. Effect of desiccation on the survival of control strains: *E. coli* DH1, *E. coli* W3110 and *D. radiodurans*

The survival fractions after desiccation were recorded and are plotted in figure 5.3. A survival fraction gradient of -2.53 was recorded over the first 3 days desiccation for *E. coli* DH1. A survival fraction gradient of -2.15 was recorded for *E. coli* W3110, however, the rates between the two *E. coli* strains were not statistically different from one another $p$=0.627 (using ANOVA statistical test and Tukey post hoc analysis, $p$<0.05 was considered as significant-

See chapter 2). *D. radiodurans* demonstrates a gradual decline in survival fraction with a gradient of -0.74 and a final cell count of $2.12x10^2$. The gradients for both *E. coli* strains were statistically steeper the gradient of *D. radiodurans*, $p>2.5x10^5$. The final viable cell counts for each *E. coli* DH1 and W3110 were $1.15x10^4$ and $2.14x10^4$ respectively, which were not significantly different from one another, nor were either significantly different from *D. radiodurans* $p>0.991$.

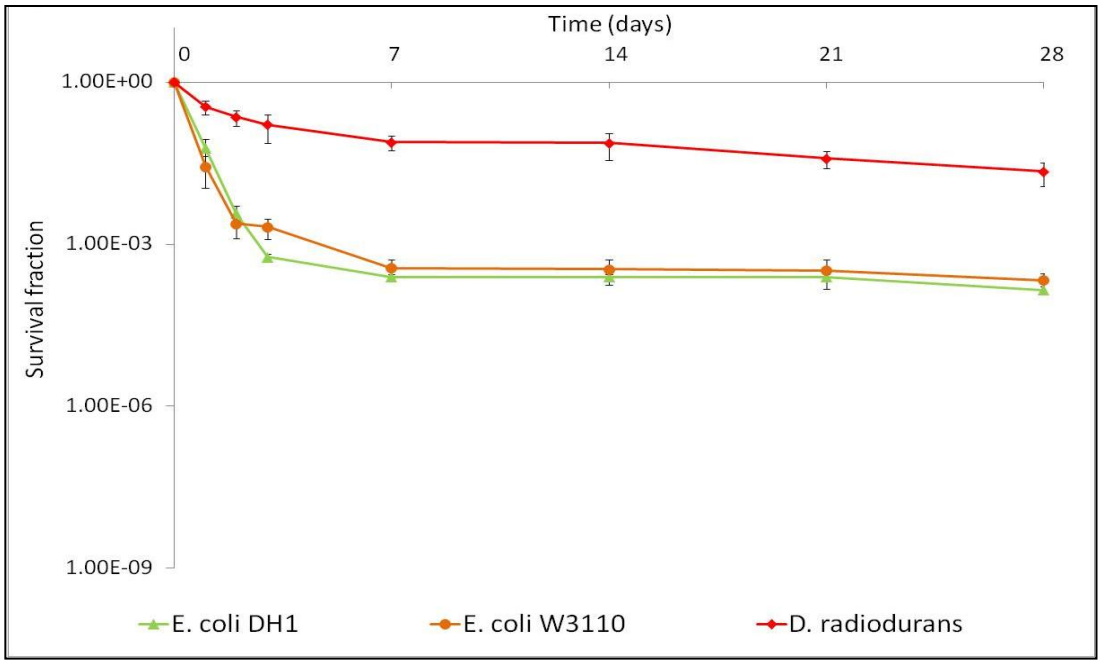



*Figure 5.3. Survival fraction of three control isolates: E. coli DH1 (Rec-), E. coli W3110 (Rec+) and D. radiodurans Error bars are 1x standard error.*

### 5.5.2.2. *Idiomarina* sp. LMLD01

*Idiomarina* sp.LMLD01 demonstrated that desiccation for two days had a statistically significant effect upon survival. The largest decrease in viable cells occurred after 1 day of incubation, with a decline in the survival fraction to $4.21 \times 10^4$ (0.04%). Figure 5.4 shows that this decrease was quite sharp, with a gradient of -3.17. This drop was not a significantly steeper decrease in survival compared to *E. coli* DH1, $p$=0.081, but was a statistically steeper gradient than recorded for *E. coli* W3110 and *D. radiodurans,* $p$<0.002. After 3 days the rate levelled off, with a gradient of -0.279. The starting cell density of $3.23 \times 10^7$ cells per ml dropped to $1.55 \times 10^3$cells per ml after 28 days desiccation. The viable cell count after 28 days for *Idiomarina* sp. LMLD01 was not statistically different to that recorded for either of the *E. coli* strains, or the *D. radiodurans* strain, $p$=0.121.

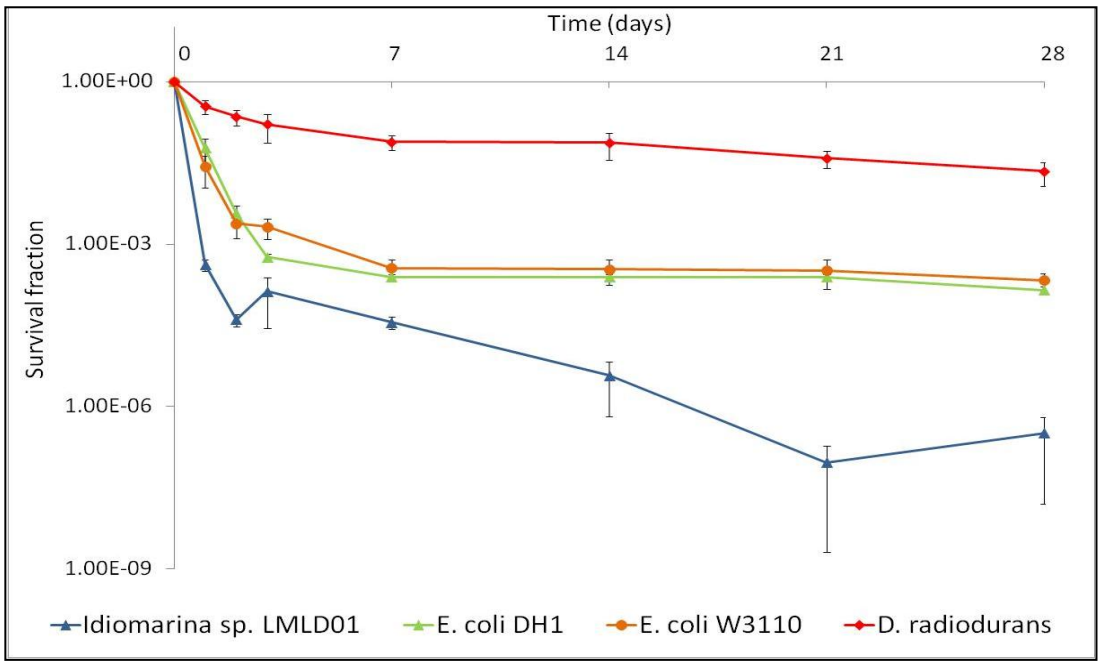



*Figure 5.4. Survival fraction of Idiomarina sp.LMLD01 over 28 days compared to E. coli DH1, E. coli W3110 and D. radiodurans. Error bars are 1x standard error.*

### 5.5.2.3. *Planococcus* sp. LMLD02

*Planococcus* sp.LMLD02 had a starting cell density of $6.29x10^8$ cells per ml. The survival curve of this strain demonstrated a decrease in viable cells count over the first three days desiccation (Figure 5.5), with a gradient of -0.62. The rate cell death was significantly lower than for either of the *E. coli* strains, $p=1.5x10^5$. The rate of cell death for *Planococcus* sp. LMLD02 was not significantly different to the survival fraction gradient recorded for *D. radiodurans* $p=0.975$. There was a decline in the number of viable cells after 2 days desiccation to a fraction of $8.53x10^1$. However there was no statistically significant difference in the viable cell numbers after 2 days desiccation, compared to the initial cell count of the sample. A further decline in the viable cell count, to a survival fraction of $1.36x10^1$, occurred after 3 days incubation. The rate of cell death decreased between 3 and 28 days, with a gradient of -0.011. *Planococcus* sp. LMLD02 had a viable cell count of $16.78x10^7$per ml, after 28 days desiccation, which was significantly greater than the cell count after 28 days for any of the control strains.

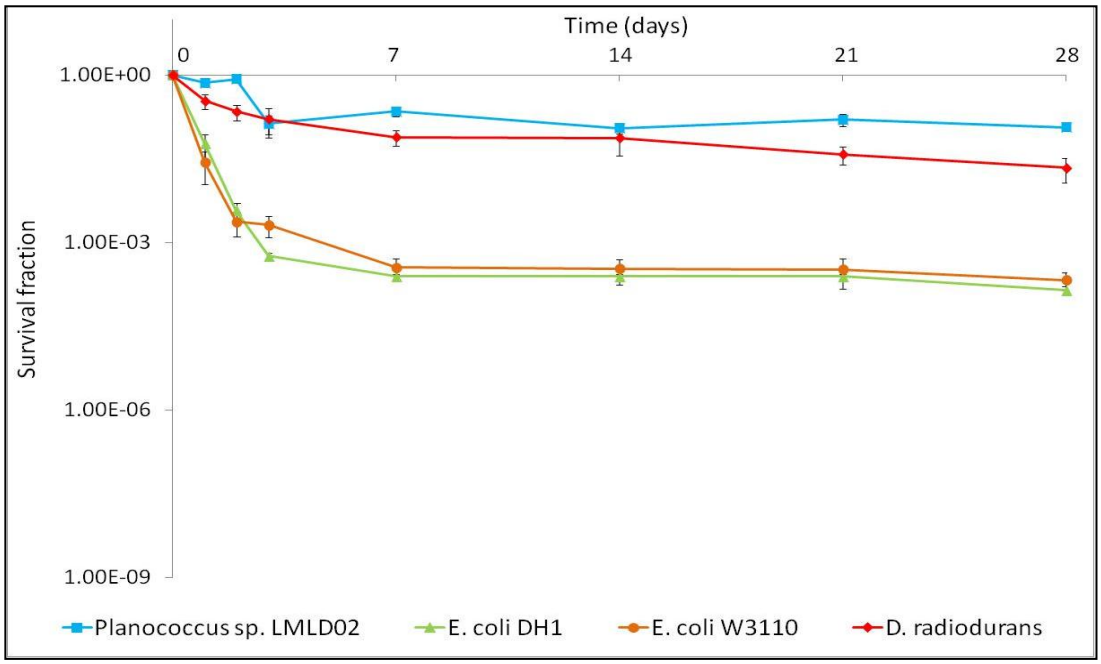



*Figure 5.5. Survival fraction of Planococcus sp.LMLD02 over 28 days compared to E. coli DH1, E. coli W3110 and D. radiodurans. Error bars are 1x standard error.*

#### 5.5.2.4. *Caldalkalibacillus* sp. LMLD03

The overnight cultures for *Caldalkalibacillus* sp.LMLD03 achieved a cell density of $4.37x10^5$ cells per ml. The results of the desiccation study showed that no colonies formed even after 1 day's incubation. This may be due to: either, that the isolate did not survive the desiccation treatment; or, that the isolate formed spores. Chapter 3 showed that *Caldalkalibacillus* sp. LMLD03 is a spore former, and that spore formation could be initiated by factors such as changes in: pH; salinity and temperature.

In order to ascertain if this strain was forming spores efforts were made to reanimate any spores which might be present in the sample. This was attempted using three different methods, firstly by heat shocking the cells in PBS, at 70 °C for 30 minutes, secondly by heat shocking the cells in PBS at 95 °C for 10 minutes (Curran and Evans, 1945). Finally an aliquot of the desiccated cells were incubated at 37 °C with 10mM Alanine (Hudson, 2001). None of the treatments tested yielded any colonies; this may indicate that the required conditions for the spore reanimation for *Caldalkalibacillus* sp.LMLD03were not identified or that no cells survived. For this reason, this isolate was removed from any subsequent experiments and no data could be gained for the desiccation resistance or UV-C tolerance of this strain.

#### 5.5.2.5. *Salinicoccus* sp. LMLD04

Results showed that there was no significant drop in *Salinicoccus* sp. LMLD04 cell number following 1 days desiccation, $p<0.005$ (Figure 5.6). The rate of cell death measured for the first three days desiccation was -0.74, which was not significantly different to that recorded for *D. radiodurans*, $p=0.999$, but was significantly shallower than both *E. coli* strains, $p=4.7\text{x}10^{5}$. As with both *E. coli* strains and *D. radiodurans,* the rate of cell death levels off between 3 days and 28 days, with a gradient of -0.021.After 28 days the strain maintained a viable cell density of $3.74\text{x}10^{7}$compared to the starting cell count. The cell count after 28 days was not significantly different to the final cell count recorded for either *E. coli* strains or *D. radiodurans*.

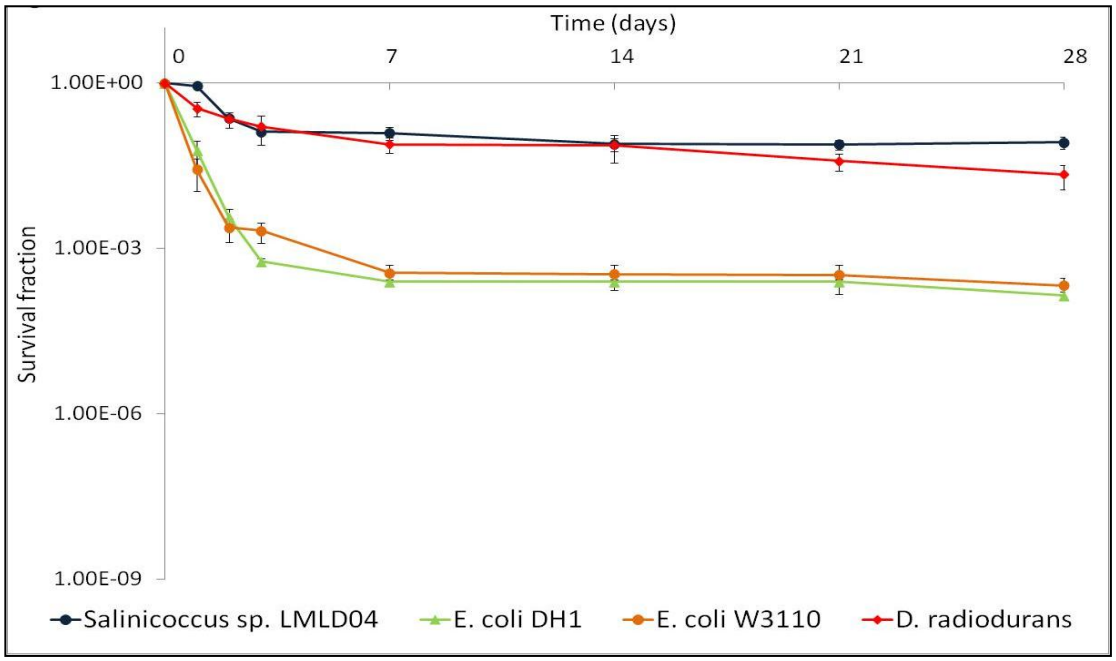



*Figure 5.6. Survival fraction of Salinicoccus sp.LMLD04 over 28 days compared to E. coli DH1, E. coli W3110 and D. radiodurans. Error bars are 1x standard error.*

### 5.5.2.6. *Bacillus* sp.LMLD05

The starting cell density for this strain was around $1.85x10^8$ cells per ml. *Bacillus* sp.LMLD05 demonstrated no significant drop in viable cell numbers after 1 day, $p$=0.112. However, there was a significant drop, by two orders of magnitude in the survival fraction between 1 and 2 days (Figure 5.7) from $7.29x10^1$ to $2.67x10^3$. After 2 days, the survival fraction remained fairly constant, with a lower rate of cell death compared between 2 and 3 days, and the final viable cell density after 28 days was $2.01x10^5$cells. This final cell density was not significantly different from either *E. coli* strains, or to *D. radiodurans*. The rate of cell death of -1.6 for *Bacillus* sp. LMLD05was significantly shallower than *E. coli* DH1, $p$=0.05 but significantly steeper than recorded for *D. radiodurans*, $p$=0.025. The rate of cell death for *Bacillus* sp. LMLD05 was not significantly different to that recorded for *E. coli* W3110 $p$=0.213.

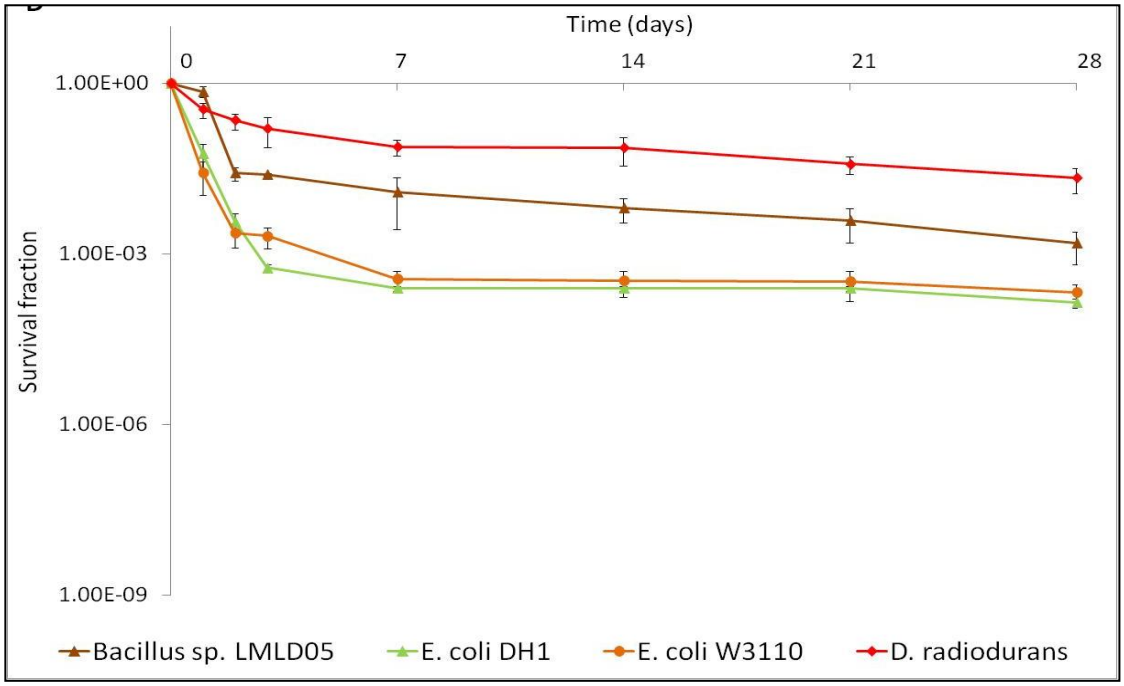



*Figure 5.7. Survival fraction of Bacillussp.LMLD02 over 28 days compared to E. coli DH1, E. coli W3110 and D. radiodurans. Error bars are 1x standard error.*

### 5.5.2.7. *Natronococcus* sp. LMLD06

The starting cell density for *Natronococcus* sp. LMLD06 was $2.4x10^6$ cells per ml, which significantly decreased to $4.4x10^5$ cells per ml after 1 day $p=1.5x10^9$. There was however no significant difference between the survival fraction after 1 days desiccation, when compared to the cell count for any subsequent time point, $p \geq 0.620$.The rate of cell death for this isolate of -0.83, was significantly shallower than the gradient for either *E. coli* strains, $p=1x10^4$, but not significantly different from *D. radiodurans* $p=0.998$. As with the control strains, the rate of cell death levelled off after 3 days. The final cell count was $8.17x10^2$, which was not significantly different to the final cell count for any of the control strains, $p<0.122$.

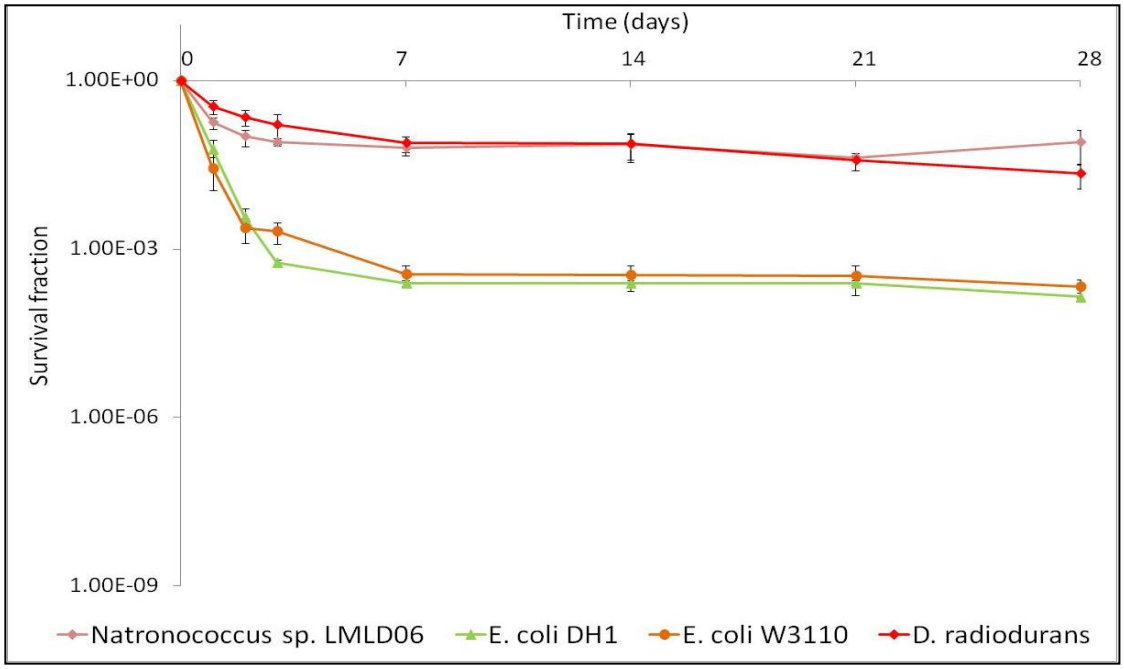



*Figure 5.8. Survival fraction of Natronococcus sp.LMLD02 over 28 days compared to E. coli DH1, E. coli W3110 and D. radiodurans. Error bars are 1x standard error.*

### 5.5.2.8. *Natrialba* sp.LMLD07

This strain starts with a cell density of $4.6x10^6$. The drop in survival fraction after 1 days desiccation to $1.21x10^0$ (Figure 5.9) was not significantly different from the survival fraction of $1x10^0$at time 0, $p$=0.235. After 2 days desiccation, a significant drop in the survival fraction occurred, down to $1.69x10^1$$p$=$1.989x10^8$. There was then no significant difference in the survival fraction between 2 days and 28 days incubation.

The rate of cell death was calculated as -0.83, which was significantly shallower than that calculated for *E. coli* DH1 and *E. coli* W3110, p≤$1x10^4$, but was not significantly different to that recorded for *D. radiodurans, p*<0.988.No data points could be obtained for time points, day 7 or 14. An error with dilution series produces uneven results. The final cell count of $4.61x10^4$ was not significantly different between *E. coli* strains, or *D. radiodurans p*≥0.051.

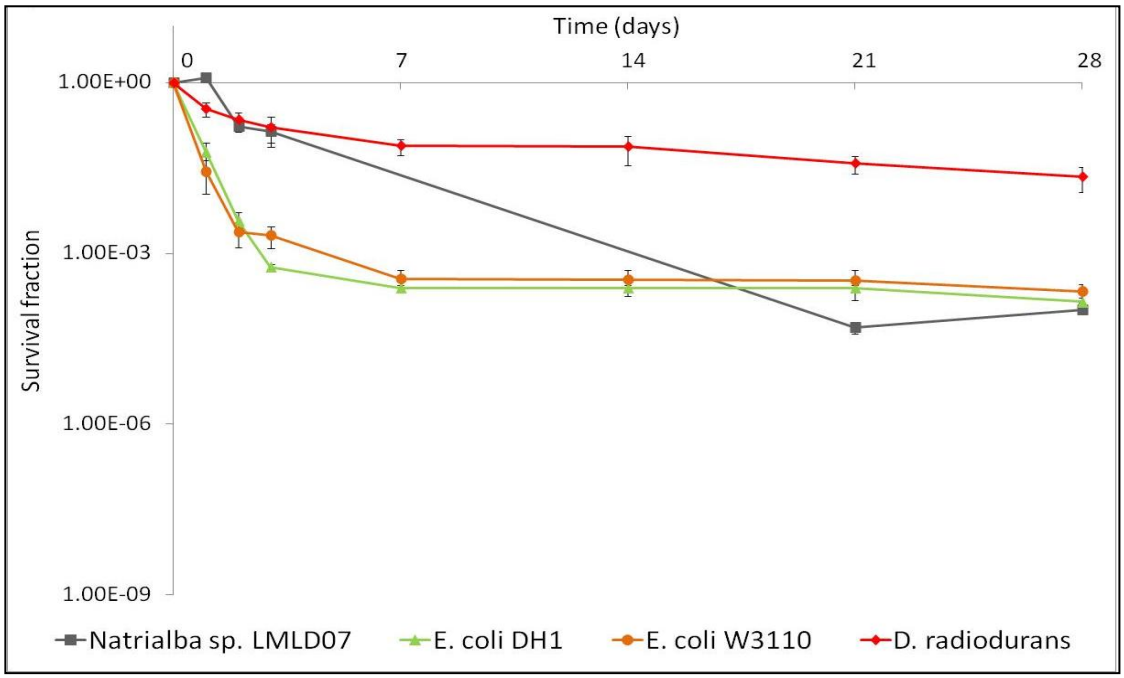



*Figure 5.9. Survival fraction of Natrialba sp. (LMLD07) over 28 days compared to E. coli DH1, E. coli W3110 and D. radiodurans. Error bars are 1x standard error.*

### 5.5.2.9. Comparison of the desiccation resistance of isolates

Plotting the survival fraction after 28 days against the survival gradient, or rate of cell death, for the first 3 days of desiccation showed, a trend of a lower survival fraction after 28 days, in association with a steep rate of cell death over the first 3 days (Figure 5.10).

*Idiomarina* sp. LMLD01 plotted with the steepest slope gradient, which was significantly steeper than any novel strain tested here, $p$<$9.6x10^6$. It also had the lowest proportion of viable cells after 28 days, but this was not found to be significantly lower, except when compared to *Planococcus* sp. LMLD02. The rate of cell death recorded for *Bacillus* sp. LMLD05 was lower than for *Idiomarina* sp. LMLD01, therefore implying a greater resistance to

the process of desiccation than these two strains, $p<9.6x10^{6}$, but it was steeper than any other novel strain tested, $p\leq0.025$.

*Planococcus* sp. LMLD02 had the highest survival fraction after 28 days and the shallowest survival gradient, both of which were significantly different to all strains tested, $p\leq0.012$, except *Salinicoccus* sp. LMLD04, *Natronococcus* sp. LMLD06 and *D. radiodurans*, where $p\geq0.051$. The rate of cell death was not significantly different when comparing the gradient between different between these four strains, $p=0.975$, and these all have significantly lower rate of cell death compared to the other strains tested, $p\leq0.004$.

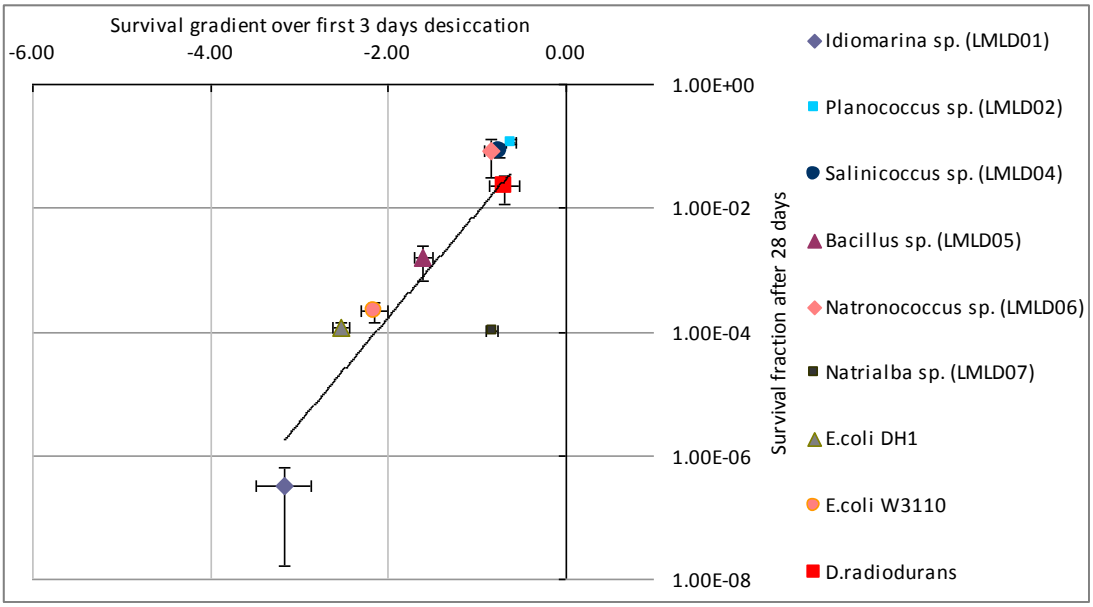



*Figure 5.10.The survival gradient over first 3 days desiccation for the novel isolates and control strains against Survival fraction after 28 days. Error bars are 1x standard error. Diagonal line is line of best fit.*

When comparing the rate of cell death between the pigmented (*Planococcus* sp. LMLD02, *Salinicoccus* sp. LMLD04, *Natronococcus* sp. LMLD06, *Natrialba* sp. LMLD07 and *D. radiodurans*) and non-pigmented strains (*Idiomarina* sp. LMLD01, *Bacillus* sp. LMLD05, *E. coli* DH1 and E. coli W3110) in this study, there was a significant difference, $p=0.0045$.

### 5.5.3. The effect of UV-C irradiation on the viability of the novel Lake Magadi isolates

#### *5.5.3.1.* The effect of UV-C irradiation on the viability of the control strains *E. coli* DH1, *E. coli* W3110 and *D. radiodurans*

*E. coli* DH1 was the least tolerant of the three control strains tested, and was unable to survive a dose higher than 10 $Jm^{-2}$, no colonies formed after a dose of 20 $Jm^{-2}$ (Figure 5.11). This was followed by *E. coli* W3110 which survived a dose of at least 30 $Jm^{-2}$ but not of 60 $Jm^{-2}$. There was a shallow rate of cell death up to 20 $Jm^{-2}$, followed by a steeper rate of

cell death between 20 and 30 $Jm^{-2}$for *E. coli* W3110. Finally, cells of *D. radiodurans* remained viable even after sample was exposed to the highest UV-C dose tested (110 $Jm^{-2}$), with a survival fraction of $4.4x10^{2}$. The survival gradient for the *E. coli* DH1 was -1.06 which was significantly steeper than the survival gradient recorded for *E. coli* W3110 of -0.25 $p=1.5x10^{8}$. The survival gradient for *D. radiodurans* was calculated as -0.12, which was significantly shallower than the survival gradients recorded for *E. coli* DHI $p=1.9x10^{9}$, but not significantly different to *E. coli* W3110 $p=0.561$.

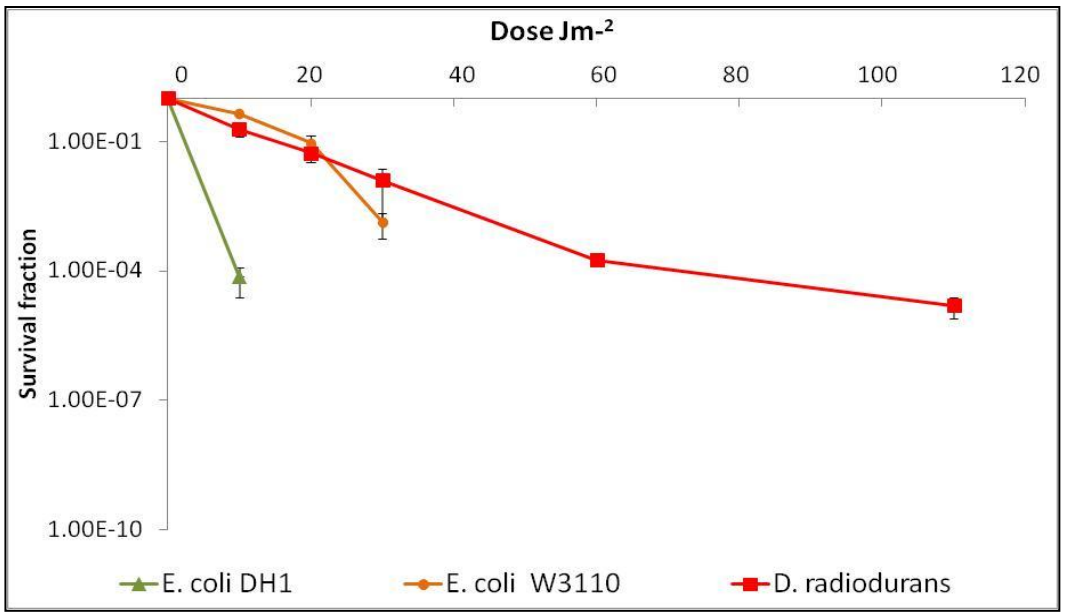



*Figure 5.11.Survival fraction of E. coli DH1, E. coli W3110 and D. radiodurans up to 110 $Jm^{-2}$. Error bars are 1x standard error.*

### *5.5.3.2. Idiomarina* sp. LMLD01

*Idiomarina* sp. LMLD01 was able to tolerate a UV-C dose up to 20 $Jm^{-2}$ but not a dose of 30 $Jm^{-2}$, for which no colonies formed, indicating it was more tolerant to UV-C irradiation than *E. coli* DH1, but less tolerant than either *E. coli* W3110 or *D. radiodurans* (Figure 5.12). The rate of cell death calculated for *Idiomarina* sp. LMLD01 was -0.59, which was significantly shallower than that recorded for *E. coli* DH1,$p=1.7x10^{5}$, but significantly steeper than recorded both *E. coli* W3110 and *D. radiodurans, p*<0.002.

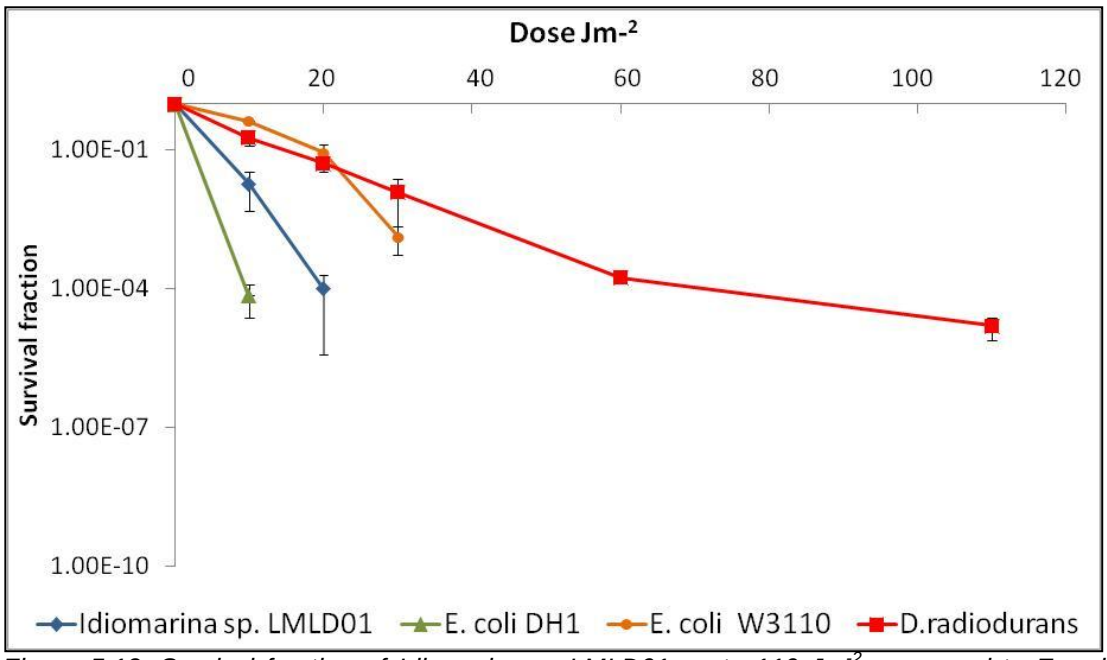



*Figure 5.12. Survival fraction of Idiomarina sp.LMLD01 up to 110 $Jm^{-2}$ compared to E. coli DH1, E. coli W3110 and D. radiodurans. Error bars are 1x standard error.*

#### 5.5.3.3. *Planococcus* sp. LMLD02

This strain was capable of surviving a dose of UV-C irradiation up to 30 $Jm^{-2}$ but not a dose of 60 $Jm^{-2}$, no colonies formed on plates inoculated with 60 $Jm^{-2}$ dose sample (Figure 5.13). The survival curve for *Planococcus* sp. LMLD02 largely followed the same trajectory as the survival curve for *D. radiodurans* until 30 $Jm^{-2}$, at which point the curves diverge. The survival gradient for this strain was calculated as -0.18, which was significantly shallower than that for *E. coli* DH1, $p=1.7x10^{9}$ but not significantly different to the survival gradients for *E. coli* W3110 or *D. radiodurans* $p>0.749$.

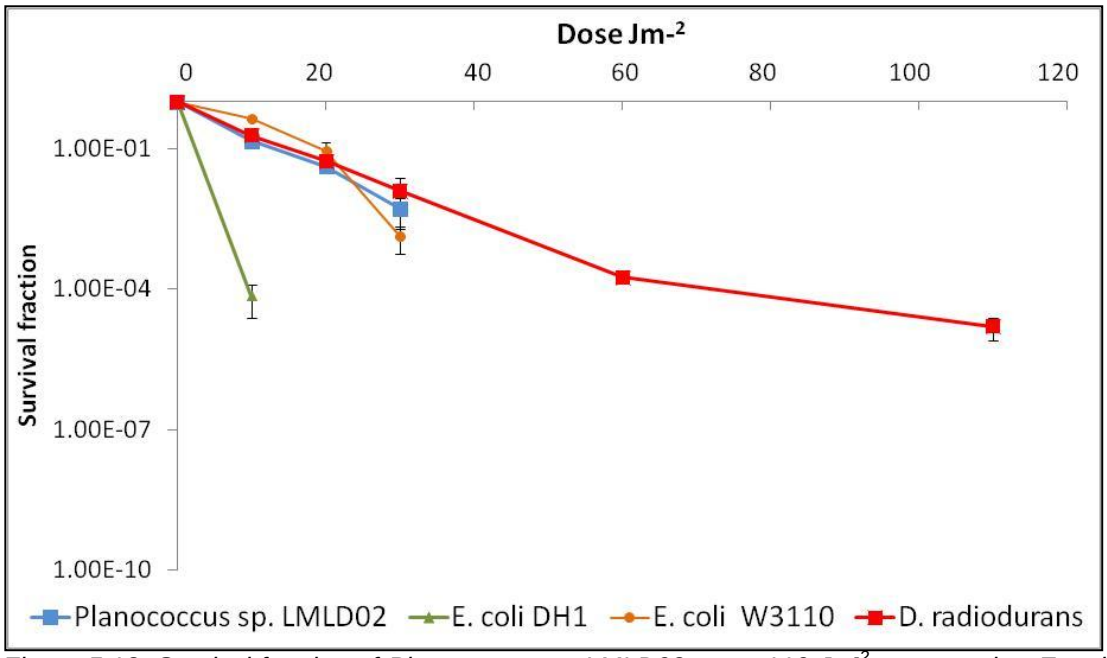



*Figure 5.13. Survival fraction of Planococcus sp.LMLD02 up to 110 $Jm^{-2}$ compared to E. coli DH1, E. coli W3110 and D. radiodurans. Error bars are 1x standard error.*

#### 5.5.3.4. *Salinicoccus* sp. LMLD04

This strain differed to those tested before it by its ability to survive up to a UV-C dose of at least 110 $Jm^{-2}$, with a survival fraction of $3.93x10^{3}$ (Figure 5.14). The rate of cell death was calculated as -0.14, which was significantly shallower than recorded for *E. coli* strain DH1, $p=1.6x10^{9}$, but not significantly different from the survival gradient for *D. radiodurans* or *E. coli* W3110, $p \geq 0.436$.

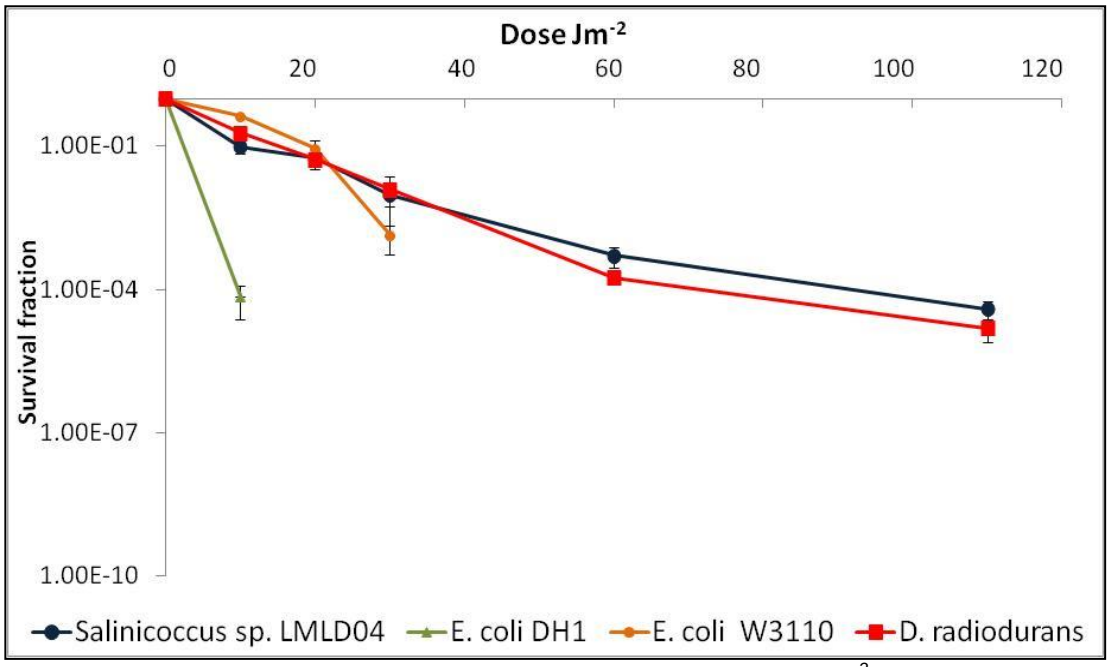



*Figure 5.14. Survival fraction of Salinicoccus sp.LMLD04 up to 110 $Jm^{-2}$ compared to E. coli DH1, E. coli W3110 and D. radiodurans. Error bars are 1x standard error.*

#### 5.5.3.5. *Bacillus* sp. LMLD05

*Bacillus* sp. LMLD05 was able to tolerate UV-C doses of up to 30 $Jm^{-2}$ but not a dose of 60 $Jm^{-2}$ (Figure 5.15). The rate of cell death of -0.46 for *Bacillus* sp. LMLD05 was not significantly different to that recorded for *E. coli* W3110, $p$=0.105. However, it was significantly different to a gradient of -1.06 for *E. coli* DH1 and -0.12 for *D. radiodurans* $p \geq 0.003$.

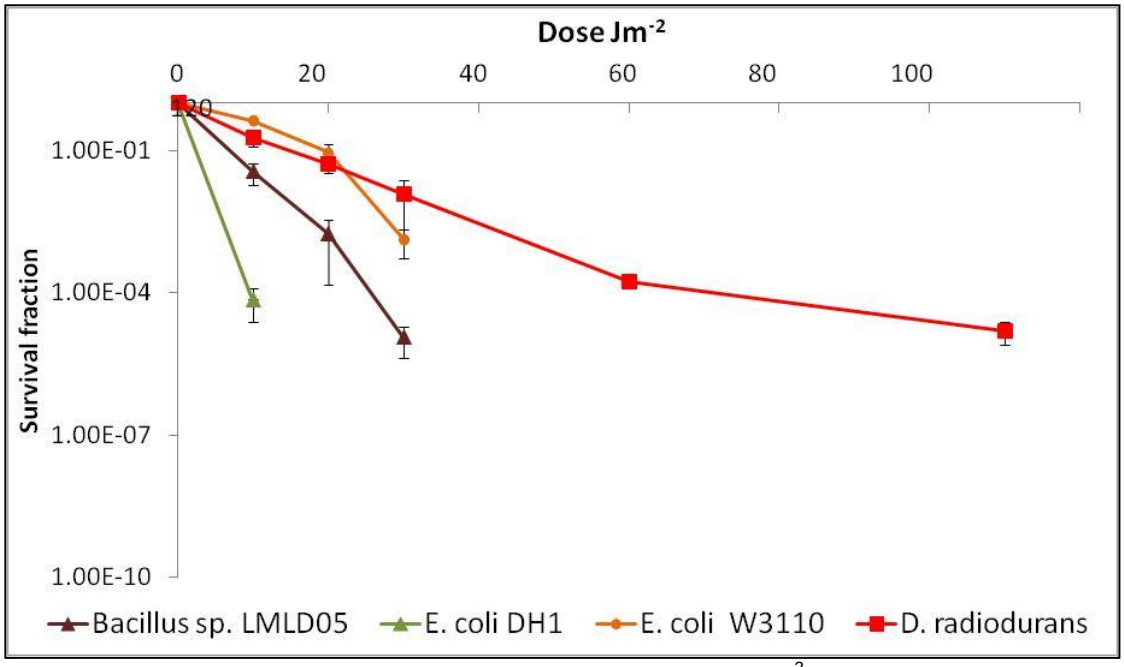



*Figure 5.15. Survival fraction of Bacillus sp.LMLD05 up to 110 $Jm^{-2}$ compared to E. coli DH1, E. coli W3110 and D. radiodurans. Error bars are 1x standard error.*

#### 5.5.3.6. *Natronococcus* sp. LMLD06

This strain had a starting cell density of $3.57x10^4$and could withstand a UV-C dose of up to at least 110 $Jm^{-2}$, in line with both *Salinicoccus* sp. LMLD04 and *D. radiodurans*. Figure 5.16 shows a higher survival fraction after a dose of 110 $Jm^{-2}$ ($5.94x10^2$ cells per ml) for *Natronococcus* sp. LMLD06, than the survival fraction recorded for *D. radiodurans* of $1.89x10^4$at 110 $Jm^{-2}$. The rate of cell death of -0.02 for this strain was significantly shallower than that recorded for either *E. coli* strains, $p \leq 0.014$, but not significantly different to the survival gradient for *D. radiodurans,* $p=0.413$.

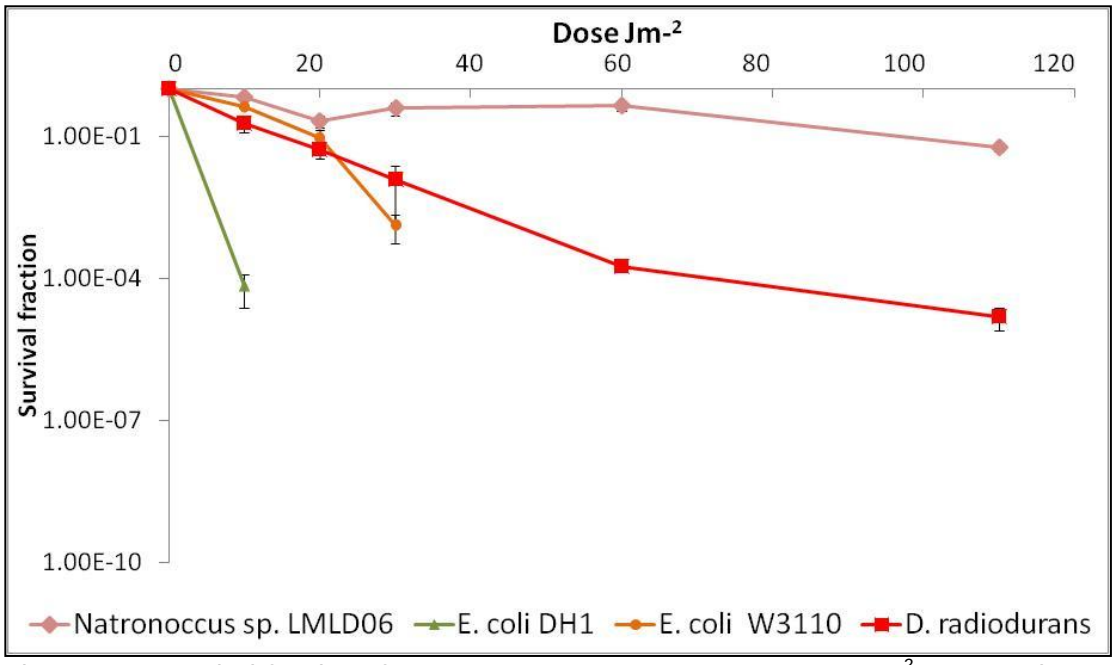



*Figure 5.16. Survival fraction of Natronococcus sp.LMLD06 up to 110 $Jm^{-2}$ compared to E. coli DH1, E. coli W3110 and D. radiodurans. Error bars are 1x standard error.*

#### 5.5.3.7. *Natrialba* sp. LMLD07

Colonies did form on all UV-C doses for this isolate, however problems with the plating of this strain after irradiation and highly uneven colony counts meant meaningful data could not be obtained.

#### 5.5.3.8. Comparison of the UV-C tolerance

The rate of cell death for *Idiomarina* sp. LMLD01 was significantly steeper than the gradient calculated for any other novel strain $p \leq 0.037$.The survival curve for *Bacillus* sp. LMLD05 was significantly steeper than for any of the other novel strains tested ($p \leq 0.037$) except *E. coli* W3110, $p=0.211$.

The survival gradient calculated for *Planococcus* sp. LMLD02 was not significantly different to the survival gradients for *D. radiodurans*, *Salinicoccus* sp. LMLD04, *Natronococcus* sp. LMLD06, nor *E. coli* W3110, $p \geq 0.126$. In turn, *E. coli* W3110, *D. radiodurans* and *Salinicoccus* sp. LMLD04 are not significantly different from one another $p>0.126$.The survival gradient

calculated for *Salinicoccus* sp. LMLD04 and *D. radiodurans* were not significantly different from the survival gradient *E. coli* W3110, $p$>0.436, however, these strains were able to survive a UV-C dose up to 110 $Jm^{-2}$ which *E. coli* W3110 was not able to do.

### 5.5.4. Desiccation resistance gradient against UV-C tolerance gradient for control strains and novel Lake Magadi isolates

The isolates *Caldalkalibacillus* sp. LMLD03 and *Natrialba* sp. LMLD07 (Refer to sections 5.3.2.4 and 5.3.3.8 of this Chapter respectively) have been excluded from this comparison due to insufficient data. Therefore eight strains have been compared in terms of their UV-C tolerance and desiccation resistance. The desiccation and UV-C survival gradients are shown in table 4.3.

*Table 4.3 Survival gradient for desiccation after 3 days, percentage survive after 28 days desiccation and survival gradient for UV-C tolerance for each isolate. * denote archaea strains. † denote control strains. – denotes absence of data.*

| Isolate | Colour | Survival gradient for desiccation | % survival after desiccation | UV-C Survival gradient |
|---|---|---|---|---|
| *Idiomarina* sp. (LMLD01) | Cream | -3.18 | 0.00003 | -0.59 |
| *Planococcus* sp. (LMLD02) | Orange | -0.62 | 11.7 | -0.18 |
| *Salinicoccus* sp. (LMLD04) | Pink | -0.74 | 8.4 | -0.14 |
| *Bacillus* sp. (LMLD05) 9-10 | Cream | -1.60 | 0.16 | -0.39 |
| *Natronococcus* sp. (LMLD06)* | Pink | -0.83 | 8.2 | -0.02 |
| *Natrialba* sp. (LMLD07)* | Pink | -0.83 | 0.01 | - |
| *Escherichia coli* DH1 (Rec-)† | Cream | -2.15 | 0.01 | -1.06 |
| *Escherichia coli* W3110 (Rec+)† | Cream | -2.17 | 0.02 | -0.25 |
| *Deinococcus radiodurans*† | Pink | -0.74 | 2.2 | -0.12 |

Those strains plotting towards the upper right corner (more positive x and y axis values) of the graph have a higher desiccation resistance and higher UV-C tolerance (Figure 5.17). Those isolates plotting towards the lower left corner (more negative x and y axis values) have a lower desiccation resistance and lower UV-C tolerance. There is a strong positive (value >0.7 (Saha and Paul, 2010)) relationship between shallow desiccation gradient and shallow UV-C gradient with a correlation coefficient of 0.76. *Planococcus* sp. LMLD02, *Salinicoccus* sp. LMLD04, *Natronococcus* sp. LMLD06 and *D. radiodurans* cluster together and plotting to the right of the graph.

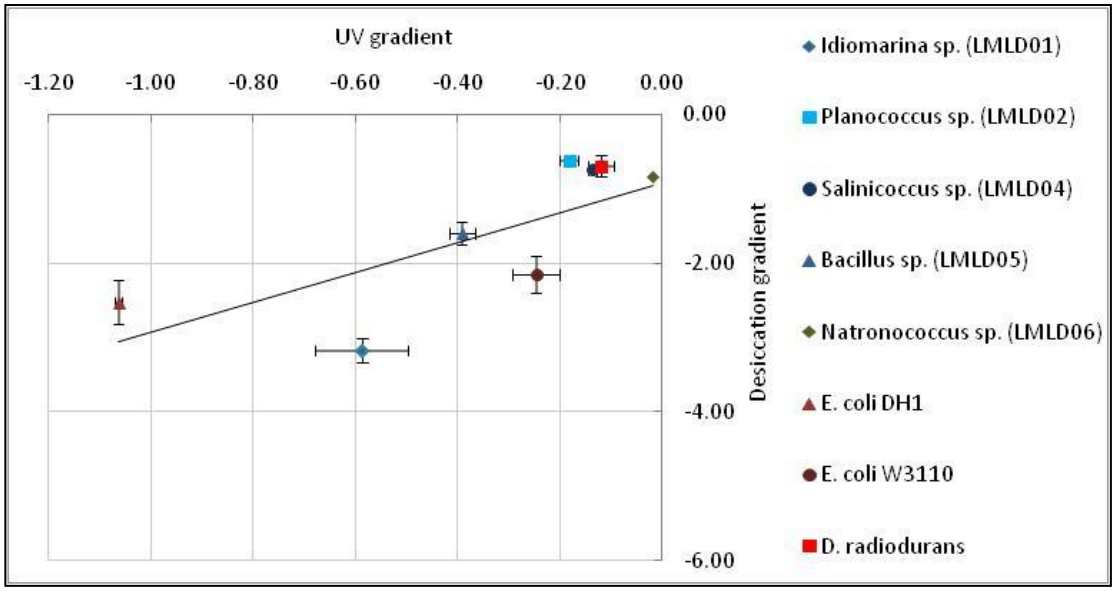



*Figure 5.17 Desiccation resistance gradient plotted against UV-C tolerance gradient. Bacterial strains are blue colours, archaea strains are green and control strains are red. Error bars are 1x standard error. Diagonal line is the line of best fit.*

**5.6. Discussion**

The focus of this study has been to assess the tolerance of seven novel strains isolated from the soda lake, Lake Magadi, to desiccation and UV-C radiation. The novel strains were compared to three control strains, two control strains of *E. coli* and one control strain of *D. radiodurans*. The desiccation survival data for nine strains was acquired, due to the absence of growth for *Caldalkalibacillus* sp. LMLD03 and for eight strains for UV-C tolerance due to the repeated uneven growth of *Natrialba* sp. LMLD07. The survival gradient for each of strain was obtained for the first 3 days of desiccation, and then between 3 and 28 days desiccation. This was judged necessary due to the apparent two stage rate of cell death observed in other studies. The UV-C survival tolerance gradient was produced using all the data points available. It was hypothesised that the novel isolates from lake Magadi would be more resistant than *E. coli*, but less resistant than *D. radiodurans* to desiccation and UV-C irradiation.

The resistance to desiccation and tolerance of UV-C (and IR) has been tested for many spore forming strains of *Bacillus*, such as *B. subtilis* and *B. odysseyii*. The resistance to these conditions has also been studied in strains *including D. radiodurans, E. coli, Micrococcus luteus, Pseudomonas fluorescens, Staphylococcus aureus, Halobacteria salinarum* and *Streptomyces* sp. (Arrage et al., 1993b; Setlow and Setlow, 1996; La Duc et al., 2004; Kottemann et al., 2005a; Chaibenjawong, 2011; Kerney and Schuerger, 2011; Dartnell et al., 2010; Osman et al., 2008). These strains have been isolated from a variety of environments, including subsurface soils, at depths of 150-500m, Antarctic Dry Valley soils, spacecraft assembly facilities, salt mines and from humans. Dartnell et al. (2010) demonstrated that the isolates from exposed, desiccating areas of the Antarctic Dry Valleys, were more resistant to

desiccation than the isolates from protected areas. This suggests that those isolates from naturally dry and relatively high levels of radiation would yield a variety of organisms which can demonstrate high levels of resistance, this would include desiccating, saline environments. Survival studies on organisms from saline/alkaline, soda environments are limited; the survival of haloarchaeon of the *Halobacteria salinarium*, isolated from a salt mine, showed it was able to survive desiccation, IR and vacuum, which is thought to be in part due to adaptation to the environment from which it was isolated (Kottemann et al., 2005b). The initial isolation and identification of strains which can survive such conditions it is then possible to explore what mechanisms they employ to survive.

### 5.6.1. Testing the desiccation and UV-C tolerance of novel bacteria and archaea from Lake Magadi

The desiccation survival fraction curves for all isolates demonstrated a reduction in the rate of cell death, generally following three days desiccation. This slowing down after an initial period of more rapid cell death has been seen in other studies on the effects of desiccation on survival, for example, in the response of the Antarctic dry valley isolates *Streptomyces* sp. and *Bacillus* sp. (Dartnell et al., 2010). A relationship between cell density and cell survival in relation to desiccation has been demonstrated with *Pseudomonas syringe*, where increase in inoculum density was shown to have a positive influence on the demonstrated resistance to desiccation (Wilson and Lindow, 1994; Monier and Lindow, 2005). It is possible, that in this study the density of cells in the sample was such that cell layering occurred as the sample desiccated, therefore cells on top provide protection to the cells beneath, explaining the slowing down in cell death observed in the survival curve of these samples. In order to explore this further the experiment would need to be repeated with different cell densities. This would confirm whether a positive correlation exists between cell survival and cell density.

The potential that a higher cell density can promote cell protection and enhance survival against both desiccation and UV would suggests that the presence of a soil matrix would also significantly enhance survival. Therefore, supporting the hypothesis that the subsurface of Mars would be the most likely place to look for evidence of life; providing an environment in which extant life is protected, or at least where evidence of life is protected from decay.

### 5.6.2. Rate of cell death during desiccation and UV-C radiation

*Idiomarina* sp. LMLD01 was the most sensitive strain tested in terms of desiccation and UV-C tolerance. Several strains of *Idiomarina* extracellular polysaccharides and work by Ophir and Gutnick demonstrated the role of extrapolysacchrides in the protection of cells from desiccation, with those organisms which form extrapolysacchrides more resistant to desiccation (Ophir and Gutnick, 1994). After incubation for several days at 37 °C, *Idiomarina* sp. LMLD01 would form a large muccoidal mass on the solid growth medium (see Chapter 3). With this in mind, *Idiomarina* sp. LMLD01 may have been expected to have survived

desiccation better than it did. However, it is possible that the growth period of 24h was not sufficient for this strain to produce enough extrapolysacchrides material for protection, by the time it was desiccated or irradiated. Further work on the survival of this organism following different incubation times is necessary for a more complete understanding its ability to produce extracellular polysaccharides (Chapter 3, section 3.1.2) and if there is benefit provided to *Idiomarina* sp. LMLD01 by its presence.

The absence of colonies of *Caldalkalibacillus* sp. LMLD03*,* even after 1 day desiccation may suggest that this strain maybe highly sensitive to the desiccation conditions imposed. Alternatively, it cannot be ruled out that this strain is forming spores as discussed in Chapter 3, and thereby surviving in this state, a common survival technique employed by *Bacilli.* The failure of the spore reanimation techniques tested to result in colony formation cannot be interpreted as evidence of complete cell death in this strain. It may instead indicate that this isolate requires different conditions for reanimation than those used in this study, and so *Caldalkalibacillus* sp. LMLD03, could potentially be surviving these stress factors in the form of a spore. Further work on the process of reversing spore formation in this strain is required for meaningful tolerance data to be obtained.

*Bacillus* sp. LMLD05 was most closely related to *Bacillus aurantiacus,* a spore forming strain of *Bacillus* (Section 5.4.2. and Chapter 3) (Borsodi et al., 2008). When considering the spore forming behaviour of *Caldalkalibacillus* sp. LMLD03 (See Chapter 4) and the absence of any colony forming units of *Caldalkalibacillus* sp. LMLD03 after 1 days desiccation, then an absence of *Bacillus* sp. LMLD05 colonies after incubation may have also been expected. Instead, there was a decline in the number of colony forming units until 3 days desiccation. It is possible that either these conditions are inducing spore formation for this strain, or simply these conditions are inducing a decline in the number of viable cells. The resistance to extreme conditions demonstrated by species of *Bacillus* is generally associated with the ability of members of this genus to form spores; however, this strain was not shown to produce spores. The comparable resistance of *Bacillus* sp. LMLD05 with both *E. coli* control strains was not expected as we had hypothesised that the strains from Lake Magadi would all be more tolerant of UV-C radiation and desiccation than the either of the *E. coli* control strains. This indicates that not all strains from saline, desiccating environments are necessarily better adapted to withstand extremes of these conditions, than organisms not isolated from such environments. *Bacillus* sp. LMLD05 and *Idiomarina* sp. LMLD01 the colonies of both isolates are cream coloured, and so lack a strong pigmentation, a factor which may contribute to their lower survival capacity when compared to the strongly pigmented strains.

The novel strains which demonstrated the lowest rate of cell death under desiccating conditions were *Planococcus* sp. LMLD02, *Salinicoccus* sp. LMLD04, *Natronococcus* sp.

LMLD06 and *Natrialba* sp. LMLD07 which had a rate of cell death which was not significantly different to *D. radiodurans,* indicating that these strains are able to survive the process of desiccation as well as the desiccation tolerant strain *D. radiodurans*. This data for archaea correlates with a study of *Natrialba magadii* ATCC 43099, which also demonstrated a high resistance to desiccation (Abrevaya et al., 2011). In turn, the slower decrease in viable cell count for these strains compared to *Bacillus* LMLD05*, Idiomarina* LMLD01 and the *E. coli* strains suggests that *Planococcus* sp. LMLD02, *Salinicoccus* sp. LMLD04, *Natronococcus* sp. LMLD06 and *Natrialba* sp. LMLD07 have superior protection mechanism/mechanisms against desiccation. These mechanisms are likely to involve protein protection, against the effects of desiccation and UVD radiation. Preservation and the continued function of proteins results in the retention of repair mechanisms which in turn allows for the repair of DNA damage, caused by desiccation (Chapter 1, section 1.5.3.3.). It is not clear what mechanisms these novel isolates employ which would require more in depth characterisation.

Of those strains tested for UV tolerance, *Bacillus* sp. LMLD04, *Idiomarina* sp. LMLD01 and *E. coli* DH1 also demonstrated the lowest tolerance. Conversely, *E. coli* W3110 demonstrated a rate of cell death which was not significantly different to the novel strains *Planococcus* sp. LMLD02 and *Salinicoccus* sp. LMLD05 or the control strain *D. radiodurans*. However, this measure does not take into account the highest dose which each strain can withstand. With the data available here, it is not possible to identify the highest UV dose *Planococcus* sp. LMLD02 can survive, or identify if that upper limit is greater than demonstrated by *E. coli* W3110. Despite a similar survival gradient *Salinicoccus* sp. LMLD04 could survive doses of at least 110 $Jm^{-2}$, in line with *D. radiodurans (*which *E. coli* could not*)*, and although *Planococcus* sp. LMLD02 cannot survive doses this high, it is possible that it could survive a higher dose than *E. coli* W3110 (somewhere between 31 $Jm^{-2}$ and 59 $Jm^{-2}$).

The archaeon *Natronococcus* sp. LMLD06 demonstrated a shallower survival gradient when irradiated with UV than any other strain tested, and was able to survive doses of at least 110 $Jm^{-2}$. Although no data was obtained in this study, a UV resistance study was conducted by *Abrevaya* et al. (2011) on halophilic archaea, including (as mentioned above) *Natrialba magadii*, this demonstrated that the UV resistances of *D. radiodurans* and *Natrialba magadii* were comparable up to a dose of 150 $Jm^{-2}$, above which *D. radiodurans* demonstrated a greater survival fraction than the archaea (Abrevaya et al., 2011), indicating that studies should be made of relatives of *Natrialba magadii*, as these may also exhibit a high resistance to UV.

The comparison of a moderately halophilic archaeon and an extremely halophilic archaeon by *Abrevaya* et al, (2011) suggested that there is a link between greater desiccation, UV resistance and extreme halophilic. This link would suggest that organisms, such as those isolated from Lake Magadi which are tolerant of higher salinities would be better adapted to

resist the detrimental factors assessed here, than organisms from a low salinity environment. The accumulation of KCl is a strategy which is employed by many halophiles to reduce the effects of osmotic pressure on cells, in a highly saline environment (Oren, 2001: Siddiqui and Thomas, 2008). The cells of some organisms, including *Halobacteria salinarium* contain intracellular salts such as; KCl, which have been noted to reduce the effects of oxidative free radicals, potentially providing a link between halophily/halotolerance and resistance to oxidation damage.

Although not all isolates demonstrated levels of resistance comparable to *D. radiodurans* the results of this chapter, plus evidence from other studies would suggest that halophilic and halotolerant archaea and bacteria should be the focus of more in depth astrobiological studies.

### 5.6.3. Correlation between desiccation and UV-C tolerance, and pigmentation

As mentioned earlier, the resistance exhibited by *Halobacteria salinarium* is thought to be fundamentally due to the hyper-saline environments from which it is isolated (Kottemann et al., 2005b). It is thought that this organism protects itself from DNA damage through the presence of red membrane proteins. Pigmentation is understood to be linked with UV-C resistance by absorbing the UV light and providing protection against free radicals (ROS) and cell membrane damage (Krinsky, 1979: Carbonneau et al., 1989: Arrage et al., 1993b: Warnecke et al., 2005: Grinstead and Lacey, 1973: Tian et al., 2009: Armstrong, 1997: Tian and Hua, 2010). Effective hydroxyl radical scavenging carotenoid pigments (such as bacterioruberin) have been identified in *Halobacteria salinarium* and other pigmented organisms from hypersaline environments. In addition, carotenoids (such as deinoxanthin) have been attributed to the resistance demonstrated by *D. radiodurans (Kottemann et al., 2005b: Tian et al., 2009).* Although pigmentation is not the sole protection mechanisms used by halophilic organisms it is an important factor (as mentioned in Chapter 1, section 1.5.3.).

The exact mechanisms by which these organisms protect themselves against oxidative stress are unclear. *Planococcus* sp. LMLD02, *Salinicoccus* sp. LMLD04, *Natronococcus* sp. LMLD06 and *Natrialba* sp. LMLD07 are all brightly pigmented strains (orange or pink). Further work would be required to identify the pigments which are present, and to ascertain exactly what mechanisms each of the novel strains employs to combat the oxidative stress experience during desiccation and UV-C irradiation. The results of this study do demonstrate a correlation between pigmentation, desiccation resistance and UV-C tolerance in these isolates. It is possible that pigmented organisms from naturally desiccating environments may be better adapted to deal with factors such as oxidative stress than unpigmented strains from these environments and therefore would make useful model organisms. However it should be noted that this is a relatively small sample size presented in this study means that the inclusion of more strains would make this result more robust. It is also unclear if these strains

would have superior survival ability in comparison to pigmented strains which have not been isolated from a saline/alkaline environment.

### 5.6.4. Implications for the presence of evidence for extinct/extant life on Mars

The ability to tolerate desiccation and levels of UV-C irradiation are key factors which could influence the search for and potential for life on Mars, which would experience both. Through the study of the resistance of strains from Lake Magadi we can further characterise the tolerance of alkaliphilic/alkalitolerant, halophilic/halotolerant strains.

Highly saline environments are often associated with high levels of desiccation, evaporative environments can also be highly alkaline as solutes concentrate. It is possible that similar situations have occurred on Mars and would potentially only be habitable for organisms with similar adaptations to those at Lake Magadi.

The results of this chapter would support the hypothesis that pigmented strains survive desiccation and UV-C irradiation better than non-pigmented strains, and promotes the concept that pigmented strains from soda lakes should be the focus of more survival studies and astrobiological analysis. In addition to the greater resistance of pigmented strains, the presence of strong pigments in these organisms could potentially make identification of their presence (extinct or extant evidence) easier. Pigments (such as bacterioruberin and deinoxanthin) will produce characteristic signatures which remote instruments such as the PanCam could detect; this will be discussed further in Chapter 7.

The next step following the assessment of the individual effects of desiccation and UVC radiation on novel strains from Lake Magadi is to evaluate the effects of the combined factors. This will be the focus of Chapter 6 where the response of one isolate and a complex soil community from Lake Magadi will be assessed.

## 5.7. Conclusions

This work increases the dataset available on the desiccation and UV-C tolerance of bacteria and archaea, in particular of organisms which are at least alkalitolerant and halotolerant.

Two novel strains from Lake Magadi, *Planococcus* sp. LMLD02 (Figure 5.5 and Figure 5.13) and *Salinicoccus* sp. LMLD04 (Figure 5.6 and Figure 5.16), pigmented isolates which vary in terms of pH tolerance and salinity tolerance, with broad NaCl range up to at least 15% NaCl survived desiccation and UV-C irradiation better than, or equally as well as, *D. radiodurans.*

In addition, the pink haloarchaeon *Natrialba* sp. LMLD07 demonstrated a resistance to desiccation which was comparable to *D. radiodurans*, and the pink haloarchaeon *Natronococcus* sp. LMLD06 could survive both desiccation and UV radiation (up 110 $Jm^{-2}$) to

a greater extent than *D. radiodurans* (Figure 5.8 and Figure 5.16) under these experimental conditions (See section 5.3.4, Table 5.4).

A correlation between UV-C and desiccation and pigmentation was identified, providing further supporting the concept that there are similarities in the repair mechanisms employed for desiccation and UV-C resistance and that pigmentation plays a role in resistance against oxidation stress.

The geology of Mars is indicating an increasing number of environments which were at one time neutral-alkaline in pH and/or saline. Terrestrial models for life on Mars should include resistant organisms which are resistant to desiccation and UV-C irradiation and could be tolerant of high salinities and varying pH, and would probably be pigmented. The analysis of samples from different environments aids with the identification of better analogue sites. The relationship between desiccation resistance, halophily and radiation tolerance would suggest that environments with high salinity are important analogue sites to study in relation to life on Mars. This is both in terms of understanding where and what to look for in the search for any evidence of native life on Mars, but also in relation to understanding the possible contamination of Mars by past and future missions.

**5.8. Further work**

This work only scratches the surface of what could be conducted using these strains. As mentioned earlier an overall increase in the dataset would be useful, providing more information of the survival of sorts of organisms from soda lake environments. This increase in sample size could include a higher number of the novel bacterial/archaeal strains isolated in Chapter 3 from Lake Magadi.

The comparison of species of the same genus isolated from non-soda environments would explore whether those species from soda lake environments have a superior tolerance to UV-C and desiccation.

All the strains in these experiments were kept in the dark as it was not possible to quantify the light intensity to which the cells could have been subjected to. Using a 1 hour dark incubation method meant that the survival gradient recorded for each strain was likely to be lower than if it was incubated in the light (Maloy, 1994), therefore this data could potentially represent the lower limit of UV-C tolerance in this strains. The repetition of this experiment, with incubation in quantifiable light intensity for 1 hour following irradiation would provide a contrasting set of data for each strain. This would allow a quantifiable comparison of the effectiveness of each mechanism against the other, for each strain.

Following on from the study of desiccation and UV-C tolerance, studies of the response of these strains to very low temperatures, varying pressures and IR would increase our

understanding of the individual effects of these stress factors on the types of organisms isolated from Lake Magadi.

Assessing the effects of the NaCl concentration of the growth medium on the resistance would be interesting, considering the connection between the NaCl concentration of the growth medium and pigmentation in species of *Planococcus* mentioned in Chapter 4. In addition, deviation from the optimal growth conditions of an isolate can have an effect upon resistance (Trigui et al., 2011).

## Chapter 6

# Response of a single isolate, and a complex soil community to simulated Martian conditions

This chapter assesses the effects of simulated Martian conditions on the novel isolate *Planococcus* sp. LMLD02 isolated in Chapter 3, and the complex soil community of sample 1A sampled from the shores of a lagoon at Lake Magadi and analysed in terms of its diversity in Chapter 3. A Mars simulation chamber can be considered simply as an extreme environmental stress chamber. These chambers are a method of assessing the combined effects of factors such as low temperature, freeze thaw cycles, UV-C irradiation, an anoxic atmosphere and hypobaria (low pressure) on a particular isolate or community. Culture based Mars chamber studies are an important method of understanding organisms and community responses to multiple environmental factors and it is necessary to access both the individual effects and combined effects of simulated Martian stresses on bacteria and archaea (Pogoda de la Vega et al., 2007). Mars chamber simulations are necessary to help define the potential habitability of certain environments on Mars, demonstrating the limits of life.

### 6.1. Mars Chamber studies

Mars chamber studies have increased in complexity since the 1950s, from simple anaerobic jars (Kooistra et al., 1958) to the more sophisticated chambers which allow the combined influence and adjustment of a variety of parameter including, low temperatures, freeze thaw cycles, hypobaria, UV-C and a reducing, $CO_2$ atmosphere (Olsson-Francis and Cockell, 2009). Previous work into Mars simulation experiments show that of these stress factors the most detrimental factors which can affect survival in these experiments are UV-C radiation, desiccation and freeze thaw cycling (Hansen et al., 2005: Hansen et al., 2009).

It is understood that subsurface communities would have protection against the direct effects of UV-C radiation; however, UV-C-generated reactive oxygen species may still be a problem for subsurface organisms. Hypobaria and desiccation can induce the unfolding of mRNA, double and single strand breaks, decreased enzyme affinity and an inability to maintain membrane fluidity (Diaz and Schulze-Makuch, 2006). Hypobaria has also been shown to inhibit cell growth and endospore germination in some species of *Bacillus* (Schuerger et al., 2006).

Cellular damage can also be caused by low temperatures, through the piercing of cell membrane by ice crystals. The process of freeze thawing is another factor which could be experienced on Mars, temperatures can cycle from well below zero to above zero in some equatorial areas resulting in freeze thawing cycling of at least the surface and near subsurface. The process of freeze thawing is a technique which is used in bacterial and

mammalian cell lysis (Ausubel, 2001) and is a potentially a significantly detrimental factor which could affect the survival of organism present in the soil surface/subsurface. Exposure to these conditions could result in a decrease in viability, unless the organisms have suitable mechanisms and/or the environment in which they placed provide sufficient protection against all the environmental factors.

Mars chamber studies have involved a variety of different analytical techniques to assess the effects of simulated conditions. Culture based analyses include assessing the survival fraction of cells before and after treatment, which will provide an indication of what proportion of the isolate or culturable community can survive incubation (Hansen et al., 2005). Colonies from community studies can subsequently be sequenced, in order to identify exactly what organisms have survived. These isolates can then be physiologically characterised in order to understand the methods they employ to survive such conditions.

Community-level physiological profiling (CLPP) can be achieved using Biolog$^{tm}$ Ecoplates, assessing changes in the metabolic activity of a soil community due to stresses applied. Differences in the metabolic activity following treatment can be identified, and therefore differences in carbon use patterns before and after incubation can be assessed. If the organisms in a sample respire a carbon source, such as glucose or tween 20, then a dye present in the well is reduced, which results in a colour change from colourless, to a shade of purple (Biolog, 2010). If the community has been affected by a treatment and therefore has changed after incubation, then changes in the pattern of carbon usage and degree of dye reduction (variation in the intensity of colour change) would be expected. This method can not only show differences in which carbon sources are utilised, but also provide a method of quantifying changes in diversity which may occur after a treatment (Chapter 1).

### 6.1.1. Single isolate survival studies

The individual study of isolated organisms provides an insight into the survivability of a particular organism and excludes any effects may be introduced by the presence of other isolates. Mars chamber studies have been conducted to assess the responses of single isolates to simulated conditions (individually and in combination), these have varied in terms of the way the isolates are prepared or expose, such as; distributing the isolate in a matrix of pre-sterilised soil (Hagen, 1967), by dispersing cells onto aluminium coupons (Mancinelli, 2000: Smith et al., 2009a) or by dispersing cells onto glass disks (Cockell, 2005). The use of a matrix such as soil provides an insight into the effects on an isolate in an ‘environment’, which may be considered as more realistic than simply exposing a strain in a liquid medium or after being dried onto a slide.

Studies to date have focussed on a variety of different bacteria including strains of *Bacillus* (in particular *B. subtilis)*, *D. radiodurans* and *E. coli*, as well as some organisms from hypersaline environments (Imshenetsky et al., 1973: Koike et al., 1992: Koike et al., 1996). Smith et al.

(2009a) studied the survival of *Psychrobacter cryohalolentis*, which was isolated from hypersaline cold temperature (-20 °C) environment. It was hypothesised that the organism would be particularly capable of withstanding low temperatures and desiccation due the conditions present in the sampling environment, but instead, the simulated conditions induced a decrease in the number of CFU. However, when the strain was placed into a medium/salt/matrix (MSM) and then subjected to UV-C, low temperatures, pressures and an anoxic atmosphere, an increase in survival was demonstrated. In contrast, other strains from hypersaline environments have demonstrated an ability to survive incubation under simulated Martian conditions believed to be in part due to adaptations to the hypersaline environment from which they were sampled (Johnson et al., 2011). The assessment of more bacteria from saline environments will contribute to our understanding of the effects of simulated Martian conditions in association with salinity.

In addition to studying isolates from the 'natural environment', Mars chamber studies have also been conducted on single strains isolated from spacecraft clean-rooms, such as *Serratia liquefaciens* (Berry et al., 2010). The protocols employed to sterilise space craft at present include dry heat treatments and vaporous hydrogen peroxide which may actually preferentially select for those organisms which are resistant to desiccation and radiation, therefore can cope with extreme protein and DNA damage. It is possible that these are the organisms which may have the greatest chance of surviving on the surface of Mars (Johnson et al., 2011). With this in mind, the question is what proportion of isolates from environments which are naturally subjected to oxidative stress can survive the combine stress factors which could be experienced on Mars.

### 6.1.2. Complex soil community survival studies

Fewer studies simulation studies have involved complex communities (Olsson-Francis and Cockell, 2009). Investigations have been conducted into the response of samples including permafrost (Hansen et al., 2009), soil (Hansen et al., 2005), basaltic lava (Cousins, 2010) and gypsum surface cracks from evaporite basin (Boston et al., 2009). These studies have demonstrated varying levels of community response to the simulated conditions, but have demonstrated that viable cell can be cultured from Mars chamber incubated samples (Hansen et al., 2005: Hansen et al., 2009). Considering the studies which demonstrate high survival rates for some single isolate from hypersaline environments, there is scope for work in relation to the response of communities which have been isolated from these environments.

The intention of this chapter is to firstly focus upon *Planococcus* sp. LMLD02 which due to the UV-C and desiccation resistance it displayed in Chapter 5, may be expected to also demonstrate a tolerance again the combined effects of sub-zero temperatures, desiccation, anoxic atmosphere and hypobaria. The response of a soil community studied in Chapter 3 to the combined factors of UV-C radiation, freeze thaw cycles, hypobaria anoxic atmosphere

and desiccation will then be assessed, with the hypothesis that this community will be able to tolerate these extreme conditions. The study of both a complex community and of a single isolate provides a more rounded study of saline alkaline environments as a potential location for the isolation of model organisms for astrobiological research.

### 6.2. Aims of this chapter

1. To investigate the survival of *Planococcus* sp. LMLD02 in a matrix of; JCS Mars-1 soil simulant, NaCl, $Na_2CO_3$ and $NaHCO_3$
2. To investigate the survival of *Planococcus* sp. LMLD02 under the multiple stress factors of temperature, hypobaria and an anoxic atmosphere
3. To investigate the survival fraction of soil community from a soda lake after incubation under the multiple stress factors of freeze thaw cycles, hypobaria, a $CO_2$ atmosphere and UV-C radiation

### 6.3. Methodology

#### 6.3.1. Single isolate Mars chamber experiment

*Planococcus* sp. LMLD02 was chosen for Mars chamber analysis because it was a relatively easy organism to grow, with colonies appearing after incubation for one day. It is halotolerant and alkaliphilic, being able to grow in up to 15% NaCl, at a pH of at least 6 and up to pH 10.3 and a temperature of at least 10 °C up to 40 °C, as described in Chapter 4. In addition, this strain was chosen due to its ability to survive desiccation and UV-C irradiation to a similar or greater extent than other strains tested in Chapter 5. It was also chosen due to its strong pigmentation, which could be of interest as a biomarker.

The effects of simulated Martian conditions on the survival of any species of *Planococcus,* or on the survival of a soda lake soil community have not been studied previously. *E. coli* DH1 was used as a control strain as in Chapter 5.

##### 6.3.1.1. Simulant preparation

The Martian regolith simulant JSC Mars-1, which is a <1 mm size fraction of a palagonitic tephra from the Pu'u Nene cinder cone, Hawaii was used as a suspension matrix (Allen et al., 1997). The JSC Mars-1 is considered a close spectral match to the bright regions of Mars (Allen et al., 1997) and was chosen because it was an available matrix, although it is recognised that the Mars has a variety of surface geology. This was combined with 6.8g NaCl, 2.72g of $Na_2CO_3$ and 0.57g of $NaHCO_3$, 90.67 ml of $H_2O$ per 100g of JSC Mars-1 simulant to create a salt, carbonate, simulant matrix (SCSM). This SCS matrix slurry was then dried in lyophilizer for 2 days. The drying of the SCSM would probably result in the formation of halite and sodium carbonate precipitates. The simulant was then autoclaved and stored on

the bench until used. The JSC Mars-1 simulant was chosen as a matrix for the cells as this is believed to be similar to the dust covering the surface of the planet.

#### 6.3.1.2. Isolate experimental setup

Three biological replicate overnight cultures of *Planococcus* sp. LMLD02 and *E. coli* DH1 were centrifuged for 10 minutes at 11000 rpm and removing the supernatant. TE buffer was added, gently vortexed to resuspend the cells, and centrifuged again for 10 minutes. The supernatant was again removed and a final 10 ml volume of TE buffer was added. The SCS matrix and suspended cells were combined in the proportions of 10 ml of TE buffer suspended cells, added to 10g of SCS matrix; this was dried in the lyophilizer overnight. The dried cell and SCS matrix was placed into sterile Nalgene containers 3 cm diameter by 4 cm depth and levelled off (Figure 6.1A). The survivability of each isolate was tested three fold, firstly survivability in the altered simulant, secondly the survival after drying in the altered simulant and finally survival after the Mars chamber treatment.

The Mars chamber sample triplicates were kept on ice until used. The samples were placed into the Mars chamber the $CO_2$ atmosphere pumped in and the chamber was pressurised. The chamber was placed into the freezer unit to maintain the temperautre at -40 °C (Figure 6.1.B). After 7 days incubation the samples were secured with fresh sterile lids and kept on ice until analysed. The samples were diluted up to $10x10^6$, both before and after treatment with *Planococcus* sp. LMLD02 plated onto 6.8% NaCl agar and *E. coli* DH1 onto NB2 agar.

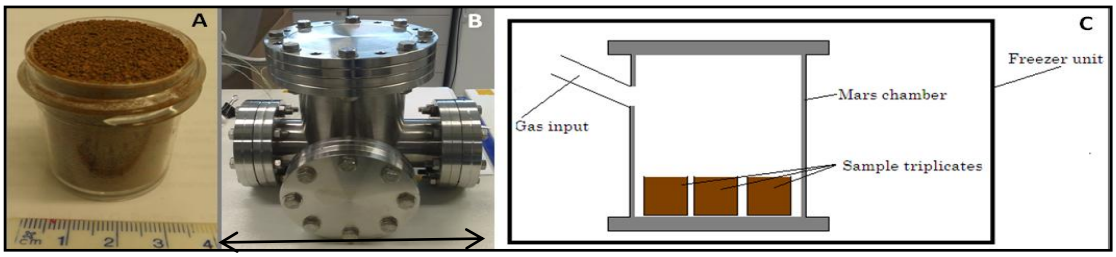



*Figure 6.1 A. Sample container containing Planococcus sp. LMLD02, and the SCS matrix. B. Mars chamber unit, scale bar represents ~30 cm C. Mars chamber experimental set up for Planococcus* sp*. LMLD02*

The *Planococcus* sp. LMLD02/SCS matrix samples were incubated for 7 days in the Mars chamber at a static temperature of -40°C, 6 mbar pressure and an anoxic atmosphere comprised predominantly of Carbon Dioxide, with 2.7% Nitrogen, 1.6% Argon, 0.13% Oxygen, and 0.07% Carbon Monoxide.

### 6.3.2. Complex soil sample Mars chamber experiment

The effect of simulated Martian conditions on the complex soil community present in soil sample 1A from Lake Magadi was studied. The lake waters nearby were measured at 32.9°C with a pH of 9.78 (Chapter 2); however the temperature of the soil was not

ascertained. As mentioned in Chapter 2, evaporation at Lake Magadi exceeds precipitation; the lake is recharged during the rainy season so that the lake levels will rise to a maximum and then decline through the year. This results in a band around the lake which is under water for some of the year, but dries out as the lake level drops. During years when the rainy season fails this region will remain dry for a prolonged period of time and will subject the organism present to periods of desiccation.

#### 6.3.2.1. Soil sample preparation

The soil used, soil sample 1A was mixed thoroughly by using a vortex at high speed for 30 minutes to homogenise the sample. A subsample of the untreated soil 1A was removed as an untreated soil sample 1A control (Figure 6.2.a). The rest of the soil was dried in a lyophilizer overnight and the soil sample 1A was again mixed to homogenise. A subsample of dried soil sample 1A was then removed and analysed as a dried soil sample 1A control replicates (Figure 6.2.b). The bench control replicates (Figure 6.2.c) and Mars chamber replicates (Figure 6.2.d) were also taken from this dried soil and then subjected to the treatments described.

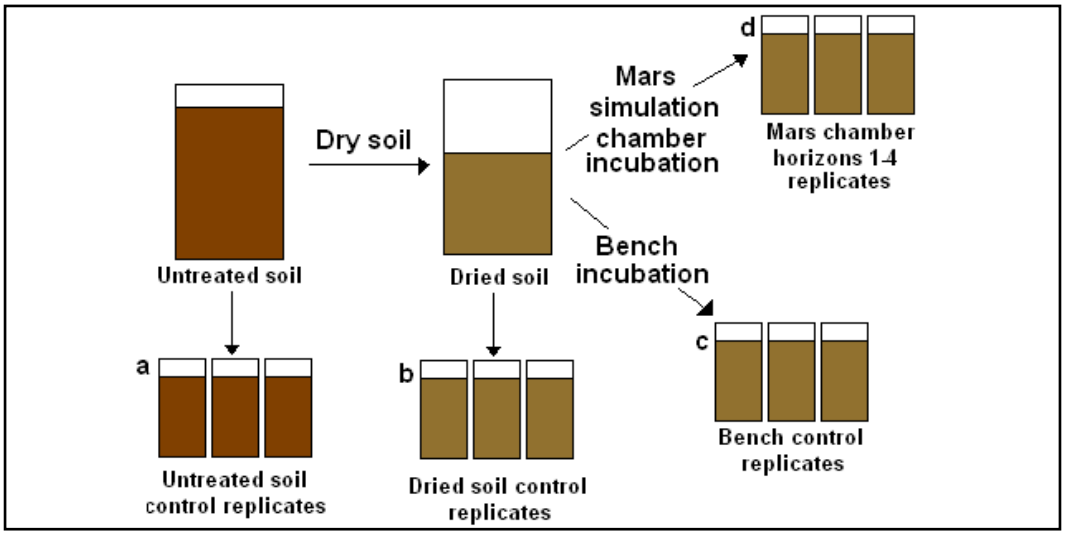



*Figure 6.2. Soil sample preparation*

#### 6.3.2.2. Soil community experimental setup

The prepared Mars chamber soil sample 1A (at the point this is simply the dried soil sample 1A from section 6.2.2.1) was placed into sterilised Nalgene containers, 3cm wide and 4cm deep and the top flattened off. Three samples were placed into the Mars chamber, the final control samples were left with loosened lids on the bench for 7 days as a soil sample 1A bench control.

The Mars chamber soil sample 1A triplicates were placed in an aluminium box wrapped in an insulating shroud of MLI, comprised of kapton, a polyimide film used as an insulator under high vacuum, and aluminium (Figure 6.3).

The Mars chamber soil samples were sub-sampled as 4 horizons, horizon 1 was 0-6mm, Horizon 2 was 6-12mm, horizon 3 was 12-22mm and horizon 4 was 22 to 32mm depth. These depths were chosen as a depth of 6mm was the shallowest depth of soil which provided sufficient material for Biolog™ Ecoplate analysis.

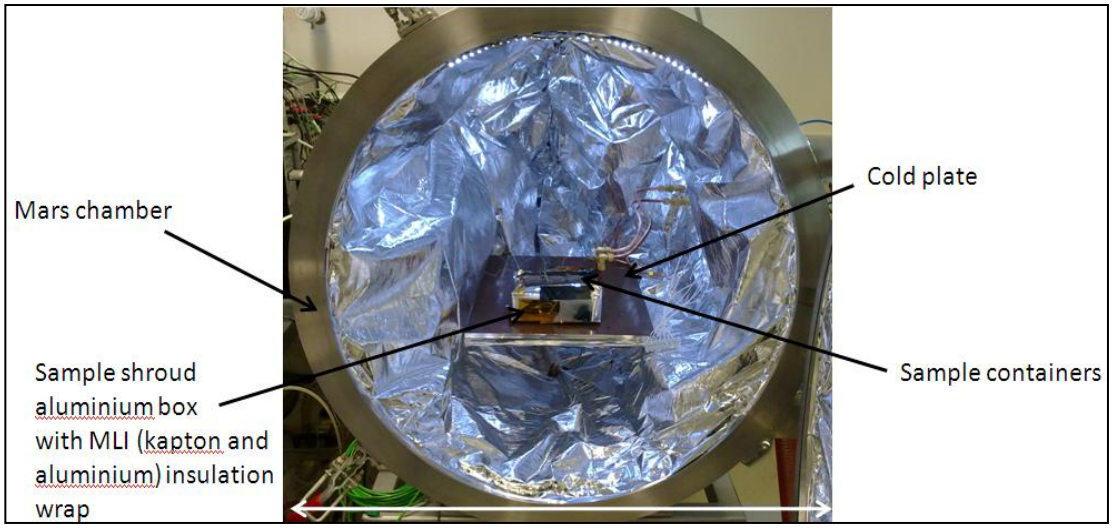



*Figure 6.3 Mars chamber set up for complex soil community experiment. Scale bar =1.5 m.*

#### 6.3.2.3. Complex community Mars simulation conditions

The samples were subjected to freeze thaw cycles between around +18 °C and -67 °C, using liquid nitrogen to cool the vessel. Once the liquid nitrogen ran out, the chamber was simply allowed to return to room temperature. Pressure was maintained at 6 mbar and the same anoxic atmospheric gas composition outlined in section 6.2.5.5.1. A 450W Xenon-arc lamp emitting >350nm wavelength was used to irradiated the sample with UV-C radiation. The samples were irradiated with UV-C radiation for one hour a day equating to the UV-C dose for one Martian sol.

Unlike other Mars chamber studies there was no temperature gradient through the soil sample. The shroud of copper foil was used to conduct the change in temperature throughout the whole sample so that the possible secondary effects of UV-C into lower layers could be studied with only the limited effect of a temperature gradient.

#### 6.3.2.4. Sample preparation

100 µl of each soil sample was suspended in PBS and 100 µl was plated onto each growth medium.

#### 6.3.2.5. Community level physiological profiling (CLPP) using Biolog™ Ecoplates

The principle of the Biolog™ Ecoplate in this study is to assess the metabolic activity of the soil community before and after incubation. Each plate contains 96 wells which contain

31 individual carbon sources, plus a water control well (in triplicate) (Figure 6.4). The presence of metabolic activity results in the reduction of the yellow tetrazole dye present in the well, into violet formazan (Garland, 1997) (Chapter 1, section 1.6.1).

2g of untreated soil or 1.5g of dried soil was added to 5ml of 6.8% (w/v) NaCl and pH 10 buffer, with ten 5 mm glass beads which was then vortexed for 2 minutes. This buffer was chosen to mimic the pH from the environment predominantly directed by the carbonate in the system and a salt concentration which may be utilised by halotolerant and some halophilic organism. The slurry was centrifuged for 5 minutes at 43000 rpm. 100 µl of the supernatant was added to each well. The plate was wrapped in Parafilm sealing film and incubated at 37 °C for 18 days. One plate was used per sample triplicate (total of 3 plates per treatment).

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A1 Water | A2 β-Methyl-D-Glucoside | A3 D-Galactonic Acid γ-Lactone | A4 L-Arginine | A1 Water | A2 β-Methyl-D-Glucoside | A3 D-Galactonic Acid γ-Lactone | A4 L-Arginine | A1 Water | A2 β-Methyl-D-Glucoside | A3 D-Galactonic Acid γ-Lactone | A4 L-Arginine |
| B1 Pyruvic Acid Methyl Ester | B2 D-Xylose | B3 D-Galacturonic Acid | B4 L-Asparagine | B1 Pyruvic Acid Methyl Ester | B2 D-Xylose | B3 D-Galacturonic Acid | B4 L-Asparagine | B1 Pyruvic Acid Methyl Ester | B2 D-Xylose | B3 D-Galacturonic Acid | B4 L-Asparagine |
| C1 Tween 40 | C2 i-Erythritol | C3 2-Hydroxy Benzoic Acid | C4 L-Phenylalanine | C1 Tween 40 | C2 i-Erythritol | C3 2-Hydroxy Benzoic Acid | C4 L-Phenylalanine | C1 Tween 40 | C2 i-Erythritol | C3 2-Hydroxy Benzoic Acid | C4 L-Phenylalanine |
| D1 Tween 80 | D2 D-Mannitol | D3 4-Hydroxy Benzoic Acid | D4 L-Serine | D1 Tween 80 | D2 D-Mannitol | D3 4-Hydroxy Benzoic Acid | D4 L-Serine | D1 Tween 80 | D2 D-Mannitol | D3 4-Hydroxy Benzoic Acid | D4 L-Serine |
| E1 α-Cyclodextrin | E2 N-Acetyl-D-Glucosamine | E3 γ-Hydroxybutyric Acid | E4 L-Threonine | E1 α-Cyclodextrin | E2 N-Acetyl-D-Glucosamine | E3 γ-Hydroxybutyric Acid | E4 L-Threonine | E1 α-Cyclodextrin | E2 N-Acetyl-D-Glucosamine | E3 γ-Hydroxybutyric Acid | E4 L-Threonine |
| F1 Glycogen | F2 D-Glucosaminic Acid | F3 Itaconic Acid | F4 Glycyl-L-Glutamic Acid | F1 Glycogen | F2 D-Glucosaminic Acid | F3 Itaconic Acid | F4 Glycyl-L-Glutamic Acid | F1 Glycogen | F2 D-Glucosaminic Acid | F3 Itaconic Acid | F4 Glycyl-L-Glutamic Acid |
| G1 D-Cellobiose | G2 Glucose-1-Phosphate | G3 α-Ketobutyric Acid | G4 Phenylethyl-amine | G1 D-Cellobiose | G2 Glucose-1-Phosphate | G3 α-Ketobutyric Acid | G4 Phenylethyl-amine | G1 D-Cellobiose | G2 Glucose-1-Phosphate | G3 α-Ketobutyric Acid | G4 Phenylethyl-amine |
| H1 α-D-Lactose | H2 D,L-α-Glycerol Phosphate | H3 D-Malic Acid | H4 Putrescine | H1 α-D-Lactose | H2 D,L-α-Glycerol Phosphate | H3 D-Malic Acid | H4 Putrescine | H1 α-D-Lactose | H2 D,L-α-Glycerol Phosphate | H3 D-Malic Acid | H4 Putrescine |

*Figure 6.4. Biolog™ Ecoplate carbon source well key (Biolog, 2010).*

Two methods can be used to acquire the data from Biolog™ Ecoplates, either by using a plate OD reader, or by manually scoring the presence or absence of colour change in wells. The OD readings at 590nm were taken for each plate after 5, 7 14 and 18 days the plates was read at 590nm on a FLUOstar OPTIMA - Fluorescence Plate Reader (Weinbauer and Hofle, 2001). The colour development for each well can be determined by subtracting the OD for the water negative control, from the OD for each well divided by the number of substrates (Choi and Dobbs, 1999).

The method of manually binary scoring well colour change produces a Biolog™ Score for each treatment by allocating a score of 1 to a well where colour change occurs (Shah and Belozerova, 2009) and 0 to a well where no colour change occurs. This Biolog™ Plate score can be calculated for each triplicate. The Biolog™ Score is calculated by averaging the

Biolog$^{TM}$ plate score for each treatment to provide a standard error for each carbon source across the biological and experimental replicates. Each carbon type can score a maximum of 1 for each treatment.

### 6.3.3. Survival fraction

The survival fraction was calculated as described in Chapter 2, section 2.3.1.8. In this chapter, the survival fraction for the dried soil replicates were calculated from the untreated soil replicates CFU. The survival fractions for the bench control replicates and the Mars simulation chamber sample replicates were calculated from the dried soil CFU, as the dried soil was what was used for these samples (See this chapter, section 6.2.2.1).

### 6.3.4. Statistical tests

Where conducted, student's t-Tests were calculated using Microsoft Excel assuming two-tailed distribution with unequal variance. ANOVA statistical tests were conducted as outlined in Chapter 2, section 2.4.2. The results were considered as significant when $p<0.05$, when using either statistical test.

## 6.4. Results

### 6.4.1. Response of *Planococcus* sp. LMLD02 to simulated Martian conditions

The initial studies showed that the introduction of *Planococcus* sp. LMLD02 cells into a slurry of the SCSM had no significant effect on the number of CFU after 7 days incubation on the bench, $p=0.316$ (Figure 6.6). Drying the cells in the SCS matrix appeared to have had a small effect on the cell count, however this again was not significant, $p=0.949$. Finally, the incubation of *Planococcus* sp. LMLD02 in the SCS matrix, under the simulated Martian conditions chosen in this study for 7 days demonstrated a slight increase in the number of CFU cells per ml, however this was not significantly different $p=0.244$.

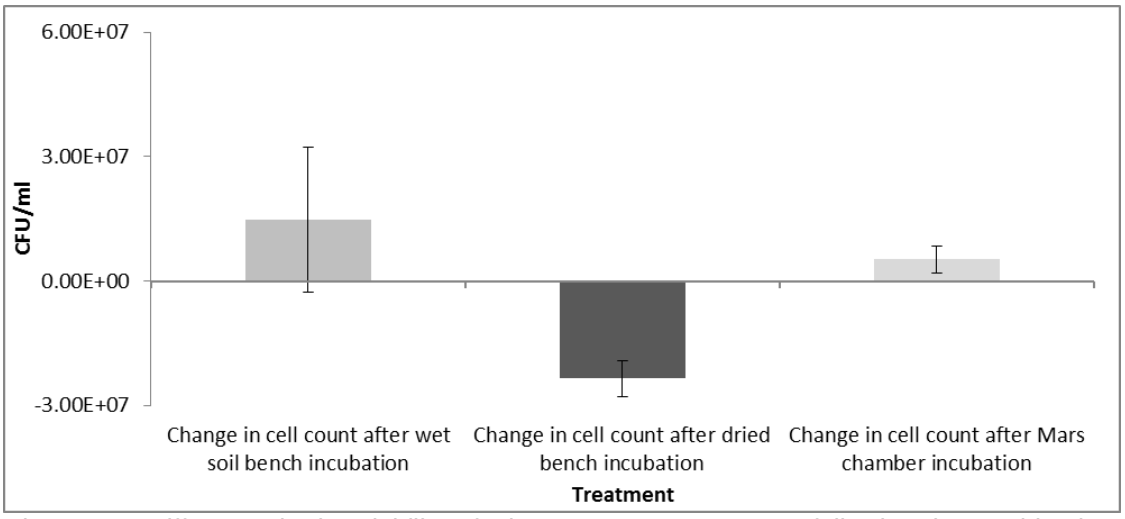



*Figure 6.5. Difference in the viability of Planococcus sp. LMLD02 following; its combination with the SCS matrix, its combination with the SCS matrix drying cell/SCS matrix and*

*incubation on the bench for 7 days and its combination with the SCS matrix drying cell/SCS matrix and incubation under simulated Martian conditions for 7 days.*

### 6.4.2. Response of *E. coli* DH1 to simulated Martian conditions

The process of lyophilising introduced airborne contaminants to the sample which could easily grow on the nutrient agar. These contaminants were not present on the AP+ plates used for *Planococcus* sp. LMLD02, most likely due to the high salinity and pH of this growth medium. The problem of contamination on the negative control plates could not be avoided, and for this reason no further studies of *E. coli* DH1 as a control could be made in this study.

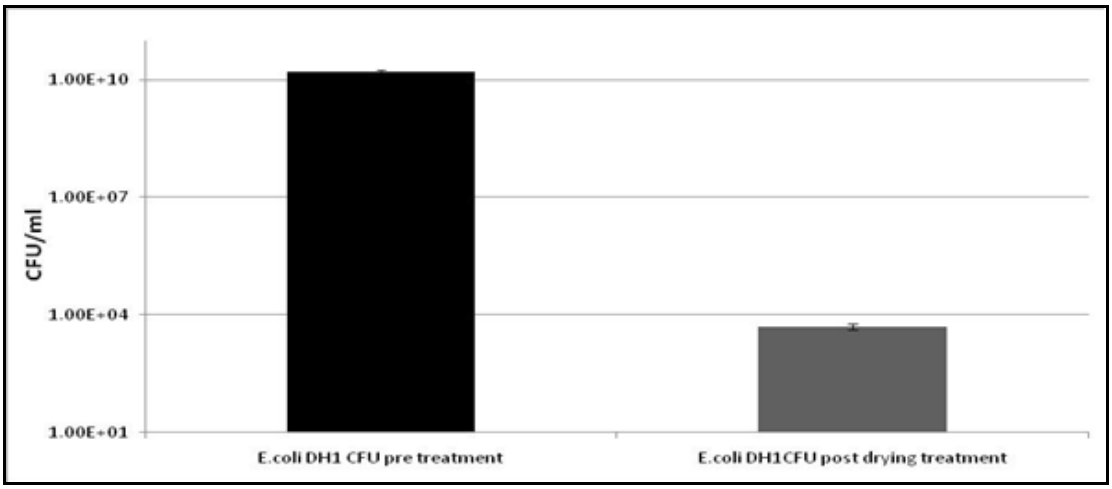



*Figure 6.6. Number of viable cells calculated for E. coli DH1, before and after drying treatment. Error bars are 1 x standard error.*

### 6.4.3. Response of complex soil community under simulated Martian conditions

#### 6.4.3.1. Temperature profile of Mars chamber

The original temperature cycling between -40 °C and around +20 °C was not achievable with this experimental setup. The method of cooling the chamber with liquid nitrogen resulted in the lowest temperature averaging at -51.64 °C (± 0.6 °C), reaching as low as -67.25 °C (Figure 6.7). Fluctuations in temperature occurred below 0 °C averagely between -26.19 °C (± 0.3 °C) and -51.64 °C (± 0.6 °C), with the highest temperature peak below 0 °C at -14.97 °C. The chamber was below 0 °C for a total of 132 hours.

The processes of bringing the chamber up to room temperature relied upon running out of the liquid nitrogen, so the chamber was simply no longer being cooled rather than the chamber being actively warmed up. The aluminium box with MLI insulation wrapped (Section 6.2.2.2.) ensured that the three samples were equally insulated and the temperature of the plate was conducted through the samples themselves. The chamber went above 0 °C a total of 8 times including the initial cooling and final bringing up to temperature spending 3.73 hours above 0 °C. The temperatures above 0 °C during the cycling process were averagely up to +9.73 °C,

with the highest temperature recorded at +18.03 °C and the lowest temperature above 0 °C recorded at +2.5 °C. The conditions provided freeze thaw cycling and cycling below zero which organism may experience on the surface or shallow subsurface of Mars.

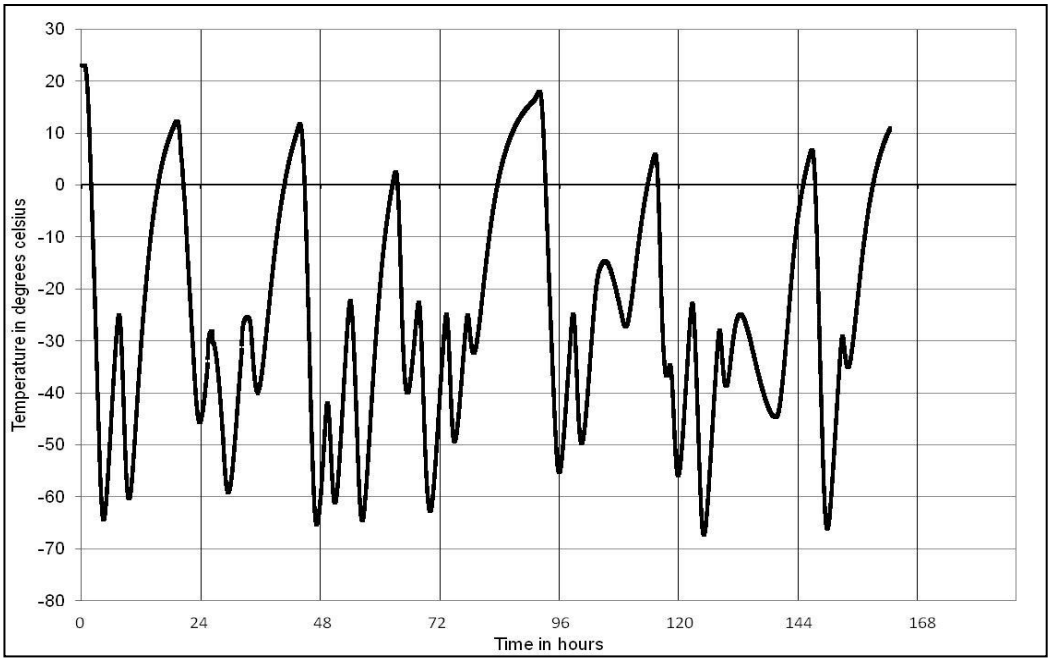



*Figure 6.7. Temperature profile for Mars chamber over 7 day (168 hour) period, showing cycling down to as low as -67.25 °C and up to 18.03 °C.*

**6.4.3.2. Survivability of complex soil community**

A comparable number of viable, culturable cells were calculated for the untreated soil sample 1A when inoculated onto the AP+0.7% NaCl agar and AP+6.8% NaCl agar, with $6.6x10^4$ CFU/ml and $6.7x10^4$ CFU/ml respectively. A culturable viable cell count an order of magnitude lower was calculated when the soil sample 1A was inoculated on the AP+15% NaCl medium (Table 6.1).

Colony morphology did not vary significantly following the different treatments. The colonies which formed on the AP+15% NaCl medium were generally smaller than on the other growth media, not developing colonies any bigger than 5mm. Colonies on all growth media, following any treatment were generally yellow, cream or whitish, with a few brown colonies present (Figure 6.8). There did not appear to be any clear decrease in colony size when comparing before and after treatment.

*Table 6.1 Colony description and cell density calculated for each growth medium and each treatment.*

| Growth medium | Treatment | Colony shape | Cell density (CFU/ml) | Diameter (mm) | Colony colour | Figure |
|---|---|---|---|---|---|---|
| AP+0.7% | Untreated | Round | $6.6x10^4$ | 3-150 | Mostly yellow or cream, with some colonies orange/brown, transparent or opaque | 6.8.A.1 |
| AP+6.8% | | | $6.7x10^4$ | 2-4 | | 6.8.A.2 |
| AP+15% | | | $8.77x10^3$ | 1-3 | | 6.8.A.2 |
| AP+0.7% | Dried | Round | $1.06x10^4$ | 3-150 | Yellow or cream | 6.8.B.1 |
| AP+6.8% | | | $6.2x10^3$ | 1-4 | Mostly, white or cream and translucent, some dark brown colonies | 6.8.B.2 |
| AP+15% | | | $3.0x10^3$ | 1-3 | Cream or yellow | 6.8.B.3 |
| AP+0.7% | Bench control | Round | $1.37x10^3$ | 2-10 | Colourless or light yellow | 6.9.C.1 |
| AP+6.8% | | | $2.42x10^3$ | 2-5 | | 6.9.C.2 |
| AP+15% | | | $4x10^2$ | <5 | | 6.9.C.3 |
| AP+0.7% | Mars Chamber | Round | - | 2-20 | Yellow, with a few brown colonies | 6.10 |
| AP+6.8% | | | - | 2-15 | Light yellow or cream with a few brown colonies | 6.10 |
| AP+15% | | | - | 1-2 | Cream or white | 6.10 |

Figure 6.9.shows the morphology of the colonies which formed on AP+6.8% medium when inoculated with; the untreated soil sample 1A (Figure 6.8.A.1), the dried soil sample 1A (Figure 6.6.A.2) and the Mars chamber horizons 1 to 4 soil sample 1A (Figure 6.9.B1-4). The colonies which formed on the AP+6.8% NaCl medium were generally cream or light yellow in colour, with a couple of brown colonies. The colony morphology was not appear to differ on the AP+6.8% medium when comparing the horizons of the Mars chamber soil sample 1A, but these horizons did have fewer colonies than the dried soil sample 1A (Figure 6.6.A.2 and B1-4). The AP+15% medium inoculated with Mars chamber soil sample 1A were cream/white coloured, with no strongly pigmented colonies.

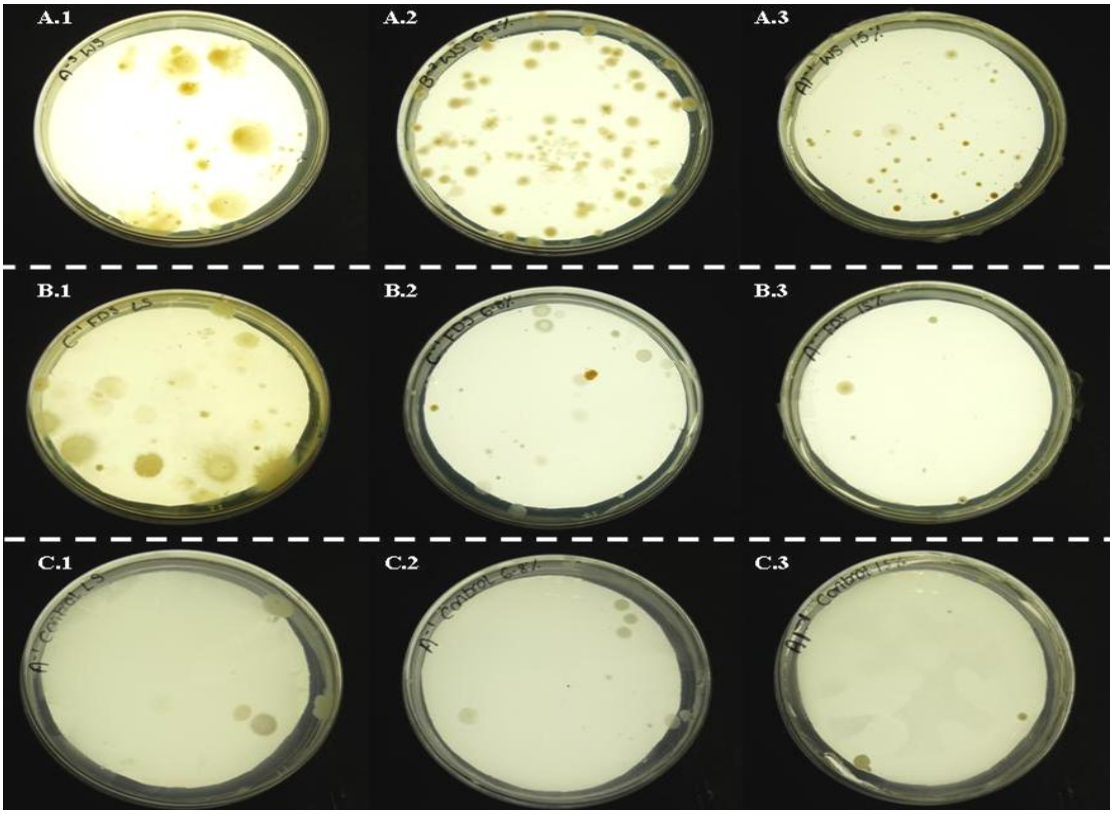



*Figure 6.8* ***(A)*** *Untreated soil sample 1A on;* ***(A.1)*** *AP+0.7% (LS) medium $10^3$ dilution,* ***(A.2)*** *AP+6.8% medium $10^2$ dilution,* ***(A.3)*** *AP+15% medium $10^1$ dilution.* ***(B)*** *Dried soil sample 1A control soil (FDS) all $10^1$ dilution, on;* ***(B.1)*** *AP+0.7% medium,* ***(B.2)*** *AP+6.8% medium,* ***(B.3)*** *AP+15% medium.* ***(C)*** *Bench soil sample 1A control soil, all $10^1$ dilution on;* ***(C.1)*** *AP+0.7% medium,* ***(C.2)*** *AP+6.8% medium,* ***(C.3)*** *AP+15% medium.*

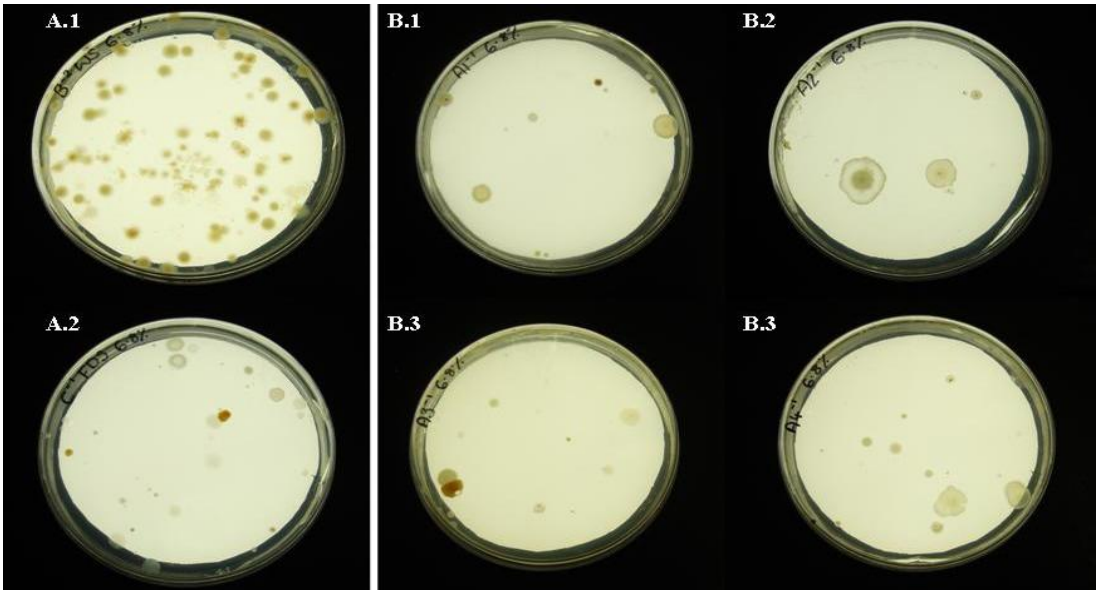



*Figure 6.9.* ***(A)*** *Untreated soil sample 1A on;* ***(A.1)*** *AP+6.8% medium,* ***(A.2)*** *Dried soil sample 1A control on AP+6.8% medium.* ***(B)*** *Mars chamber soil sample 1A Horizons* ***(B1-4)*** *correspond to horizons 1 to 4 on AP+6.8% medium.*

#### 6.4.3.2.1. Survival fraction of CFU on AP+0.7% medium solid medium

Using ANOVA and Tukey Post-hoc test (Chapter 2, section 2.4.2), the survival fraction of cells able to grow on 0.7% (w/v) NaCl agar plates indicated a significant decrease ($p$<0.05 was considered significant, raw data see appendix J) in the survival fraction after drying, when compared to the untreated soil sample 1A, $p$=0.0027 (Figure 6.10.A). A significant decrease in the survival fraction of cells cultured on the 0.7% NaCl medium for the bench soil sample 1A control was observed when compared to the dried soil sample 1A control on the same growth medium, $p$=2.2x10$^4$ (Figure 6.10.B).

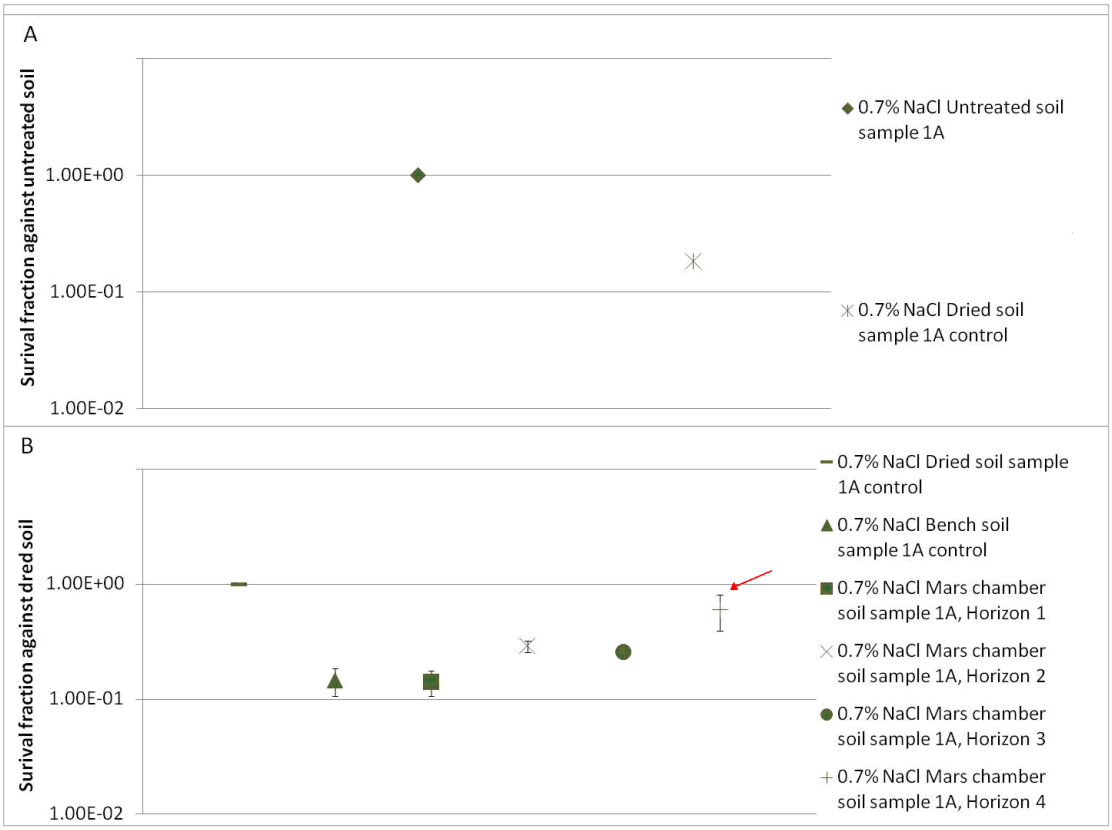



*Figure 6.10. Survival fraction for culturable community on AP+0.7% NaCl medium* ***(A)*** *Survival fraction (calculated from the untreated soil sample 1A culturable cells per ml) of culturable soil community for the untreated soil sample 1A and dried soil sample 1A control.* ***(B)*** *Survival fraction (calculated from the dried soil sample 1A control culturable cells per ml) of dried soil sample 1A control, bench soil sample 1A control and for four depths in the Mars chamber soil sample 1A replicates. Arrow highlights point with no significant difference to the dried soil control using ANOVA and Tukey Post-hoc test (Chapter 2, section 2.4.2). A p<0.05 was considered as significant. Error bars are 1 x standard error.*

There was also a significant decline in survival fraction between the dried soil sample 1A control and the Mars chamber soil sample 1A, Horizons 1, 2 and 3, $p$<0.001 (Figure 6.11.A). In contrast, there was no significant difference between the survival fraction of the cells able to grow on 0.7% NaCl in the dried soil sample 1A control, and the Mars chamber soil sample 1A, Horizon 4, $p$=0.066. There was no significant difference between the survival fractions of

the bench soil sample 1A control, compared to the Mars chamber soil sample 1A, Horizons 1 to 3, $p$>0.855, however the survival fraction for Mars chamber soil sample 1A, Horizons 4 was significantly higher than the bench soil sample 1A control, $p$=0.034.

#### 6.4.3.2.2. Survival fraction of CFU on AP+6.8% medium solid medium

As with the culturable cell count on AP+0.7% medium there was a significant decrease in the survival fraction (calculated as described in Chapter 6, section 6.3.3.2.1) of organisms able to grow on AP+6.8% medium for the dried soil compared to the wet soil colony count, $p$=0.002 (Figure 6.11).

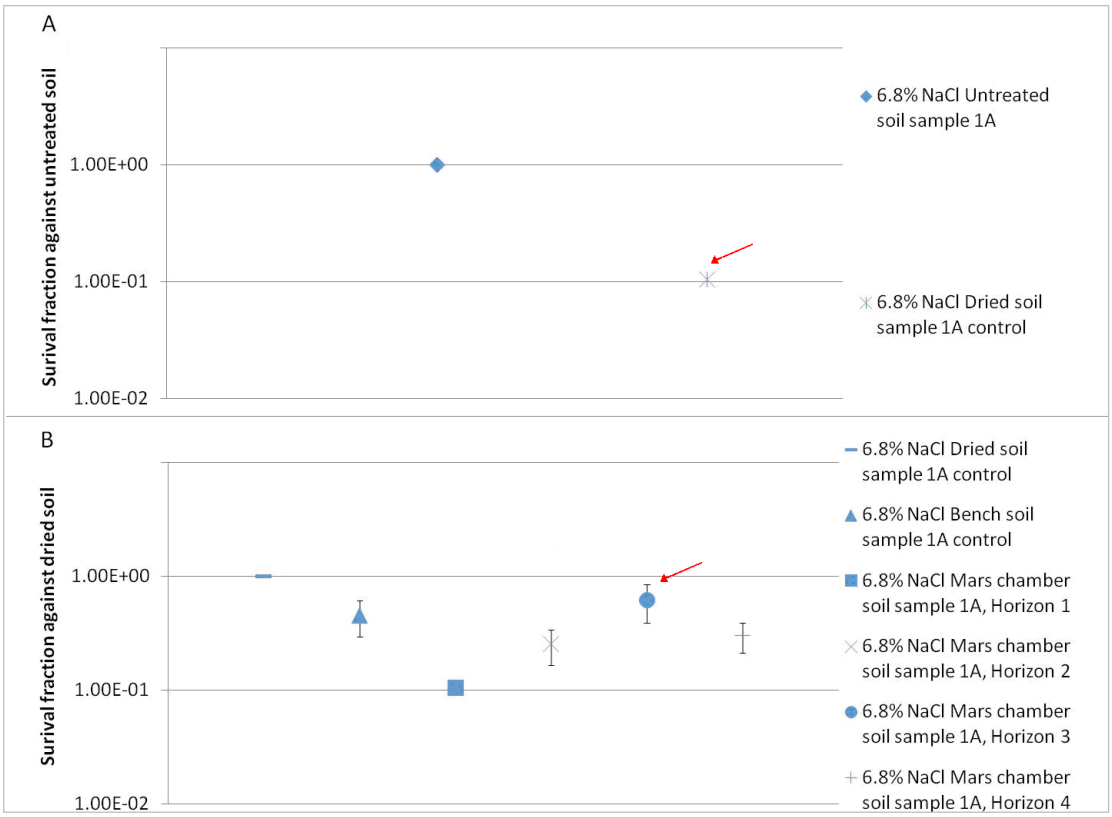



*Figure 6.11. Survival fraction for culturable community on AP+6.8% NaCl medium* ***(A)*** *Survival fraction (calculated from the untreated soil sample 1A culturable cells per ml) of culturable soil community for the untreated soil sample 1A and dried soil sample 1A control.* ***(B)*** *Survival fraction (calculated from the dried soil sample 1A control culturable cells per ml) of dried soil sample 1A control, bench soil sample 1A control and for four depths in the Mars chamber soil sample 1A replicates. Arrows highlight points with no significant difference to the base control (A. wet soil or B. dried soil control) using ANOVA and Tukey Post-hoc test (Chapter 2, section 2.4.2). A p<0.05 was considered as significant. Error bars are 1 x standard error.*

There was no significant difference in the survival fraction calculated for the dried soil sample 1A control when compared to that calculated for the bench soil sample 1A control on AP+6.8% medium, $p$=0.082. There was a significant difference between the survival fraction

calculated for dried soil sample 1A control and the Mars chamber soil sample 1A replicates for Horizons 1, 2 and 4, $p>0.019$. Only the survival fraction calculated for the Mars chamber soil sample 1A, Horizon 3 was not significantly lower than the dried soil sample 1A control, $p=0.328$, although it was not statistically different from the bench soil sample 1A control, $p=0.935$ (Figure 6.11). There was no significant difference between the survival fraction calculated for any of the Mars chamber horizons and the bench soil sample 1A control, $p>0.098$.

#### 6.4.3.2.3. Survival fraction of CFU on AP+15% medium solid medium

There was a significant drop in the survival fraction calculated from the AP+15% medium colony count, for the dried soil sample 1A control, $p=0.00006$. There was then no significant change in survival fraction calculated from the AP+15% medium colony count after the Mars chamber treatment, or after the bench incubation, when compared to the survival fraction calculated for the dried soil sample 1A control, $p>0.081$.

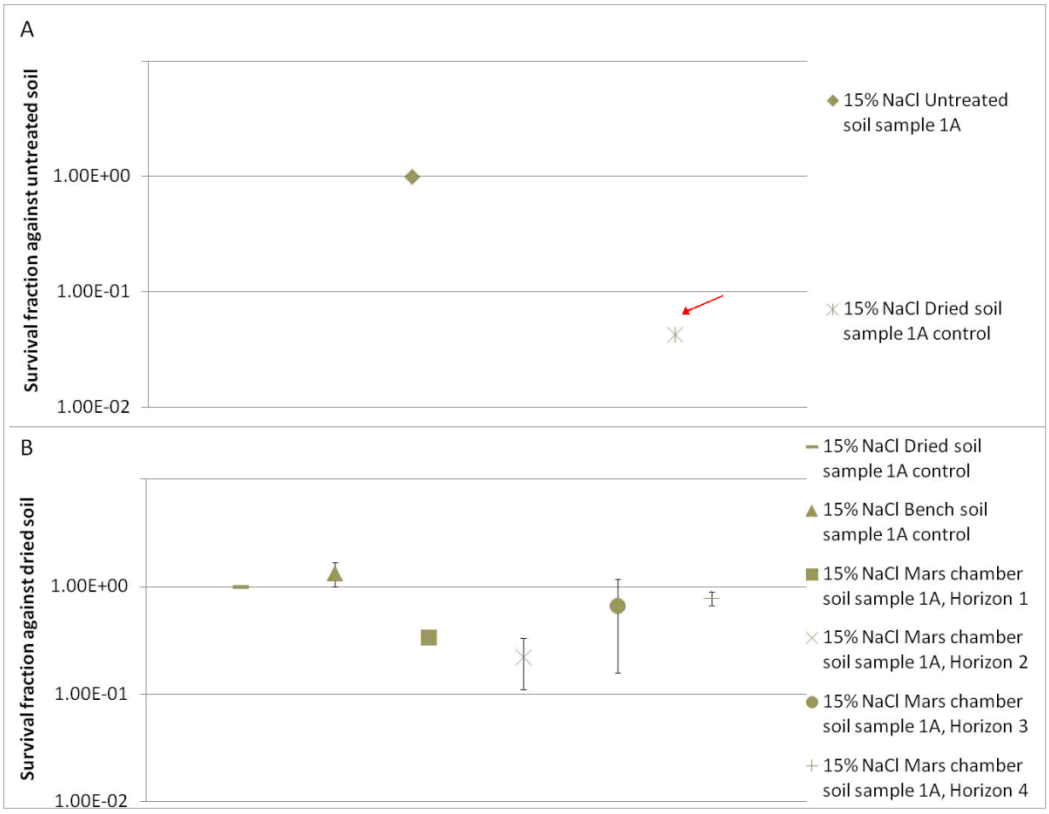



*Figure 6.12. Survival fraction for culturable community on AP+15% NaCl medium* ***(A)*** *Survival fraction (calculated from the untreated soil sample 1A culturable cells per ml) of culturable soil community for the untreated soil sample 1A and dried soil sample 1A control.* ***(B)*** *Survival fraction (calculated from the dried soil sample 1A control culturable cells per ml) of dried soil sample 1A control, bench soil sample 1A control and for four depths in the Mars chamber soil sample 1A replicates. Arrows highlight points with no significant difference to the wet soil control using ANOVA and Tukey Post-hoc test (Chapter 2, section 2.4.2). A p<0.05 was considered as significant. Error bars are 1 x standard error.*

#### 6.4.3.3. Biolog$^{TM}$ Ecoplate analysis

##### 6.4.3.3.1. Carbon usage of soil community before and after each treatment

Biolog$^{TM}$ Ecoplates were used to ascertain any changes in types of carbon sources metabolised by the culturable community after each treatment compared to the untreated soil sample 1A community (Figure. 6.16). See section 6.2.2.5, Figure 6.4 for carbon source map.

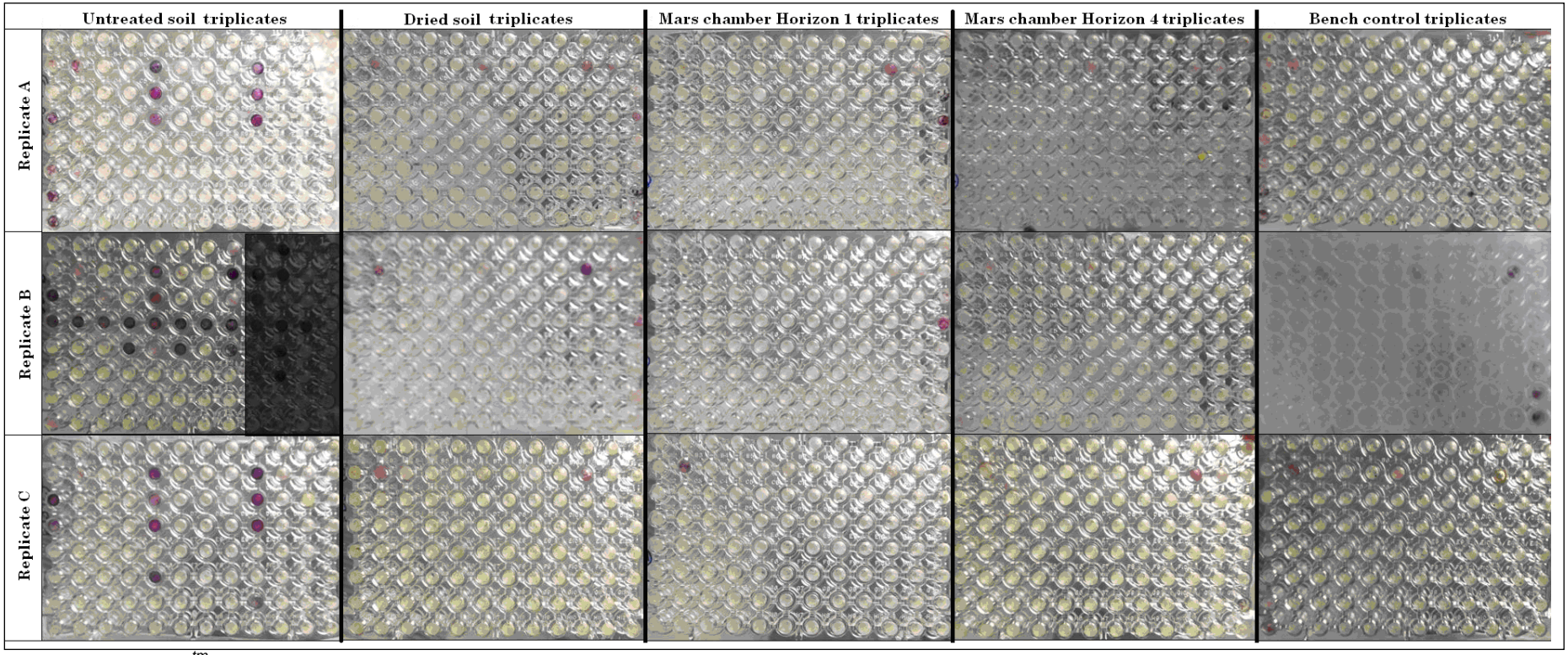



*Figure 6.13. Biolog[tm] Ecoplate for; Untreated soil, Dried soil control, Mars chamber horizon 1, Mars chamber Horizon 4 and Dried bench contol. The the third assay replicate for rample replicate B has been darkened as this was not used in the analysis, due to a positive colour change in the control water well.*

Due to the presence of opaque precipitant at the bottom of the wells it was not possible to read the plates using a Fluorescence Plate Reader; therefore the manual method of positive or negative score for colour change was used. As a result, the diversity of the community, assessed by the degree of colour change in the wells and changes in the development of colour before and after incubation, could not be accurately studied in this chapter. A positive colour change in the water control occurred in one replicate of the triplicate untreated soil B, so this was excluded from analysis. The degree of colour change could not be quantified in this study due to problems with precipitants in the wells.

The innoculation of the Biolog[tm] Ecoplate with the untreated soil control resulted in the positive colour change in 18 of the wells (Figure 6.15). This differed to the 10 carbon sources utilised by the viable culturable community present in the dried soil control. The number of different carbon sources which recorded a positive colour change dropped further to 6 different carbon sources for the Ecoplates incolulated with Mars chamber soil Horizon 4, and only 4 different carbon sources were demonstrated a positive colour change when the plate was inncolated with soil from Horizon 1. Finally, the Biolog[tm] Ecoplates innoculated with the bench soil control only demonstrated a positive colour change for 5 different carbon sources.

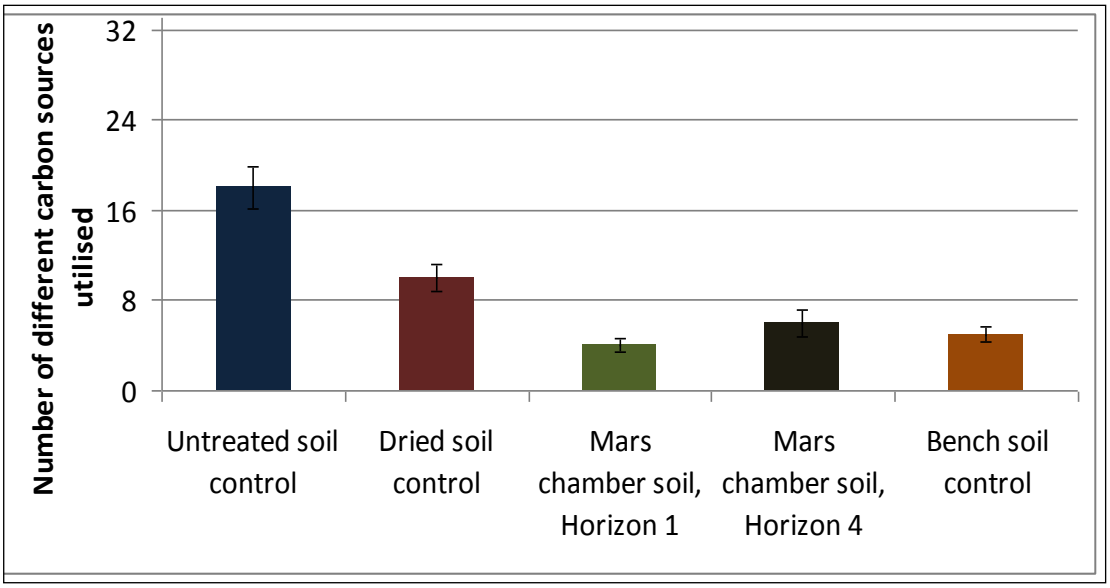



*Figure 6.14 Number of different carbon sources utilised after each treatment across replicates. Error bars are 1x standard error.*

The only carbon source to be utilised after every treatment was D-xylose, suggesting that the community which can utilise this carbon source is capable of remaning viable after all treatments (Table 6.2). A positive colour change was recorded in the Pyruvic acid methyl ester, Tween 40, Tween 80, beta methyl- D-glucoside, D-manitol, N-acetyl-D-Glucosamine, L-arginine, L-asparagine, L-phenylanine and Glyl-L-glutamis acid wells, but only when inoculated with the untreated soil sample 1A (Figure 6.16).

The carbon sources utilised by the two horizons of the Mars chamber soils differed by the fact that Horizon 4 was able to utilise putrescine and phenylethylamine in addition to alpha cyclodextrin, D-xylose, D-galacturonic acid and I-threonine which was utilised by Horizon 1 (Table 6.2).

*Table 6.2 Carbon sources utilised in the Biolog™ Ecoplate by the viable culturable community in the untreated soil, and after each treatment.*

| Treatment | Carbon source |
|---|---|
| Untreated | Pyruvic acid methyl ester, Tween 40, Tween 80, alpha cylodextrin, glycogen, d-cellobiose, alpha D lactose, D-xylose, D-manitol, N-acetyl-D-Glucosamine, D-galacturonic acid, 4-hydrobenzonoic acid, 2-hydroxybutryic acid, L-asparagine,L-Serine, I-threonine, L-phenylanine, Glyl-L-glutamis acid |
| Dried control | d-cellobiose, alpha D-lactose, D-xylose, D-galacturonic acid, 2-hydroxybutryic acid, 4-hydrobenzonoic acid, L-Serine,I-threonine, Phenylethylamine, Putrescine |
| Mars chamber soil Horizon 1 | alpha cylodextrin, D-xylose, D-galacturonic acid, I-threonine |
| Mars chamber soil Horizon 4 | alpha cylodextrin, D-xylose, D-galacturonic acid, I-threonine, Phenylethylamine, Putrescine |
| Bench control | alpha cylodextrin, d-cellobiose, alpha D lactose, D-xylose, glycogen |

The carbon sources utilised by the dried and bench control were a smaller subset of the carbon sources utilised by the untreated soil community, and were a slightly different variety of carbon sources differ to those utilised by the community viable in the Mars chamber soil samples. In addition, innoculation with the dried control resulted in the additional positive response in the phenylethylamine and putrescine wells.

No colour change occured after innoculation with any soil type in the; Beta methyl-D-glucose, L-erthritol, D-glcoasminic acid, Glucose alpha phosphate, D.L alpha gylcerol phosphate, D-galactonic acid lamder lactose, Lamder hydroxybutryic acid, Itaconic acid, alpha ketobutryic acid, malic acid or L-arginine wells (Figure 6.16).

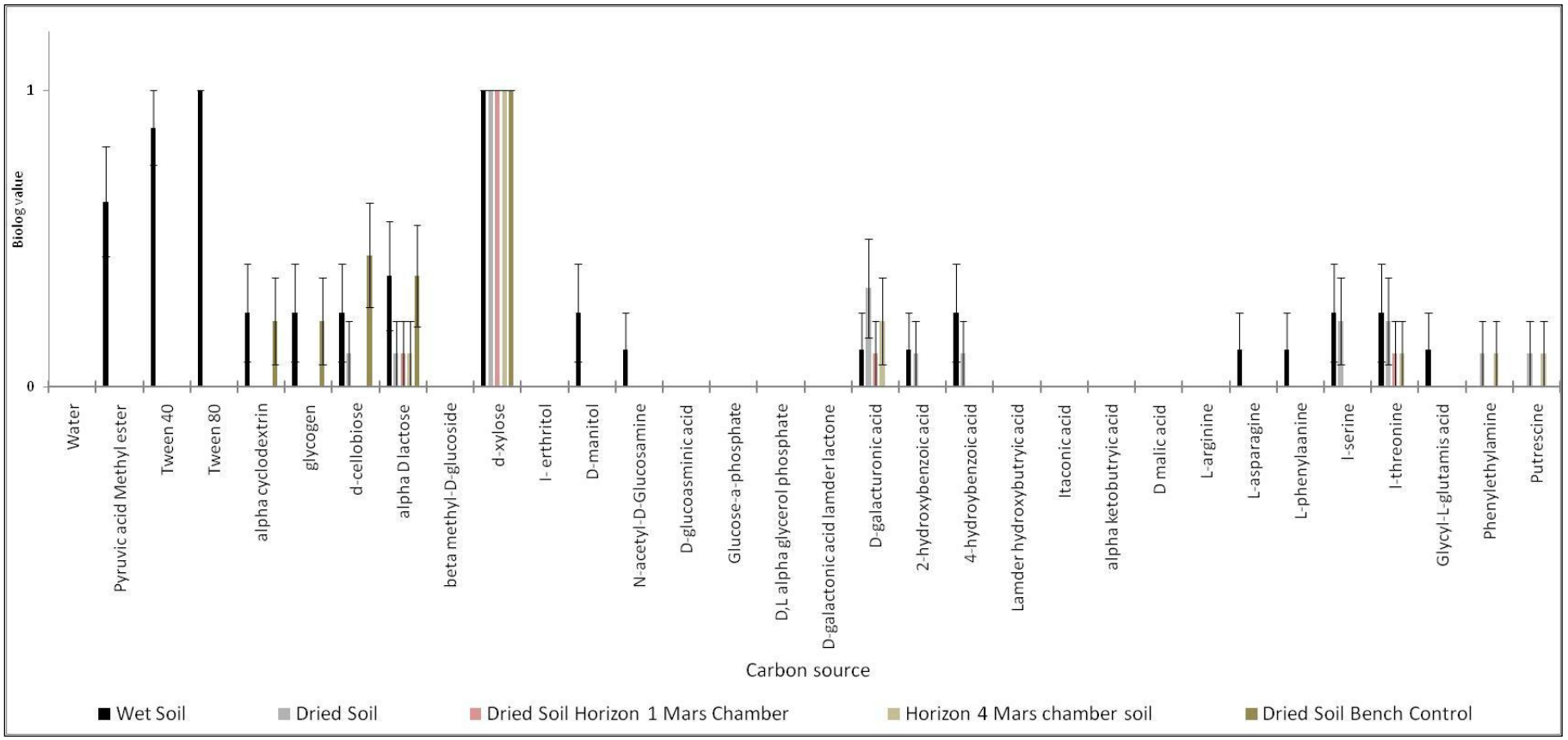



*Figure 6.15. Graph of Biolog™ score for each carbon source, value of 1 indicate presence of colour change for that carbon type in all wells across all triplicates, value of 0 means no colour change for that carbon type in any wells across any triplicates*

#### 6.4.3.3.2. PCA analysis of Biolog[tm] Ecoplate data

PCA analysis of the Biolog[tm] Ecoplate data showed that the untreated soil sample 1A control replicates all plotted with a positive axis 1 value and plotted away from the data points representing the other treatment replicates (Figure 6.17). The replicates for the other treatments all plot in a similar area with a negative axis 1 value, with the exception of one dried soil sample 1A control datum point. The position of this datum point was calculated from the dried soils sample 1A control replicate with the highest Biolog[tm] Ecoplate score. However, this point is still separated from the the untreated soil sample 1A control points.

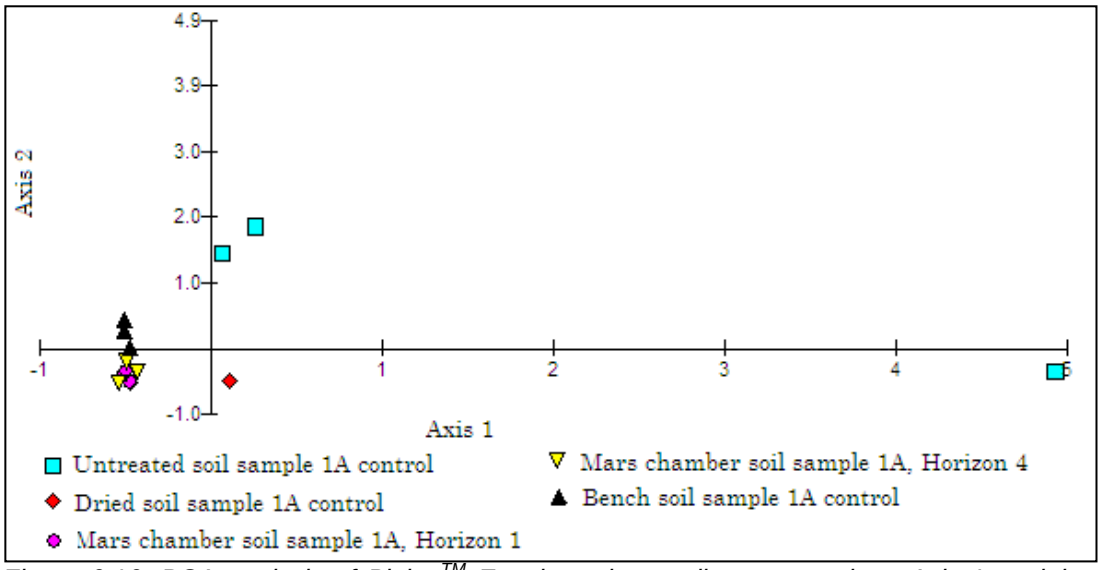



*Figure 6.16. PCA analysis of Biolog[TM] Ecoplate plate well response data. Axis 1 explained 55% of the variance and axis 2, 15% of the variance.*

#### 6.4.3.4. Identification of cultures

Colonies from the Mars chamber and control plates were sequenced to identify those organisms capable of surviving the drying and Mars chamber incubation. A total of 51 isolates were successfully sequenced from three different NaCl concentrations. 30 isolated strains were most closely related to members of the phylum Firmicute, 14 were related to members of the phylum Proteobacteria and 1 isolate was related to members of the phylum Bacteroidetes (Figure 6.19). The sequences were then run through the Greengenes 16s rRNA gene database to obtain the near neighbours and nearest neighbours for each sequence as described in Chapter 3. Partial sequences ~840bp in length were used and sequencing was conducted in the forward direction. The description of each isolate sequenced is given in table 6.3. The culturable diversity of the untreated soil sample 1A control is displayed in Figure 6.19 and was described in Chapter 2.

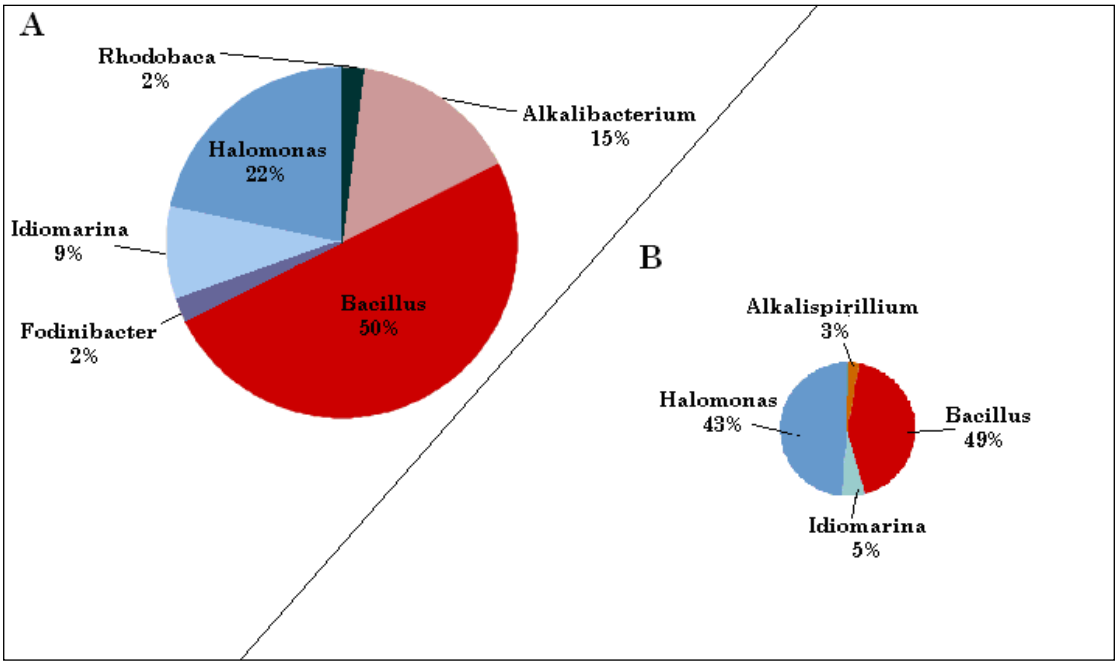



*Figure 6.17.* ***(A)*** *Proportions of cultured genera after Mars chamber incubation.* ***(B)*** *Proportions of cultured genera isolated from the untreated soil. Genera of the phylum Firmicute in shades of red, genera of the phylum Proteobacteria in shades of blue and genera of the phylum Bacteroidetes in purple.*

#### 6.4.3.4.1. Isolates related to members of the phylum Firmicute

The highest proportion of isolates was for the phylum Firmicute, with 23 isolates being most closely related to members of the genus *Bacillus* (Figure 6.20) and 7 isolates were related to members of the genus *Alkalibacterium*, relatives of which were not isolated from the untreated soil (Table 6.3). The proportion of *Bacillus* strains isolated after incubation is comparable to the proportion isolated from the untreated soil.

The *Bacillus* isolates were closely related to organisms identified in a range of locations, all of which were either saline or alkaline environments. The closest cultured database strains separated into 10 different species of *Bacillus*, one isolate, 0.7%_B1-1F, from horizon 1 was most closely related to *B. alkalinitrilicus* a spore forming organism, isolated form high salt, poorly drained marsh land in Russia (Table 6.3).


*Figure 6.18. The Lake Magadi isolates closely related to members of the phylum (pink)*

*Firmicute, their near neighbour and nearest neighbour matches (black) using Greengenes alignment software (DeSantis et al., 2006). Numbers at each node are the percentage bootstrap value of 1000 replicates. Bootstrap >45%.*

Isolates related to strains from Lake Elementia, a soda lake in Kenya have been cultured. In addition, relatives of *B. hemicellulosilyticus,* an obligatory alkaliphilic, spore forming bacterium, *B. okhensis* from salt pan and *B. wakoensis* which are both alkaliphilic, halotolerant strains (Nogi et al., 2005: Nowlan et al., 2006) and *Bacillus* sp. from calcite/gypsum rock were also isolated. This suggests that some strain from this environment may be capable of living in both an alkaline soil and an environment where gypsum can form.

Isolate 0.7_B4-1F was most closely related to an uncultured organism from hydrocarbon contaminated saline-alkali soil and the closest strain match to *Bacillus arseniciselenatis. B. arseniciselenatis* is an obligatory anaerobic strain and is capable of reducing arsenate ($AsO_4^{3-}$) to arsenite ($AsO_3^{3-}$), and can use selenate ($SeO_4^{3-}$) and Fe III as ion acceptors (Switzer Blum et al., 1998: Zavarzina et al., 2009). This species was first isolate from Mono Lake, a shallow saline, alkaline lake in California which differs to Lake Magadi by its higher calcium concentration. Calcium levels in Mono Lake allows for the precipitation of calcium carbonate columns or tufas. Mono Lake has been studied as an analogue environment on Mars, with the concept that with the evaporation rates exceeding precipitation or water input then the bodies of water would become highly saline and possibly have a high pH (Forsythe and Zimbelman, 1995: McDonald et al., 1999). Strains related to *B. arseniciselenatis* were isolated from a sample from the northern end of Little Magadi and Lake Magadi (Chapter 3).

Strains related to *B. vedderi* JaH were isolated from every horizon of the soil cores, on both the 0.7% and 6.8% NaCl agar plates. There was a similarity of 95% to 97% between *B. vedderi* JaH and the isolated strains from the Mars chamber. *B. vedderi* is a spore forming organisms first isolated from mud associated with bauxite processing waste and was isolated from samples to the south of Lake Magadi in Chapter 3 (Agnew et al., 1995).

Several isolates including 6.8%_B1-1B and 15%_C1-1A were most closely related to *Bacillus aurantiacus* which is an orange pigmented, alkaliphilic and moderately halophilic strain first isolated in Hungarian soda lakes (Borsodi et al., 2008). Four strains of *B. agardhaerens* were isolated from the upper most horizon of the soil core and were isolated on both the 6.8% and the 15% NaCl, but it was not isolated on the 0.7% medium. The type strain is an obligate alkaliphile isolated from undefined ‘soil’ and is capable of utilising D-xylose as a carbohydrate substrate (Nielsen et al., 1995).

*Table 6.3. BLAST database closest relatives and closest cultured species based on 16S rDNA similarity (Accession code).*

| Isolate | Sequence length | ID closest relative | % match | ID closest cultured strain relative | % match |
|---|---|---|---|---|---|
| 0.7%_B1-1C | 831 | Hailaer soda lake bacterium F24 (AF275702.1) | 99 | *Alkalibacterium psychrotolerans* JCM 12281 (AB294176.1) | 99 |
| 0.7%_B1-1F | 823 | *Bacillus* sp. ISO_06_Kulunda (EU676884.1) | 99 | *Bacillus alkalinitrilicus** ANL-iso4 (EF422411.1) | 98 |
| 0.7%_A2-1D | 844 | Hailaer soda lake bacterium F24 (AF275702.1) | 98 | *Alkalibacterium psychrotolerans* JCM 12281 (AB294176.1) | 98 |
| 0.7%_A2-1E | 833 | *Bacillus* sp. GSP69 (AY553128.1) | 94 | *Bacillus alcaliinulinus* AM31 (AB018595.1) | 94 |
| 0.7%_A2-1F | 818 | *Bacillus* sp. Zby6 (GU583650.1) | 97 | *Bacillus vedderi** JaH (NR_026526.1) | 96 |
| 0.7%_B2-1E | 817 | *Bacillus* sp. E-127 (FJ764772.1) | 98 | *Bacillus pseudofirmus** *s*RAB (AB595898.1) | 96 |
| 0.7%_B2-1F | 918 | Hailaer soda lake bacterium F24 (AF275702.1) | 98 | *Alkalibacterium psychrotolerans* IDR2-2 (NR_024830.1) | 97 |
| | | *Alkalibacterium* sp 10011 (EU432578.1) | 98 | | |
| 0.7%_A3-1A | 824 | *Bacillus okhensis* (DQ026060.1) | 98 | *Bacillus wakoensis** (AB043851.1) | 98 |
| 0.7%_B4-1D | 824 | Uncultured *Bacillus* sp. clone ENR_before_Playa (EU676872.1) | 98 | *Bacillus cohnii** D7023 (FJ161329.1) | 98 |
| 0.7%_B4-1 1F | 828 | Bacterium clone Bms_CK325 (HQ697807.1) | 98 | *Bacillus arseniciselenatis** DSM 15340T (AJ865469.1) | 97 |
| 6.8%_B1-1 | 818 | bacterium clone LCKS000B19 (EF201718.1) | 97 | *Bacillus vedderi** JaH (NR_026526.1) | 97 |
| 6.8%_B1-1B | 827 | Hailaer soda lake bacterium T10 (AF275707.1) | 99 | *Bacillus aurantiacus* * K1-10 (AJ605772.2) | 97 |
| 6.8%_B-1C | 811 | Bacillaceae bacterium halo-2 (EU581836.1) | 99 | *Bacillus agaradhaerens** DSM 8721 (NR_026142.1) | 98 |
| | | Bacterium 103NT4 (X92163.1) | 98 | | |
| 6.8%_A2-1 | 818 | bacterium clone LCKS000B19 (EF201718.1) | 97 | *Bacillus vedderi** JaH (NR_026526.1) | 97 |
| 6.8%_A2-1D | 829 | *Bacillus cellulosilyticus** DSM 2522 (CP002394.1) | 96 | *Bacillus vedderi** JaH (NR_026526.1) | 95 |
| 6.8%_B3-1D | 827 | Hailaer soda lake bacterium F24 (AF275702.1) | 97 | *Alkalibacterium psychrotolerans* JCM 12281 (AB294176.1) | 97 |
| 6.8%_B3-1G | 815 | *Bacillus hemicellulosilyticus** (AB043846.1) | 99 | *Bacillus krulwichiae** T-28 (HQ202852.1) | 95 |
| 6.8%_C3-1B | 822 | Hailaer soda lake bacterium F24 (AF275702.1) | 99 | *Alkalibacterium psychrotolerans* JCM 12281 (AB294176.1) | 98 |
| 6.8%_C31E | 829 | *Bacillus cellulosilyticus** DSM 2522 (CP002394.1) | 98 | *Bacillus vedderi** JaH (NR_026526.1) | 97 |

*Table 6.3. continued*

| Isolate | Sequence length | ID closest relative | % match | ID closest cultured relative | % match |
|---|---|---|---|---|---|
| 6.8%_A4-1C | 830 | Hailaer soda lake bacterium F24 (AF275702.1) | 98 | *Alkalibacterium psychrotolerans* JCM 12281 (AB294176.1) | 98 |
| 6.8%_A4-1B | 820 | Hailaer soda lake bacterium F24 (AF275702.1) | 98 | *Alkalibacterium psychrotolerans* IDR2-2 (NR_024830.1) | 97 |
| | | *Alkalibacterium* sp. 10011 (EU432578.1) | 98 | | |
| 6.8%_A4-1E | 828 | *Bacillus cellulosilyticus** DSM 2522 (CP002394.1) | 95 | *Bacillus vedderi** JaH (NR_026526.1) | 95 |
| 6.8%_B4-1 | 844 | Bacterial species 3E1 (X92162.1) | 86 | *Bacillus saliphilus* 6AG 1 (NR_025554.1) | 88 |
| 6.8%_C4-1C | 827 | Bacterium clone LCKS000B19 (EF201718.1) | 98 | *Bacillus vedderi** JaH (NR_026526.1) | 98 |
| 15%_C1-1A | 824 | Hailaer soda lake bacterium T10 (AF275707.1) | 99 | *Bacillus aurantiacus** K1-10 (AJ605772.2) | 97 |
| 15%_C-1A | 816 | *Bacillus* sp. X5B(2010) (HQ433452.1) | 94 | *Bacillus clarkii** DSM 8720 (NR_026141.1) | 94 |
| 15%_C-1B | 815 | *Bacillus* sp. clone EK CK628 (JN038220.1) | 97 | *Bacillus agaradhaerens** Mi102(GQ121032.1) | 98 |
| 15%_C3-1B | 817 | Bacillaceae bacterium halo-2 (EU581836.1) | 99 | *Bacillus agaradhaerens** DSM 8721 (NR_026142.1) | 98 |
| 15%_C3-1C | 811 | *Bacillus agaradhaerens** (AB211544.1) | 99 | *Bacillus agaradhaerens**IB-S7 (FN432808.1) | 98 |
| 15%_A4-1B | 826 | Hailaer soda lake bacterium T10 (AF275707.1) | 99 | *Bacillus aurantiacus** K1-10 (AJ605772.2) | 97 |
| 15%_Ccontrol | 811 | *Bacillus* sp. clone ML615J-19 (AF454300.1) | 91 | *Caldalkalibacillus uzonensis** JW/WZ-YB58 (DQ221694.1) | 91 |
| 15%_Ccontrol | 819 | *Bacillus* sp. ISO_02_Chiprana (EU676882.1) | 99 | *Bacillus polygoni* YN-1 (NR_041571.1) | 99 |
| 15%_FD_A1-1C | 830 | Bacterium clone LCKS000B19 (EF201718.1) | 97 | *Bacillus vedderi** JaH (NR_026526.1) | 97 |
| 15%_C1_FD | 810 | *Bacillus* sp. clone EK CK628 (JN038220.1) | 97 | *Bacillus agaradhaerens** Mi10-62 (GQ121032.1) | 98 |
| 15%_A1-1FD | 816 | *Bacillus* sp. X10-7 (HM598405.1) | 99 | *Bacillus saliphilus* 6AG 1 (NR_025554.1) | 96 |
| 6.8%_B3-1B | 815 | *Aquiflexum* sp. DL6 (JF812063.1) | 96 | *Fodinibacter* sp. S2 (FR681898.1) | 92 |
| 0.7%_A2-1B | 811 | Gamma proteobacterium E-410(FJ764791.1) | 98 | *Idiomarina seosinensis* CL-SP19 (NR_025826.1) | 93 |
| 0.7%_B2-1A | 800 | *Halomonas* sp. 4AB3 (HM587243.1) | 95 | *Halomonas campisalis* HB10.1(GU228480.1) | 95 |
| 0.7%_B2-1C | 811 | Gamma proteobacterium E-410(FJ764791.1) | 99 | *Idiomarina seosinensis* CL-SP19 (NR_025826.1) | 93 |
| 0.7%_A3-1D | 815 | *Halomonas* sp. 4AB3 (HM587243.1) | 94 | *Halomonas campisalis* HB10.1(GU228480.1) | 94 |
| 0.7%_B4-1B | 821 | *Halomonas* sp. 4AB3 (HM587243.1) | 99 | *Halomonas campisalis* HB10.1(GU228480.1) | 99 |

*Table 6.3. continued*

| Isolate | Sequence length | ID closest relative | % match | ID closest cultured relative | % match |
|---|---|---|---|---|---|
| 0.7%_B4-1E | 822 | *Halomonas* sp. 4AB3 (HM587243.1) | 95 | *Halomonas campisalis* HB10.1(GU228480.1) | 95 |
| 6.8%_B1-1C | 831 | *Halomonas* sp. 4AB3 (HM587243.1) | 97 | *Halomonas campisalis* HB10.1(GU228480.1) | 97 |
| 6.8%_B1-1D | 794 | *Halomonas* sp. 4AB3 (HM587243.1) | 99 | *Halomonas campisalis* LL6 (DQ077911.1) | 99 |
| 6.8%_A2-1E | 814 | Gamma proteobacterium E-410(FJ764791.1) | 99 | *Idiomarina seosinensis* strain 2PR51-17 (EU440983.1) | 92 |
| 6.8%_B3-1E | 812 | *Idiomarina* sp. JK38 (EF554896.1) | 99 | *Idiomarina salinarum* ISL-52 (EF486355.1) | 93 |
| 6.8%_B3-1F | 795 | *Halomonas* sp. 4AB3 (HM587243.1) | 99 | *Halomonas campisalis* LL6 (DQ077911.1) | 99 |
| 6.8%_C3-1A | 794 | Uncultured clone TX1A_96 (FJ152648.1) | 97 | *Halomonas salina* 5-5-10 (GU113001.1) | 97 |
| 6.8%_A4-1A | 772 | Uncultured alpha proteobacterium clone WN-HSB-198 (DQ432305.1) | 98 | *Rhodobaca barguzinensis* VKM B-2406 (EF554833.1) | 97 |
| 6.8%_C4-1A | 794 | *Halomonas* sp. 4AB3 (HM587243.1) | 99 | *Halomonas campisalis* LL6 (DQ077911.1) | 99 |
| 15%_C1-1C | 788 | Halomonas sp. E-069 (FJ764763.1) | 100 | Halomonas venusta strain NBSL25 (FJ973522.1) | 99 |

Strain 15%_C-1A was a 94% match for an uncultured *Bacillus* sp. strain from a hypersaline lake and 94% similarity to *B. clarkii* an obligate alkaliphile, 6.8%_B4-1 had the lowest database match of any isolate from the Mars chamber. This strain has 86% match to a bacterial strain from Lake Elementia and an 88% match to *Bacillus saliphilus* a haloalkaliphilic organism which does not form spores. *B. saliphilus* is a haloalkaliphilic bacterium with a wide salinity tolerance. This low database match would indicate that this is a new species and possibly a new bacterial genus.

Those strains related to the genus *Alkalibacterium* were most closely related an unidentified Hailaer soda lake bacterium from an Inner Mongolian soda lake and/or *Alkalibacterium* from mud volcanoes in China. The closest strain matches to these isolates were strains of *Alkalibacterium psychrotolerans,* with between 97% and 99% match, isolated from the indigo fermenting process*. A. psychrotolerans* is a non-spore forming bacterium and an obligate alkaliphile and a facilitative anaerobe, which can utilise D-Xylose and D-Arabinose (Yumoto et al., 2004). Strains of *Alkalibacterium* and in particular close relatives to *A. psychrotolerans* were commonly cultured isolates from several samples in Chapter 3.

Two strains from the control plate were closely related to *Caldalkalibacillus uzonensis and B. clarkii* with none of the Mars chamber isolates sequenced being related to these two strains of Bacilli. Isolates of the class Bacilli and specifically of the family Bacillaceae were isolated from each horizon of the Mars chamber samples. The isolates from the dried soil were most closely related to strains present in hypersaline lakes, petroleum contaminated soil and from soda soil with 97% to 99% match). The dried isolates were most closely related to cultured isolates of *B. vedderi*, *B. agardhaerens* and finally *B. saliphilus*. Only strains from the controls were described as most closely related to *B. saliphilus* and the other two strains were the most common closest database match to the Mars chamber isolates.

#### 6.4.3.4.2. Isolates related to members of the phylum Proteobacteria and Actinobacteria

The 15 isolates of the phylum Proteobacteria were of the genus *Halomonas*, *Idiomarina or Rhodobaca*, with strains related to these genera being isolated from many samples in Chapter 3. Interestingly, the proportion of isolates closely related to *Idiomarina* increased, whilst the proportion of isolates related to *Halomonas* decreased. 8 isolates matched to Halomonads were most closely related to *Halomonas campisalis*. All these strains were most closely related to the same strain for closest relative, and again for the closest cultured relative (See table 6.3). *H. campisalis* is an obligatory alkaliphilic halophile (Boltyanskaya et al., 2004), but the type strain is not able to utilise D-xylose as a carbohydrate substrate. *H. campisalis* is able to grow over a very wide range of NaCl concentrations (Table 6.3) and was commonly isolated in Chapter 3.

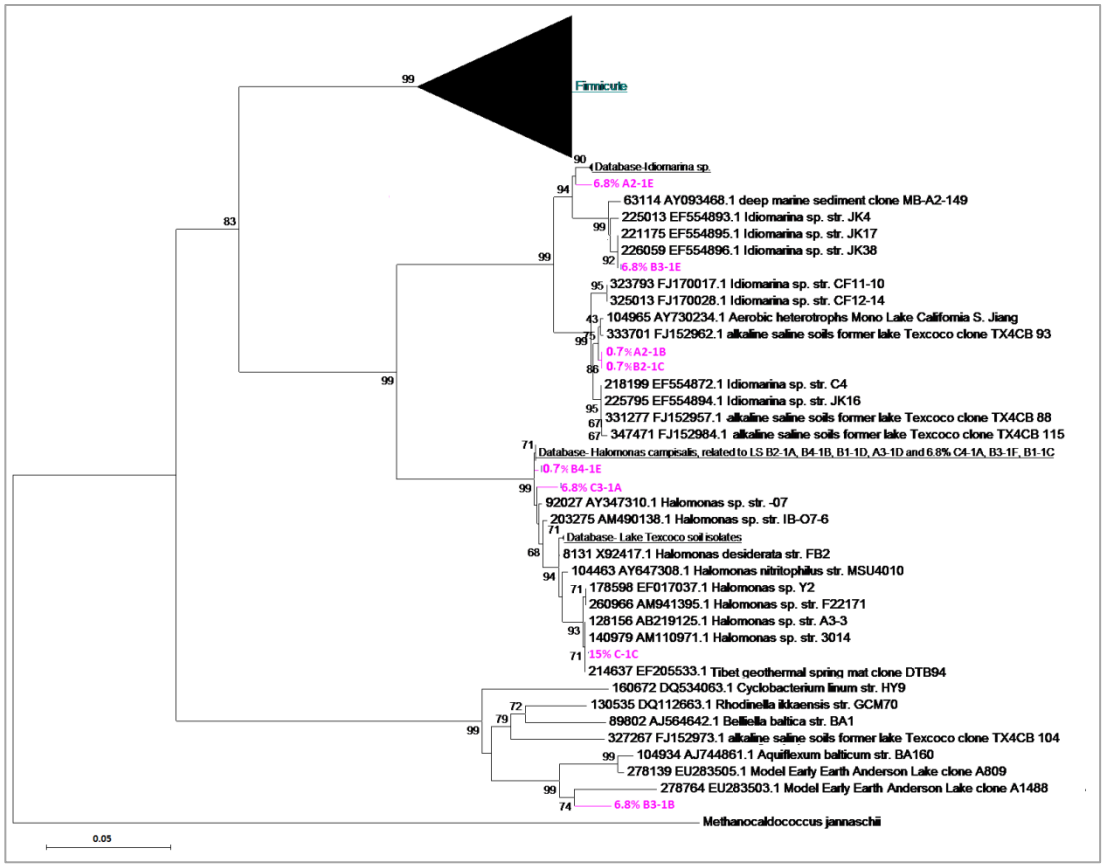



*Figure 6.19. The Lake Magadi isolates closely related to members of the phylum (pink) Proteobacteria and Bacteroidetes, their near neighbour and nearest neighbour matches (black) using Greengenes alignment software (DeSantis et al., 2006). Numbers at each node are the percentage bootstrap value of 1000 replicates. Bootstrap value > 50% are shown.*

*H. campisalis* has been isolated from a variety of environments including bioreactors treating Bayer liquor (Boltyanskaya et al., 2004), alkali soil and the soda, lake Lonar Lake (Joshi et al., 2008) and does not form spores. A strain closely related to; uncultured clone from saline alkaline soil, a close relative to the cultured strain *H. salina*, a strain related to a Lake Elementia strain of *Halomonas* sp. and to the cultured strain *H. venusta* were also identified.

Four strains most closely related to *Idiomarina* were isolated from second and third horizons. Three strains were most closely related to Gamma Proteobacterium from Lake Elementia with 98% - 99% similarity and are most closely related to the cultured strain *Idiomarina seosinensis* with 92% - 93% similarity. The other isolate related to *Idiomarina* had 99% similarity to *Idiomarina* sp. isolated from a Hungarian soda lake, and 93% similarity to the cultured strain *Idiomarina salinarum.* These are non-spore forming strains which can grow at a range of salinities.

Strain 6.8%_A4-1A was most closely related to alpha proteobacterium clone from an alkaline/hypersaline lake in Egypt with 98% similarity. This strain was most closely related to the cultured strain *Rhodobaca barguzinensis* isolated from a soda lake with 97% similarity, *Rca. barguzinensis* is a purple non-sulphur bacteria (Boldareva et al., 2008).

Finally strain 6.8%_B3-1B is most closely related to a red bacterium *Aquiflexum* sp. (96% match) a member of the phylum Bacteroidetes, isolated from Lonar Lake (Brettar et al., 2004). Lonar Lake is a saline lake with a pH of 9.8 (Kanekar et al., 2008). Members of the genus *Aquiflexum* do not require NaCl to grow but their growth is improved in its presence up to 6% NaCl and can grow on a range of temperatures from 4 °C to 40 °C and a pH 7 to pH 9 (Brettar et al., 2004). Strain 6.8%_B3-1B only has a 92% match to a cultured isolate, *Fodinibacter* sp., cultured from a salt mine, which is of the phylum Actinobacteria. *Fodinibacter* species so far are non-spore forming (Wang et al., 2009) and the genera *Aquiflexum* and *Fodinibacter* do not use D-xylose (Brettar et al., 2004). Isolates closely related to these strains were also isolated from samples in Chapter 3.

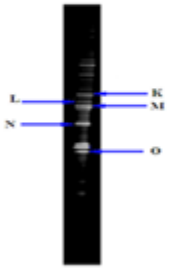
The phylogenetic relationship of the isolates most closely related to strains belonging to the phylum Proteobacteria are shown in figure 6.21. This shows that four of the strains most closely related to strains of *H. campisalis* (99%) cluster together. The *Halomonas* strains which have a lower database match than 99% to *H. campisalis*, cluster separately, as do *Halomonas* strains related to other species of the genus.

The two strains LS_A2-1B and LS_B1-1C cluster with strains from soil from the former Lake Texcoco and marine sediments, including deep sea sediments. Isolate 6.8%_B3-1E and 6.8%_A2-1E were more closely related to strains from soda pond environments (Kiesel et al., 2007) and isolates from mud volcanoes in Xijiang. 6.8%_A4-1A is most closely related to strains from alkaline/saline/soda lakes in Serbia (Boldareva et al., 2008) and Egypt. Finally 6.8%_B3-1B clustered closely with strains of *Aquiflexum*, including two uncultured clones from Anderson Lake, a saline, alkaline arsenic enriched system.

#### 6.4.3.5. Comparison of the cultured Mars chamber isolate ladder, to the DGGE profile of samples from Chapter 3

Finally, cultured isolates from the Mars chamber were used to produce a cultured ladder (Table 6.4), which was run along with the samples analysed in Chapter 3. This was done in order to identify any if any bands which are comprised of the strains which survived the Mars chamber treatment, are also present in other samples from Lake Magadi.

*Table 6.4 Ladder Key. DGGE ladder with bands assigned, blue arrows indicate bands assigned to Mars chamber isolates. Names assigned were the closest match from the NCBI database.*




| Key | Closest uncultured database match | % | Isolate | Closest strain match | % |
|---|---|---|---|---|---|
| K | *Halomonas* sp. 4AB3. HM587243.1 | 94 | 6.8%_B3-1F | *Halomonas campisalis* HB10.1 gu22848.01 | 99 |
| | *Bacillus* sp. GSP69. AY552128.1 | 99 | LS_A2-1E | *Bacillus alcalitrudinus* M31. AB018595.1 | 94 |
| I | *Bacillus* sp. ISO_02_Chiprana EU676882.1 | 99 | 15%_CcontrolB | *Bacillus polygoni* YN-1 NR_041571.1 | 99 |
| M | *Halomonas* sp. 4AB3. HM587243.1 | 94 | LS_B2_1A | *Halmonas salina* LLM DQ333297.1 | 93 |
| | *Aquifexum* sp. DL6. JF812063.1 | 96 | 6.8%_B3-1B | *Fodinibacter* sp. S2. FR681898.1 | 98 |
| N | Unidentified Hailer soda lake bacterium T10. AF275707.1 | 99 | 6.8%_B1-1B | *Bacillus aurantiacus* K1-10. AJ605772.2 | 97 |
| O | Bacterium clone Bms_CK325 HQ697807.1 | 98 | LS_B4-1F | *Bacillus alkalidiazotrophicus* MSNR_044420.1 | 98 |
| | Bacterium clone nod2068g08c1 JF177875.1 | 98 | LS_B4-1D | *Bacillus cohnii* DT025 FJ161329.1 | 98 |

The isolates 6.8%_B3-1F and 0.7%_A2-1E from the Mars chamber contribute to bands marked in royal blue in Figure 6.22. This band can also be traced in the samples 2A, 58, 72.2, 113, 125 and 94. The strain 15%_110A, related to *H. pantelleriensis* from Chapter 2 also migrates to this position. The band containing the strain 15%_CcontrolB, related to *B. polygoni* (Figure 6.22, light brown dashed line) is present in almost all samples, except sample 72.2 which was from the lagoon area of the lake.

Isolates 0.7%_B2-1A and 6.8%_B3-1B migrate to the same position in the gel (Figure 6.22, purple dashed line), isolates 6.8%_106A, related to *C. uzonensis*, isolate 0.7%_58C, related to *B. cohnii* and strain 6.8%_86C, related to *Idiomarina,* from Chapter 3 also migrate to this same position. This indicates that the resolution of the gel is limited to a certain extent. The band corresponding to these strains can be traced in samples 72.2, 76, 133, 125 and 94.

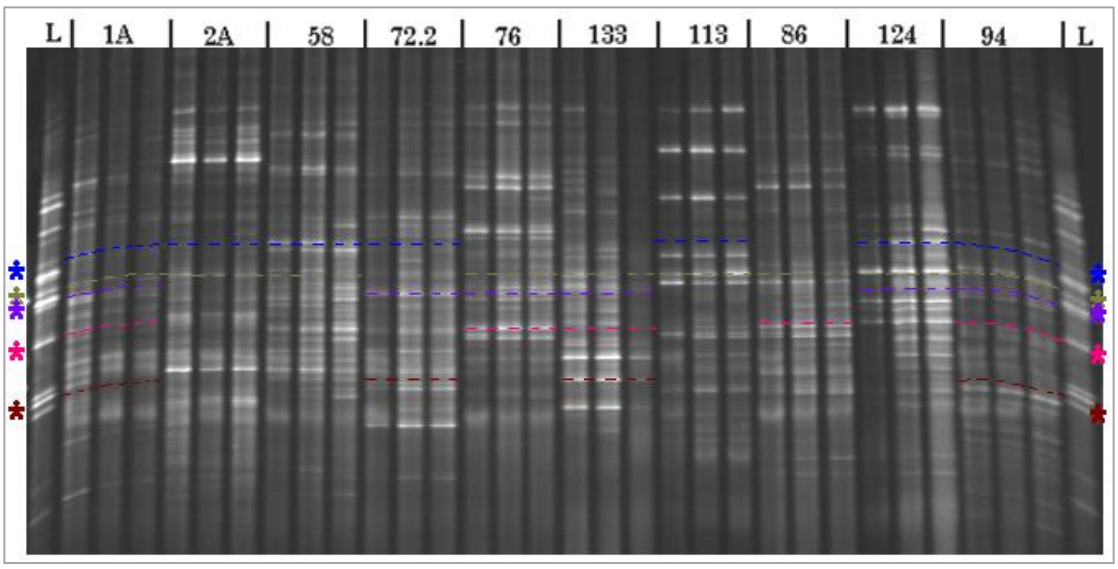



*Figure 6.20. DGGE gel of the bacterial community of soil, biological material and rock samples around Lake Magadi. Royal blue dashed line and ✱ denotes bands in corresponding location to the band for 6.8%_B3-1F, 0.7%_A21E and (Table 6.4 K, Light brown dashed line and ✱ denotes bands in corresponding location to the band for 15%_ControlB (Table 6.4 I),*

*Purple dashed line and* *denotes bands in corresponding location to the band for 0.7%_B2-1A and 6.8%_B3-1B (Table 6.4.M). Pink dashed line and* *denotes bands in corresponding location to the band for 6.8%_B1-1B (Table 6.4.N). Dark red dashed line and* *denotes bands in corresponding location to the band for 0.7%_B4-1F and 0.7%_B4-1D (Table 6.4.O). L denotes ladder lane*

The band containing isolate 6.8%_B1-1B (Figure 6.22, pink dashed line), related to *B. aurantiacus* was identified in samples 76, 133, 86 and 94, three strains most closely related to this species of *Bacillus* were isolated from the Mars chamber samples.

Finally, the strains 0.7%_B4-1F and 0.7%_B4-D, which were more closely related to two species of *Bacillus*, *B. alkalidiazotrophicus* and *B. cohnii* contribute to a band which could be traced in samples 72.2, 133 and 94.

## 6.5. Discussion

When considering which terrestrial environments and organisms might be best suited for analogue studies, it would be logical to select those that inhabit environmental conditions which may have been similar to those on past and present on Mars. Organisms living in those environments would require cellular adaptations, for example, adaptations against different types of oxidative stress, which would also be beneficial on Mars. This chapter has assessed the effects of simulated Martian conditions on the survivability of a halotolerant, alkaliphilic novel isolate, and a complex soil community from a saline alkaline environment, which had not previously been studied in an astrobiological context.

### 6.5.1. Survival of *Planococcus* sp. LMLD02

The data presented in this chapter demonstrates that *Planococcus* sp. LMLD02, isolated from the northern end of Little Magadi (see Chapters 2 and 3), can survive short term exposure to at least some of the extreme conditions which would be experienced on Mars. Some degree of survival by *Planococcus* sp. LMLD02 after incubation under simulated Martian conditions was expected due to the desiccation and UV resistance it exhibited in Chapter 5 Sections 5.3.2.9 and 5.3.3.4. Studies conducted on other non-spore forming strains, including *Deinococcus radiodurans*, *Escherichia coli* and *Psychrobacter cryohalolentis* have also demonstrated that the bacteria were able to survive incubation under various simulated Martian conditions (including desiccation, anoxic atmosphere and low pressure), although they did display a decrease in viability (Section 6.1.1, this Chapter) (Pogoda de la Vega et al., 2007: Smith et al., 2009a: Berry et al., 2010). *Planococcus* sp. LMLD02, however, demonstrated no significant decrease in viability following incubation for 7 days. In order to fully understand the response of *Planococcus* sp.LMLD02 it would be necessary to compare this isolate against a control strain. The development of the drying procedure to eliminate

contaminants such as fitting a filter to the lyophilizer, or by using growth media to select specifically for the control strain could assist with this.

The ability of *Planococcus* sp. LMLD02 to survive the simulated conditions may be a product of the fact that it is adapted to grow in a highly alkaline/saline environment. Studies have suggested that the resistance to simulated Martian surface conditions including freeze thaw cycles and UV radiation exhibited by some halophiles (*Halorubrum chaoviator* and *Halobacterium salinarium* (Chapter 5 section 5.4.2.)) is a factor of the hypersaline environment from which they are isolated (Kottemann et al., 2005b: Johnson et al., 2011: Gygli et al., 2009: Baxter et al., 2007b).

The contrast between the decline in cell numbers during desiccation demonstrated in Chapter 5 and the results described in this chapter could be the result of the SCSM providing protection to *Planococcus* sp. LMLD02, as discussed in Chapter 5, section 5.4.1. In addition, a study on *D. radiodurans*, comparing the individual effects of desiccation (71% survival) and temperature (72% survival) to their combined effects on cell viability, showed an increase in cell survival (98% survival) when the desiccation and temperature were combined. A similar pattern was also observed with *E. coli,* and it is thought the strains are responding synergistically to both stresses (Diaz and Schulze-Makuch, 2006).

Low temperature resistance requires an ability to maintain membrane fluidity and continue metabolic activities and protein synthesis (Hebraud and Potier, 1999). The methods used to combat desiccation such as compatible solutes, can also play a role in limiting the effects of freezing conditions (da Costa et al., 1998). Mechanisms which are triggered by the presence of one factor can produce the additional advantage of protecting the organism against another stress which provokes similar cellular damage. The organisms could then benefit from the combined effects of several mechanisms which work synergistically and would result in a superior survival against the combined detrimental factors. Again, it is not clear what mechanisms are involved in the resistance of *Planococcus* sp. LMLD02, which would require in depth analysis of the functional genes and proteins.

### 6.5.2. Response of complex soil community stress factors

#### 6.5.2.1. Effects of desiccation on a complex soil community

Desiccating soil 1A resulted in a significant decrease in the number of colony forming units calculated from each growth medium, as well as a decrease in substrate utilisation identified through Biolog$^{TM}$ Ecoplate analysis. However, this result is in contrast to what we might have expected considering the desiccating nature of the sampling environment and the response of *Planococcus* sp. LMLD02. Other studies assessing the effects of simulated Martian conditions on complex microbial communities have demonstrated a decrease in CFU and substrate utilisation following desiccation (Hansen et al., 2005) and in addition, Chapter 5

demonstrated a decrease in cell viability due to desiccation in both archaea and bacteria from Lake Magadi. This suggests that in spite of the desiccating conditions from which the soil was sampled, the community was still sensitive to the extreme desiccation of the simulated Martian environment. It is important to note however that the soil was not dry when it was sampled and so organisms which are not necessarily so desiccation resistant may also have been present and where then affected by the subsequent desiccation. The most detrimental factors of a simulated Martian environment on biology are desiccation, as well as freeze thaw cycles and UV-C irradiation, so a decline in viability and a decrease in carbon usage might have been expected. (Hagen, 1967: Hansen et al., 2009).

There was no apparent difference in the survival fraction between the different NaCl concentrations incubated with the dried soil control, suggesting that those organisms able to grow in the presence of 15% NaCl were no more resistant to this method of desiccation than the community able to grow on 0.7% NaCl. Alternatively, it could be that the culturable community present in this soil sample have a broad NaCl growth range and so could grow on in the presence of all three NaCl concentrations (therefore were represented on all three media). This hypothesis maybe supported by the absence of any clear distinction between the genera and species of cultured organisms, identified on the three growth media in Chapter 3. To address this, the use of a growth medium with an even greater salinity, such as 25% may restrict growth to more extreme halophiles.

Although the cell count does decrease, between 4-18% of the original culturable community were able to survive the drying process on the different NaCl concentrations. Therefore, some of the organisms present in soil samples from Lake Magadi would be able to survive desiccating conditions, such as those present on Mars. However, this is just one detrimental factor which these communities would face and the community which could survive desiccation would also have to withstand prolonged desiccation, along with hypobaric conditions, UV radiation and freeze thaw cycles.

It is important to consider however that a drawback of culture based techniques is that typically the culturable community only represents <1%of the whole microbial community (as mentioned in Chapter 3). However, culture based do provide an initial indication of changes induced by environmental factors. The addition of culture independent techniques, such as Live/Dead® staining would allow the assessment of viability survial for the whole microbial community and not just the culturable one.

#### 6.5.2.2. Combined effects of freeze thaw cycles, desiccation, hypobaria, UV-C radiation and anoxic atmosphere on complex soil community

Incubation under simulated Martian conditions resulted in a significant decrease in the culturable community present in most horizons. However, there was no significant difference between the Mars chamber horizons, suggesting that the simulated Martian

conditions had a detrimental effect upon the culturable community down to a depth of at least 23mm. This change in community after Mars chamber incubation was accompanied by a decrease in the number of carbonate substrates utilised. These community changes could have been due to the prolonged desiccation, freeze thaw cycles, hypobaria and/or UV radiation (be that a direct result, or due to the secondary effects of UV radiation, such as photochemcially produced free oxygen species) (Hansen et al., 2005). Significantly, the community able to grow on 15% NaCl appeared less affected by the simulated Martian conditions, with no horizon demonstrating a decline in cell count following incubation compared to the desiccated control. This would suggest that the community able to grow on this higher salinity is better adapted to survive the conditions outlined above than those able to grow on 0.7% or 6.8% NaCl. This could be because the adaptations required to grow at high salinity are also beneficial in the presence of these simulated Martian conditions.

Mars chamber simulations are only able to reproduce the Martian environment to a certain extent. The absence of factors such as IR in the simulation means that the viability identified by *Planococcus* sp. LMLD02 and the complex soil community is probably only an upper limit. However, it still provides an indication of what maybe possible if an organism or community were protected from other factors. In addition these experiments were all conducted on a short, 7 day timescale due to the experimental constraints of running Mars chamber simulations for longer periods of time.

#### 6.5.2.3. Effects of bench control conditions on complex soil community

This study showed that there was no significant difference between the bench control and the Mars chamber samples when incubated on 6.8% and 15% NaCl. This indicates that where a significant decline in viability occurred, the conditions experienced were no more detrimental than the ambient temperatures and humidity experienced on the bench. A decline in the culturable cell count was only demonstrated in the bench control incubated on the 0.7% NaCl medium, suggesting that the community able to grow on higher salinities were less affected by the conditions experienced on the bench than experience in the Mars chamber. This could suggest that these organisms are more tolerant of a range of environmental conditions, which could mean that they would be able occupy a broader variety of habitats on Mars.

#### 6.5.2.4. CLPP following each treatment

A decline in the number of carbon sources utilised was recorded after desiccation and following the Mars chamber and bench treatment as mentioned above. Although the types of organisms isolated in the untreated soil and following the Mars chamber incubation were similar, the decrease in the number of single carbon sources which can be metabolised by the community does change. This could have been a reflection of changes in the metabolic activity of these isolates due to the conditions they have been exposed to. A positive response was noted in every xylose well, this carbon source is a major constituent of

hemicellulose plant cell wall material and one of the most abundant carbon sources in nature (Johnsen et al., 2009). Its metabolism following each treatment might be expected as it is a readily available carbon source and so that when a stress such as desiccation is applied, metabolism of a readily available carbon source would be beneficial.

The use of Biolog$^{TM}$ EcoPlates was problematic due to the low cell density of the untreated soil sample and the even lower cell densities recorded after treatment. The Biolog$^{TM}$ EcoPlates do provide useful data on changes in metabolism following desiccation and Mars chamber incubation and did provide details on changes in the metabolic profile of the soil community in this study, however they maybe more suitable for use with samples which have a high culturable cell density, at least in the untreated control.

#### 6.5.2.5. Isolates capable of surviving simulated Martian conditions

Following, following Mars chamber incubation the isolates were most closely related to members of the main bacteria genera which were isolated from the untreated soil, *Bacillus, Idiomarina* and *Halomonas* (Chapter 3), plus isolates of the genera *Alkalibacterium*. This would suggest that although the culturable viability of the community decreases, representatives of the common culturable genera from soda lakes are capable of surviving exposure to some of the detrimental conditions which would be experienced on Mars, at least on a short time scale. In addition, the DGGE gel demonstrated that the band corresponding to strains isolated from the Mars chamber could be traced in a variety of samples from around Lake Magadi, again suggesting they maybe a common isolate present in a soda lake environment. However, it is possible that the band represents more than just the one species and sequencing of the band would be needed for confirmation (Chapter 3).

The presence of isolates related to *Alkalibacterium* from the treated soil, despite the absence of any isolates from the untreated control (Chapter 3) demonstrates another limitation of culture based techniques. Despite attempts at complete sample homogenisation, it is still possible that the sample will not be completely homogenised, and that species in a community are not represented in a sample. It is also possible that the dynamics of the microbial community present in that particular sample restrict the growth of a certain isolate. An alternative would be to use a culture-independent method such as pyrosequencing to determine the community structure (as mentioned in Chapter 3). However, its application in this type of study has its own issues. Firstly the problem of sample homogenisation discussed above could still apply and secondly, due to the preservation of DNA under simulated Martian conditions (Hansen et al., 2005), sequencing after Mars chamber incubation could result in the identification of both viable and dead cells, where the DNA has been preserved.

The strains which were isolated from the Mars chamber samples were related to halotolerant and alkaliphilic or alkalitolerant and indicate those organisms adapted to living in an alkaline environment are able to survive exposure to simulated Martian conditions, which is significant

considering the evidence for alkaline environments on Mars (Ehlmann et al., 2008b: Bishop et al., 2008: Ehlmann et al., 2010a: Ehlmann et al., 2010b).Provided with additional protection against ionizing radiation, and the availability of nutrients and water, then alkaliphilic/alkalitolerant halophiles could potentially survive on Mars, most probably in the subsurface.

### 6.6. Conclusions

Studies on the affects of simulated Martian conditions on soil communities from a soda lake environment have not been widley reported in the literature. The increasing evidence indicating the presence of alkaline and saline environments on Mars means that studying the survivability, repsonse and biosignatures of the communities of such environments is necessary.

A change in carbon usage was identified using Biolog$^{TM}$ Ecoplates following desiccation and Mars chamber treatment however, Biolog$^{TM}$ Ecoplates do have limited use in Mars chamber studies when utilising samples wich have a naturally low culturable cell count in the untreated samples. The subsequent treatment may lower the cell count to a point where the Biolog$^{TM}$ Ecoplate cease to perform at their fullest potential.

A decline in the number of colony forming units recorded on 0.7%, 6.8% and 15% NaCl growth media occurred following desiccation, along with a decline in the number of carbon sources being utilised. Desiccation was the biggest factor affecting survival of the complex soil community, the conditions in the Mars chamber including freeze thaw cycles and UVC radiation had no more effect upon the culturable cell viability than the bench conditions. The community able to grow on a medium of 15% NaCl was unaffected by neither the bench conditions nor the Mars chamber conditions.

The isolate *Planococcus* sp. LMLD02 and species of the genera *Idiomarina*, *Alkalibacterium* and *Halomonas* (as part of a complex soil community) all demonstrated an ability to survive simulated Martian conditions for at least 7 days, and could be useful model organisms for further survival studies.

Studying the response of a single isolate and a soil community to simulated Martian conditions or extreme environmental parameters are both important in adding to our understanding of the limits of life. The combination of colony counting, sequencing of isolates and Biolog$^{TM}$ Ecoplates provides a more complete view of the effects of drying and simulated Martian conditions.

### 6.7. Further work

There are several lines of enquiry which could be pursued following this study using Mars chamber systems.

Firstly, *Planococcus* sp. LMLD02 survived as well when placed into the SCSM, but does the presence of NaCl and carbonate in the simulant aid survival? If the presence of these minerals in a soil does enhance survival, then this would lend further support to the importance of identifying environments with such minerals (soda environments) when looking for past or present signs of life on Mars. Identification of such environments is the focus of Chapter 7.

Secondly, an interesting comparison to this study would be to compare the response of soil 2A, sampled further from the lake edge to simulated Martian conditions. It may be that the culturable community present in this sample would be desiccated for a high proportion of the year, therefore it would be expected that those organisms from a naturally highly desiccating environment would be even more resistant to the types of stresses applied in this study.

The survival of organisms following incubation under simulated Martian conditions is one aspect of considering the contamination of Mars and the possibility for any terrestrial life on Mars. However, it is not enough to simply look at survival, following the identification of organisms capable of survivng such conditions it is then necessary to identify and understand if any of those strains are capable of reproducing under these conditons, or if factors such as low temperature and hypobaria inhibit growth/germination in these isolates.

Finally, the analysis of changes in the functional genes for strains after incubation under different extreme parameters would indicate how the organisms respond to these conditions. Functional gene arrays could be used to analyse the functional genes before and after incubation, looking at changes in biogeochemical processes including carbon, nitrogen or phosphorus cycling and resistance (He et al., 2008) in response to the environmental stresses imposed. This will be discussed in more depth in Chapter 8.

## Chapter 7

# Testing PanCam multispectral filter sets on geological samples typical of alkaline environments

The primary focus of both the ESA ExoMars mission and the NASA Mars Science Laboratory mission is to find evidence of present life and/or past life on Mars. Gaining an understanding of what types of life occupy highly saline, alkaline and desiccating environments, and to what extent these organisms can withstand the types of environmental factors which would be experience on Mars has been the focus of this thesis so far. In the context of searching for signs of extraterrestrial life, it is vital that we have an understanding of exactly what types of environments should be the focus of missions and then understand how to identify those environments with the resources and equipment available to astrobiological missions.

### 7.1. The role of multispectral imaging in Mars missions

Multispectral imaging instruments have been present on past and present missions to Mars, such as the Beagle 2 Stereo Camera System, MSL MastCam filter set and MER PanCam instrument and for the future ExoMars mission (Chapter 1, section 1.8.3.1). The panoramic camera, instruments provide remote analysis of the surface geology. This system has an important role to play and on the ExoMars rover it is currently the only analytical instrument (apart from the ground penetrating radar system WISDOM), which does not rely upon the drill system (Cousins et al., 2010). The remote sensing instruments on a rover must provide the science team with sufficient information to allow for the priority scientific targets to be identified. The ability to do this remotely reduces the risk of wasting time and power on moving the rover to explore targets of no significant interest. It is therefore important that we understand the type and quality of multispectral data which may be produced during the missions. The NASA strategy of; “follow the water” when looking for life on Mars demands a focus by missions, upon minerals which form in association with water.

Water is highly significant in the search for life as we know it. Any geological evidence for past or present existence of water, through mineralogical composition (i.e. the presence of OH or $H_2O$), or those minerals which require water to form, but do not require liquid water to remain stable are prime targets for astrobiology missions (Bishop, 2005: Murchie et al., 2009: Cousins et al., 2010).

### 7.2. Multispectral Imaging filter sets

A multispectral camera system captures image data at specific wavelengths across the electromagnetic spectrum (Chapter 1, section 1.8.3.1). The images at different wavelengths are produced using a series of narrowband filters placed in front of the camera lens. The

panoramic camera instruments on the Pathfinder/Beagle 2, Mars Exploration Rover (MER), Mars Science Laboratory (MSL) and ExoMars can produce visible to near infrared multispectral images between 400-1010nm wavelengths (Smith et al., 1997: Bell et al., 2004: Griffiths et al., 2006: Malin et al., 2010: Cousins et al., 2010).

The choice of the centre filter wavelengths for these filters is key to ensuring the maximum amount of information is gathered by the multispectral imaging instrument. The filter sets designed for missions to Mars, both past and present and are outlined in table 7.1. These filter sets have been designed based on a set of criteria related to mineral identification, for example, wavelengths of iron oxidation, Fe and/or $H_2O$/HO absorptions features have been used, these being main features of interest for the Mars Pathfinder mission and the MER mission (Smith et al., 1997: Bell et al., 2003).

*Table 7.1. Filter centre wavelengths (λ) and bandpass in nm for the Pathfinder, Beagle 2, MER, MSL and ExoMars multispectral filter sets. All data are in nm. N/D no data*

| Pathfinder (Smith et al., 1997) | | Beagle 2 (Griffiths et al., 2006) | | MER (Bell et al., 2003) | | MSL (Malin et al., 2010) | | ExoMars F2-12 (Cousins et al., 2010) | | ExoMars Ferric (Cousins, Pers. Comm.) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| λ (nm) | Band pass | λ (nm) | Band pass | λ (nm) | Band pass | λ (nm) | Band pass | λ (nm) | Band pass | λ (nm) | Band pass |
| 443 | 22 | 440 | 22 | 432 | 25 | 440 | 12.5 | 440 | 22 | 440 | N/D |
| 479 | 28 | 480 | 28 | 482 | 27 | 525 | 10 | 470 | 26 | 500 | |
| 530 | 32 | 530 | 32 | 535 | 19 | 675 | 10 | 510 | 30 | 530 | |
| 499 | 21 | 600 | 21 | 601 | 17 | 750 | 10 | 560 | 27 | 570 | |
| 671 | 17 | 670 | 17 | 673 | 16 | 800 | 10 | 590 | 21 | 610 | |
| 752 | 18 | 750 | 18 | 753 | 20 | 865 | 10 | 650 | 18 | 670 | |
| 801 | 20 | 800 | 20 | 803 | 20 | 905 | 10 | 710 | 17 | 740 | |
| 858 | 34 | 860 | 34 | 864 | 17 | 935 | 12.5 | 750 | 18 | 780 | |
| 897 | 42 | 900 | 42 | 904 | 25 | 1035 | 12.5 | 820 | 27 | 840 | |
| 931 | 32 | 930 | 32 | 934 | 24 | - | 50 | 890 | 38 | 900 | |
| 966 | 29 | 965 | 29 | 1009 | 28 | - | - | 960 | 30 | 950 | |
| 1002 | 28 | 1000 | 28 | - | - | - | - | 1000 | 28 | 1000 | |

The basis for the ExoMars PanCam filter set selection was to assist with the detection of environments of astrobiological interest in the Martian geological record, in particular hydrates minerals including phyllosilicates and sulphates. The use of different criteria methods by each mission (Pathfinder, Beagle 2, MER, MSL, ExoMars) for the choice of filter wavelengths results in a series of subtlety different filter sets. (Figure 7.1). The 12 filters provisionally devised for

the ExoMars PanCam instrument, filter set “F2-12” were designed using a suite of phyllosilicates, sulphates and a carbonate mineral (Cousins et al., 2010). Following this, an alternative “Ferric” filter set has been designed, using the ferric minerals haematite, goethite, ferrihydrate and magnetite (Cousins. Pers. Comm.)

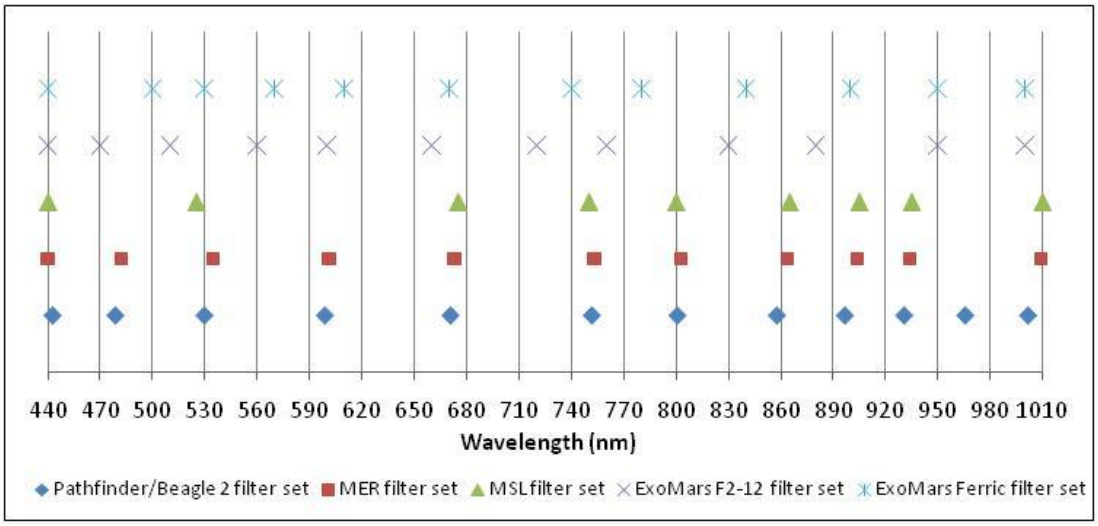



*Figure 7.1 Distribution of centre wavelengths for each of the filter sets.*

Most distinguishing features for mineral samples occur beyond 1010nm (Cousins et al., 2010). The limitation of multispectral imaging to the range 400-1000nm, means that many spectral features beyond the VIS-NIR region are missed. It is therefore necessary to use a range of analytical techniques, which can work together to produce complementary data in order to identify both the mineralogy and lithology of the outcrops.

ExoMars will use a Raman spectrometer (Ellery et al., 2004: Courrèges-Lacoste et al., 2007) and X-ray diffraction (Marinangeli et al., 2007) to assist with identification of mineralogy, (via a drill sample) at a site selected, based upon PanCam data. It is by the remote analysis and identification of sites of potential interest by the PanCam instrument, coupled with the in-situ analysis by other instruments, that an understanding of the geology and any potential biosignatures in the area can be obtained.

### 7.3. Geology of alkaline/saline environments using Multispectral Imaging.

Evidence of mineral deposits on Mars which are suggestive of alkaline or saline environments (or both), has been provided by in-situ rover analysis (Morris, 2010: Boyton, 2009) and remote sensing observations (Ehlmann et al., 2008b). Minerals including carbonates, phyllosilicates and zeolites have been identified on the surface in varying quantities (Table 7.2) (Mustard, 2008: Boynton et al., 2009: Ehlmann et al., 2009: Morris, 2010).

*Table 7.2 Table of minerals identified or hypothesised to be present on Mars which indicate periods of neutral-alkaline aqueous alteration. The table outlines the pH range for formation of each mineral on Earth, and where it has been identified on Mars.*

| Mineral type | Mineral name | pH formation range | Reference for identification or prediction of presence on Mars |
|---|---|---|---|
| Zeolite | Chabazite | pH>9 (Gottardi, 1985) | Hypothesised to be present (Bish et al., 2003: Ruff, 2004b) |
| | Phillipsite | pH 9-11 and stable at pH 7-10 at 5°C (Iijima and Utada, 1966) | Hypothesised to be present and possibly identified phillipsite (Ruff, 2004b: Bishop, 2005) |
| | Analcime | pH>9 (Iijima and Utada, 1966: Kalló, | Nili Fossae (Ehlmann et al., 2009) |
| Carbonate | Siderite | Neutral to alkaline pH (Ehlmann et al., 2008b) | Columbia Hills of Gusev crater (Morris, 2010) Nakhla meteorite (Bridges and Grady, 1999), |
| | Calcite | | Phoenix landing site (Boynton et al., 2009) |
| | Magnesite | | Columbia Hills of Gusev crater (Ehlmann et al., 2009) |
| Phyllosilicate | Montmorillonite | pH>7.5 (Weaver, 1989) | Smectite clays in Mwarth Vallis (Mustard, 2008) and Nili Fossae (Ehlmann et al., 2009) |
| Halide (evaporite) | Halite | pH1.7- >10 (Jagniecki and Benison, 2010) | Nakhla meteorite (Bridges and Grady, 1999) |

On Mars these minerals, which require alkaline aqueous environments to form, were predominantly deposited during the Noachian to early Hesperian periods (Ehlmann et al., 2008b: Morris, 2010) and suggest that localised areas on the surface of Mars were once neutral to alkaline (Chapter 1, section 1.8.3.3). Another point of interest is that in some areas minerals have survived which we would have expected to have been eroded away if subjected to the acidic conditions hypothesised for the whole surface of the planet from the Hesperian period (Ehlmann et al., 2008b). This may indicate that Mars was not globally environmentally homogeneous and that areas of both acidic and alkaline environments could have been present on the planet since the Noachian (Ehlmann et al., 2008b).

It is becoming clear that there would have been areas of more neutral/alkaline conditions on Mars until more recently (Morris, 2010). Analogue studies are being conducted in areas with a

less acidic pH (Ehlmann et al., 2011a), but there is much scope for further studies on alkaline environments with an astrobiological focus.

### 7.4. Spectral features of minerals which can form under alkaline/neutral conditions

#### 7.4.1. Spectral features of carbonate minerals

As discussed in Chapter 1, section 1.4, alkaline/saline systems can produce different minerals depending upon the water chemistry they precipitate from. Magnesite ($MgCO_3$), calcite ($CaCO_3$) and trona ($Na_3H(CO_3)_2{\cdot}2H_2O$) all form in alkaline environments. However the precipitation of sodium carbonate minerals such as trona requires very low levels of calcium and magnesium in the system (Warren, 2006). Therefore it is necessary to be able to distinguish between different types of carbonates.

The iron carbonate siderite ($FeO_3$) has distinctive absorption features around 600nm and 1000nm that are attributed to the presence of iron (Figure 7.2.A). The diagnostic spectral features of trona are generally at wavelengths >1000nm, trona has a small absorption around 1008nm (Drake, 1995) which could not be defined, water absorption features generally occur from about 950-1000nm (Figure 7.2.B) due to the OH stretching vibrations (Cousins et al., 2010). Anhydrous carbonates such as calcite and magnesite (Figure 7.2.A) contain no distinct absorption features in the visible and NIR region (Drake, 1995).

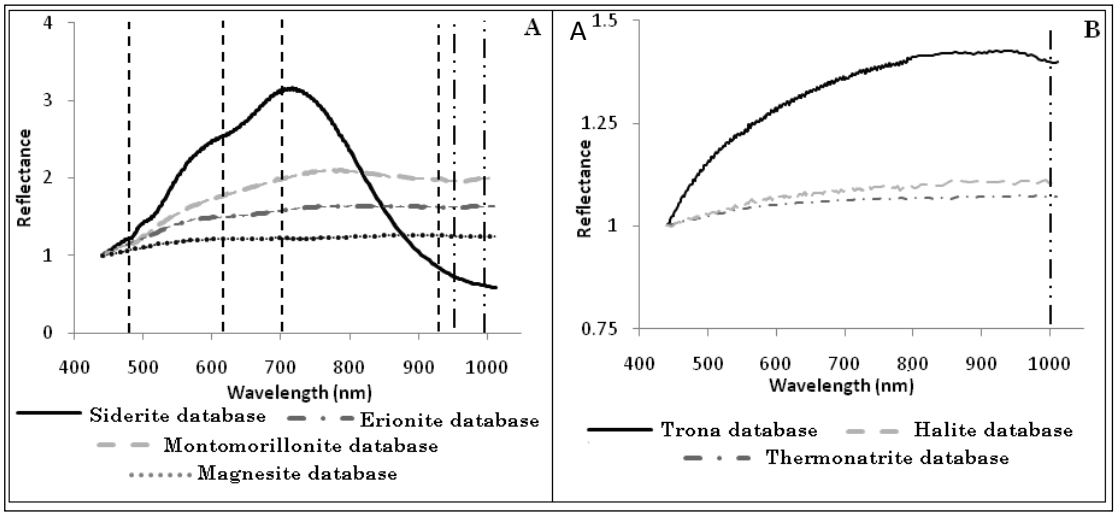



*Figure 7.2. Database spectra for minerals which form under alkaline conditions, showing the position of iron (dashed line) and water features (dash and dotted line) within the spectra.* ***(A)*** *Example database reflectance spectra for iron carbonate Siderite (Baldridge et al., 2009b), zeolite mineral Erionite (RELAB-Spectral-Database, 2008) and phyllosilicate montmorillonite. (Clark et al., 2007)* ***(B)*** *Example database reflectance spectra for evaporite minerals Trona (Baldridge et al., 2009b), Thermonatrite (Baldridge et al., 2009b) and halite (Clark et al., 2007).*

The formation of evaporite carbonate minerals in playa environments is complex, with a range of minerals forming with varying levels of hydration (Warren, 2006). Table 5.3 outlines evaporite carbonates of differing hydrations which can form, with the spectral signature for each sodium carbonate species (except natrite) are shown in Figure 7.3.

*Table 7.3 Sodium carbonate species variation indicating differences between levels of hydration.*

| Mineral | Formula | Reference |
|---|---|---|
| Natrite | $Na_2CO_3$ | (Warren, 2006) |
| Nahcolite | $NaHCO_3$ | |
| Thermonatrite | $Na_2CO_3 \cdot H_2O$ | (Fall, 2005) |
| Trona | $Na_3(CO_3)(HCO_3) \cdot 2(H_2O)$ | (Crowley, 1991) |
| Natron | $Na_2CO_3 \cdot 10H_2O$ | |

The features in the reflectance spectra for these different species are limited but do show variation in absorption features around 650nm and 990nm, which is assigned as a hydration feature (Cousins, 2010).

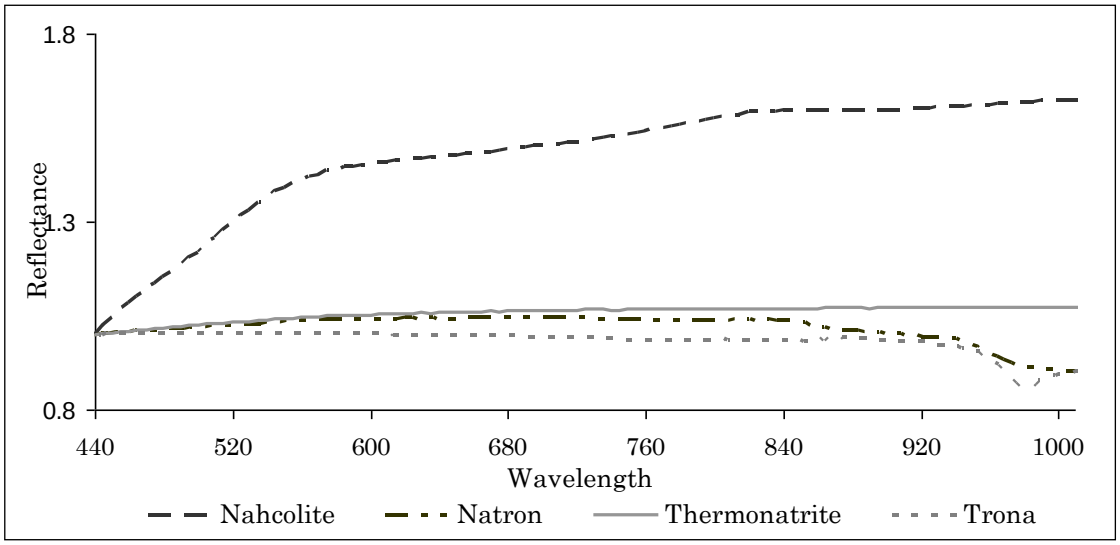



*Figure 7.3. Reflectance spectra for different carbonate species; Nahcolite, natron, trona (Clark et al., 2007) and thermonatrite (RELAB-Spectral-Database, 2008).*

### 7.4.2. Spectral features of evaporite minerals

Saline minerals generally exhibit a number of diagnostic features in the near-infrared (700-25000 nm). However the mineral halite, which is potentially an important mineral for astrobiology, as discussed in Chapter 1, section 1.7.3.1, exhibits no diagnostic features between 440-1010nm (Figure 7.3.B) (Crowley, 1991).

Hydration and dehydration of a mineral has an effect on the structural properties of evaporite minerals (including hydrated carbonates) and on the reflectance spectra they produce (Crowley, 1991). The formation of evaporite minerals and the interaction of different minerals in a mixture of particles of different sizes is complicated. The combinations of different minerals show non-linear mixing relationships and all these factors can result in the displacement of features and is particularly evident in the VIS-NIR region (Crowley, 1991). For this reason it is important to study geological samples and the natural mixtures of minerals.

### 7.4.3. Spectral features of zeolite minerals

Zeolites are hydrated aluminosilicate minerals and have been described in Chapter 1, section 1.8.3.4.4. On Earth zeolites form in saline-alkaline lakes and soils, and commonly in hydrothermal systems in basaltic and volcanic regions as well cold polar deserts (Hay, 1986: Cloutis, 2002). Zeolites can form through the gradual alteration of volcanic ash and tephra and require alkaline/neutral water to form (Warren, 2006).

In general zeolitic water between the layered structure of the zeolites is responsible for the absorption bands in zeolite spectra (Cloutis, 2002). The Vis-NIR reflectance spectra of zeolites exhibit some key features, An absorption band at 970nm, attributed to the presence of the O-H stretch which may be due to either bound or absorbed water in the zeolite structure (Cloutis, 2002).

The presence of iron in the form of FeO and/or $Fe_2O_3$ in a zeolite structure results in absorption features near 460nm, 600nm, 660nm, 700nm and/or ~900nm-920nm wavelengths (Cloutis, 2002) (Figure 7.1.A). A positive absorption edge from 400-600nm and absorption bands at 520nm and 900nm are due to the presence of ferric iron and a band around 1100nm is due to the presence of ferrous iron in some minerals (Bailin, 1991). The presence of ferric iron can also produce bands at 650nm and 950nm (Cloutis, 2006: Cousins et al., 2010).

### 7.4.4. Spectral features of phyllosilicate (smectite) minerals

The presence of clays on Mars is important not only for the indication of water present for their formation, but also due to their capacity to potentially preserve any organic material (Weaver, 1989: Mustard, 2008: Murchie et al., 2009). The phyllosilicate clay nontronite, an iron rich phyllosilicate with the typical formula of $\underline{Ca}_{.5}(\underline{Si}_7Al_{.8}Fe_{.2})(Fe_{3.5}Al_{.4}\underline{Mg}_{.1})O_{20}(O\underline{H})_4$ exhibits ferric absorption features around 480, 650 and 950nm (Stewart et al., 2006). Montmorillonite, a hydrated sodium calcium aluminium magnesium silicate hydroxide ($(Na,Ca)_{0.33}(Al,Mg)_2(Si_4O_{10})(OH)_2{\cdot}nH_2O$) may also contain iron within its structure. The spectrum for montmorillonite (both Na and Fe forms) contains no distinct absorption features in the 440-1010nm region, the only features being an increase in absorption from 440-750nm and a change in slope from 500-600nm (Figure 7.3.A) (Orenberg, 1992).

### 7.4.5. Spectral features of biological material

It is important to remember that geological and biological material is not always distinct, for example, endolithic organisms occupy niches within the rocks themselves (Konhauser, 2007). Analysis of those samples from Lake Magadi where endolithic organisms have probably colonised would provide important information on the spectral signatures produced in these associations. In addition biomats, although extremely unlikely to be discovered on the surface of Mars, will also provide information on the sorts of biosignatures such as preserved photosynthetic pigments, which organisms from soda lake environments produce (Ellery et al., 2004).

Absorption features occur between 450 nm and 520 nm which are attributed to chlorophyll and carotenoids, these compounds also result in a reflectance peak around 520 nm to 600nm (Figure 7.4). The maximum absorption for chlorophyll occurs at 630 nm to 690 nm (Hale, 2000). Liquid water in the structure can result in absorption overtones for water at 970 nm and towards 1190 nm (Toselli, 1992). Bacteriochlorophyll absorbs at wavelengths of 590 nm and 800 nm (Sparks et al., 2009). The pigment bacterioruberin, identified in some halophilic archaea (Chapter 5, section 5.4.3), exhibit reflectance peaks around 497 nm and minor peaks at 533 nm, 475 nm, and 392 nm (Dummer et al., 2011).

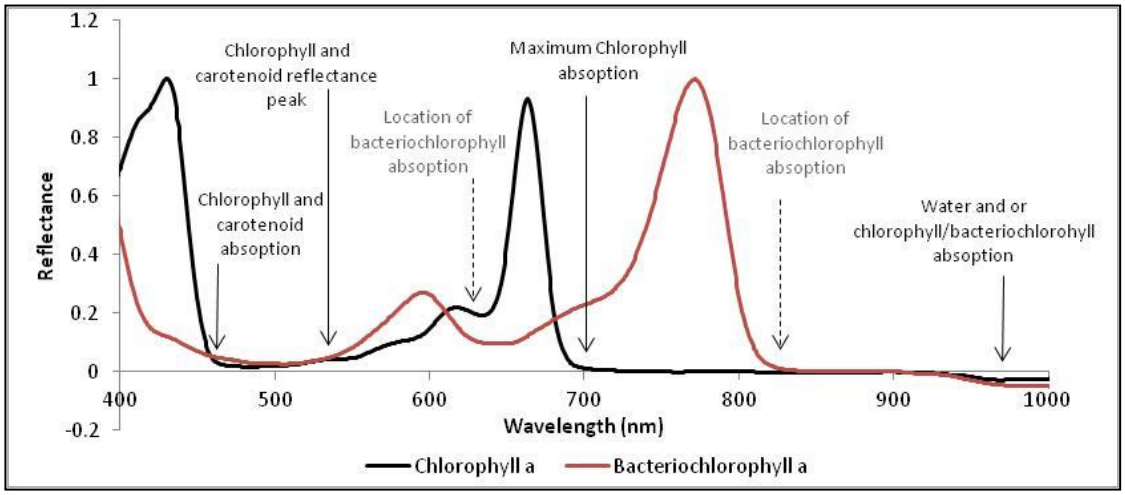



*Figure 7.4 Reflectance spectra chlorophyll and bacteriochlorophyll, with adsorptions and reflection peaks labelled (Toselli, 1992: Hale, 2000: Sparks et al., 2009).*

### 7.5. Aims of this chapter

1. To test filter sets designed for rover missions to Mars on a range of geological samples which form in alkaline environments
2. To test the filter sets designed for rover missions to Mars on biological material from Lake Magadi

3. To identify those features present in each spectrum, which can be used to maximise the information from the data sets.
4. To identify the filter set that is potentially most capable of identifying alkaline/saline forming minerals and biosignatures from organisms in these environments.

## 7.6. Methods

### 7.6.1. Samples from alkaline/neutral, saline environments

A variety of samples have been used to test the ability of the filter sets designed for the Pathfinder/ Beagle 2, MER, MSL and ExoMars to detect and distinguish between minerals which form in alkaline/saline environments. Two sets of samples were used (Table 7.4) with a total of 15 samples.

*Table 7.4 Description and sampling location for each sample from Lake Magadi (group 1) and description of samples from Geology Collections UCL.*

| Sample type | Sample name | Location and conditions | Sample description |
|---|---|---|---|
| **Sample group 1**- Geological and biological samples from Lake Magadi | SR1 | North of Lake Magadi | Weathered brittle and light rock |
| | GR1 | South of Lake Magadi | A dense but light rock, light green coloured with dark green bands |
| | MGS1 | Trona platform | Dark grey material with crystals of translucent/vitreous mineral |
| | MGS2 | Trona platform | Black and vitreous hard mineral. Massive |
| | MGS3 | Trona platform | Black and vitreous hard mineral with opaque white patches on the surface. Massive |
| | MGS11 | Trona platform | White/grey opaque mineral |
| | MGS17 | Trona platform | Vitreous brown/cream with monoclinic elongate crystals |
| | MudS41 | Mud flat area to south of Lake Magadi | Brown, black dried and semi-consolidated soil |
| | S133 | Embedded in the soil to the southern end of the lake | Cream brittle material, has pink areas around the top and bottom. A harder white region is present in the middle of the sample. |
| | BM108 | Northern end of Lake Magadi, 64.4 °C and a pH of 9. | Unconsolidated biological material, red in colour |
| | BM81 | North East, 82 °C, and a pH of 9.51, collected from a pool at 38 °C | Dark green and dark pink biomat |
| **Sample group 2**- Samples from UCL Collections, Department of Earth Sciences | GCS1 (Siderite) | - | Carbonate pinky, brown sample with some bladed crystals. Fine grained |
| | GCS2 (Magnesite) | - | Carbonate, white and opaque. Fine grained |
| | GCS3 (Analcime) | - | Zeolite, pinky cream opaque with trapezohedral crystals and matrix remnants in some areas. |
| | GCS4 (Phillipsite) | - | Zeolite, cream- grey colouration, translucent monoclinic crystals |

Sample group 1, containing 9 geological samples and 2 biological samples were collected from around Lake Magadi or collected from the trona layer within the lake by the Magadi Soda Company. . Sample group 2 contained 4 samples, magnesite and siderite, two carbonates which form in alkaline/non-soda environment and two zeolites phillipsite and analcime, which are known to form around Lake Magadi but were not sampled. These were loaned from The Geological Collection, UCL.

### 7.6.2. Sample preparation for SEM/EDS

Sample SR1 was analysed as a powder. Sample MGS3 was mounted on a stub but not gold coated, this was to allow use of the sample after SEM/EDS analysis, these samples were not polished. Sample GR1 was impregnated with resin as the sample was very brittle and analysed as a polished thin section, again this was not coated before analysis.

### 7.6.3. Sample preparation for multispectral analysis

All samples were imaged as whole, untreated samples to provide a more natural spectra, it being noted that spectral differences, such as loss of absorption features, occur when samples are crushed (Drake, 1995).

### 7.6.4. Multispectral imaging

The process of multispectral imaging and subsequent analysis undertaken here follows the method outlined in Cousins *et al*. (2010). Images were gathered using a Foculus FO432SB camera (1.4Mpixles, 8- bits/pixel greyscale, 15° field of view lens, exposure time 1 ms to 65 s). The camera had a 1024 x 1024 pixel CCD which responded to wavelengths between 400 and 1010nm. Connected to the camera was either a visible wavelength (440-750nm, bandpass 20nm) CRI Varispec liquid crystal tuneable filter (LCTF) or a LCTF covering the near infrared wavelengths (650 – 1100nm, bandpass of 10nm). Images were taken with the filters every 10nm from 440nm-720nm (visible) and 710nm to 1010nm (near infrared).

#### 7.6.4.1. Multispectral image protocol

Images were processed using the ImageJ program; (http://rsbweb.nih.gov/ij/), the reflectance spectra were calculated using: Reflectance = (T/C), where T is the brightness value for the target area of the sample, and C is the brightness value for the calibration target in the background of each image. The size of the region analysed from the target region varied depending upon the area of interest. Where appropriate a spectrum was sampled for different coloured regions of the sample.

#### 7.6.4.2. Reflectance spectra

The full reflectance spectrum obtained was sub-sampled to the specific filter wavelengths, representing the filter set to be tested: a 12 point spectrum for Pathfinder, Beagle 2 and ExoMars filter sets, an 11 point spectrum for the MER and a 9 point spectrum for the

MSL filter sets (Table 7.1). Each filter for the MER and MSL filters sets were represented by rounding up or down to the nearest 10nm.

#### 7.6.4.3. Spectral parameter plots

Spectral parameter plots were used to identify variability between filter sets. Band slope and band depth are demonstrated in figure 7.5 (Farrand, 2008: Cousins, 2010). The spectral parameters analysed are described in table 7.5.

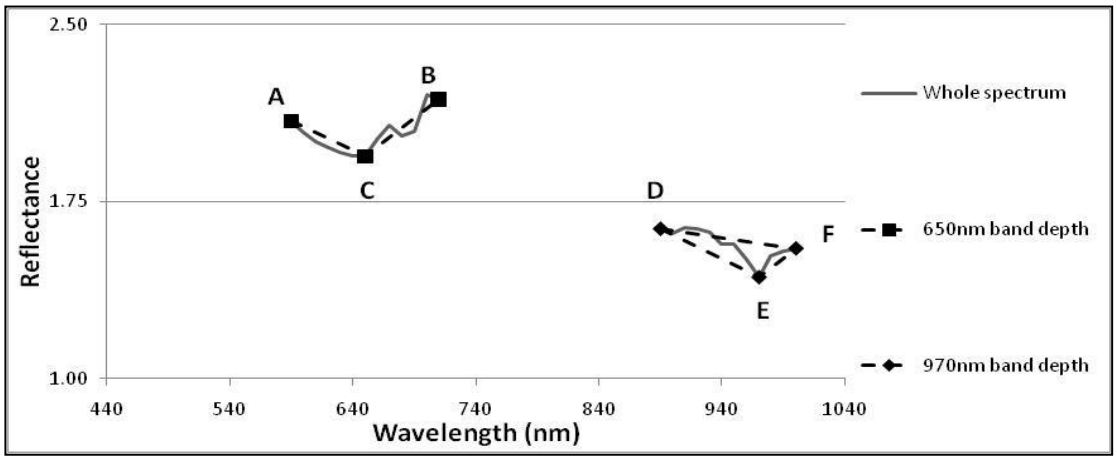



*Figure 7.5. Example of all spectral features analysed; slope (D to F) and band depth features (A, C, B and D, E, F) for analysis, indicating possible filter positions used for each analysis.*

For band depth analysis the wavelength and reflectance value for three points are used, a lower filter, an upper filter, (Figure 7.5, points A to B), and a central filter between the two (Figure 7.5., point C). The band depth was calculated as; Central filter reflectance value/ (a*Lower filter reflectance value) + (b*Upper filter reflectance value), where a= (Middle filter wavelength (in nm) - Lower filter wavelength) / (upper filter wavelength - lower filter wavelength) and b = 1-a.

#### 7.6.4.4. Database reference spectra

*Table 7.5 Spectral parameters used to assess the filter sets*

| Parameter | Filter set | Filter sets used (nm) | Description of possible explanation |
|---|---|---|---|
| 950-1000nm slope | Pathfinder/Beagle 2<br>MER<br>MSL<br>ExoMars F2-12<br>ExoMars Ferric | 970-1000<br>930-1010<br>940-1010<br>960-1000<br>950-1000 | Presence of water or iron |
| 650nm band depth | Pathfinder/Beagle 2<br>MER<br>MSL<br>ExoMars F2-12<br>ExoMars Ferric | 670<br>670<br>680<br>650<br>670 | Presence of Iron |
| 970nm band depth | Pathfinder/Beagle 2<br>MER<br>MSL<br>ExoMars F2-12<br>ExoMars Ferric | 970<br>930<br>940<br>960<br>950 | Hydration feature in zeolites |

For each spectral parameter plot 2-3 reference spectra from either the USGS (Clark et al., 2007), Aster JPL database (Baldridge et al., 2009b) or Brown University database (RELAB-Spectral-Database, 2008) were also plotted.

#### 7.6.4.5. Calculating error score

The absolute difference in reflectance between the whole spectrum and the estimate spectrum produced by each filter set was calculated to assess the quality of the sub-sampled spectra for each wavelength compared to the whole spectra at every 10 nm. The sum of the absolute difference for each 10 nm wavelength was the error score for that particular filter set and mineral. Low error score indicates a good fit of the whole spectrum compared to the estimated filter set spectrum. The error score for each mineral are then summed together to produce an average error score for each filter set.

## 7.7. Results

### 7.7.1. Detection of samples from alkaline/saline depositional environments from the Geological Collections- UCL

### 7.7.1.1. Sample GCS1

Sample GCS1 (Figure 7.6.A) was confirmed as the iron carbonate siderite, the raman spectrum resembled the reference spectrum for siderite, with diagnostic fundamental vibrations around1089cm$^{-1}$, 300cm$^{-1}$ and 194cm$^{-1}$ (Figure 7.6.B) (Bonelli, 2003).

The reflectance spectrum shows a diagnostic emission feature at ~720nm (Figure 7.6.C). The multispectral features of sample GCS1 are dominated by ferric absorption features at ~460nm, ~650nm and ~960nm (Figure 7.6.D). There was an increase in absorption from the 960nm to the 1010nm filters which is not apparent in the reference spectrum for siderite (Figure 7.6.C).

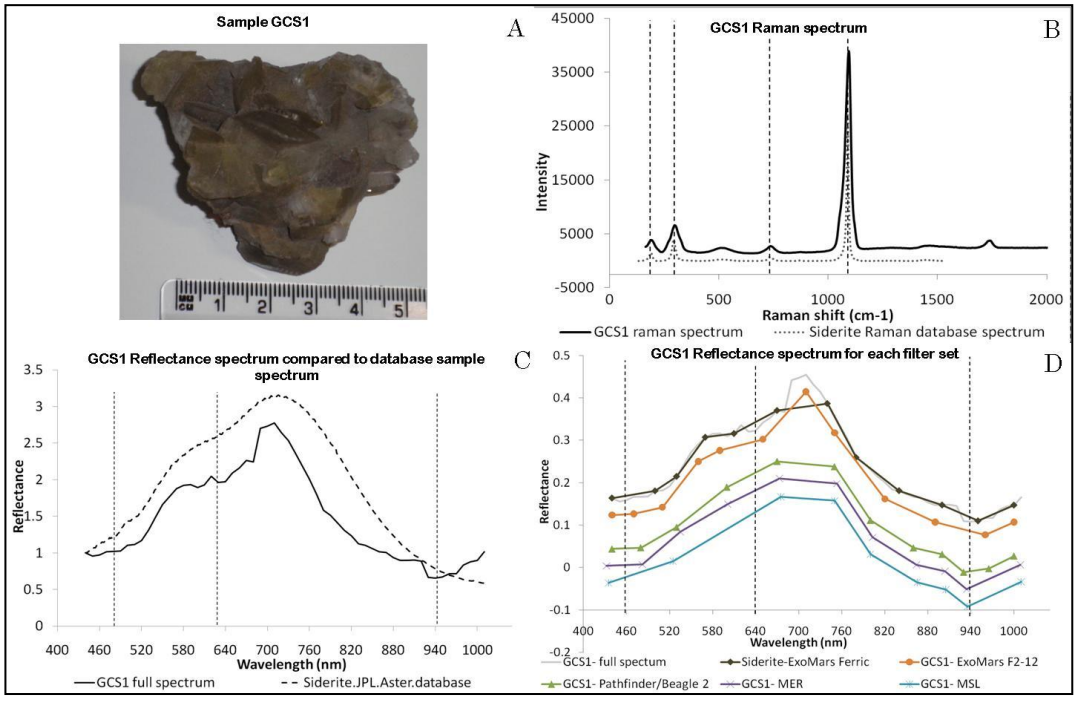



*Figure 7.6.* ***(A)*** *Sample GCS1.* ***(B)*** *Raman spectrum for GCS1 against RRUFF Project reference spectrum (Downs, 2006).* ***(C)*** *Comparison of GCS1 reflectance spectrum and a database reference spectrum (Baldridge et al., 2009b) both normalised to 1.* ***(D)*** *Reflectance spectrum for GCS1 and the sub-sampled spectrum for each filter set which have been offset for clarity.*

The only filter set to pick up the emission peak at ~720nm was the ExoMars F2-12 filter set (Figure 6.6.D). All the other filter sets miss this feature, although an overall trend of higher reflectance in this central region was generally picked up by all filter sets. The absorption features at 460nm and ~960nm are reproduced by most filter sets however the MSL filter set misses the 460nm absorption feature. Finally the 650nm absorption was only reproduced by the ExoMars PanCam F2-12 and Ferric filter sets.

### 7.7.1.2. Sample GCS2

Sample GCS2 (figure 6.7.A) was confirmed as the magnesium carbonate magnesite ($MgCO_3$) with fundamental vibrations at about 1092cm$^{-1}$ 316cm$^{-1}$ and 204cm$^{-1}$ (figure 6.7.B) (Bonelli, 2003).

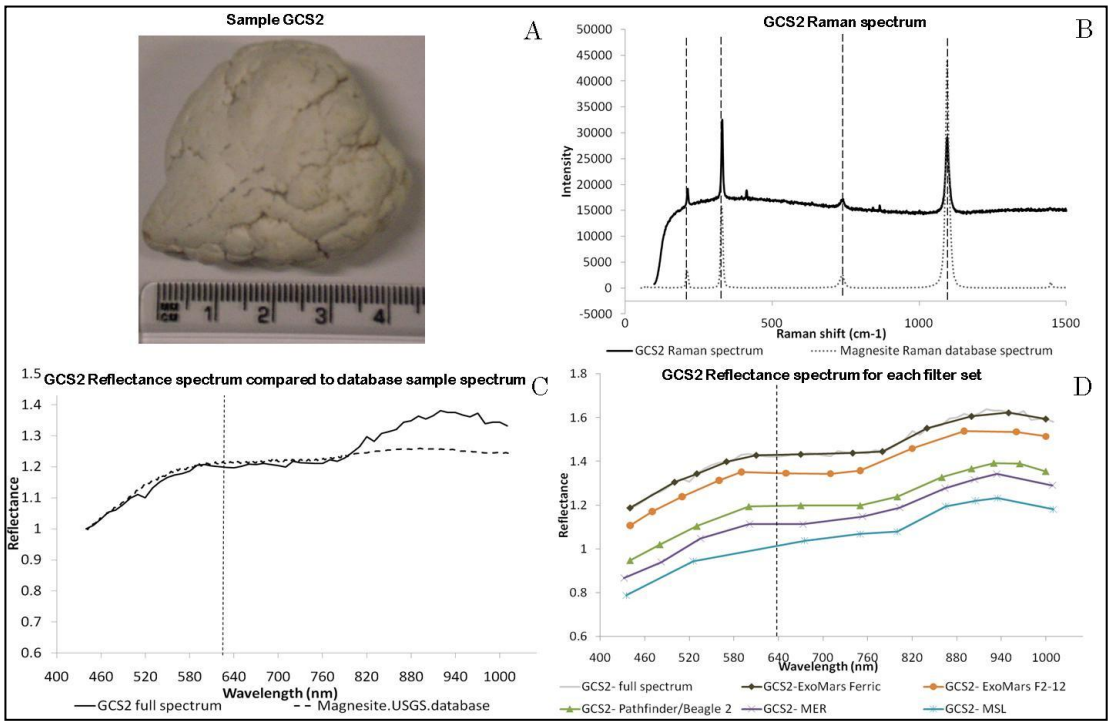



*Figure 7.7**(A)** Sample GCS2. **(B)** Raman spectrum for GCS2 against RRUFF Project reference spectrum (Downs, 2006). **(C)** Comparison of GCS2 reflectance spectrum and a database reference spectrum normalised to 1 (Clark et al., 2007). **(D)** Reflectance spectrum for GCS2 and the sub-sampled spectrum for each filter set which have been offset for clarity.*

The reflectance spectra for sample GCS2-magnesite (figure 6.7.C, solid line) resembles the magnesite database spectra (figure 6.7.C, dashed line), however a higher relative reflectance occurs in the NIR wavelengths from ~790nm. In addition, the sample spectrum demonstrated a plateau ~650nm and the beginning of an absorption feature from ~960nm. (Figure 6.7.C)

Figure 6.8.A shows the comparison of the database spectra from the USGS database (Baldridge et al., 2009b) for magnesite (figure 6.8.A, dashed line) with two spectra taken from sample GCS2. Other spectra obtained from different regions of sample GCS2 shows a larger absorption feature at ~730nm, indicated by a dashed line. This suggests possibly an additional trace element/mineral component within the magnesite, most likely an iron containing mineral.

Microprobe analysis confirmed the presence of trace amounts of iron in the sample, although this was very low, with FeO content up to 0.033 ± 0.0005 mass percent. Reflectance

spectroscopy is capable of detecting trace amounts of iron down to 0.01wt% content (Gaffey, 1985). Figure 6.8.B compares GCS2 whole spectrum 2 to the Aster Database spectrum (Baldridge et al., 2009b) for the nickel, iron carbonate gaspeite ($(Ni.Fe.Mg)CO_3$). A mixing model example which includes 0.5% gaspeite and 95.5% magnesite results in a small absorption feature occurring ~730nm (figure 6.4B). The presence of iron and the 730nm absorption feature for GCS2-magnesite are both thought to indicate the presence of trace amounts of gaspeite (Cloutis pers. Comm.).

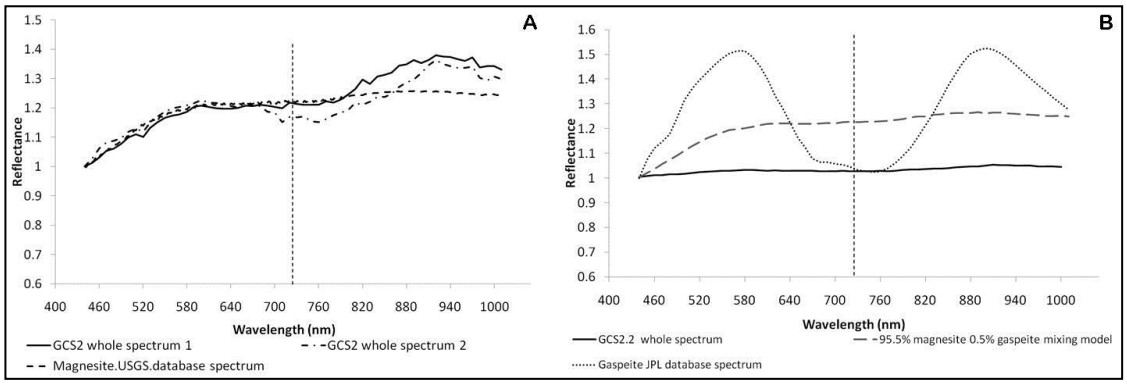



*Figure 7.8 Reflectance spectra of sample GCS2* ***(A)*** *Comparison of the reference spectra for magnesite from the database with two sample spectra for GCS2. Solid line spectrum is the spectrum used for filter analysis in GCS2-magnesite. GCS2 whole spectrum 2 (dot dashed line) is a spectrum taken from another region of the sample.* ***(B)*** *Comparison of the database spectra for magnesite and gaspeite, with a mixing model which mixes the two minerals. All spectra have been normalised to 1.*

Due to the relatively featureless nature of the VNIR region in carbonate minerals all filter sets are capable of reproducing the sample spectrum (figure 6.7.D). All the filter sets reproduce the plateau and absorption edge seen in the whole spectrum.

#### 7.7.1.3. Sample GCS3

Sample GCS3 (Figure 6.9.A) was confirmed as the sodium zeolite analcime ($NaAlSi_2O_6 \cdot H_2O$), with fundamental raman vibrations at 484cm$^{-1}$ (Bonelli, 2003), 1100cm$^{-1}$ and 300cm$^{-1}$ (Figure 6.9.B).

The overall shape of the reflectance spectrum (figure 6.9.C) resembles that of the database reflectance spectrum for analcime, although the absorption edge between 440nm and 600nm was much steeper than in the reference spectrum (Figure 6.9.C). Figure 6.9.D shows an increase in reflectance between 470nm and 610nm with a change in slope (shallower slope) from 610nm to 920nm. A distinct absorption feature occurs around 970nm and can be attributed to the second overtone of a $H_2O$ or OH absorption feature at 1400nm (Cloutis,

2002). A small absorption feature was present around 480nm which may indicate the presence of iron which is often present in zeolites (Cloutis, 2002).

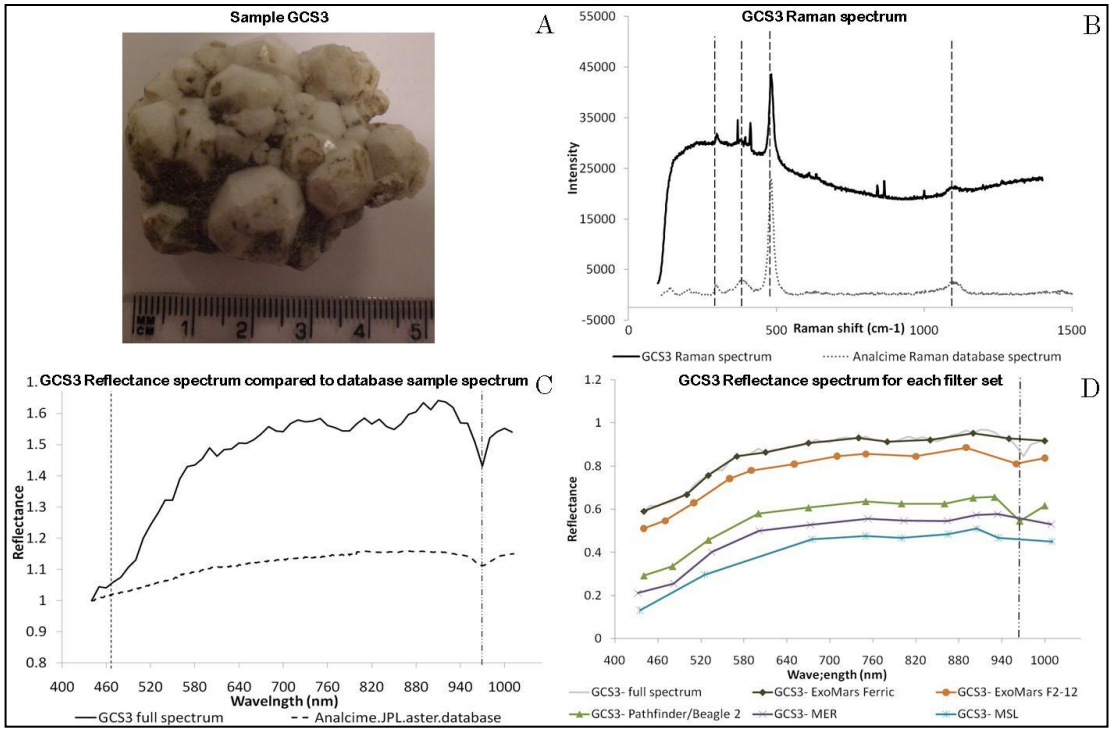



*Figure 7.9 (**A**) Sample GCS3. **(B)** Raman spectrum for GCS3 against RRUFF Project reference spectrum (Downs, 2006). **(C)** Comparison of GCS3 reflectance spectrum and a database reference spectrum normalised to 1 (Clark et al., 2007). **(D)** Reflectance spectrum for GCS3 and the sub-sampled spectrum for each filter set which have been offset for clarity.*

Only the Pathfinder/Beagle 2 filter sets are capable of reproducing the absorption feature at 970nm (figure 6.9.D). The ExoMars F2-12 filter set does reproduce a slight absorption around 970nm, however it was far less pronounced. This loss of detail in the MER, MSL and ExoMars Ferric filter set spectra was due to the spacing of filters in the 700-1010nm region. The Pathfinder/Beagle 2, MSL and ExoMars F2-12 filters all fail to reproduce the 490nm absorption feature present in this sample spectrum.

### 7.7.1.4. Sample GCS4

Sample GCS4 was described as phillipsite by the Geology Collections UCL and was analysed with Raman spectroscopy. The raman spectrum suggested it was a zeolite mineral; however is not easy to distinguish between phillipsite and analcime using Raman spectroscopy (Figure 6.10.B). It was not possible to sub-sample for XRD.

The absorption seen at 970nm in the GCS4 sample was not present in the reference spectra for phillipsite used here, but was seen in analcime reference spectra (Figure 6.10.C). This feature was attributed to the presence of $H_2O$ or OH in the minerals structure (Cloutis, 2002).

The steep 440-600nm absorption edge and small absorption feature at 650nm may be due to the presence of iron in the structure (Cloutis, 2002).

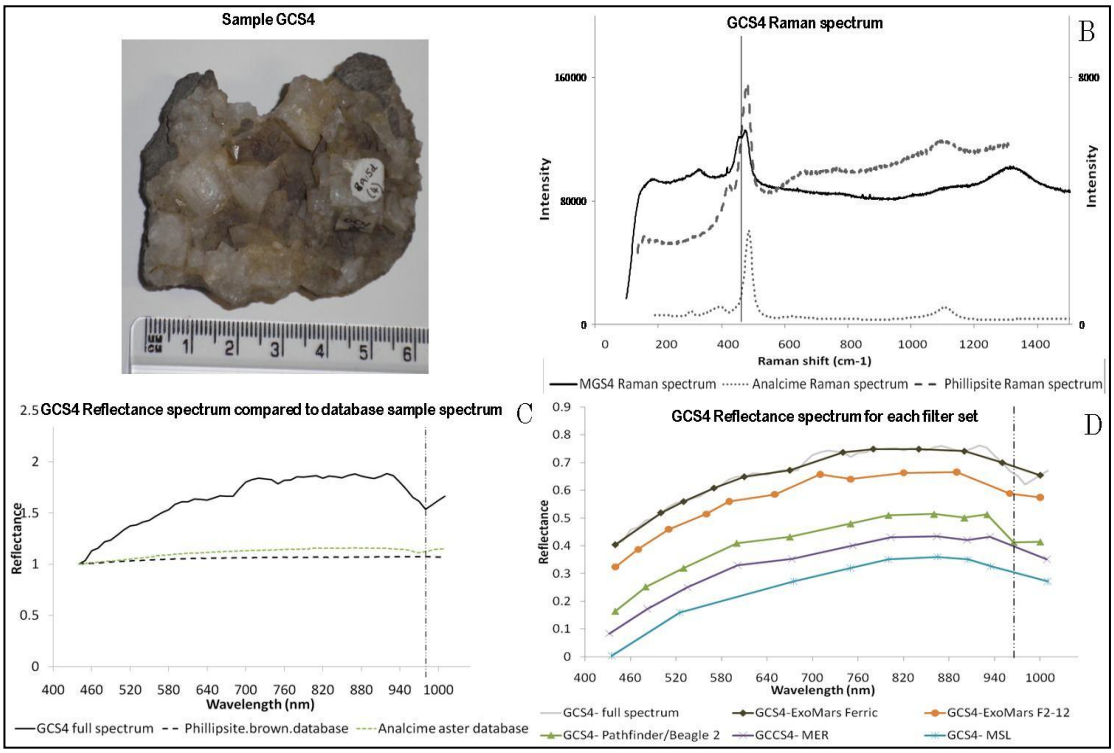



*Figure 7.10* ***(A)*** *Sample GCS4.* ***(B)*** *Raman spectrum for GCS4 against RRUFF Project reference spectrum (Downs, 2006).* ***(C)*** *Comparison of GCS4 reflectance spectrum and a database reference spectrum and normalised to 1 (Clark et al., 2007).* ***(D)*** *Reflectance spectrum for GCS4 the sub-sampled spectrum for each filter set which have been offset for clarity.*

As with the 970nm hydration feature in GCS3-analcime, only the Pathfinder/Beagle 2 filter set are able to reproduce the feature, with the other filter sets missing the feature completely (Figure 710.D). The small absorption at 650nm was picked up by all filter sets with the exception of the MSL filter set. This 650nm feature was slightly deeper than the absorption feature in a similar region seen in GCS1-siderite, this may explain why it was replicated in GC4 but not GCS1-siderite.

### 7.7.2. Detection of samples from Lake Magadi

#### 7.7.2.1. Sample GR1

The XRD data indicates this light green sample with dark green bands (Figure 7.11.A) contains the zeolite mineral erionite ($(Na_2, K2, Ca)_2Al_4Si_{14}O_{36}{\cdot}15H_2O$) and the evaporite halite (NaCl), with almost all peaks accounted for (Figure 7.11.B). EDS analysis of GR1 indicated the presence of 11.3 wt% iron and 1.99 wt% magnesium.

The reflectance spectrum for sample GR1-erionite exhibits an absorption features at about 490nm, 650nm and possibly a small absorption ~940nm (Figure 7.11.C). The sample spectrum exhibits a greater increase in reflectance from 440nm to 560nm compared to that seen for the database reference. The absorption features are also much less pronounced and relatively shallower in the reference spectrum. The 490nm absorption feature was attributed to ferrous iron (Cloutis, 2002), with the 650nm feature the result of ferric iron in the sample (Cousins et al., 2010). The absorption at 940nm could be attributed to iron, a 940nm absorption is present in both ferric minerals goethite and hematite (Leone and Sommer, 2000: Bishop and Murad, 2005).

A comparison of the reflectance spectrum taken from the dark banded area to the spectrum taken from the light banded area showed no differences in spectral features (figure 7.11C).

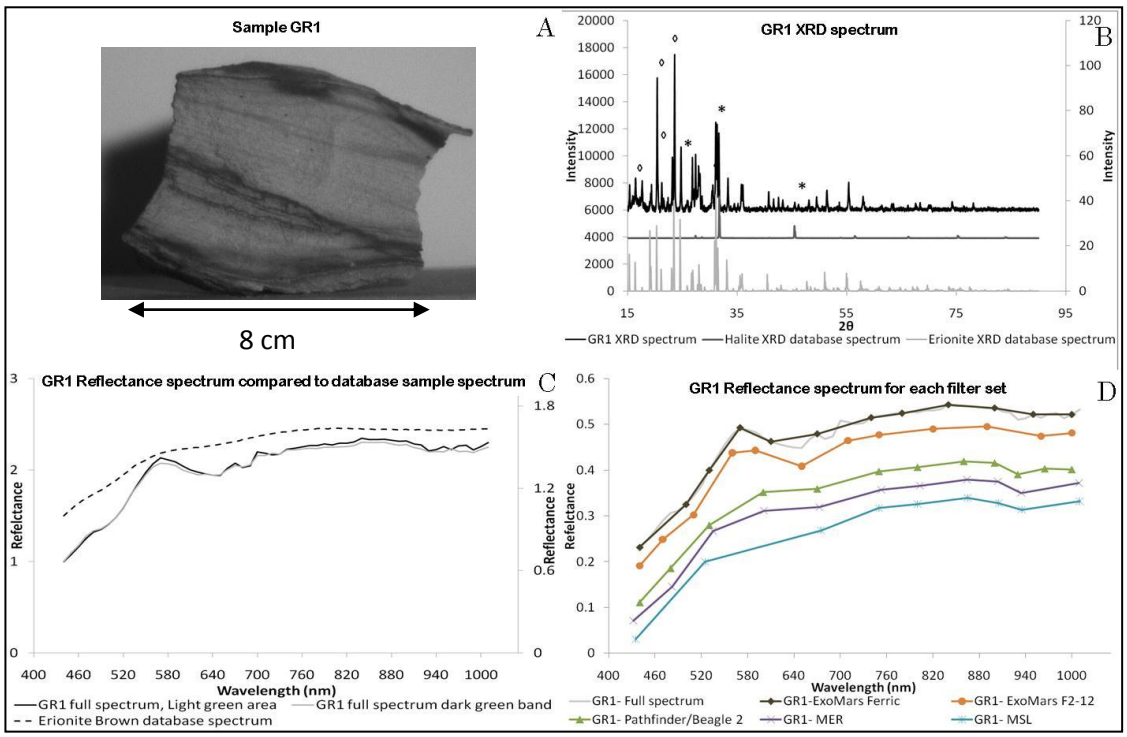



*Figure 7.11* ***(A)*** *Sample GR1.* ***(B)*** *XRD spectrum for GR1 against RRUFF Project reference spectrum (Downs, 2006).* ***(C)*** *Comparison of GR1 reflectance spectrum and a database reference spectrum (Clark et al., 2007).* ***(D)*** *Reflectance spectrum for GR1 the sub-sampled spectrum for each filter set which have been offset for clarity.*

The MER and ExoMars F2-12 filter sets replicate the 490nm absorption feature in the spectrum which was missed by Pathfinder /Beagle 2 and MSL. The ExoMars Ferric filter set reproduces a very minor absorption in this region. The emission feature at 560nm and the following 660nm absorption are distinctly replicated by both ExoMars filter sets (figure 7.8.D). These features are greatly diminished by the Pathfinder/Beagle 2 and MER filter sets although

a small absorption was replicated in the 660nm region. The 490nm absorption and the 560nm emission are both missed by MSL. The final feature around 940nm was replicated by all filter sets.

#### 7.7.2.2. Sample SR1

Sample SR1, a light brown yellow sample (Figure 7.12.A) was analysed using XRD, which confirmed the sample contains the zeolite chabazite ((Ca, Na2, K2, Mg)Al2$Si_4O_{12}$·6$H_2O$) and halite, with most peaks accounted for (figure 7.12.B). EDS analysis of SR1-chabazite indicated the presence of small amounts of calcium, 1.05 wt% and 2.1 wt% iron.

The reflectance spectrum for SR1 has absorption features at 490nm, 650nm, due to iron and around 1000nm probably due to $H_2O$ or OH. The 650nm absorption corresponds to an absorption feature in the reference spectrum, however, the reference spectrum for chabazite lacks the strong absorption feature at 1000nm observed in the sample SR1.

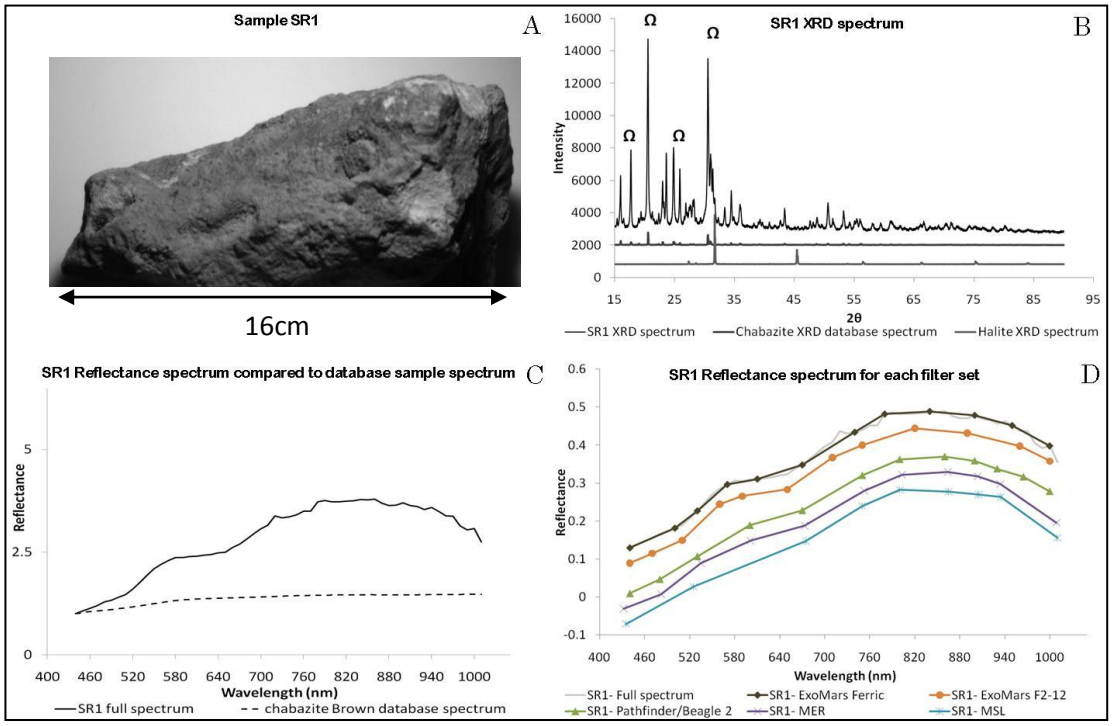



*Figure 7.12* ***(A)*** *Sample SR1.* ***(B)*** *XRD spectrum for SR1 against RRUFF Project reference spectrum (Downs, 2006).* ***(C)*** *Comparison of SR1 reflectance spectrum and a database reference spectrum, normalised to 1 (Clark et al., 2007).* ***(D)*** *Reflectance spectrum for SR1 and the sub-sampled spectrum for each filter set which have been offset for clarity.*

The ExoMars Ferric and F2-12 filter sets can replicate all of the spectral features for this mineral sample (Figure 7.12.D). The MER filter set can replicate the 490nm absorption band but still misses the feature around 650nm The Pathfinder/Beagle 2 filter sets are capable of

reproducing the 1000nm absorption edge; however they miss the spectral features around 490nm and 650nm.

### 7.7.2.3. Sample MGS1

XRD analysis suggests that MGS1 contains a mix of trona, thermonatrite, halite, montmorillonite and erionite (Figure 7.13.B). This sample could be an amalgamation of fine grained material blown onto the trona platform area within the lake along with larger crystals of broken up trona (Figure 7.13.A). The MGS1 sample spectrum was compared to the minerals trona and erionite (figure 7.13.C), the spectrum follows the steep increase in reflectance from 440 to 600nm which was present in erionite (Cloutis, 2002). It also exhibits absorption features at 660nm and 940nm, which correspond to similar absorption features in erionite and could be due to iron, and 1000nm probably due to the presence of $H_2O$ in trona (Drake, 1995).

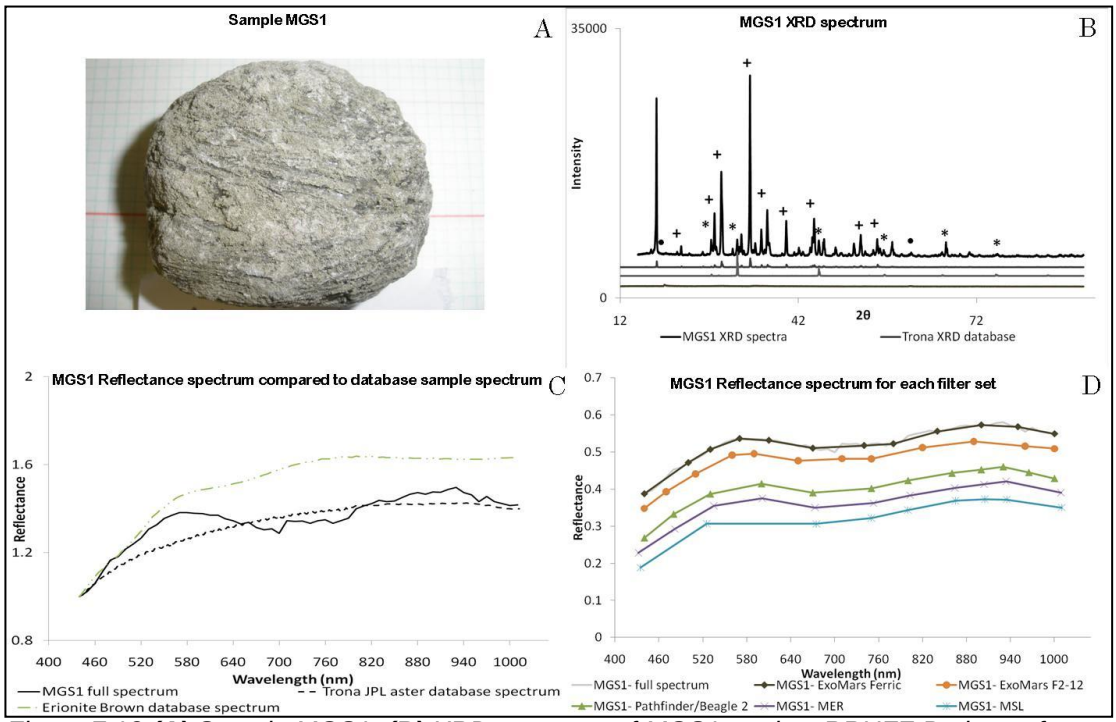



*Figure 7.13* ***(A)*** *Sample MGS1.* ***(B)*** *XRD spectrum of MGS1 against RRUFF Project reference spectrum (Downs, 2006).* ***(C)*** *Comparison of MGS1 reflectance spectrum and a database reference spectrum, normalised to 1 (Clark et al., 2007).* ***(D)*** *Reflectance spectrum for MGS1 and the sub-sampled spectrum for each filter set which have been offset for clarity.*

All filter sets can replicate the absorption feature at 700nm equally well (figure 7.7.D). The emission peak at around 580nm was missed by the MSL filter set, but reproduced by all the other filter sets. The hydration feature around 1000nm was replicated by all filter sets.

### 7.7.2.4. Sample MGS2

XRD analysis confirms the sample as containing trona, thermonatrite and halite, with all peaks accounted for (figure 7.14.B). EDS analysis of the white are of the sample identified the

presence of chlorine (45.8 wt %) as well as smaller amounts of potassium (0.98 wt %), aluminium (1.06 wt %) and iron (1.77 wt %). The darker area of the sample contains less chlorine (0.98 wt %), more iron (5.67 wt %) and fluorine (3.72 wt %) in comparison. It was not possible to obtain a reflectance spectrum from the two different regions of the sample as the spectrum was too noisy for any meaningful analysis to be conducted. The spectrum from the darker area exhibits an absorption feature at about 650nm and again in the 1000nm region with an increase in absorption towards the 1010nm filter (Figure 7.14.C). The 1000nm absorption was similar to that seen in the reference spectrum of trona, however there is an absence of a 650nm absorption in trona (Figure 7.13.C). The 650nm absorption in the sample spectrum could be a factor of iron being present in the sample.

The sub sampling to each filter set demonstrates they are all capable of replicating the 650nm and 1000nm absorption features, although the MER filter set does flatten off more around 1000nm (Figure 7.13.D).

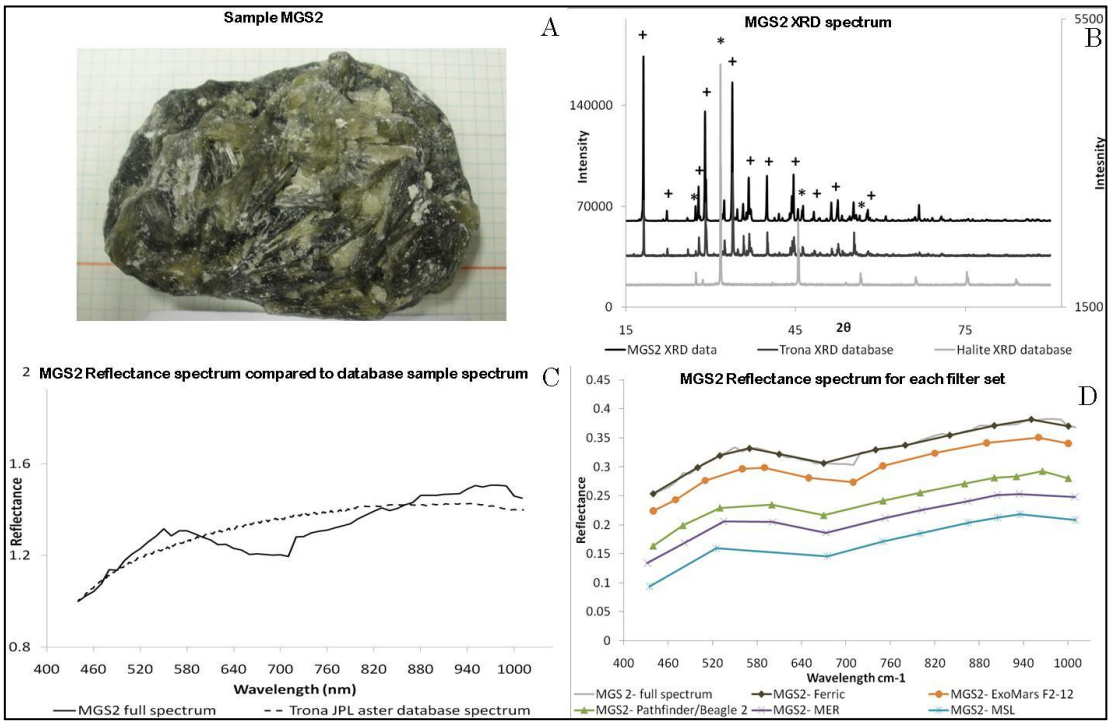



*Figure 7.14* ***(A)*** *Sample MGS2.* ***(B)*** *XRD spectrum of MGS2 against RRUFF Project reference spectrum (Downs, 2006). (C) Comparison of MGS2 reflectance spectrum and a database reference spectrum, normalised to 1 (Clark et al., 2007).* ***(D)*** *Reflectance spectrum for MGS2 and the sub-sampled spectrum for each filter set which have been offset for clarity.*

### 7.7.2.5. Sample MGS3

The composition of MGS3 was confirmed using Raman spectroscopy (figure 7.15.B) and most peaks align with the peaks present in the trona reference spectrum with fundamental vibrations occurring at 1060cm-$^{1}$, 184cm-$^{1}$ and 175cm-$^{1}$ (Downs, 2006). The vibration seen in

the sample spectrum at ~156m-$^{1}$ corresponds with the reference spectrum for thermonatrite, which has fundamental vibrations at 1062cm-$^{1}$, 185cm-$^{1}$ and 156cm-$^{1}$ (Downs, 2006).

Energy dispersive X-ray analysis indicated that the sample contained iron (2.44 wt %), with traces of magnesium (0.31 wt %). The presence of sodium and chloride (37.88 wt%) would indicate that halite maybe present, however, halite has no activity in the raman spectrum (Edwards, 2006) and so its presence cannot be confirmed with this technique.

As with sample MGS2 it was not possible to take reflectance spectra from both the light and dark areas of the sample as the spectra were too noisy. The spectrum obtained (Figure 7.15.C) shows a general increase in reflectance from 440 to 940nm, and an absorption feature from 940nm to 1010nm. It has a greater overall reflectance compared to the database reference spectrum for trona, but does exhibit the same absorption at the longer wavelengths. There was possibly an absorption feature around 670nm, however the spectrum for this sample has a degree of noise which makes any small absorption features difficult to discern.

All filter sets can reproduce this spectrum equally well all reproducing the water hydration feature around 950nm to 1010nm (Figure 7.14.D).

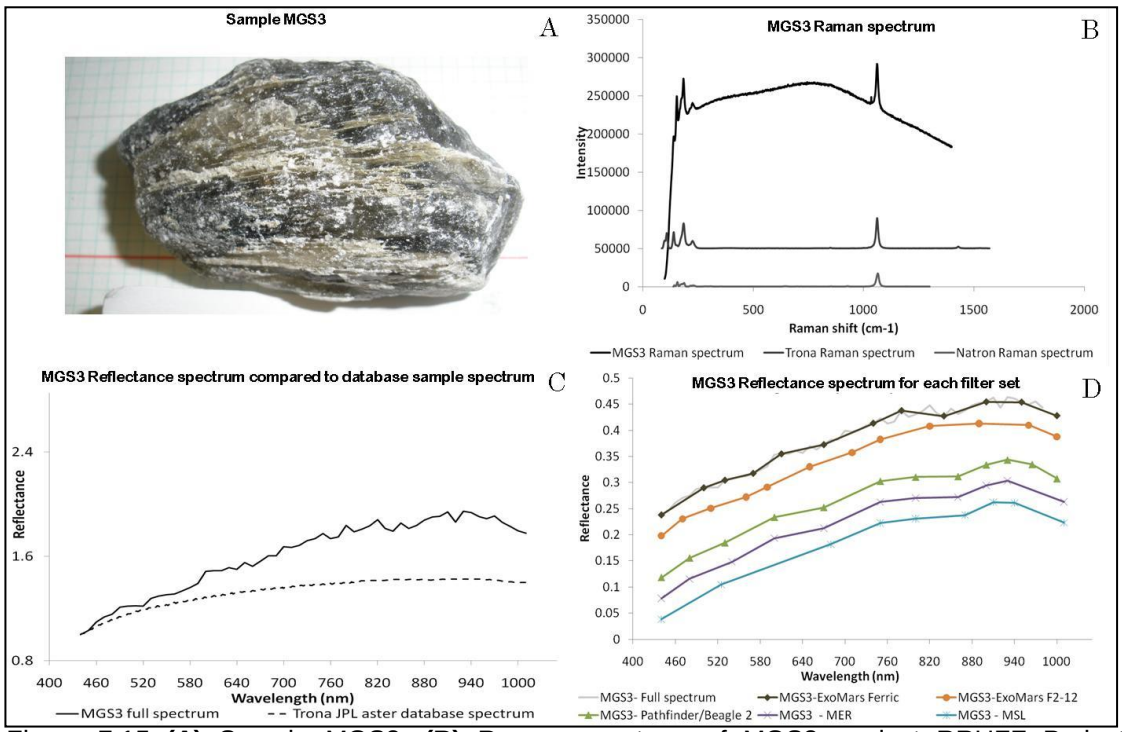



Figure 7.15 **(A)** Sample MGS3. **(B)** Raman spectrum of MGS3 against RRUFF Project reference spectrum (Downs, 2006). **(C)** Comparison of MGS3 reflectance spectrum and a database reference spectrum, normalised to 1 (Clark et al., 2007). **(D)** Reflectance spectrum for MGS3 and the sub-sampled spectrum for each filter set which have been offset for clarity.

### 7.7.2.6. Sample MGS11

XRD analysis identified that MGS11 contains trona and halite, with most peaks accounted for (Figure 7.16.B). The reflectance spectrum for sample MGS11 exhibits a 1000nm absorption feature which was the beginning of a feature occurs further into the NIR (Crowley, 1991) (Figure 7.16.C). This spectrum differs to the spectra for MGS2 and MGS3 by the absence of an absorption feature below 900nm. The reflectance spectrum for MGS11-trona has similarities to the database reference spectrum for trona, with the overall trend of increasing reflectance from 440 to 830nm followed by a decrease in reflectance towards 1010nm (figure 7.16.C).

The filter sets for Pathfinder/Beagle 2, and ExoMars replicate the sample spectrum equally well, however MER and MSL filter sets reduce the amount of absorption seen in the 1000nm region and almost flatten the spectrum off in this area (Figure 7.16.D).

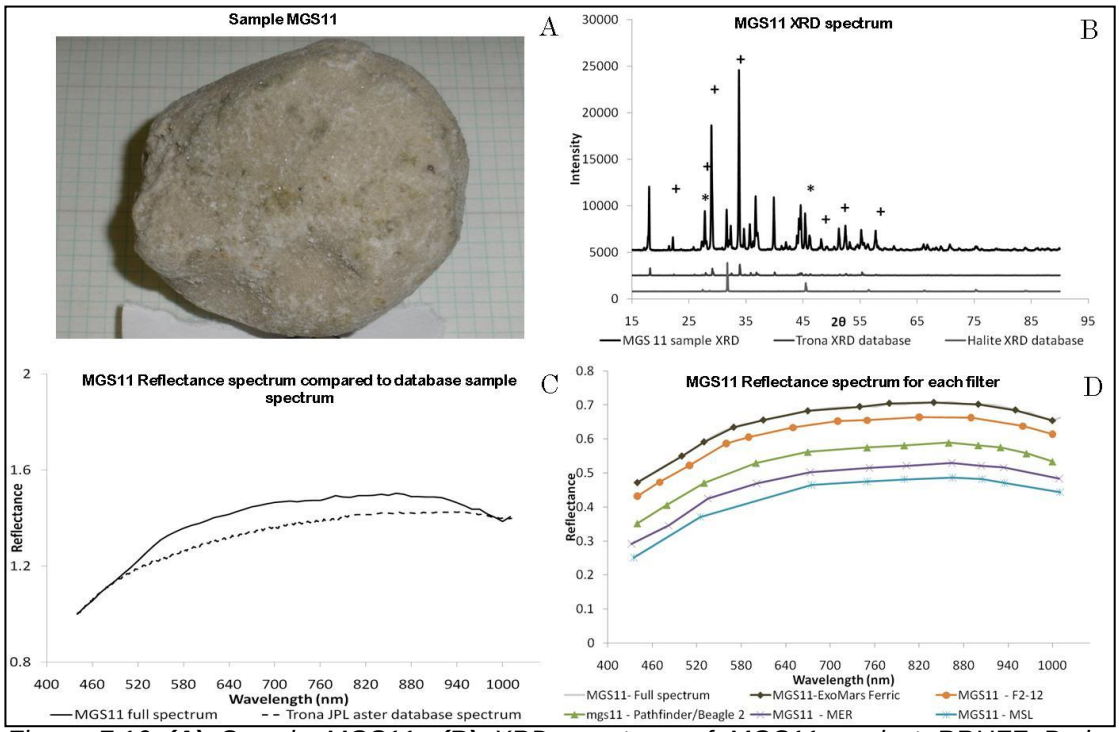



*Figure 7.16* ***(A)*** *Sample MGS11.* ***(B)*** *XRD spectrum of MGS11 against RRUFF Project reference spectrum (Downs, 2006).* ***(C)*** *Comparison of MGS11 reflectance spectrum and a database reference spectrum, normalised to 1 (Clark et al., 2007).* ***(D)*** *Reflectance spectrum for MGS11 and the sub-sampled spectrum for each filter set which have been offset for clarity.*

### 7.7.2.7. Sample MGS17

The morphology of MGS17 differs to the previous samples with the crystals having a more elongate habit and which stick together (Figure 7.17.A). The raman spectrum of this

sample indicates that it is predominantly trona and thermonatrite with fundamental vibrations at 1060cm$^{-1}$, 184cm$^{-1}$ 175cm$^{-1}$ and 156m$^{-1}$ (Figure 7.17.B).

The reflectance spectrum for this sample demonstrates absorption features around 490nm, 650nm and ~1000nm (figure 7.17.D). The absorption feature around 1000nm again is likely to be due to water in the minerals structure. The absorption feature around 650nm may again be due to the presence of iron present in the waters from which the evaporite forms, washed in from the surrounding geology. This feature was very similar to the pronounced absorption in MGS1 sample spectrum (figure 7.13.C), and was absent from the reference spectra for trona (figure 7.17.C).

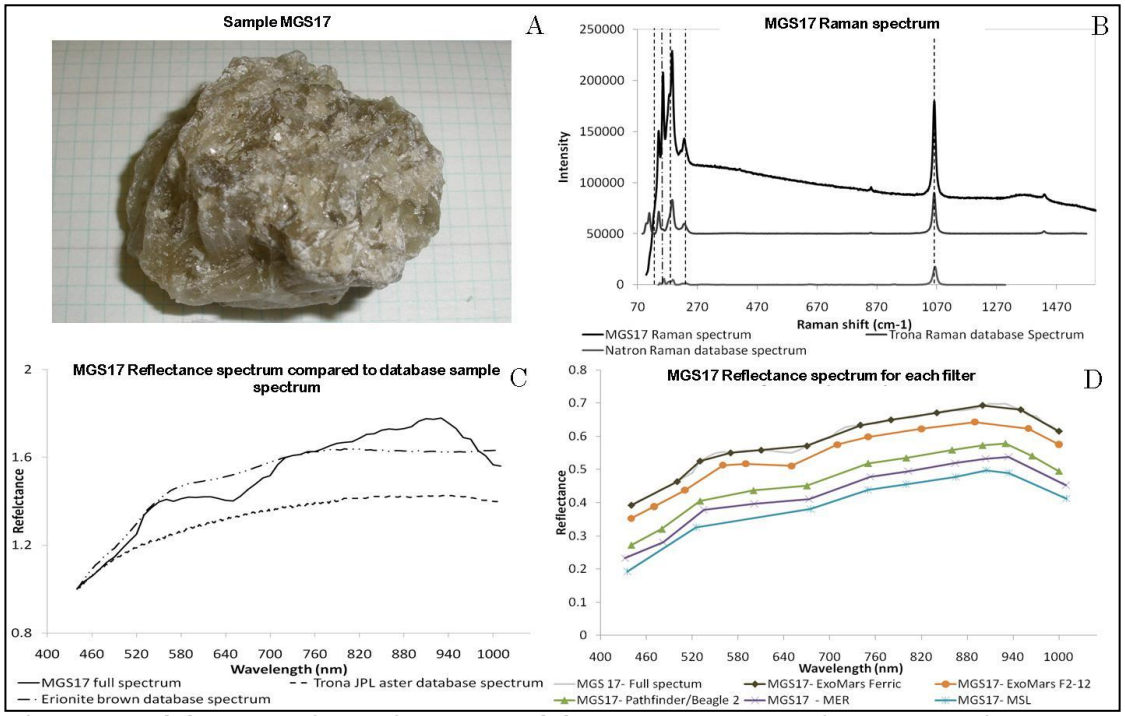



*Figure 7.17* ***(A)*** *Image of sample MGS17.* ***(B)*** *Raman spectrum of MGS17 against RRUFF Project reference spectrum (Downs, 2006).* ***(C)*** *Comparison of MGS17 reflectance spectrum and a database reference spectrum normalised to 1 (Clark et al., 2007).* ***(D)*** *Reflectance spectrum for MGS17 and the sub-sampled spectrum for each filter set which have been offset for clarity.*

All filter sets are able to reproduce the absorption features at 1000nm, however the 650nm absorption band was only clearly replicated by the ExoMars F2-12 and Ferric filter set (figure 7.17.D). The 650nm feature was only slightly reproduced by the Pathfinder/Beagle 2, MER and MSL filter sets. The 490nm absorption feature was also missed by several filter sets with only the MER and ExoMars Ferric filter sets reproducing it.

### 7.7.2.8. MudS41

The composition of MudS41 (Figure 7.18.A) was ascertain through XRD as a mixture of sanadine, a potassium feldspar mineral which forms at high temperatures in volcanic regions, the phyllosilicate montmorillonite and halite (Figure 7.18.B). Soils have a complex composition and so other minor components will be present in the sample which will influence the spectral features produced by the sample.

The spectra for the sample in figure 7.18.C shows absorption features around 490nm and 1000nm, the rest of the spectrum demonstrates an arched absorbance pattern with a peak around 860nm. All the filters are again capable of reproducing the 1000nm absorption feature, however again the small 490nm feature was only replicated by the ExoMars Ferric and F2-12 filter sets (Figure 7.18.D).

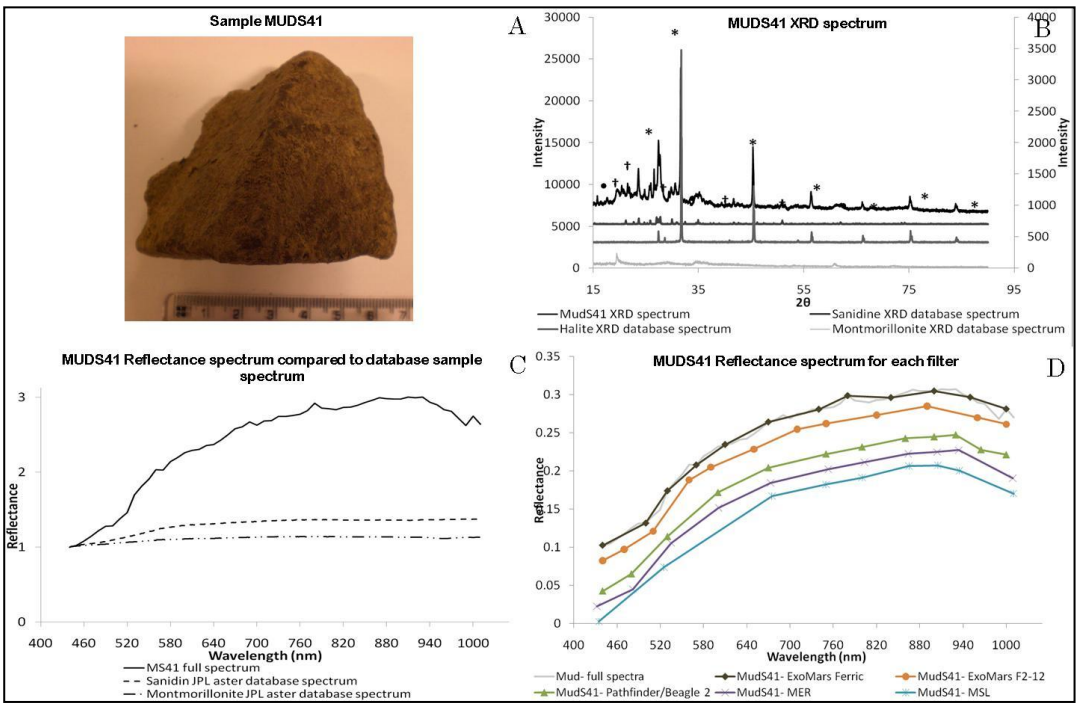



*Figure 7.18* ***(A)*** *Sample MUDS41.* ***(B)*** *XRD spectrum of MUDS41 against RRUFF Project reference spectrum (Downs, 2006).* ***(C)*** *Comparison of MUDS41 reflectance spectrum and a database reference spectrum normalised to 1 (Clark et al., 2007).* ***(D)*** *Reflectance spectrum for MUDS41 and the sub-sampled spectrum for each filter set which have been offset for clarity.*

### 7.7.2.9. S133

S133 was characterised through XRD analysis as the hydrous sodium silicate mineral magadiite, hydrous sodium silicate mineral ($NaSi_7O_{13}(OH)_3 \cdot 4(H_2O)$) which forms in alkaline brines (Figure 7.19.B) (Gunn, Pers. Comm.) (Warren, 2006).

The sample shows absorption features at 440nm and 500nm, 660nm with a small feature around 760nm, 970nm and an absorption towards 1000nm. The 970nm absorption was seen in both the middle region of the sample (II) and peach/pink area of the sample (I) (Figure 7.19.C). This again is likely to be a hydration feature, as seen in GCS3 and GCS4.

The other absorption features present occur in regions where features typical of biological pigments occur. The presence of carotenoid pigments could explain the absorption features between 400nm and 500nm (Lichtenthaler and Buschmann, 2001). Chlorophyll *a* and bacteriochlorophyll *a* absorb at 430nm-450nm,660nm, 760nm and 1020nm (Lichtenthaler and Buschmann, 2001: Oren, 2009) (Figure 7.19 C and D). Bacterioruberin produces absorption peak at 497nm and could explain the 500nm feature (Dummer et al., 2011).

The overall shape of the spectrum was replicated by most filter sets reproducing the main features around 440-500nm, 680nm and towards 1000nm. However the MSL filter set does not reproduce the 440-500nm absorption feature. The small feature at 760nm and 970nm are missed by all filter sets (Figure 7.19.D) as these are both shallow features.

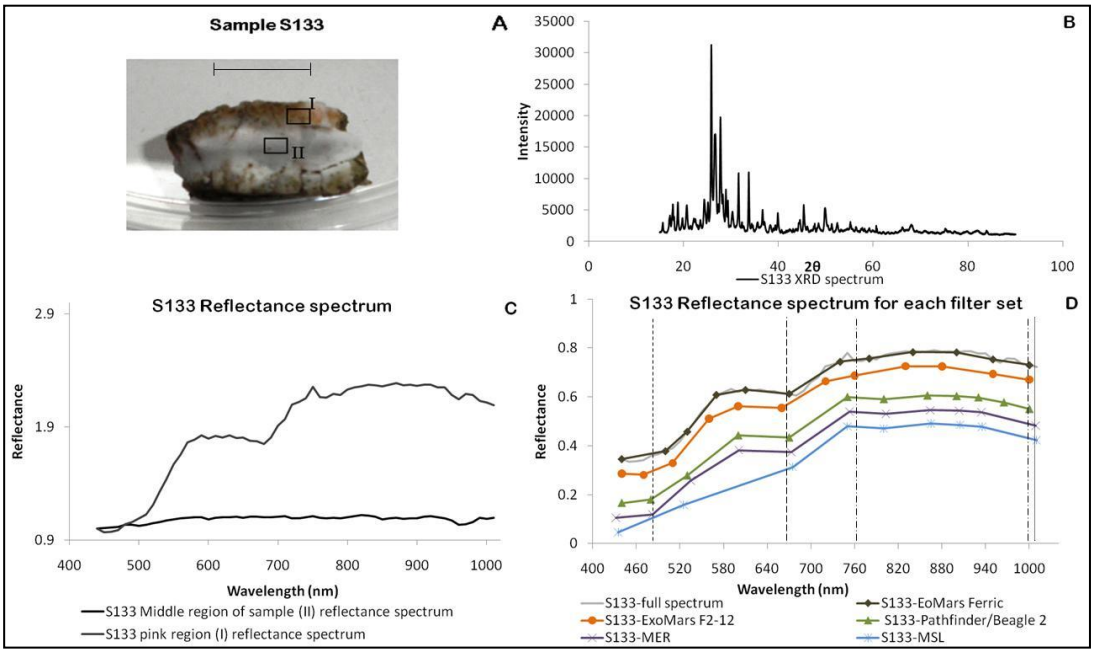



*Figure 7.19* ***(A)*** *Image of sample S133, with regions used for spectral acquisition. Scale bar is 1.5cm.* ***(B)*** *XRD spectrum for S133, no reference spectrum available, both spectra are normalised to 1.* ***(C)*** *S133 reflectance spectrum of white middle areas of sample S133 (See A, II), against the peach/pink region of sample S133 (See A, I).* ***(D)*** *Reflectance spectrum for peach area of S133 then sub-sampled for each filter set and offset for clarity. Short dashed line indicates position of absorption due to carotenoids/chlorophyll, long dashed line indicates position of chlorophyll absorption region. Dot dashed line indicates absorption for bacteriochlorophyll. Dotted line indicates region of possible hydration absorption feature.*

### 7.7.2.10. BM81

A reflectance spectrum was acquired for both the green (I) and pink (II) regions of the sample and outlined in figure 7.34.B. The overall pattern of the spectra was largely similar with small absorptions occurring at 440-500nm, 600nm, and significantly larger absorptions at 660-680nm, between 780nm and 900nm and towards 1010nm (Figure 7.33.B). The 440-500, 660, 800 and 1010nm absorptions can all be attributed to bacteriochlorophyll *a* and/or chlorophyll *a,* with the absorption at 600nm and 850nm could be attributed to the presence of chlorophyll *a* (Oren, 2009).(Laisk, 2000: Lichtenthaler and Buschmann, 2001). The presence of carotenoids again may explain the 440-500nm absorption.

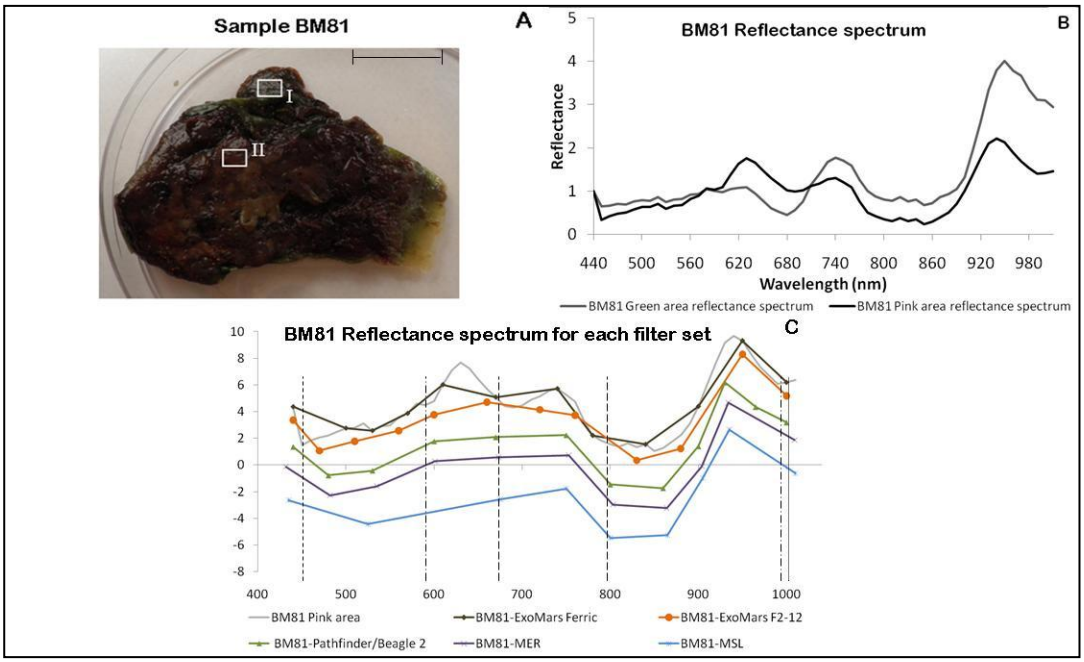



*Figure 7.20* ***(A)*** *Image of sample BM81, with regions used for spectral acquisition. Scale bar is 3cm.* ***(B)*** *BM81 reflectance spectrum of green area of biomat (See A, II), against the pink region of sample BM81 (See A, I).* ***(C)*** *Reflectance spectrum for pink area of BM81 then subsampled for each filter set and offset for clarity. Short dashed line indicates position of absorption due to carotenoids/chlorophyll, long dashed line indicates position of chlorophyll absorption region. Dot dashed line indicates absorption for bacteriochlorophyll. Dotted line indicates region of possible hydration absorption feature.*

The ExoMars Ferric filter set can reproduce the 660nm absorbance which was missed by all the other filter sets. The filter positions in the visible however result in the reproduction of an absorbance feature at ~510nm, but missed the large absorbance feature around 440-500nm. This was also the case for the MSL, MER and Pathfinder/Beagle 2 filter sets, however ExoMars F2-12 does replicate the feature to a certain extent. The 600nm absorbance was not

replicated by any filter set. Most of the features at wavelengths greater than 750 nm are reproduced by all filter sets.

**7.7.2.11. Sample BM108**

The reflectance spectrum for sample BM108 (Figure 7.21.A) showed absorptions at ~450nm, 520nm, 600nm, 660nm, 760nm and ~850nm (Figure 7.21.B). There also appears to be the beginnings of an absorbance around 1010nm, this was only reproduced by the Pathfinder/ Beagle 2 filter sets (Figure 7.21.C). The ExoMars Ferric filter set reproduces 520nm, 660nm, 850nm absorption bands along with the Pathfinder/Beagle 2 filter sets. The ExoMars F2-12 filter set reproduced the 440-500nm absorption and was the only filter set to do so, as well as the 660nm and 850nm features. No filter set was capable of reproducing the 600nm absorption or that at 760nm, or the 630nm emission.

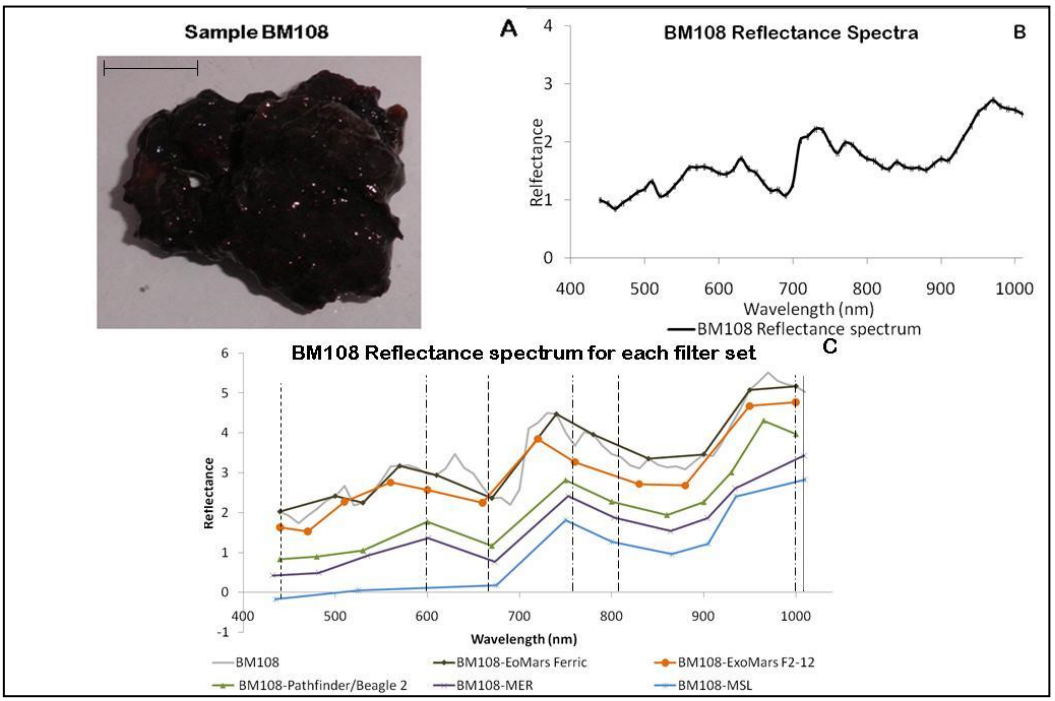



*Figure 7.21* ***(A)*** *Image of sample BM108, with regions used for spectral acquisition. Scale bar is 2cm.* ***(B)*** *BM108 reflectance spectrum of* ***(C)*** *Reflectance spectrum for BM108, then subsampled for each filter set and offset for clarity. Short dashed line indicates position of absorption due to carotenoids/chlorophyll, long dashed line indicates position of chlorophyll absorption region. Dot dashed line indicates absorption for bacteriochlorophyll. Dotted line indicates region of possible hydration absorption feature.*

**7.7.3. Analysis of the capacity of each filter set to reproduce sample spectral features.**

Each filter set was scored one point for its ability to reproduce a key spectral feature in a sample (Table 7.6). The replication value is the sum for each filter set when 1 point is allocated for every key spectral feature present in each sample which the filter can replicate (Figure 7.7). The MSL filter set scores the lowest replication value with a score of 17 (Table 7.6).

*Table 7.6 Table of key spectral features for each sample with a + or - symbol denoting a filter sets ability to replicate a feature. + scores 1 point, - scores 0 point.*

| Sample | Feature | Pathfinder/Beagle 2 | MER | MSL | ExoMars F2-12 | ExoMars Ferric |
|---|---|---|---|---|---|---|
| GCS1 | 490nm abs | + | + | - | + | + |
| | 650nm abs | - | - | - | + | + |
| | 720nm peak | - | - | - | + | - |
| | 960nm abs | + | + | + | + | + |
| GCS2 | 650nm abs | + | + | + | + | + |
| | 1000nm abs | + | + | + | + | + |
| GCS3 | 490nm abs | - | + | - | - | + |
| | 970nm abs | + | - | - | + | - |
| GCS4 | 650nm abs | + | + | - | + | + |
| | 970nm abs | + | - | - | + | - |
| MGS1 | 650nm abs | + | + | + | + | + |
| | 1000nm abs | + | + | + | + | + |
| MGS2, MGS3 & MGS11 | 1000nm abs edge | + | + | + | + | + |
| MGS17 | 490nm abs | - | + | - | + | + |
| | 650nm abs | + | + | - | + | + |
| | 1000nm abs | + | + | + | + | + |
| GR1 | 490nm abs | - | + | - | + | + |
| | 650nm abs | + | + | - | + | + |
| | 580nm peak | - | - | - | + | + |
| | 930nm abs | + | + | + | + | + |
| SR1 | 490nm abs | - | + | - | + | + |
| | 650nm abs | + | + | + | + | + |
| | 1000nm abs | + | + | + | + | + |
| MudS41 | 490nm abs | - | + | - | + | + |
| | 1000nm abs | + | + | + | + | + |
| S133 | 440-500nm | + | + | - | + | + |
| | 680nm abs | + | + | + | + | + |
| | 760nm abs | + | + | + | + | + |
| | 970nm abs | - | - | - | - | - |
| | 1010nm abs | + | + | + | + | + |
| BM81 | 440-500nm | - | - | - | + | - |
| | 520nm abs | - | - | - | - | - |
| | 600nm abs | - | - | - | - | - |
| | 850nm abs | + | + | + | + | + |
| | 1010nm abs | + | + | + | + | + |
| BM108 | 440-500nm | - | - | - | + | + |
| | 520nm abs | - | - | - | - | + |
| | 600nm abs | - | - | - | - | - |
| | 680nm abs | - | - | - | - | - |
| | 780nm abs | - | - | - | - | - |
| | 850nm abs | + | + | + | + | + |
| | 1010nm abs | + | - | - | - | - |
| Replication value (out of 41) | | **25** | **27** | **17** | **33** | **31** |

The ExoMars F2-12 and Ferric filter sets both outperform the other filter sets with the ferric filter set scoring 30 but the F2-12 filter set performs the best, with a score of 33. The better performance of the F2-12 filter set was due to the reproduction of the 740nm peak in the GCS1 sample and the reproduction, although only by a small amount, of the 970nm absorption in the GCS3 and GCS4 samples. This would indicate that the filter distribution for the ExoMars filter sets are superior to the filter distribution of the other filter sets when looking to identify these sorts of mineral deposits.

### 7.7.4. Error score

The error score was calculated for each sample for the Pathfinder Beagle 2 filter set, MER ExoMars F2-12 and ExoMars Ferric filter sets. The program did not allow as few as 9 filters to be processed so the MSL filter set was omitted. The four sets which have been tested are the top performers in terms of their replication value.

#### 7.7.4.1. Error score calculated for different samples

The Pathfinder/Beagle 2 and MER filter sets performed the worst for sample GCS1-siderite due to the loss of resolution at the 710nm emission peak. The samples GCS3-analcime and GCS4-phillispite score of 0.46 and 0.45 using the Pathfinder/Beagle 2 filter sets due to their ability to replicate the 970nm absorption feature, this contrast to the performance of either ExoMars filter sets for the two samples which scored over 0.5. The ExoMars filters perform the worst of all filter sets on the S81 biological sample, with an error score of 0.71 and 0.73 compared to a value of 0.57 and 0.56 for the Pathfinder/Beagle 2 and MER filter sets respectively.

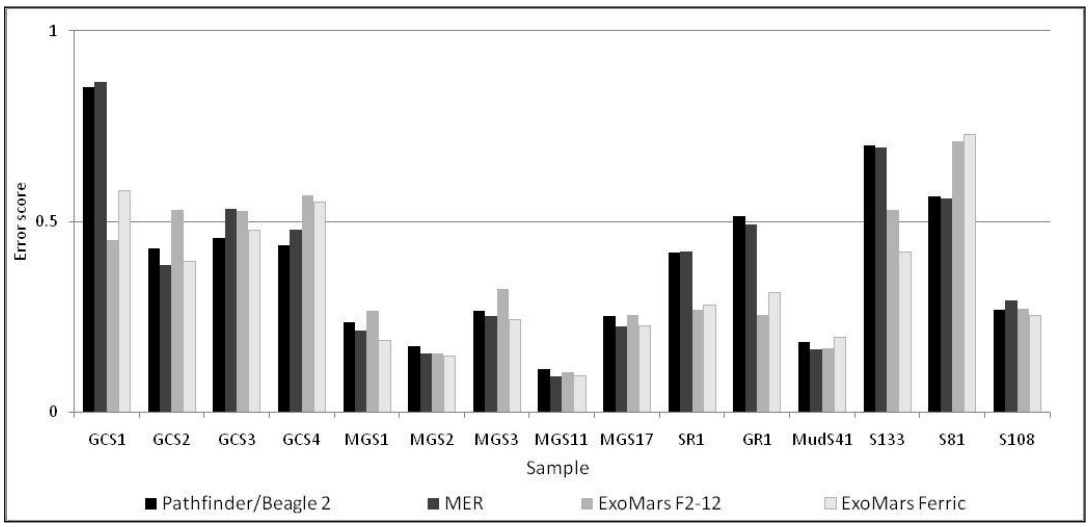



*Figure 7.22 Bar chart of error score for the Pathfinder/Beagle, MER and ExoMars F2-12 and Ferric filter sets, a score of 0 would indicate complete replication of the sample spectrum by the filter set.*

Generally the Lake Magadi samples MGS1, MGS2, MGS3, MGS11, MGS17 and the soil sample MudS41 produce an error score below 0.32 with all filter sets performing to a similar

degree. The lowest error scored for all filter sets was for MGS11, with a value <0.11. MGS11 was a trona sample with a fairly featureless spectrum. Samples GR1, SR1, S133 and GCS1 produce a higher error score for each sample using the Pathfinder/Beagle 2 and MER filter sets, compared to the ExoMars filter sets.

#### 7.7.4.2. Error score calculated for each filter set

The average error scores were calculated as 0.39 for the Pathfinder and Beagle 2 and the MER filter sets, 0.36 for the ExoMars F2-12 and finally 0.34 for the ExoMars Ferric filter set. This demonstrates that overall the ExoMars filter sets produce better estimated reflectance spectra for this suite of samples than the Pathfinder/Beagle 2 or MER filter sets. This confirms the findings through the replication value calculated for each filter set for the Pathfinder/Beagle 2 and MER filter sets, however does contrast to the replication value data for the ExoMars filter sets. This mainly due to the closer fit to the whole spectrum for each sample by the ferric filter set, in spite of the loss of some spectral features which are replicated by the ExoMars Ferric filter set.

### 7.7.5. Spectral Parameter plots

The parameters used were the 950 - 1000nm slope and the 970nm band depth in the region for hydration and bacteriochlorophyll absorption features, and the 650nm band depth for iron and chlorophyll absorption features.

The samples are plotted the following sets;

1. All samples to look for the capacity of the filter set to distinguish between different types of minerals which form in neutral-alkaline/saline environment.

2. Only those samples containing carbonate or halide minerals to look at ability to distinguish between minerals of a similar type

3. Only those samples containing zeolite or feldspar minerals again to look at the distinction between different minerals of a similar type

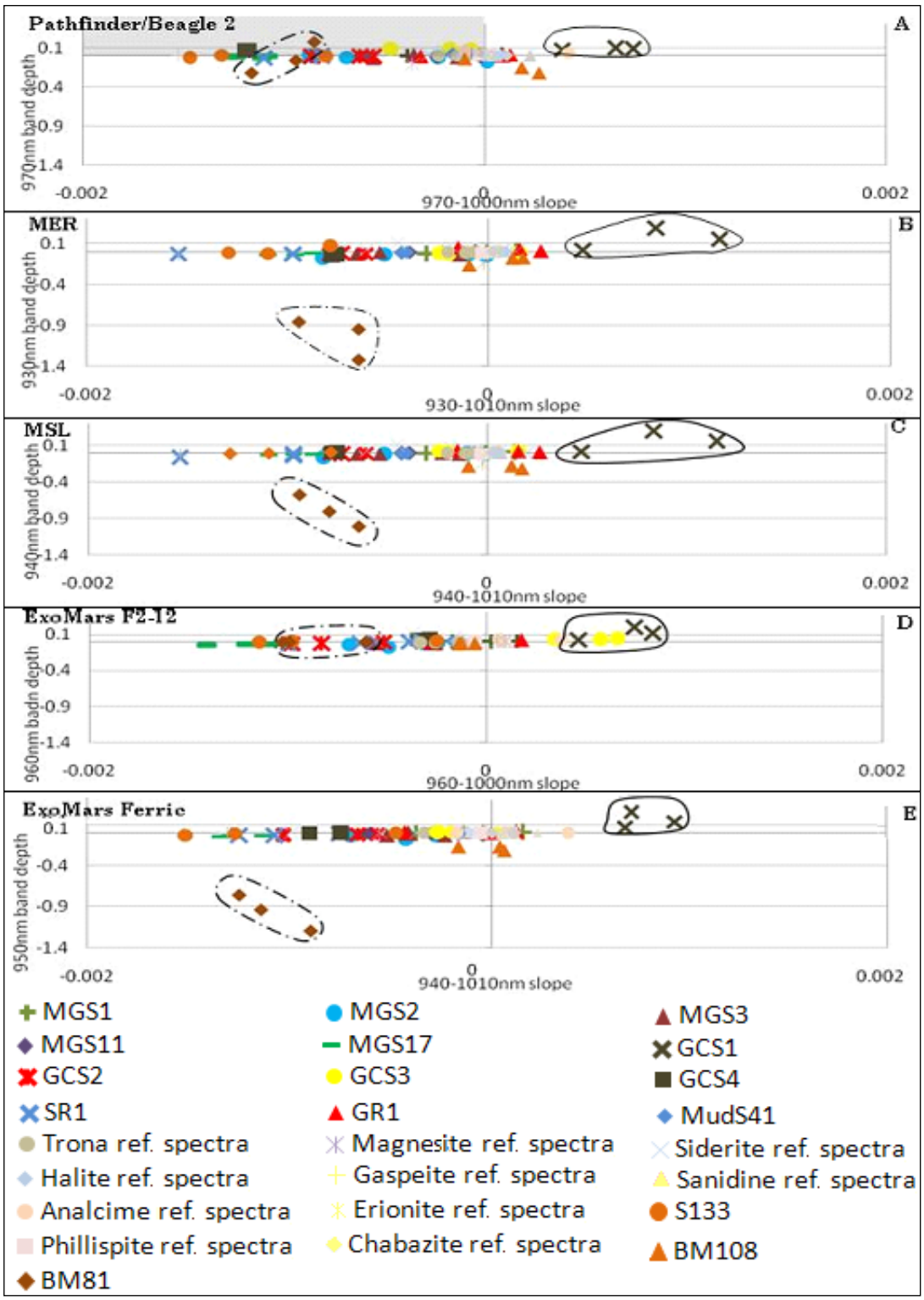



*Figure 7.23 Plots of all samples together with the spectral parameters 440-600nm slope and 960-1000nm slope for each filter set. Solid line outlines the compositional group containing GCS1 samples. Dot - dashed line outlines BM81 compositional group. Those samples plotting in the grey area have a downwards slope from 960-1000nm and an absorption feature at 970nm.*

#### 7.7.5.1. 970nm band depth versus 950nm – 1000nm slope spectral parameter plots

Despite the presence of a 970nm absorption band in zeolites it was not possible to clearly distinguish between zeolites and sodium carbonates or magnesium carbonates using these parameters (Figures 7.24.A to E).

The samples, which have or may have a biological component, show some distinction from one another and from the other samples. S133 plots indistinctly from the trona containing samples due to the increase in absorption from the 950nm to 1000nm filters and the lack of a 970nm absorption feature occurs in sample S133 and MGS1, MGS2, MGS3, MGS11 and MGS17. Sample BM81 plots separated when using the MER, MSL or ExoMars Ferric filter sets. This was due to an emission peak around 960nm which these filters reproduce. The position of the filters in the Pathfinder/Beagle 2 and ExoMars F2-12 filter sets means that less of the emission was reproduced producing a more positive 970nm absorption value (Figure 7.24 dot-dash line).

##### 7.7.5.1.1. Carbonate, halide and biomat samples using 970nm band depth and 950nm – 1000nm slope parameters

There was some distinction between the different types of carbonates when using the Pathfinder/Beagle 2, MSL, MER or ExoMars Ferric filter sets. GCS1-siderite has a distinct absorption feature between 950nm and 1000nm. Where GCS1-siderite plots distinctly this was due to a more positive 950-1000nm slope value produced by the presence of a greater reflectance value at the higher wavelength filter (1010nm or 1000nm) compared to lower wavelength filter (930nm, 940nm, 950nm, 960nm or 970nm) by these filter sets. The only exception was when using the ExoMars F-12 filter set, in this case the samples plot overlapping the spectra for GCS3-analcime (Figure 7.25. dotted line).

There was no compositional definition between GCS2-magnesite and the trona containing samples GCS1, GCS2, GCS3, GCS11 and GCS17 (figure 7.25, A-E). The samples which are morphologically similar, MGS2 and MGS3 again plot in a similar region with all filter sets. The carbonates exhibit no absorption band specifically in the 970nm region, demonstrating more of a peak instead. The trona containing samples and GCS2-magnesite have an absorption edge in the 950 – 1000nm slope region, the beginning of an absorption featured occurring further into the near infrared.

##### 7.7.5.1.2. Zeolite and feldspar containing samples using 440-600nm and 970nm band depth parameters

Distinction between zeolite minerals can be seen with these parameters using certain filter sets. The Pathfinder/Beagle and ExoMars F-12 filter sets plot GCS3-analcime separately from the other zeolites. The Pathfinder/Beagle 2, MER and MSL filter sets all plot GCS4-philipsite/analcime distinctly from the other zeolites. Only the Pathfinder/Beagle 2 filter set can

clearly separate those samples with a 970nm absorption feature (GCS3-analcime and GCS4-philipsite see table 7.6) from those which do not (Figure 7.26.A, dotted line).

This was because the Pathfinder/Beagle 2 filter sets actually have a filter at 970nm the others have filters between 10nm and 50nm away from this point, which results in the loss of information on the 970nm feature.

Less distinction between the zeolite and mud samples can be seen when using either the ExoMars F2-12 or ferric filter sets when compared to Pathfinder/Beagle 2, MER or MSL filter set. When only considering the zeolite minerals (excluding MudS41) the Pathfinder/Beagle 2 filter set plots each of the four zeolite samples separately from one another. The MER filter set also distributes the different sample types to a certain degree although does group GCS3-analcime and GR1-erionite quite close together. Finally, although GCS4 was not confirmed as either analcime or phillipsite it does plot distinctly from GCS3, confirmed as analcime due to differences in 950nm – 1000nm slope, although the 970nm absorption values are similar.

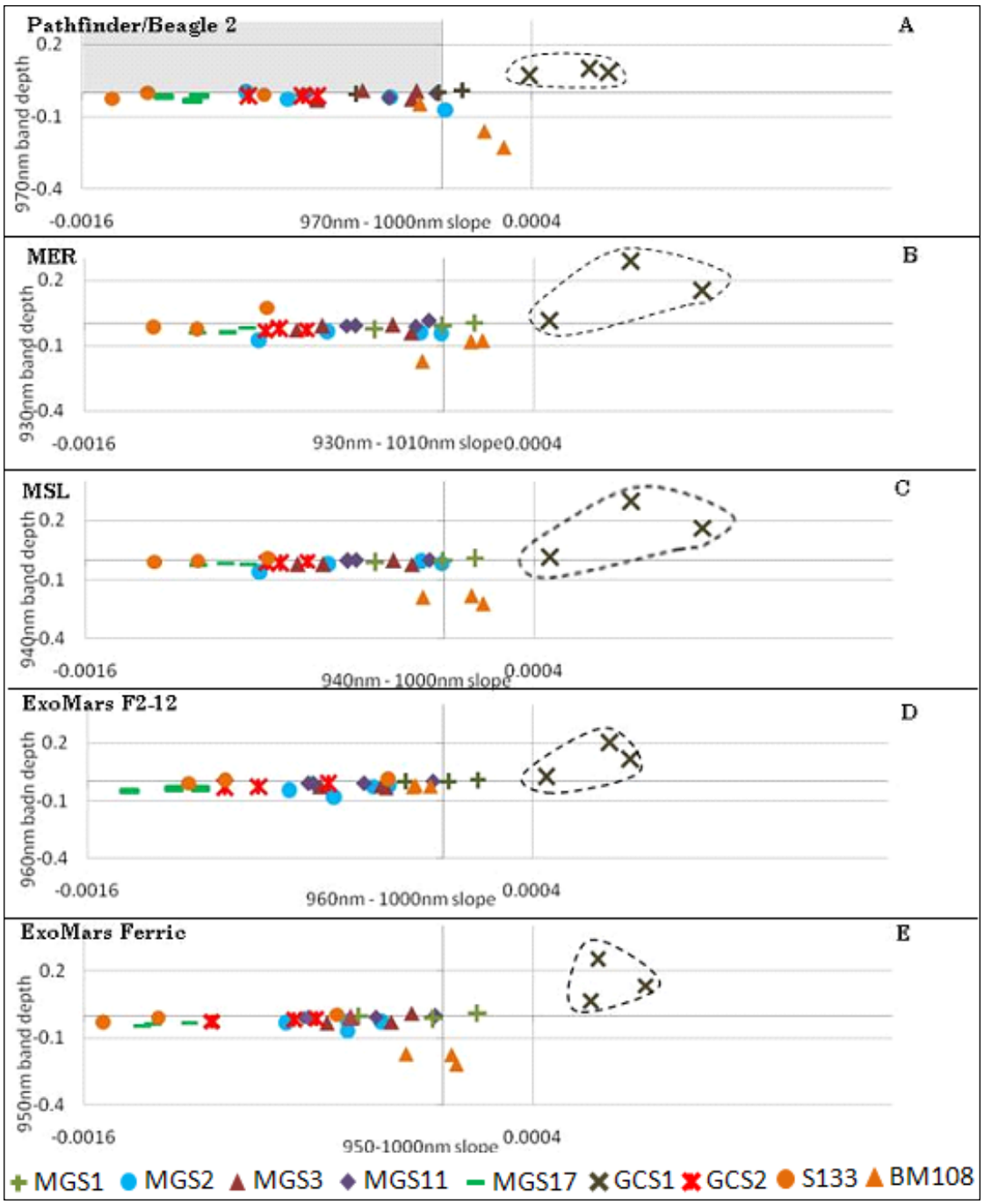



*Figure 7.24 Plots of all carbonate and biological samples (minus BM81) with the spectral parameters 970nm band depth and 950nm - 1000nm slope for each filter set. Dot dash line outlines the region containing both carbonate and zeolite minerals in close association. Solid line outlines the compositional group containing GCS1 samples. Those samples plotting in the grey area have a downwards slope from 960nm - 1000nm and an absorption feature at 970nm.*

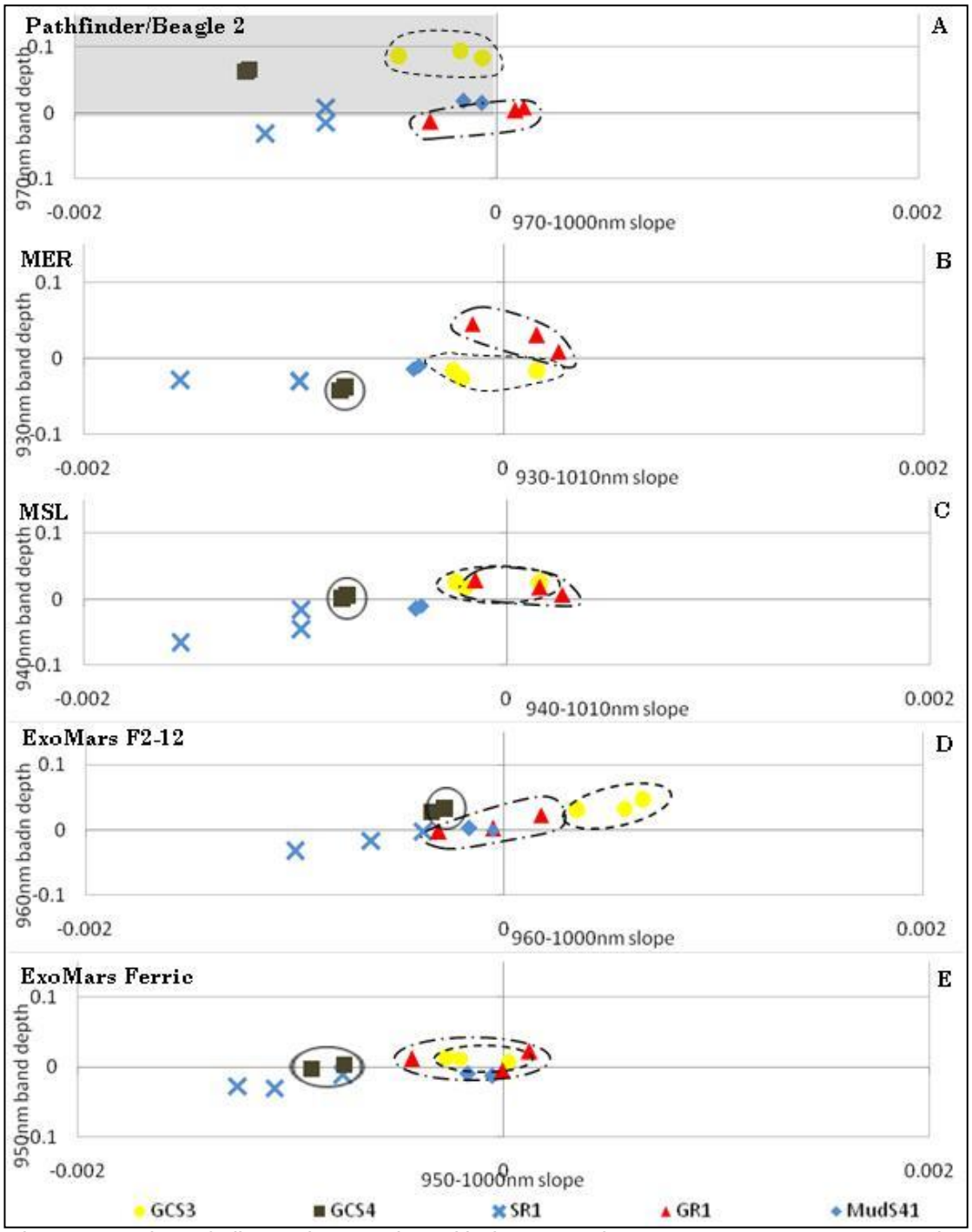



*Figure 7.25 Plots of all samples together with the spectral parameters 960nm - 1000nm slope and 970nm band depth for each filter set. Dot dash line GR1 samples, dashed line outlines GCS3 spectra. Solid line outlines GCS4 spectra. Those samples plotting in the grey area have a downwards slope from 950nm – 1000nm and a 970nm absorption.*

#### 7.7.5.2. 650nm band depth versus 970nm band depth spectral parameter plots

The previous section indicates the importance of the 970 nm absorption in zeolite minerals; we now will look at using this feature along with the 650nm iron absorption band depth.

##### 7.7.5.2.1. Carbonate and halide containing samples and biomat samples

Figure 7.27 shows that the sample BM81 generally plots separately this was due to an emission around 970nm as mentioned before resulting in a more negative 970nm value for all filter sets except Pathfinder/Beagle 2 (Figure 7.27, solid line). The samples S133 and BM108 plot less distinctly situated amongst the other samples analyzed.

In Figure 7.28, plotting only the carbonate containing samples GCS1-siderite once again plots without overlapping with other samples with a more negative 680nm band depth value for all filter sets, with the exception of the ExoMars F2-12 filter set. The ExoMars F-12 filter set plots the samples with a more positive value due to the presence of an absorption feature in the 650nm region which only the ExoMars F2-12 filter set was capable of reproducing. The position of a filter at 740nm in the ExoMars Ferric filter set, compared to 720nm in the F2-12 filter set causes the Ferric filter set to miss the absorption feature. All filter sets are capable of reproducing the 960nm absorption feature in sample GCS1-siderite.

Figure 7.27 shows that none of the filter sets allow for the distinction between the trona containing Lake Magadi samples and the GCS2-magnesite which is outline by dot dashed line. The compositional grouping containing magnesite spectra overlaps with some trona containing samples with every filter set (Figure 7.28).

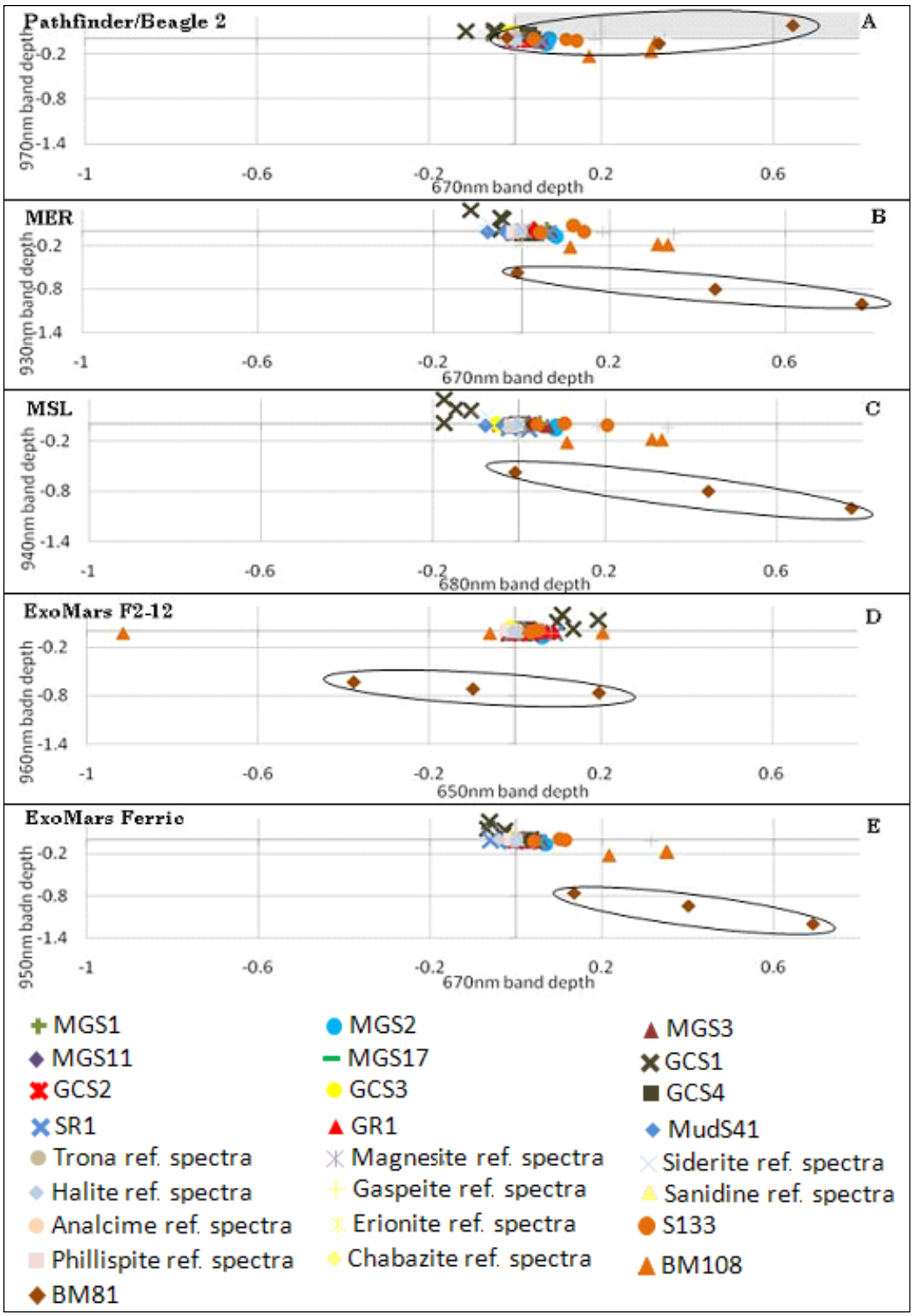



*Figure 7.26 Plots of all samples together with the spectral parameters 650nm band depth and 970nm band depth for each filter set. Dashed line outlines GCS3 compositional group. Dotted line outlines GCS2 compositional group. The grey region indicates area where 970nm absorption but no 650nm absorption would occur. Those samples plotting in the grey area have an absorption at both the 970nm and 650nm regions.*

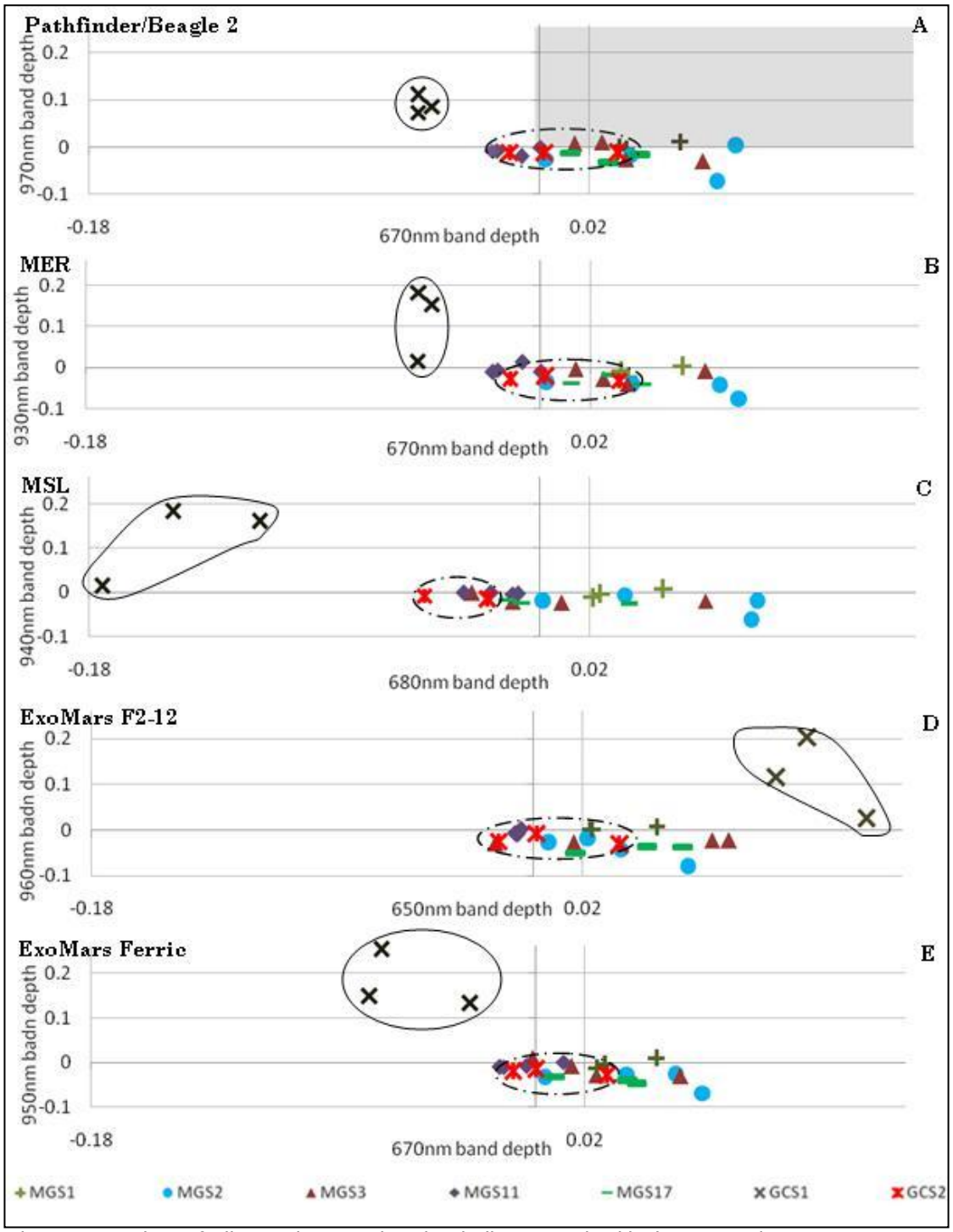



*Figure 7.27 Plots of all samples together (excluding GCS1) with the spectral parameters 650nm band depth slope and 970nm band depth for each filter set. Dashed line outlines GCS3 compositional group. Dot-dashed outlines compositional group containing GCS3 and GCS4. The grey region indicates area where 970nm absorption but no 650nm absorption would occur. Please note that figure is plotted on a different axis as no axis could be plotted to allow all plots to be clear.*

#### 7.7.5.2.2. Zeolite and feldspar containing samples using 650nm band depth versus 970nm band depth

For every filter set the sample SR1-chabazite plots quite broadly due to variation in the intensity of the 650nm absorption band in different areas of the sample. The SR1-chabazite spectra plot either side of the GR1-erionite, MudS41 and GCS4-philipsite/analcime. It would be difficult to group these compositionally to produce distinct clusters as the spectra plot close together or overlap (Figure 29.A to E).

Distinct compositional groups form for the samples GCS3-analcime and GCS4-philipsite/analcime using the Pathfinder/Beagle 2 filter sets, plotting away from the other samples in this group due to a more positive 970nm absorption value. The only other groupings are the MudS41 samples when using the MER filter set which position further to the left of the plot than any other sample spectra. The samples GR1 and SR1 overlap one another and other sample spectra with every filter set tested (Figure 7.29).

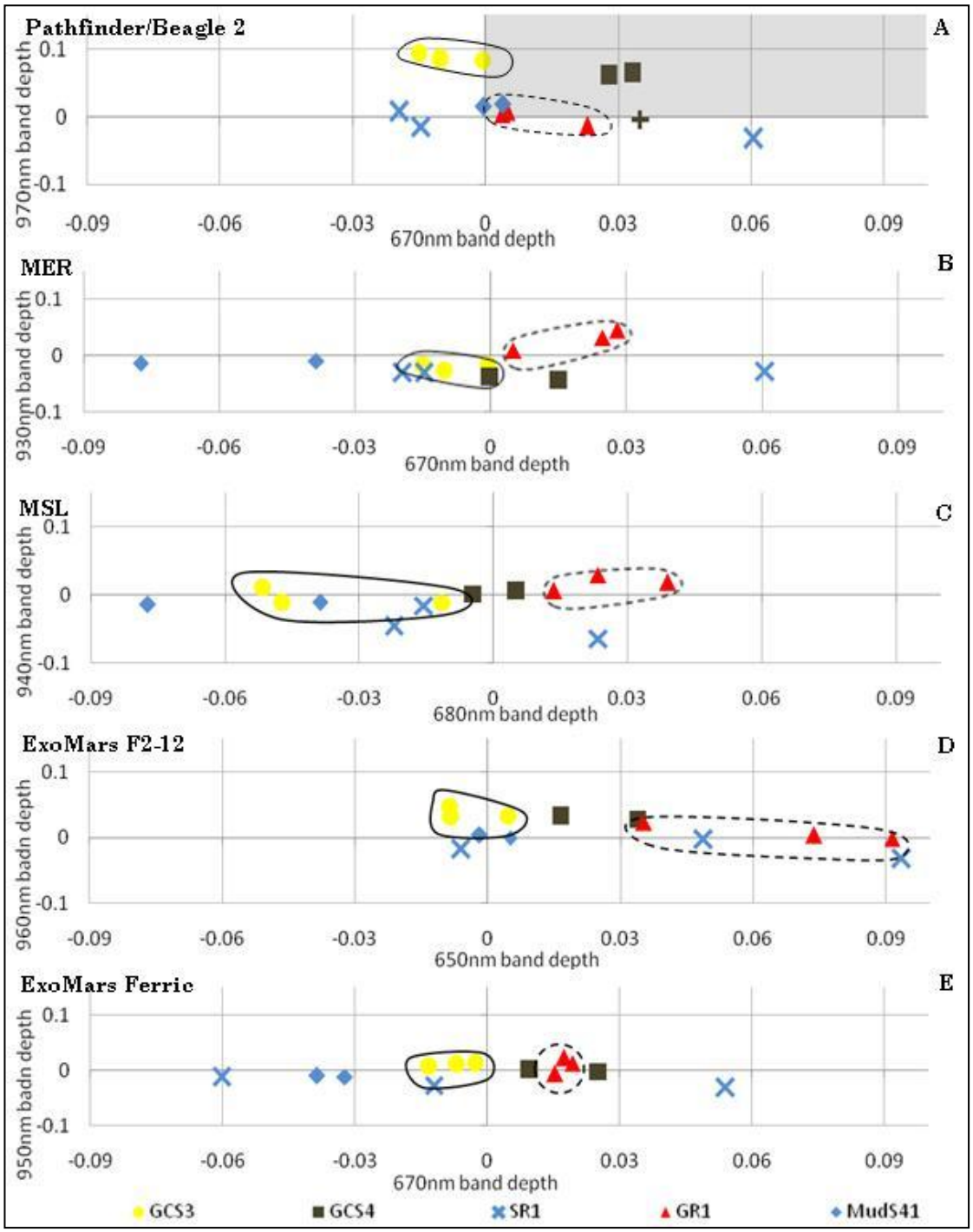



*Figure 7.28 Plots of all samples together with the spectral parameters 650nm band depth and 970nm band depth for each filter set. Solid line outlines GCS3 compositional group. Dotted line outlines GR1 compositional group. The grey region indicates area where 970nm absorption but no 650nm absorption would occur.*

## 7.8. Discussion

This chapter focuses upon the capacity of missions to Mars, past, present and future to identify minerals which typically form in an alkaline-saline environment. The filter sets chosen for the Mars missions to date were not designed using minerals which form in alkaline environments, with the exception of the ExoMars F2-12 filter set which included two types of phyllosilicates and one carbonate. The purpose of this study was to assess whether or not excluding minerals from alkaline-saline environments such as the zeolite analcime, which is known to form on Mars, has a detrimental effect upon each filter sets ability to replicate the spectra of these sorts of minerals. This analysis highlighted some important points when considering the detection of these types of hydrated minerals on Mars.

### 7.8.1. Comparing the performances of different filter sets

The replication capacity of the filter sets designed for Pathfinder/Beagle 2, MER, MSL and the two filter sets proposed for ExoMars: F2-12 and Ferric was examined. The ExoMars filter sets achieve the best results when using samples from alkaline-saline environments. The replication capacity of each filter set was analysed in two ways, 1) identifying the ability of each filter to reproduce each spectral feature for each mineral spectrum, defined as the replication value and 2) measuring the error score for each filter set, which was the difference between the original spectrum and the subsampled filter spectrum. This chapter would have benefitted from the addition of replicated acquisition of spectral data for each data point, for each sample to provide error bars, which was not done for this study.

Of the filter sets tested here, the two filter sets designed for the ExoMars mission were more suitable for identifying the mineralogy of deposits from a neural/alkaline-saline environment than the Pathfinder/Beagle 2, MSL or MER filter sets. The ExoMars F2-12 filter set scored the highest replication value of 31 and the second lowest error score of 0.36, the ExoMars Ferric filter set scores the second highest replication value of 33 and the lowest error score 0.34. This superior replication capacity of the ExoMars filter sets was due to a combination of the even distribution of filters across the whole spectral range being studied, which was particularly relevant to <700nm and 930-1010nm regions, and the use of 12 filters. The overall better performance of the ExoMars filter sets compared to the Pathfinder/Beagle 2 filter set supports the decision to redesign the filter set for the ExoMars mission.

The MSL filter set scored the lowest replication value of 17. This poor score compared to the ExoMars filter sets was due to the lower number of filters available to the MSL system (9 filters compared to the 12 for the ExoMars and Pathfinder/Beagle 2 filter sets), and in addition the limited number of filters covering the visible wavelength range (3 filters covering 440-700nm, compared to 6 for the ExoMars filter sets). These differences have implications for the data to be obtained by the MSL multispectral instrument. The number and distribution pattern of the

filters may limit the MSL instrument as an analytical technique, particularly when looking for/identifying minerals which have features in the 440-700 and 934-1010nm regions.

It is important to remember that the bandpass for each filter will provide some scope for identifying features beyond the specific wavelength of each filter, however the effects of the bandpass breath for each filter, in each filter set was not within the scope of this study.

### 7.8.2. The ability of the filter sets tested to sufficiently distinguish between different carbonates

Moving the focus to assessing the individual replication capacity of each sample spectrum by each filter set produced some interesting results, particularly concerning those samples containing trona, magnesite, erionite, phillipsite and analcime. The reference spectra for trona are generally featureless, except for the presence of an absorption towards from ~980nm to 1010nm, one limb of a ~1000nm absorption band (Drake, 1995). The spectrum for MGS11-trona was very similar to the database spectrum for trona (although a slightly higher reflectance between 520nm and 970nm) and its overall smooth shape was easily replicated by all filter sets, as demonstrated by its having the lowest error value of any sample. Problems arose when using the PanCam instrument to distinguish between different types of carbonates.

Siderite has a relatively complex spectrum in the 440-1010nm region, with several absorption and emission features this makes it quite distinct. The reflectance spectrum for magnesite however was generally featureless, except for a slight levelling off between 610nm and 850nm and the spectrum for sample GCS2-magnesite contains an absorption from 970nm towards 1010nm. The similar spectral profiles of trona and magnesite produced in this study rendered the differentiation between the two carbonates difficult. In addition, despite the purpose of spectral parameter plots to highlight differences between the spectral features of different minerals, there was no clear differentiation between the two types of carbonate with any of the spectral parameters analysed. The different chemistry required for the formation of trona and magnesite, coupled with the inability of any of the multispectral filter sets to distinguish between the two minerals means that the presence of different types of alkaline deposit or depositional environments maybe overlooked.

### 7.8.3. The effects of heterogeneous samples on mineral identification when using reflectance spectroscopy

This study benefitted from the inclusion of geological samples rather than using pure minerals for filter testing, this meant that real variation in the spectra for each sample could be seen in the spectral profiles. Heterogeneous samples may contain spectral features which may not occur in the spectrum of the pure mineral, or even when using basic mixing models. The mixing of minerals is not linear, particularly not in the visible/NIR region and so it is important

to look at spectra from naturally heterogeneous samples (Crowley, 1991). The problems which can occur with mineral identification due to variation between the reference spectrum and the spectrum for a natural sample was demonstrated in the multispectral analysis of some of the Lake Magadi samples. The spectrum for GR1-erionite contained an iron absorption at ~660nm. MGS2-magnesite and the Lake Magadi samples MGS2, MGS3 and MGS17, which predominantly contain trona and halite, also exhibited a feature around this wavelength. This feature was also attributed to the presence of iron in the samples and was not present in the reference spectra for trona or magnesite (Clark et al., 2007: RELAB-Spectral-Database, 2008: Baldridge et al., 2009b). This 660nm absorption means that the identification of these deposits maybe compromised if compared to reference spectra of pure minerals, this was especially important when considering the presence of dust which contains iron on the surface of Mars.

### 7.8.4. The reproducibility of hydration features of minerals from alkaline saline environments

One of the most important limitations of some of the filter sets identified by this study relates to the 970nm hydration feature present in some samples. Absent in carbonates and some hydrated aluminosilicate samples such as GCS3-analcime, GCS4-phillispite/analcime and S133-magadiite exhibit an absorption feature at 970nm, which was missed or diminished if there was an absence of a filter at precisely this wavelength. Only the Pathfinder/Beagle 2 filter set has a filter exactly at 970nm, the ExoMars F2-12 filter set has a filter at 960nm which means that the depth of the feature was reduced. The absence of this feature then renders the spectrum as featureless, and similar to the spectra of trona and magnesite, an important issue when considering the role of PanCam in remote identification of outcrops of interest. The association of the 970nm band with hydration and the filter distribution in this area makes this a particularly important disadvantage to the MER, MSL and ExoMars filter sets. Even though the ExoMars F2-12 filter set does have a filter at 960nm, it was still only replicated by a small amount which may still be overlooked.

This problem was also illustrated by the multispectral imaging by the MER PanCam at Gusev crater. Hydrated minerals have been identified, however the MER PanCam data did not allow the resolution of the type of mineral beyond a list of 18, including borates, chlorides, sulphates and the carbonates natron and trona (Rice et al., 2010). These all have relatively featureless spectra except for an absorption around 1000nm, demonstrating as we have in this study the limitation of the MER filter set. The interpretation of the MER PanCam data of the Gusev Crater has also suggested the detection of silica-rich, hydrated mineral deposit, identified by the presence of a negative slope from the 934nm to 1009nm filter. The spectral data was analysed in relation to the full spectra for hydrated sodium silicates: magadiite, sodium metasilicate pentahydrate ($Na_2SiO_3.5H_2O$) and sodium metasilicate nonahydrate ($Na_2SiO_3.9H_2O$), all of which exhibit an absorption around 970nm attributed to $H_2O$, a feature that the MER filter set could not replicate (Rice et al., 2010). The hydration feature in sodium

metasilicate nonahydrate was slightly broader and so occurs at slightly longer wavelengths than in the other two minerals. The MER filter set replicates this band as a negative slope in the 934 to 1009nm range, similar to what was seen in the MER PanCam data (Rice et al., 2010). This replication of at least a negative slope indicating the presence of a feature was not possible for the samples analysed in this study which have a 970nm feature, this was because the feature was too narrow, so the filter set skims over the top. This is a working example of the limitations of the filter sets which lack a filter in the 970nm region, supporting the issue of identifying hydrated silicates highlighted by this study.

The minerals present in the samples used in this study from Lake Magadi have all been documented from this area previously and are good samples to use for testing the ExoMars PanCam equipment with reference to alkaline-saline depositional environments. It was unfortunate that none of the samples from Lake Magadi contained analcime or phillipsite, however the prior identification of these minerals in the Lake Magadi area (Eugster, 1967) was considered sufficient to include Geological Collection-UCL examples of them in the filter testing.. It would still have been interesting to have included samples of these zeolites from Lake Magadi itself.

The inclusion of biological material although not to be found on the surface of Mars provides details on the spectral signatures of pigments present in biomats from alkaline-saline environments. If life were present on Mars, and if pigmented, these pigments maybe identifiable on Mars. The filter set testing showed that most of the features are reproducible because they are generally quite large scale and broad. We also see that when using a spectral parameter plot for the 650 nm and 970 nm band depths we do get some differentiation between the geological samples and the purely biological material with the ExoMars, MER and MSL filter sets.

The problems of missed hydration features and featureless spectra in the 440-1010nm region are not issues for the orbiting visible-infrared spectrometers CRISM or OMEGA. These instruments can image over a much wider range from 362nm to 3920nm and 400nm to 5100nm respectively (Langevin et al., 2005: Calvin et al., 2009). This means that they can cover wavelengths where the major diagnostic features occur, at wavelengths longer than 1000nm. These instruments do have their own limitations in terms of their capacity to detect small amounts of minerals such as carbonate and also in identifying the presence of more than one mineral. For example, the masking of a 2300nm carbonate absorption feature by the presence of certain clay minerals (Orofino et al., 2009). In spite of the spectral constraints of the surface multispectral instruments such as ExoMars PanCam, these instruments do provide the benefit of spectral data on a much finer geographical scale. These multispectral instruments on the surface can potentially pick up small scale depositional variation which the

orbital instruments may not, and indicate areas where more in-depth analysis by in-situ instrumentation would be required.

This study contributes to the data available on the study of alkaline-saline environments with a focus on early Martian deposits. Orbital and lander/rover observations of Mars are constantly providing data indicating that it is a geologically heterogeneous planet, with a variety of depositional environments. The number of Mars analogue studies being conducted on neutral-alkaline environments is increasing; with the recognition that the study of neutral-alkaline environments has been largely neglected in favour of acidic environments (Ehlmann et al., 2011a). Work is now being conducted by Ehlmann et al. on the aqueous alteration of basalts in Iceland as a neutral-alkaline analogue for early Mars in recognition of the identification of smectite clays, silica and zeolites. Although deposits such as trona have not yet been identified on Mars the argument that absence of evidence is not evidence of absence is particularly pertinent. Without a full understanding of the multispectral characteristics of a wide range of alkaline-saline environments the misidentification of samples may occur. Particularly if not all the necessary information is available, or an understanding of the limitation of the instruments is not understood.

The multispectral imaging systems are necessary instrumentation of rover missions to Mars and can help focus time and energy on the more interesting areas of scientific exploration. It is important they are designed with the consideration of a variety of different depositional environments, in order to ensure that they perform at their highest capacity. This study provides an indication of the limitations of the PanCam multispectral instrument in relation to the hydration features of minerals from alkaline-saline environments. These results will hopeful mean that the characteristics of neutral-alkaline-saline depositional environments are understood and that the potential diversity of these environments on Mars is not overlooked.

### 7.9. Conclusions

The analysis of 15 different samples highlighted that one of the most significant features relating to hydrated minerals from alkaline saline lakes is the 970nm hydration absorption associated with several hydrated silicate minerals.

This study also showed that in general the spectral features carbonate minerals such as magnesite and trona are not sufficiently distinct to allow for differentiation. This is important because of the depositional environment in which trona can form does not allow for the formation of magnesite due to low levels of Mg. The presence of both carbonates would indicate a more diverse set of neutral-alkaline environments than thought if just one type of carbonate is identified. It also showed how the heterogeneity of a sample, for example the presence of iron in a sample can have a significant effect upon the spectral profile.

This study demonstrated that both of the ExoMars filter sets developed outperform the Pathfinder/Beagle 2, MSL and MER filter sets and the presence of a 960nm filter in the ExoMars F2-12 filter set provides this filter set with an advantage in relation it displaying some capacity to replicate the 970nm hydration features in hydrated silicate minerals, an ability which the subsequently designed Ferric filter set does not have.

The poor performance in relation to reproducing spectral features for alkaline saline minerals by the MER filter set may be in part due to the limited number of filter sets as well as the distribution of the filter sets it does have so that it misses the 970nm region.

This study indicates the importance of using a variety of minerals in the design of filter sets for missions with or without an astrobiological objective. The absence of minerals which form in an alkaline setting in the design and initial testing of the filters could result in the overlooking of certain minerals of scientific interest. The design constraints on multispectral instruments with respect to filter numbers available mean that some spectral resolution will be lost. However this study shows how important it is that this very diagnostic feature of hydrated silicate minerals, which we know occur on the surface of Mars is being missed by most of the filter sets tested and may have implications for the accuracy of multispectral imaging and the geological interpretation of Mars.

### 7.10. Further work

The study of exactly how the reflectance spectrum changes with the proportions of minerals, for example increased amounts of halite and iron content in a predominantly trona sample would be of interest and would require data on the proportions of minerals through XRD analysis. This would provide a better understanding of the behaviour of spectral data in relation to composition and a database of mixed minerals from different environments. In addition the significant variation between the reference spectra available from different databases, which in turn differ from the spectra produced by this study, may be problematic when analysing spectral data. The continued addition of the spectra of geological samples to multispectral databases will provide a vital reference point for the data obtained from current and future missions.

An interesting further study would be to test of the ExoMars filter sets replacing the 950nm and 960nm filters with a 970nm filter. This would examine whether the data obtained with the ExoMars filter sets with a filter at 970nm is superior to that obtained when there is a filter at 950nm or 960nm. Performing this analysis would indicate if the quality of a spectra produced by the original ExoMars F2-12 and Ferric filter sets with a 950nm or 960nm filter respectively is superior across a wider suite of minerals than when the filter is at 970nm.

Lake Magadi has provided one alkaline-saline analogue environment with carbonate minerals and hydrothermal springs. The continuation of this sort of study on the carbonate deposits and associated hydrothermal springs in a cold environmental setting such as Iceland would provide an interesting contrast. Iceland has a large number of hydrothermally active site and some of those further away from the spreading ridge have an alkaline pH. This environment could then be studied in terms of the mineralogy which forms in this type of environment which would have a water chemistry compared to Lake Magadi. The in-situ spectral imaging of these sorts of sites, plus this imaging of Lake Magadi would complete this sort of multispectral analysis of these analogue environments.

## Chapter 8

# Discussion

The aim of this PhD was to explore an alkaline/saline hydrothermal environment in relation to habitable environments on Mars. This work was intended to provide details on the microbial communities present in such environments, characterising novel isolates, assessing the survivability of organisms and finally, understand how to identify similar environments in the rock record using remote instruments. Through the biological and geological study of Lake Magadi it is hoped that the findings presented here will contribute to the field of astrobiology through the study of the potential habitability of Mars and other planetary bodies.

### 8.1. Terrestrial alkaline/saline environments

Soda lakes are the most stable, naturally occurring alkaline environments on Earth (Rees, 2004: Wani et al., 2006: Lefèvre et al., 2011); Lake Magadi was chosen as the study site because of its associated hydrothermal system, the high pH, high salinity of the lake and hydrothermal springs, and because of the high rate of evaporation compared to precipitation, resulting in an arid environment (Chapter 1, section 1.3). This combination of extreme conditions requires a bacterial and archaeal community which can survive not just one, but several unfavourable environmental conditions simultaneously.

The variability between the lake and spring salinity and the temperature gradient along the lake provides a variable sampling environment, which occurs across a relatively small area. The hydrothermal system which feeds the lake varies in temperature from north to south (over ~32km), between 82.7 ºC and 36.6 ºC (Chapter 1, Section 1.3 and Chapter 3, Section 3.4.1). An alkaline pH >9, was recorded for all sampling environments and is the result of groundwater interacting with the surrounding geology. The water chemistry of the springs flowing into lake Magadi have a dominance of Na and $HCO_3$ over Ca and Mg (Chapter 3), resulting in the formation of sodium carbonate minerals (trona) and an absence of calcium and magnesium minerals (calcite, gypsum, magnesite) (Chapter 7). The salinity of the springs was generally around ~0.7% NaCl (Chapter 2) but in spite of this low salinity halotolerant and some obligate halophilic archaea were isolated from all the environments sampled.

### 8.2. The applications of alkaline/saline analogue environments

The astrobiological study of alkaline/saline environments has not been widely reported in the literature, possibly in part due to the predominant focus of analogue research on lower pH environments in relation to Mars including Rio Tinto and acid saline lakes in Australia (Benison and Laclair, 2003: Fernández-Remolar et al., 2005: Benison and Bowen, 2006: Bowen et al., 2008). Evidence suggesting the presence of alkaline hydrothermal subsurface

waters on Mars during its past and the possibility of contemporary saline liquid water means there is a need for more astrobiological studies of such environments (Abramov and Kring, 2005: Ehlmann et al., 2008b: Ehlmann et al., 2011b).

The conditions on Early Mars are hypothesised to have been similar to early Earth; possibly warm, wet and a $CO_2$ atmosphere (Chapter 1.8.3.3). It could be considered that given the parameters outline above, life potentially could have also developed on Mars. If that is the case, what needs to be addressed is where could life have occurred and where are such environments on Mars? Studies on communities and model organisms can provide details on what types of extant or extinct life could be, or have been present on Mars (either life which originated on the planet or life that is present as a result of contamination), as well as where to look for evidence of life. It is also possible that habitable environments do exist on Mars, but no life has colonized there, which could be due to number of reasons such as life not having evolved, or that any habitable environments which could be present are too transient for life to take hold (Cockell, 2011: Cockell et al., 2012). In this instance, the environment has to be firstly identified as potentially habitable through biological and Mars simulation studies, and the reasons why the environment is uninhabited evaluated. This clarification of why a given environment is uninhabited is important to understand when considering the potential habitability of other planetary bodies.

The possible application of soda lake astrobiological research extends beyond Mars: the hypothesised oceans of Enceladus and Europa, with their potentially alkaline/saline chemistry, means results of this work may have implications for the habitability of these planetary bodies.

### 8.3. Habitability of alkaline hydrothermal environments on Mars

Alkaline hydrothermal systems have been at the centre of research focussing on the origins of life. One hypothesis centres on the origin of terrestrial life in an environment where alkaline hydrothermal systems and colder more acidic waters (i.e. an ocean) interacted. This mixing would have provided a chemical gradient to supply energy, supply and concentrate necessary molecules and allow for the removal of waste (See Chapter 1) (Lane, 2010: Mellersh and Smith, 2010). If life originated in alkaline hydrothermal environments on Earth then it could be possible that life could also have developed on Mars, in similar environments. This would suggest that alkaline environments on Mars should be a high priority for astrobiological missions, therefore it is important to understand the capacity of missions to identify such environments, as discussed in Chapter 7.

Subsurface alkaline hydrothermal activity has been suggested as the mechanism for phyllosilicate formation on Mars in areas including Nili Fossae, Houldon and Eberswalde craters (Ehlmann et al., 2011b: Fairén et al., 2010). On Mars a subsurface alkaline

hydrothermal system (including impact induced hydrothermal systems) would have provided a warm, wet environment sheltered from any radiation which could have been habitable. The presence, in this system of a chemical gradient, the continued supply of nutrients and the removal of waste products (toxins) are conducive to life and necessary processes for established communities (See Chapter 1, section 1.1). In addition, the circulation in a subsurface hydrothermal system would allow for the movement and circulation of organisms, which could then result in a more widespread distribution around the planet. It is possible that the subsurface of Mars has remained a habitable environment to the present, although this is much less likely. It is also possible that present day impacts may generate hydrothermal activity in association with subsurface ice (Abramov and Kring, 2005).

Although the chemistry involved in the alkaline hydrothermal ground waters on Mars identified so far may differ to those studied here, it should be noted that many of the genera isolated from the springs and associated areas at Lake Magadi were not restricted to just soda lake environments. This suggests that these types of organisms are capable of exploiting a variety of niches, for example, species of the genera *Idiomarina, Halomonas* and *Bacillus* have been isolated from environments including; bauxite deposits, soil, seawater, salt pans, the deep sea, and deep sea hydrothermal systems (Chapter 3) (Ivanova, 2000: Donachie et al., 2003: Boltyanskaya et al., 2004: Joshi et al., 2007).

The organisms cultured in this study were most closely related to heterotrophic bacteria which rely, in part, upon the photosynthesising phototrophic community to provide organic carbon. Lake Magadi has a thriving phototrophic community. With the radiation levels associated with the surface of Mars making it largely uninhabitable, it is highly unlikely that phototrophic bacteria would play a role in any community on Mars today, although they may have done early on in Mars' history. In the subsurface, the absence of light means that the community would have to be comprised of chemotrophic, organotrophic, lithotrophic and chemolithotrophic organisms, without a phototrophic component (Shock, 2009). In the absence of phototrophs, chemotrophic organisms could provide organic material to any heterotrophic organisms within the community. The metabolic activity of bacteria and archaea is diverse(Shock, 2009).

The subsurface environment is more suited to anaerobic organisms. Although the culturing environment provided in this study was aerobic, facultative anaerobic strains including *Amphibacillus fermentum, Alkalibacterium psychrotolerans, Bacillus vedderi* and *Bacillus pseudofirmus* were isolated (Chapter 3), with relatives of the last three strains surviving the process of Mars chamber incubation (Chapter 6). Strains related to the arsenic respiring *Bacillus arseniciselenatis* and *Bacillus macyae* were isolated in this study (Chapter 3) and relatives of both these strains were isolated from the soil community after incubation under

simulated Martian conditions for 7 days (refer to Chapter 6). However, these organisms would still all rely upon a primary producer in the community. Anaerobic chemotrophs such as methanogens have been isolated from Lake Magadi, including *Methanosalsus zhilinae* (Oren, 2002a) and could provide material for a heterotrophic community.

### 8.4. Habitability of the alkaline environments on the surface of Mars

It has been suggested that some of the clay minerals on Mars formed from the subsurface interaction between the alkaline hydrothermal system and the surrounding geology, with transient surface water which means that the surface could have been mostly cold and arid (Ehlmann et al., 2011b). This contrasts to the warm and wet concept of the surface of early Mars (Pollack et al., 1987: Craddock and Howard, 2002), and would situate a warm and wet Mars in the subsurface instead. However, if the surface was cold and intermittently wet this could still be a habitable environment, if only temporarily.

The movement of organisms by hydrothermal fluids is not necessarily restricted to the subsurface; groundwater could potentially seep to the surface, taking with it any bacterial/archaeal community. Sedimentary clay deposits have been identified in association with possible fluvial basins and palaeolakes (Ehlmann et al., 2008a: Wray et al., 2008: Ehlmann et al., 2011b), this material could be the product of in-situ weathering, but it is also possible that the minerals have been transported, having been formed through hydrothermal activity or surface weathering elsewhere (Milliken and Bish, 2010: Ehlmann et al., 2011b).

The introduction of life from the subsurface to the surface could either result in the death of those organisms, or the proliferation of those able to survive the surface conditions present (hypobaria, radiation, cold, arid, freeze thaw cycles etc). At Lake Magadi, areas around the springs and lake are only wet during and after the rain season, followed by periods of aridity and desiccation. The Mars chamber studies in this thesis also demonstrated that even as shallow as 32mm alkaliphilic/alkalitolerant, halotolerant organisms could survive desiccation, hypobaria, freeze thaw cycles and UV radiation for at least short periods of time (Chapter 6), although it is not known if growth can occur.

It was also noted that the genera isolated in this study were not limited to only inhabiting soda lake environments like Lake Magadi. Isolates related to the genera; *Alkalibacterium*, *Halomonas, Planococcus* and *Idiomarina* isolated in this study, have been isolated from cold environments such as Antarctica (Christner et al., 2001: Reddy et al., 2002: Alam et al., 2003: Gilbert et al., 2004: Naganuma et al., 2005: Chattopadhyay, 2006). The environmental range of these genera indicates that these organisms are capable of occupying a wide range of niches and so relatives could be identified in an alkaline/saline/cold environment (Chapter 3). If these types of organisms can inhabit these environments, the next question is, can they

survive desiccation, and what are the effects of freeze thaw cycles and radiation on these particular organisms?

This work has shown that isolates of the genera: *Bacillus, Idiomarina, Planococcus, Salinicoccus* and the archaea *Natronococcus* and *Natrialba* from Lake Magadi can survive desiccation for up to 28 days. The strains *Planococcus, Salinicoccus* and *Natronococcus* were more resistant than some strains of *E. coli* to UVC radiation, with *Salinicoccus* and *Natronococcus* (along with D*. radiodurans*) able to survive longer than 7.9 seconds exposed on the surface of Mars with its present UV flux (calculated from the highest UV dose tested, using the Xenon lamp, equated to Martian UV flux, refer to Chapter 5). It is important to note that a high cell density or layer of soil can protect cells, and so increase survival (Osman et al., 2008). The study of *Planococcus* sp. LMLD02 in this thesis, a strain related to *Planococcus maritimus* (Chapter 3 and 4) demonstrated that *Planococcus* sp. LMLD02 was able to survive being placed into the Mars simulant and incubated under simulated Martian conditions for up to 7 days (Chapter 6).

With this in mind, it is possible to imagine a situation where a bacterial/archaeal community may be present in an alkaline subsurface hydrothermal environment on Mars (in its history, or possibly at present). In addition, it might be feasible that a community could colonise the surface when the environment was suitable and remain (protected from radiation) once the conditions turned more arid, in order to reanimate once conditions become more favourable once again. This could result in the preservation of evidence of life in the shallow subsurface, as well as the deeper subsurface of Mars.

### 8.5. Relevance of alkaline/saline analogue studies to the contamination of Mars

An important issue for planetary exploration and astrobiology is the potential for contamination of planetary bodies with foreign life. An important point to note is the identification of alkalitolerant and halophilic/halotolerant organisms from spacecraft clean-rooms (Ghosh et al., 2010: Vaishampayan et al., 2010). The culture based study of the Phoenix spacecraft clean-room demonstrated that alkalitolerant strains (such as *Bacillus pumilus* and *Oceanobacillus iheyensis*) were present and were not a minor part of the cultured community, being frequently detected (Ghosh et al., 2010). This indicates that these alkalitolerant organisms have survived the sterilisation procedure applied, and there is no reason why the strains could not also be present on the space craft itself. If this is the case, and the organisms were shielded during the flight to Mars, then theoretically they could be transferred to the surface/subsurface of the planet. This would have implications for the discovery of life on Mars.

These organisms would not necessarily be strictly defined as extremophiles, but are tolerant of extreme conditions such as pH. Together with the fact that alkalitolerant isolates from Lake Magadi could survive desiccation, oxidative atmosphere and low temperatures, as long as they are provided with some protection from UV-C irradiation (and ionizing radiation), makes the potential contamination of Mars with alkalitolerant organisms a real consideration. The additional tolerance of these organisms to high salinity and temperatures makes them resistant to a wider range of environmental stresses.

This study identified both extremophilic and extremotolerant organisms from this extreme environment (Chapter 3). Extremotolerant organisms are particularly interesting due to their capacity to occupy a wider range of niches than organisms which are extremophilic. For example, the cells of some hyper-halophilic archaea will lyse in the presence of less than 15% NaCl, due to their cell adaptations for high salinity (Chapter 5). In contrast, the isolate *Salinicoccus alkaliphilus* (a close database match for a strain isolated in this study-Chapter 3 and studied in Chapter 5) can grow in the presence of 0% NaCl and in the presence of up to 25% NaCl (Zhang, 2002). This is an excellent example of how a tolerance for a wide range of environmental conditions can be more beneficial than only being able to tolerate the most extreme conditions. Further study into the survivability and resistance of these possibly more adaptable, tolerant organisms could be extremely beneficial for the field of astrobiology.

Alkalitolerant strains including *Paenibacillus* sp. and *Bacillus flexus*, were isolated from the Phoenix spacecraft clean-room and have also been isolated from the sodic Lonar Lake (Kanekar et al., 2008). This study identified several strains from Lake Magadi which were related to isolates from Lonar Lake, indicating a similarity between the culturable communities of the two environments (Chapter 3). The presence of strains in sterilised clean-rooms which have also been isolated from soda lakes emphasises the importance in understanding the resistance and survivability of organisms from these environments under simulated Martian conditions.

### 8.6. Preservation of biosignatures on Mars

There are several ways in which evidence of life could be preserved on Mars, on Earth clay deposits preserve organic material (Ehlmann et al., 2008a: Summons et al., 2011). The alkaline/neutral pH environment required for smectite clay formation would suggest that any life preserved in these deposits would be alkaliphilic/alkalitolerant. Areas like the Jezero crater, where the clay deposits are associated with deltaic deposits are considered possible areas for organic preservation (Ehlmann et al., 2008a). Depending upon the depositional environment, carbonate deposits could also serve to preserve chemical biosignatures (Bada et al., 2009). In addition, the hydrous sodium silicate mineral magadiite was identified in this study in association with a prokaryotic community within several mm of the surface of the

mineral (Chapter 7). This mineral is not very stable and on Earth and will lithify into a type of chert. It would be interesting to ascertain if any evidence of the bacterial/archaeal community could be preserved and identified in the chert. If it could, then this would provide another mineral of interest to astrobiology.

Halite is another interesting mineral with a potential for biological preservation. On Earth, evaporitic minerals are able to encapsulate organic material (Mancinelli et al., 2004). Several studies conducted by Vreeland *et al*, have identified the presence of bacteria and archaea preserved in halite crystals, with contentious reports of the viability of some organisms even after geological periods of time (Vreeland et al., 2000: Vreeland et al., 2007). The study of pigmented organisms has shown their capacity to survive desiccation, periods of UV irradiation and the combined effects of simulated Martian conditions (Chapter 5 and 6). If a pigmented strain (such as a pink haloarchaeon) is encapsulated in halite, the pigmentation can still be seen through the crystals, and can be recognized (Vítek et al., 2010: Edwards et al., 2010). In addition, it has been suggested that the hygroscopic nature of the mineral could lead to small amounts of liquid water being available, potentially creating a habitable microenvironment (Hovorka et al., 2007). These factors would all suggest that the identification of halite on Mars is very relevant to astrobiology and supports the possibility that halite, and the environments in which it forms, are another possible preservation environment (Bada et al., 2009).

### 8.7. The ability of multispectral instruments to detect and identify saline/alkaline environments on Mars

It is essential to test that alkaline/saline environments are identifiable using the equipment available to missions. With this taken into consideration, plus the purpose of the ExoMars PanCam instrument to identify particular outcrops of significance through remote analysis, there is a risk that different types of environments or particularly interesting outcrops, for example, those containing zeolite minerals, could be overlooked.

A range of geological samples were collected from the trona pavement at Lake Magadi and, along with Fe and Mg carbonates and zeolite minerals associated with alkaline/saline environments, were used to test the geological filters proposed for the ExoMars PanCam instrument. Trona and zeolite minerals were not included in filter set development. In addition, the filter sets chosen for the Pathfinder/Beagle 2, MER PanCam instruments and the MSL MastCam were also tested. The purpose of this part of the study was to assess the capacity of each filter set to reproduce the key features associated with the types of minerals associated with saline/alkaline environments, in order to assess whether or not these types of environments can be identified in situ, using multispectral remote instruments.

Several conclusions were drawn from this study. The most significant are associated with the inability of the filter sets to identify and/or distinguish between different minerals which form in alkaline environments. It is difficult to distinguish between different types of carbonates (Na and Mg) due to their relatively featureless spectra at wavelengths between 440 and 1010nm (Cousins et al., 2010), with the exception of siderite. The absence of a filter in the proposed ExoMars, MER and MSL instruments at 970nm could detrimentally affect their ability to zeolites, whose formation is associated with water. Therefore this has two possible implications, firstly for the geological characterisation of Mars in-situ, and secondly, for the identification of locations of water-rock interactions.

The filter set designed for Mars Science Laboratory (MSL) has nine filters compared to the twelve filter sets proposed for the ExoMars instrument (Chapter 7, section 7.1.2). The poor performance of the MSL filter set in comparison could be due to the limited number of filters, as well as the differences in filter distribution, although these are in part linked. The lower number of filters means results in larger gaps between filters, therefore increasing the chances of missing any small, but still potentially significant features in the spectrum of a mineral. This would suggest that the number of filters is also an important factor to consider.

Finally, comparing the database spectra from RELAB, USGS Spectroscopy Lab- Spectral Library and the ASTER Spectral Library demonstrated that often, due to the heterogeneity of unprepared geological samples, the spectra showed spectral differences. This is important when considering the datasets to be used when interpreting the multispectral data from Mars. It also reinforces why ground testing of instruments using unprepared, geological samples is so necessary.

To address these points several suggestions have been made:

1. Minerals from alkaline/saline environments, particularly zeolites must be considered during the design of filter sets for multispectral instruments
2. Include a 970nm filter in the filter set
3. There should be as many filters for geological analysis as is possible, given any technical constraints
4. Untreated, geological samples should be used to test equipment rather than prepared samples of uniform size etc. providing more ‘realistic’ comparisons for data obtained by instruments

### 8.8. Future work

This thesis has served as a grounding in the study of Lake Magadi as an analogue environment for Mars. It has provided an initial study into the geology, environmental

chemistry and biology of the lake, and provided details of the effects of desiccation, UV-C radiation and the combined Mars simulated conditions on the prokaryotic community and individual isolates. These preliminary studies can potentially lead onto further exploration and experimentation, which will now be discussed.

There is always the desire to start studying a new location, assessing the diversity or types of organisms present and comparing those to other environments. However, there is still a great deal which can be done using the samples and data collected in this study. The time constraints meant that although a number of initial ideas were developed, these could not all be put into practice during this PhD.

### 8.8.1. Continued study of Lake Magadi isolates

#### 8.8.1.1. Gene expression and protein synthesis

The ultimate assay of cell viability is growth. However, assessing the effects of desiccation and UV radiation on cell viability through CFU counts is but one step towards understanding the effects of these factors on organisms (Rochelle et al., 2011). The next stage involves assessing the enzyme and protein expression of different strains reflecting changes due to the application of stress, or environmental factors. Bacteria respond to variation in their environment through the transcriptional activation of genes (Ramos, 2001: Schumann, 2006). The presence of a stress can result in the triggering of different genes, these genes can code for products which will assist the organisms to cope with the stress being applied (Ramos, 2001: Schumann, 2006: Cytryn et al., 2007). Gene expression, and therefore protein expression, can be altered by a variety of factors including the application of solvents and desiccation, and changes in temperature, salinity, and pH (Booth, 1985: McLaggan et al., 1998: Cytryn et al., 2007).

The effects of desiccation and UV radiation on protein expression and gene regulation has been studied in strains including some cyanobacteria (Potts, 1986: Fleming and Castenholz, 2007) haloarchaea (comparing light and dark repair conditions) (Leuko et al., 2011) and bacteria from Antarctica (Yergeau et al., 2007: Yergeau and Kowalchuk, 2008: Yergeau et al., 2008). The exact genes which can be expressed differ between species, so that the response of a specific organism can only be identified by studying that organism (Burg et al., 1996).

The bacterium *Bradyrhizobium japonicum* up-regulates up to 405 genes in response to desiccation. In particular, an increase in the intracellular concentration of trehalose, a sugar involved in osmoresistance/desiccation resistance, was observed (Booth, 1985: McLaggan et al., 1998). By studying protein and gene expression before and after exposure to stress

factors involved in a Martian environmental simulation have been applied, the effect of different stresses on gene expression could be assessed.

Studies on gene expression have used 2-dimensional gel electrophoresis to separate proteins and produce protein profiles, quantitative real time (qRT) PCR to identify and assess changes in expression levels of genes and functional gene arrays, which are used to assess the functional activities of complex communities (He et al., 2007). The use of 2D protein gel would allow for the identification of differences in the protein profiles. Proteins of particular interest (those proteins with a notable change), can be sequenced, and the genes involved in expression identified. The increase or decrease in gene expression can then be quantified using qRT PCR.

This work would aim to answer questions such as: what are the gene profiles for strains like *Salinicoccus* sp. LMLD05 and *Planococcus* sp. LMLD02? How do the protein profiles differ between the different strains and how do they differ when comparing before and after exposure? This would provide a greater understanding of the effects of these stresses on the types of organisms isolated from Lake Magadi. These studies could look at both short and long term influence on gene expression and could be applied to organisms which have been incubated under one stress factor and/or after incubation under combined simulated Martian conditions, to assess the influence of these factors on gene expression.

#### 8.8.1.2. Preservation and viability of archaea and bacteria in halite crystals

As mentioned earlier, halite crystals potentially have a role in the preservation of biosignatures on Mars. Preliminary work has been conducted on the ability of *Planococcus sp*. LMLD02 and *Natronococcus* sp. LMLD06 to survive being preserved in halite crystals over a 7 day period whilst being air dried at 37 ºC. The cell surface was sterilised using the protocol outlined in Rosenzweig (2000) and each crystal surface was smeared on to appropriate solid medium (Chapter 3 and 5) to check for growth and ensure sterility.

No growth was recorded for *Planococcus* sp. LMLD02 after this procedure indicating that this strain was not able to survive being trapped in fluid inclusions within halite crystals. However, it is not clear if *Planococcus* sp. LMLD02 could survive on the surface of the halite crystal, but not the entrapment in fluid inclusions, or that it could not survive any of the protocol. This suggests that an unsterilized crystal surface control would be useful indicating what part of the procedure maybe affecting *Planococcus* sp. LMLD02.

In contrast, *Natronococcus* sp. LMLD06 was also able to survive being placed into halite crystals, in accordance with other researchers studies of relatives of Natronococcus (Norton and Grant, 1988). *Natronococcus* sp. LMLD06 was more desiccation resistant and UV-C

tolerant than many of the other strains. The equally high survival of *Salinicoccus* sp. LMLD05 to desiccation and UV-C radiation (Chapter 5) makes this another interesting candidate for halite preservation studies. In addition, the survival of *D. radiodurans* in halite crystals would be of greater interest.

The purpose of this work would be two fold, firstly to assess the effects of halite encapsulation on different archaea and bacteria, identifying those which could potentially be preserved in halite. Secondly, the types of biosignatures and the degradation biosignatures produced by cells encapsulation within halite could be assessed. This could be, for example, biosignatures produced through the presence of pigments visible through the halite crystals.

### 8.8.2. Other sampling environments

#### 8.8.2.1. Alkaline springs, Iceland

The study of the alkaline hydrothermal springs in Iceland would be a fascinating comparison to this study, contrasting hydrothermal systems in cold and hot environments. Reykjanes in the Ísafjarðardjúp Bay, north western Iceland have alkaline hydrothermal springs around 2-3m below sea level at low tide (Kristjansson et al., 1986: ALFREDSSON et al., 1988: Hobel et al., 2005) (Figure 8.1). These springs vary between 45 ºC and 95 ºC and have an alkaline pH (Kristjansson et al., 1986: Hobel et al., 2005).

In addition to these submarine alkaline hydrothermal springs, terrestrial springs, such as those at the Hveragerdi-Hengill area, and the Biskupstungur-Geysir area have pH's measured up to 10.1 (Kristjansson et al., 1986). A few culture based studies have been conducted on these springs, as well as bacterial 16S rRNA clone libraries being compiled for waste water drains from Hveragerdi and Reykjanes Power Stations, which have a pH and salinity of 9.1 and <0.1%, and 7.5 and 4.5% respectively (Tobler and Benning, 2011). The Reykjanes site also has a higher silica content of 695 ppm and so forms silica sinters very quickly (Tobler and Benning, 2011).

*(Figure removed for copyright reasons)*

*Figure 8.1 Map of Iceland with approximate areas of alkaline hydrothermal springs (Yellow marker). The Hvalfjordur area was used as analogue for Noachian Mars by Ehlmann et al. (2011a) (Red marker). From Google Earth.*

These sites have not been explored as a potential Martian analogue environment. An initial culture based study, followed by assessing the effects of simulated Martian conditions again on individual isolates and on communities would be very interesting. Such a study could answer questions such as, do these organisms survive the simulated conditions as well as or better than those isolated and tested in this thesis? The different water chemistry and geology means that this environment and its microbiology will differ when compared to Lake Magadi. Any organisms isolated which are similar to those from Lake Magadi may have genotypic and phenotypic difference, being adapted to a different habitat.

Alkaline Mars analogue studies have begun in areas such as Iceland, with the study of altered basaltic lavas by Ehlmann et al. (2011a) in the Hvalfjordur area (Figure 8.1), but it is important to continue along this vein, increasing the number of astrobiological studies of these types of alkaline environments.

#### 8.8.2.2. Black Mountains, Wales

Some preliminary work was conducted in February 2009 in the Black Mountain area of Wales. The sites of interest are located below a disused limestone quarry. At the quarry, limestone was heated to convert the $CaCO_3$ to produce CaO, or quick lime, a highly alkaline

product. The presence of these deposits resulted in the high pH spring waters in the area and areas of carbonate re-deposition, some of which is dark orange red, possibly due to iron (Figure 8.2.A, and Figure 8.3 A-D).

Five sites were explored in the preliminary study, the springs at the sites were between 3.5 ºC and 8.2 ºC, with a pH between 8.3 and 10.8 and flowed into pools, which were iced over during the field trip. Biomats have developed over limestone blocks, partially submerged in the pools (Figure 8.3.E-F). The organisms which can inhabit such an environment would have to tolerate both the high pH and high Ca content of the spring water, as well as the relatively low temperature springs as well. This environment contrasts to the Lake Magadi samples through the water chemistry and environmental conditions, i.e. temperature.

*(Figure removed for copyright reasons)*

*Figure 8.2 Satellite image of Black Mountain sampling areas (A) 51º 51' 29.62"N, 3 º 51'26.34"W (B) 51 º 51'23.97"N, 3 º 50'17.99"W. Images taken from Google Earth. Scale bar is 25km and images are in North-South orientation.*

In addition to using this site for microbiological analysis, the carbonate aprons would be of use for further testing of the PanCam instrument. This would involve a compositional analysis of the carbonate aprons followed by the acquisition of laboratory spectra for samples as well as field testing of the ExoMars PanCam instrument.

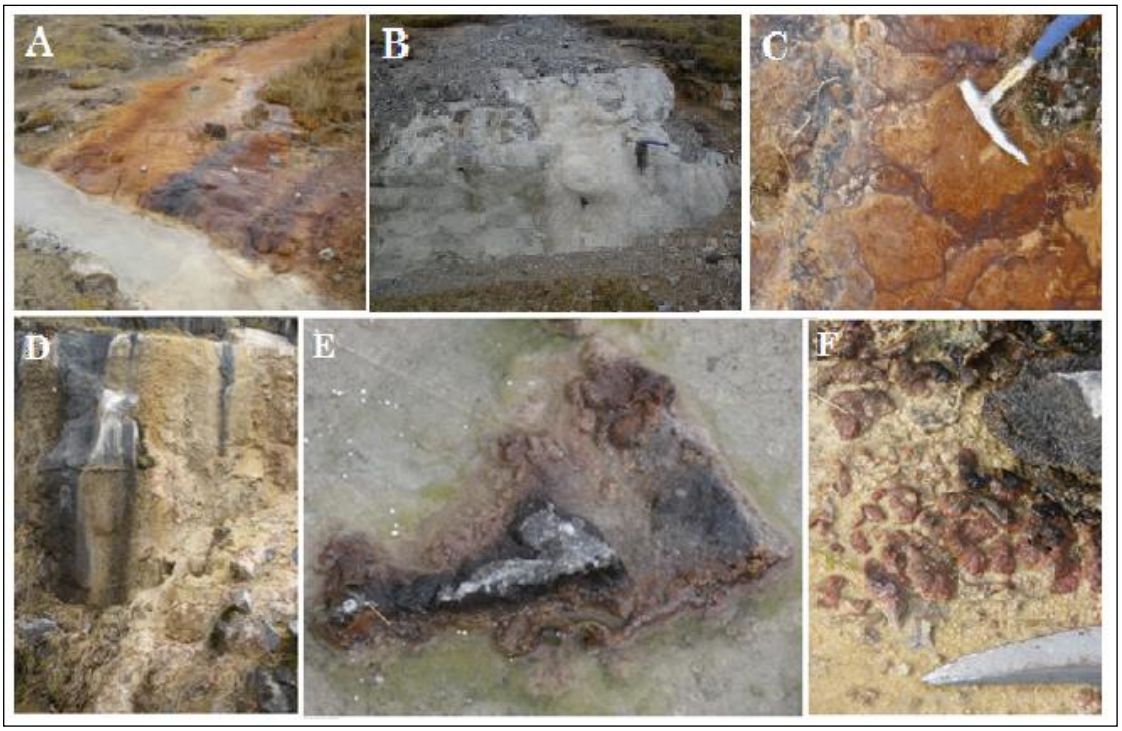



*Figure8.3 Carbonate deposits in the Black Mountain area. Images A to D are areas of carbonate precipitation, displaying orange colouration to the deposits in some areas. Images E and F show pink and black biomat material which forms in the pools, on limestone blocks and on the floor.*

### 8.8.3. Alkalitolerant/ halotolerant strains from non-extreme environments

Finally, more research needs to be conducted on the types of alkalitolerant/alkaliphilic and halotolerant/halophilic organisms which are isolated from non-extreme environments, these could simply be using garden soil samples from the UK, or sea water samples. Olsson-Francis *et al*. (2010) describe using exposure to low Earth orbit as a method of isolating novel extremotolerant strains. The same principle could be applied to Mars chamber studies, subjecting soil samples to a range of environmental factors, and then isolating strains from these samples to characterisation in terms of growth range and tolerance to desiccation etc. It is documented that alkalitolerant and halotolerant strains can be isolated from non-extreme environments (Al'Abri, 2011: Wiegel, 2011), including spacecraft clean-rooms, and indicates that these types of organisms can survive a in a broad range of environmental conditions. More astrobiology-related studies should be done on strains from non-extreme environments in order to assess what alkalitolerant/halotolerant organisms exist in these communities, and what their resistance and tolerances are. These strains have the benefit of a being more flexible in terms of adapting to changing environmental conditions (i.e. high and low salinity etc). The potential contamination of Mars is more likely to be from microorganisms from non-extreme environments which are in the vicinity of the laboratories. Therefore, the study of

these organisms in relation to simulated Martian conditions would be valuable when considering the potential contamination of Mars.

### 8.9. Final conclusions

The search for life beyond Earth firstly requires an understanding of where to look and then what to look for. Only then are we equipped to potentially identify life present on planetary bodies such as Mars. Analogue studies must start with the characterisation of an environment in terms of the chemistry, geology and types of environmental factors and conditions which are present. This produces an environmental profile upon which a biological profile can be built. This process of analogue research requires the use of a variety of techniques, which has been conducted in this study. The results of this work are summarised into the following conclusions:

- More analogue research needs to be conducted on alkaline hydrothermal environments and the organisms associated with them. This will ensure that we have as clear an understanding of possible environments which could be analogous to areas identified on planetary bodies such as Mars, Europa and Enceladus. Recent data indicates that Mars has been an environmentally diverse planetary body during its history and has hot alkaline, neutral and acidic environments. As the study of planetary bodies continues, the variety and number of environments which could be considered habitable may also increase, as will our understanding of the specific chemistry and geology associated with these environments.
- Culture-based studies are still a necessary part of the microbiological characterisation of environments, the characterisation of novel isolates and the identification of resistant, potentially model organisms for astrobiological study. By testing the resistance of organisms against different environmental stresses, it is possible to identify novel strains which have a particularly high resistance to different stresses, such as *Planococcus* sp. LMLD02, *Salinicoccus* sp. LMLD05, *Natronococcus* sp. LMLD06 and *Natrialba* sp. LMLD07. These organisms can then be studied in terms of the mechanisms they employ to combat high pH, high salinity and/or oxidative stress through desiccation or UV-C radiation. This will increase our understanding of these mechanisms and the limits of life.
- Many of the organisms isolated in this study are related to strains which would be classed as halo-tolerant (i.e. *Salinicoccus* sp. LMLD04), alkali-tolerant (i.e. *Planococcus* sp. LMLD02), with relatively broad growth ranges. An ability to grow under extreme and non-extreme conditions increases the number of environments which they can inhabit, and halophile has been linked to desiccation and UV resistance. In addition, the isolation of halotolerant and alkalitolerant strains from clean-room environments demonstrates an ability of some tolerant strains to withstand sterilisation procedures and therefore could represent potential contaminants for the surface of Mars.

- Culture based study of different areas around Lake Magadi demonstrated a dominance of *Bacillus, Idiomarina, Halomonas* and *Alkalibacillus* strains, from all parts of the Lake, with no clear differentiation in the culturable diversity along the temperature gradient when using the media AP+0.7%, AP+6.8% or AP+15%. This identification of similar organisms from the higher (~82 ºC) and lower temperature (~35 ºC) areas of the lake indicate an ability of these strains to occupy a range of hydrothermal environments.
- Isolates of *Bacillus, Idiomarina, Halomonas* and *Alkalibacillus* were able to survive being subjected to simulated Martian conditions, including UV-C radiation, freeze thaw cycles and low pressure for up to seven days when part of a complex soil community. The combination of these findings potentially highlights a small number of genera which could be the focus of further astrobiological study.
- The process of desiccation has a significant effect on organisms which can grow on NaCl concentration of 0.7%, 6.8% and 15%, but following this the isolates which can grow on 15% NaCl are not affected by the simulated Martian conditions tested here. In contrast, those able to grow on 0.7% and 6.8% NaCl were affected by the bench incubation and the simulated Martian conditions, suggesting that those organisms which can grow on these lower salinities were more sensitive than those able to grow on 15% NaCl. Therefore, those organisms which are adapted to grow on 15% NaCl maybe better suited to surviving the conditions on the surface of Mars.
- Pigments produced by halophilic and halotolerant organisms probably aid their resistance to oxidative stress, induced by several factors which are present on Mars. The link between desiccation, UV radiation and pigmentation in the survival of isolates in this thesis would suggest that organisms which could survive on Mars may be pigmented. Pigments would then provide a biosignature which could be identified by instruments including PanCam.
- The ability of multispectral filter sets, such as that present on the ExoMars PanCam instrument, to reproduce the spectra of alkaline/saline minerals can is impaired by the failure to consider minerals associated with alkaline/saline environments during their design and testing. Filter set designs may benefit from; including representative key minerals from a variety of environments (i.e. zeolites), and from considering key features of particular minerals of interest (i.e. 970nm absorption in analcime), ensuring that these features can be detected.
- It is important to test equipment and instruments for missions using ‘raw’ geological samples in order to understand their capacity and limitations. It is necessary that astrobiological missions are able to identify and distinguish between as many different types of environments as possible. It is not enough to simply be able to identify an alkaline environment on Mars if we do not understand the habitability, and equally, it is pointless to understand the habitability of an environment if it cannot be identified on the surface for in situ analysis or sample return.

# References


ABRAMOV, O. & KRING, D. A. 2005. Impact-induced hydrothermal activity on early Mars. *J. Geophys. Res.,* 110**,** E12S09.

ABREVAYA, X. C., PAULINO-LIMA, I. G., GALANTE, D., RODRIGUES, F., MAUAS, P. J., CORTÓN, E. & LAGE CDE, A. 2011. Comparative survival analysis of *Deinococcus radiodurans* and the haloarchaea *Natrialba magadii* And *Haloferax volcanii*, exposed to vacuum ultraviolet irradiation. *Astrobiology,* 11**,** 1034-40.

ACINAS, S. G., SARMA-RUPAVTARM, R., KLEPAC-CERAJ, V. & POLZ, M. F. 2005. PCR-Induced Sequence Artifacts and Bias: Insights from Comparison of Two 16S rRNA Clone Libraries Constructed from the Same Sample. *Applied and Environmental Microbiology,* 71**,** 8966-8969.

AGNEW, M. D., KOVAL, S. F. & JARRELL, K. F. 1995. *Isolation and characterization of novel alkaliphiles from bauxite-processing waste and description of Bacillus vedderi sp. nov., a new oblique alkaliphile,* München, ALLEMAGNE, Elsevier.

AL'ABRI, K. 2011. *Use of molecular approaches to study the occurrence of Extremophiles and extremodures in non-extreme environments.* PhD, University of Sheffield.

ALAIN, K., MARTEINSSON, V. T., MIROSHNICHENKO, M. L., BONCH-OSMOLOVSKAYA, E. A., PRIEUR, D. & BIRRIEN, J. 2002. *Marinitoga piezophila* sp. nov., a rod-shaped, thermo-piezophilic bacterium isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. *International Journal of Systematic and Evolutionary Microbiology,* 52**,** 1331-9.

ALAM, S., SINGH, L., DUBE, S., REDDY, G. & SHIVAJI, S. 2003. Psychrophilic *Planococcus* maitriensis sp.nov from Antarctica. *Systematic and Applied Microbiology,* 26**,** 505-510.

ALBEE, A. L. 1992. Mars Observer Mission. *Journal of geophysical research,* 97**,** 7665.

ALBEE, A. L., PALLUCONI, F. D. & ARVIDSON, R. E. 1998. Mars Global Surveyor Mission: Overview and Status. *Science,* 279**,** 1671-1672.

ALFREDSSON, G. A., KRISTJANSSON, J. K., HJORLEIFSDOTTIR, S. & STETTER, K. O. 1988. *Rhodothermus marinus,* gen. nov., sp. nov., a Thermophibic, Halophilic Bacterium from Submarine Hot Springs in Iceland. *Journal of' General Microbiology,* 134**,** 199-306.

ALLEN, C. C., MORRIS, R. V., LINDSTROM, D. J., LINDSTROM, M. M. & LOCKWOOD, J. P. 1997. JSC MARS-1: Martian regolith simulant. *Lunar and Planetary Science Conference.* Houston, Texas.

ALTSCHUL, S. F., GISH, W., MILLER, W., MYERS, E. W. & LIPMAN, D. J. 1990. Basic local alignment search tool. *Journal of Molecular Biology,* 215**,** 403-410.

AMANN, R. I., BINDER, B. J., OLSON, R. J., CHISHOLM, S. W., DEVEREUX, R. & STAHL, D. A. 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Applied and environmental microbiology,* 56**,** 1919-1925.

AMILS, R., GONZALEZ TORIL, E., FERNANDEZ REMOLAR, D., GOMEZ, F., AGUILERA, A. & RODRIGUEZ, N. 2007. Extreme environments as mars terrestrial analogs: The Rio Tinto case. *Planetary and space science,* 55**,** 370-381.

ANDERSON, A., NORDON, H. C., CAIN, R. F., PARRISH, G. & DUGGAN, D. 1956. Studies on a radio-resistant micrococcus. I. Isolation, morphology, cultural characteristics and resistance to gamma radiation. *Food Technology,* 10**,** 575– 578.

ANDREWS HANNA, J., PHILLIPS, R. & ZUBER, M. 2007. Meridiani Planum and the global hydrology of Mars. *Nature,* 446**,** 163-166.

ANJEM, A., VARGHESE, S. & IMLAY, J. A. 2009. Manganese import is a key element of the OxyR response to hydrogen peroxide in Escherichia coli. *Molecular microbiology,* 72**,** 844-858.

ARMSTRONG, G. A. 1997. Genetics of eubacterial carotenoid biosynthesis: a colorful tale. *Annual Review of Microbiology,* 51**,** 629-659.

ARORA, M. 1998. Alkaliphilic microorganisms. *In:* ARORA, M. (ed.) *Biological Control Of Environmental Pollution.* New Dehli: Anmol Publications Pvt. Ltd.

ARRAGE, A., PHELPS, T., BENOIT, R., PALUMBO, A. & WHITE, D. 1993a. Bacterial sensitivity to UV-light as a model for ionizing-radiation resistance. *Journal of microbiological methods,* 18**,** 127-136.

ARRAGE, A. A., PHELPS, T. J., BENOIT, R. E. & WHITE, D. C. 1993b. Survival of subsurface microorganisms exposed to UV radiation and hydrogen peroxide. *Applied and Environmental Microbiology,* 59**,** 3545-3550.

ARVIDSON, R., ASHLEY, J., BELL, J., CHOJNACKI, M., COHEN, J. & ECONOMOU, T. 2011. Opportunity Mars Rover mission: Overview and selected results from Purgatory ripple to traverses to Endeavour crater. *Journal of geophysical research,* 116.

ASHLEY, G. M., MWORIA, J. M., MUASYA, A. M., OWEN, R. B. & DRIESE, S. G. 2004. Sedimentation and recent history of a freshwater wetland in a semi-arid environment: Loboi Swamp, Kenya, East Africa. *Sedimentology,* 51**,** 1301-1321.

AUSUBEL, F. M. 2001. *Current protocols in molecular biology* [Online]. New York: Wiley, InterScience. [Accessed].

BADA, J. L., AUBREY, A. D., GRUNTHANER. F.J, HECHT, M., QUINN, R. C., MATHIES, R., ZENT, A. P. & CHALMERS, J. H. 2009. Seeking signs of life on mars: In situ investigations as prerequisites to sample return missions. *Planetary science decadal survey White Paper.*

BAILIN, Y. 1991. Spectral reflectance features of rocks and ores and their applications. *Chinese Journal of Geochemistry,* 10**,** 188.

BAKER, B. H. 1955. Geology of the Magadi Area. *Report of the Geological Survey of Kenya,.* Nairobi

BAKER, B. H. 1958. Geology of the Magadi Area. *Report of the Geological Survey of Kenya,.* Nairobi

BAKER, V. R. 1979. Erosional Processes in Channelized Water Flows on Mars. *J. Geophys. Res.,* 84**,** 7985-7993.

BAKER, V. R. 2001. Water and the martian landscape. *Nature,* 412**,** 228-236.

BALDRIDGE, A. M., HOOK, S. J., CROWLEY, J. K., MARION, G. M. & KARGEL, J. S. 2009a. Contemporaneous deposition of phyllosilicates and sulfates: Using Australian acidic saline lake deposits to describe geochemical variability on Mars. *Geophysical Research Letters,* 36.

BALDRIDGE, A. M., HOOK, S. J., GROVE, C. I. & RIVERA, G. 2009b. The ASTER spectral library version 2.0. *Remote Sensing of Environment,* 113**,** 711-715.

BANDFIELD, C. L. 2002. Global mineral distributions on Mars. *Journal of geophysical research: planets,* 107**,** 9.

BARTHOLOMEW, J. W. 1950. A simplified bacterial spore stain. *Biotechnic & histochemistry,* 25**,** 153.

BATTISTA, J. R. 1997. AGAINST ALL ODDS:The Survival Strategies of Deinococcus radiodurans. *Annual Review of Microbiology,* 51**,** 203-224.

BAUMGARTE, S. 2003. *Microbial Diversity of Soda Lake Habitats.* Doktors der Naturwissenschaften, Universität Carolo-Wilhelmina zu Braunschweig.

BAXTER, B., EDDINGTON, B., RIDDLE, M., WEBSTER, T. & AVERY, B. 2007a. Great Salt Lake halophilic microorganisms as models for astrobiology: Evidence for desiccation tolerance and ultraviolet irradiation resistance. *Proceedings of SPIE--the international society for optical engineering,* 6694**,** 669415.

BAXTER, B., EDDINGTON, B., RIDDLE, M., WEBSTER, T. & AVERY, B. 2007b. Great Salt Lake halophilic microorganisms as models for astrobiology: Evidence for desiccation tolerance and ultraviolet irradiation resistance

Proceedings of SPIE. *Proceedings of SPIE--the international society for optical engineering,* 6694**,** 669415.

BEBLO, K., DOUKI, T., SCHMALZ, G., RACHEL, R., WIRTH, R., HUBER, H., REITZ, G. & RETTBERG, P. 2011. Survival of thermophilic and hyperthermophilic microorganisms after exposure to UV-C, ionizing radiation and desiccation. *Archives of Microbiology***,** 1-13.

BEBLO, K., RABBOW, E., RACHEL, R., HUBER, H. & RETTBERG, P. 2009. Tolerance of thermophilic and hyperthermophilic microorganisms to desiccation. *Extremophiles,* 13**,** 521-531.

BELL, J., SQUYRES, S., ARVIDSON, R., ARNESON, H., BASS, D. & CALVIN, W. 2004. Pancam multispectral imaging results from the Opportunity Rover at Meridiani Planum. *Science,* 306**,** 1703-1709.

BELL, J. F., SQUYRES, S. W., HERKENHOFF, K. E., MAKI, J. N., ARNESON, H. M., BROWN, D., COLLINS, S. A., DINGIZIAN, A., ELLIOT, S. T., HAGEROTT, E. C., HAYES, A. G., JOHNSON, M. J., JOHNSON, J. R., JOSEPH, J., KINCH, K., LEMMON, M. T., MORRIS, R. V., SCHERR, L., SCHWOCHERT, M., SHEPARD, M. K., SMITH, G. H., SOHL-DICKSTEIN, J. N., SULLIVAN, R. J., SULLIVAN, W. T. & WADSWORTH, M. 2003. Mars Exploration Rover Athena Panoramic Camera (Pancam) investigation. *Journal of geophysical research,* 108**,** 8063.

BENISON, K. C. & BOWEN, B. B. 2006. Acid saline lake systems give clues about past environments and the search for life on Mars. *Icarus,* 183**,** 225-229.

BENISON, K. C. & LACLAIR, D. A. 2003. Modern and ancient extremely acid saline deposits: Terrestrial analogs for martian environments? *Astrobiology,* 3**,** 609-618.

BENLLOCH, S., ACINAS, S. G., ANTÓN, J., LÓPEZ-LÓPEZ, A., LUZ, S. P. & RODRÍGUEZ-VALERA, F. 2001. Archaeal Biodiversity in Crystallizer Ponds from a Solar Saltern: Culture versus PCR. *Microbial ecology,* 41**,** 12-19.

BERRY, B., JENKINS, D. G. & SCHUERGER, A. 2010. Effects of simulated Mars conditions on the survival and growth of *Escherichia coli* and *Serratia liquefaciens*. *Applied and Environmental Microbiology,* 76**,** 2377-2386.

BIBRING, J.-P., LANGEVIN, Y., GENDRIN, A., GONDET, B., POULET, F., BERTHE, M., SOUFFLOT, A., ARVIDSON, R., MANGOLD, N., MUSTARD, J., DROSSART, P. & THE

OMEGA TEAM 2005. Mars Surface Diversity as Revealed by the OMEGA/Mars Express Observations. *Science,* 307**,** 1576-1581.

BIBRING, J. P., LANGEVIN, Y., MUSTARD, J. F., POULET, F., ARVIDSON, R., GENDRIN, A., GONDET, B., MANGOLD, N., PINET, P., FORGET, F. & TEAM, O. 2006. Global mineralogical and aqueous mars history derived from OMEGA/Mars express data. *Science,* 312**,** 400-404.

BIGHAM, J. M., SCHWERTMANN, U., TRAINA, S. J., WINLAND, R. L. & WOLF, M. 1996. Schwertmannite and the chemical modeling of iron in acid sulfate waters. *Geochimica Et Cosmochimica Acta,* 60**,** 2111-2121.

BIOLOG, I. 2010. *Microbial Community Analysis EcoPlate™ - Biolog, Inc* [Online]. Hayward. [Accessed].

BISH, D. L. 2003. Stability of hydrous minerals on the Martian surface. *Icarus,* 164**,** 96.

BISH, D. L., CAREY, J. W. & FIALIPS, C. 2003. Water-bearing minerals on Mars: source of observed mid-latitude water? *Lunar and Planetary Science Conference.* Houston Texas.

BISHOP, J., MURAD, E., LANE, M. & MANCINELLI, R. 2004. Multiple techniques for mineral identification on Mars: a study of hydrothermal rocks as potential analogues for astrobiology sites on Mars. *Icarus,* 169**,** 311-323.

BISHOP, J. L. 2005. Hydrated Minerals on Mars. *In:* TOKANO, T. (ed.) *Water on Mars and Life.* Berlin Heidelberg: Springer.

BISHOP, J. L., MCKEOWN, N. K., NOE DOBREA, E. Z., EHLMANN, B. L., MICHALSKI, J. R., MILLIKEN, R. E., POULET, F., MUSTARD, J. F., SWAYZE, G., MURCHIE, S. L., BIBRING, J.-P. & TEAM, C. Year. Phyllosilicate Diversity Observed by CRISM in Mawrth Vallis: Identification of Nontronite, Montmorillonite, Kaolinite, and Hydrated Silica. *In:* Lunar and Planetary Institute Conference Abstracts, March 1, 2008 2008. 2124.

BISHOP, J. L. & MURAD, E. 2005. The visible and infrared spectral properties of jarosite and alunite. *American Mineralogist,* 90**,** 1100-1107.

BOLDAREVA, E. N., AKIMOV, V. N., BOYCHENKO, V. A., STADNICHUK, I. N. & MOSKALENKO, A. A. 2008. Rhodobaca barguzinensis sp nov., a new alkaliphilic purple nonsulfur bacterium isolated from a soda lake of the Barguzin Valley (Buryat Republic, Eastern Siberia). *Microbiology,* 77**,** 206-218.

BOLTYANSKAYA, Y., ANTIPOV, A., KOLGANOVA, T., LYSENKO, A., KOSTRIKINA, N. & ZHILINA, T. 2004. Halomonas campisalis, an obligatorily alkaliphilic, nitrous oxide-reducing denitrifier with a molybdenum cofactor-lacking nitrate reductase. *Microbiology,* 73**,** 271-278.

BONELLI, R., FREZZOTTI, M.L.,. 2003. *Raman Spectroscopy* [Online]. [Accessed].

BOOTH, I. R. 1985. Regulation of Cytoplasmic pH in Bacteria. *Microbiological Reviews,* 49**,** 359-37.

BORSODI, A. K., MÁRIALIGETI, K., SZABÓ, G., PALATINSZKY, M., POLLÁK, B., KÉKI, Z., KOVÁCS, A. L., SCHUMANN, P. & TÓTH, E. M. 2008. *Bacillus* aurantiacus sp. nov., an alkaliphilic and moderately halophilic bacterium isolated from Hungarian soda lakes. *International Journal of Systematic and Evolutionary Microbiology,* 58**,** 845-851.

BOSSIO, D. & SCOW, K. 1995. Impact of Carbon and Flooding on the Metabolic Diversity of Microbial Communities in Soils. *Applied and Environmental Microbiology,* 61**,** 4043-4050.

BOSTON, P., TODD, P., VAN DE CAMP, J., NORTHUP, D. & SPILDE, M. 2009. Mars simulation challenge experiments: microorganisms from natural rock and cave communities. *American Society for Gravitational and Space Biology,* 22**,** 39.

BOWEN, B. B., BENISON, K. C., OBOH-IKUENOBE, F. E., STORY, S. & MORMILE, M. R. 2008. Active hematite concretion formation in modern acid saline lake sediments, Lake Brown, Western Australia. *Earth and Planetary Science Letters,* 268**,** 52-63.

BOYNTON, W. V., MING, D. W., KOUNAVES, S. P., YOUNG, S. M. M., ARVIDSON, R. E., HECHT, M. H., HOFFMAN, J., NILES, P. B., HAMARA, D. K., QUINN, R. C., SMITH, P. H., SUTTER, B., CATLING, D. C. & MORRIS, R. V. 2009. Evidence for Calcium Carbonate at the Mars Phoenix Landing Site. *Science,* 325**,** 61-64.

BOYTON, W. V., MING, D.W., KOUNAVES, S.P., YOUNG, S.M.M., ARVIDSON, R.E., HECHT, M.H., HOFFMAN, J., NILES, P.B., HAMARA, D.K., QUINN, R.C., SMITH, P.H., SUTTER, B., CATLING, D,C., MORRIS, R.V., 2009. Evidence for calcium carbonate at the Mars Pheonix Landing Site. *Science,* 325**,** 60-64.

BRAZELTON, W. J., SCHRENK, M. O., KELLEY, D. S. & BAROSS, J. A. 2006. Methane- and Sulfur-Metabolizing Microbial Communities Dominate the Lost City Hydrothermal Field Ecosystem. *Applied and Environmental Microbiology,* 72**,** 6257-6270.

BRETTAR, I., CHRISTEN, R. & HOFLE, M. 2003. *Idiomarina* baltica sp nov., a marine bacterium with a high optimum growth temperature isolated from surface water of the central Baltic Sea. *International Journal of Systematic and Evolutionary Microbiology,* 53**,** 407-413.

BRETTAR, I., CHRISTEN, R. & HOFLE, M. G. 2004. Aquiflexum balticum gen. nov., sp. nov., a novel marine bacterium of the Cytophaga-Flavobacterium-Bacteroides group isolated from surface water of the central Baltic Sea. *International Journal of Systematic and Evolutionary Microbiology,* 54**,** 2335-2341.

BRIDGE, J. S. 2008. *Earth Surface Processes, Landforms and Sediment Deposits*.

BRIDGES, J. & GRADY, M. 1999. A halite-siderite-anhydrite-chlarapatite assemblage in Nakhla: Mineralogical evidence for evaporites on Mars. *Meteoritics,* 34**,** 407-415.

BROCK, T. D. & FREEZE, H. 1969. Thermus aquaticus gen. n. and sp. n., a Nonsporulating Extreme Thermophile. *Journal of Bacteriology,* 98**,** 289-297.

BROWN, R. H. 2006. Composition and physical properties of Enceladus' surface. *Science,* 311**,** 1425.

BRYANTSEVA, I., GORLENKO, V. M., KOMPANTSEVA, E. I., IMHOFF, J. F., SULING, J. & MITYUSHINA, L. 1999. Thiorhodospira sibirica gen. nov., sp. nov., a new alkaliphilic purple sulfur bacterium from a Siberian soda lake. *Int J Syst Bacteriol,* 49**,** 697-703.

BURG, M., KWON, E. & KULTZ, D. 1996. Osmotic regulation of gene expression. *The FASEB Journal,* 10**,** 1598-1606.

BURNS, D. G., JANSSEN, P. H., ITOH, T., KAMEKURA, M., LI, Z., JENSEN, G., RODRÃ-GUEZ-VALERA, F., BOLHUIS, H. & DYALL-SMITH, M. L. 2007. Haloquadratum walsbyi gen. nov., sp. nov., the square haloarchaeon of Walsby, isolated from saltern crystallizers in Australia and Spain. *International Journal of Systematic and Evolutionary Microbiology,* 57**,** 387-392.

CADET, J. & DOUKI, T. 2010. Molecular effects of UV and ionizng radiations on DNA. *In:* GARGAUD, M., LÓPEZ-GARCÌA, P. & MARTIN, H. (eds.) *Origins and evolution of life an astrobiological perspective.* Cambridge; New York: Cambridge University Press.

CAESAR-TONTHAT, T. C., CAESAR, A. J., GASKIN, J., F.,, SAINJU, U. M. & STEVENS, W. B. 2008. Ecology and function of culturable microbes in soil aggregation. *In:* LIU, T.-X. (ed.) *Soil ecology research developments.* New York: Nova Science Publishers.

CALVIN, W. M., ROACH, L. H., SEELOS, F. P., SEELOS, K. D. & GREEN, R. O. 2009. Compact Reconnaissance Imaging Spectrometer for Mars observations of northern Martian latitudes in summer. *Journal of geophysical research,* 114.

CARBONNEAU, M. A., MELIN, A. M., PERROMAT, A. & CLERC, M. 1989. The action of free radicals on Deinococcus radiodurans carotenoids. *Archives of Biochemistry and Biophysics,* 275**,** 244-251.

CARR, M. H. 1998. Evidence for a subsurface ocean on Europa. *Nature,* 391**,** 363.

CARR, M. H. 2006. *The surface of Mars,* Cambridge [u.a.], Cambridge Univ. Press.

CAVICCHIOLI, R. 2002. Extremophiles and the search for extraterrestrial life. *Astrobiology,* 3**,** 281-292.

CHADWICK, D. 1999. *Novartis Foundation Symposium 221 - Bacterial Responses to pH*.

CHAIBENJAWONG, P. 2011. Desiccation tolerance in Staphylococcus aureus. *Archives of Microbiology,* 193**,** 125.

CHAN, H.-L., GAFFNEY, P. R., WATERFIELD, M. D., ANDERLE, H., PETER MATTHIESSEN, H., SCHWARZ, H.-P., TURECEK, P. L. & TIMMS, J. F. 2006. Proteomic analysis of UVC irradiation-induced damage of plasma proteins: Serum amyloid P component as a major target of photolysis. *FEBS Letters,* 580**,** 3229-3236.

CHASSEFIERE, E. & LEBLANC, F. 2011. Methane release and the carbon cycle on Mars. *Planetary and space science,* 59**,** 207-217.

CHATTOPADHYAY, M. K. 2006. Mechanism of bacterial adaptation to low temperature. *Journal of Biosciences,* 31**,** 157-165.

CHELA-FLORES, J. 2010. From the Moon to the Moons: Encedalus and Europa. The Search for Life and Reliable Biomarkers. *Journal of Cosmology,* 5**,** 971-981.

CHELA FLORES, J. 2010. Instrumentation for the search for habitable ecosystems in the future exploration of Europa and Ganymede. *International Journal of Astrobiology,* 9**,** 101-108.

CHEN, M. 1973. Survival of soil bacteria during prolonged desiccation. *Soil biology & biochemistry,* 5**,** 213.

CHOI, D. H. 2005. *Idiomarina* seosinensis sp. nov., isolated from hypersaline water of a solar saltern in Korea. *International Journal of Systematic and Evolutionary Microbiology,* 55**,** 379.

CHOI, J.-H., IM, W.-T., LIU, Q.-M., YOO, J.-S., SHIN, J.-H., RHEE, S.-K. & ROH, D.-H. 2007. *Planococcus* donghaensis sp. nov., a starch-degrading bacterium isolated from the East Sea, South Korea. *Int J Syst Evol Microbiol,* 57**,** 2645-2650.

CHOI, K. & DOBBS, F. 1999. Comparison of two kinds of Biolog microplates (GN and ECO) in their ability to distinguish among aquatic microbial communities. *Journal of microbiological methods,* 36**,** 203-213.

CHRISTNER, B. C., MOSLEY-THOMPSON, E., THOMPSON, L. G. & REEVE, J. N. 2001. Isolation of bacteria and 16S rDNAs from Lake Vostok accretion ice. *Environmental Microbiology,* 3**,** 570-577.

CLARK, R. N., SWAYZE, G. A., WISE, R., LIVO, K. E., HOEFEN, T. M., KOKALY, R. F. & SUTLEY, S. J. 2007. USGS Digital Spectral Library splib06a *In:* U.S. GEOLOGICAL SURVEY (ed.) *Data Series 231.*

CLARRIDGE, J. 2004. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical microbiology reviews,* 17**,** 840.

CLOUTIS, E. A. 2002. Spectral reflectance properties of zeolites and remote sensing implications. *Journal of geophysical research,* 107**,** 5067.

CLOUTIS, E. A. 2006. Detection and discrimination of sulfate minerals using reflectance spectroscopy. *Icarus,* 184**,** 121.

COCKELL, C. 2011. Vacant habitats in the Universe. *Trends in ecology & evolution,* 26**,** 73-80.

COCKELL, C. & RAVEN, J. 2004. Zones of photosynthetic potential on Mars and the early Earth. *Icarus,* 169**,** 300-310.

COCKELL, C. S. 2005. Effects of a simulated martian UV flux on the cyanobacterium, Chroococcidiopsis sp. 029. *Astrobiology,* 5**,** 127.

COCKELL, C. S., BALME, M., BRIDGES, J. C., DAVILA, A. F. & SCHWENZER, S. P. 2012. Uninhabited habitats on Mars. *Icarus,* 217**,** 184-193.

COURRÈGES-LACOSTE, G. B., AHLERS, B. & PÉREZ, F. R. 2007. Combined Raman spectrometer/laser-induced breakdown spectrometer for the next ESA mission to Mars. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* 68**,** 1023-1028.

COUSINS, C. R. 2010. *An Astrobiological Study of High Latitude Martian Analogue Environments.* PhD, University College London

COUSINS, C. R., GRIFFITHS, A. D., CRAWFORD, I., A., , PROSSER, B. J., STORRIE-LOMADI, M. C., DAVIS, L. E., GUNN, M., COATES., A., J., , JONES, A. P. & WARD, J. M. 2010. Astrobiological considerations for the selection of the geological filters on the exomars PanCam instrument. *Astrobiology,* 10**,** 933-951.

COX, M. M. & BATTISTA, J. R. 2005. Deinococcus radiodurans - The consummate survivor. *Nature Reviews Microbiology,* 3**,** 882-892.

CRADDOCK, R. A. & HOWARD, A. D. 2002. The case for rainfall on a warm, wet early Mars. *J. Geophys. Res.,* 107**,** 5111.

CROWLEY, J. K. 1991. Visible and near-infrared (0.4-2.5 microns) reflectance spectra of playa evaporite minerals. *Journal of geophysical research,* 96**,** 16231.

CURRAN, H. & EVANS, F. 1945. Heat Activation Inducing Germination In The Spores Of Thermotolerant And Thermophilic Aerobic Bacteria. *Journal of bacteriology,* 49**,** 335-346.

CYTRYN, E. J., SANGURDEKAR, D. P., STREETER, J. G., FRANCK, W. L., CHANG, W.-S., STACEY, G., EMERICH, D. W., JOSHI, T., XU, D. & SADOWSKY, M. J. 2007. Transcriptional and Physiological Responses of Bradyrhizobium japonicum to Desiccation-Induced Stress. *Journal of Bacteriology,* 189**,** 6751-6762.

DA COSTA, M., SANTOS, H. & GALINSKI, E. 1998. An overview of the role and diversity of compatible solutes in *Bacteria* and *Archaea* Biotechnology of Extremophiles. *In:* ANTRANIKIAN, G. (ed.). Springer Berlin / Heidelberg.

DALLAS, S. S. 1997. *Mars global surveyor mission*

*1997 IEEE Aerospace Conference*.

DALY, M. J. 2009. A new perspective on radiation resistance based on *Deinococcus radiodurans*. *Nature Reviews Microbiology,* 7**,** 237-245.

DALY, M. J. 2011. Death by protein damage in irradiated cells. *DNA Repair*.

DALY, M. J., GAIDAMAKOVA, E. K., MATROSOVA, V. Y., VASILENKO, A., ZHAI, M., LEAPMAN, R. D., LAI, B., RAVEL, B., LI, S.-M. W., KEMNER, K. M. & FREDRICKSON, J. K. 2007. Protein Oxidation Implicated as the Primary Determinant of Bacterial Radioresistance. *PLoS Biol,* 5**,** e92.

DALY, M. J., GAIDAMAKOVA, E. K., MATROSOVA, V. Y., VASILENKO, A., ZHAI, M., VENKATESWARAN, A., HESS, M., OMELCHENKO, M. V., KOSTANDARITHES, H. M., MAKAROVA, K. S., WACKETT, L. P., FREDRICKSON, J. K. & GHOSAL, D. 2004. Accumulation of Mn(II) in Deinococcus radiodurans Facilitates Gamma-Radiation Resistance. *Science,* 306**,** 1025-1028.

DARLING, W. G., GIZAW, B. & ARUSEI, M. K. 1996. Lake-groundwater relationships and fluid-rock interaction in the East African Rift Valley: isotopic evidence. *Journal of African Earth Sciences,* 22**,** 423-431.

DARMODY, R. G., THORN, C. E. & DIXON, J. C. 2007. Pyrite-enhanced chemical weathering in Karkevagge, Swedish Lapland. *Geological Society of America bulletin,* 119**,** 1477-1485.

DARTNELL, L. R., DESORGHER, L., WARD, J. M. & COATES, A. J. 2007a. Martian sub-surface ionising radiation: biosignatures and geology. *Biogeosciences,* 4**,** 545-558.

DARTNELL, L. R., DESORGHER, L., WARD, J. M. & COATES, A. J. 2007b. Modelling the surface and subsurface Martian radiation environment: Implications for astrobiology. *Geophysical Research Letters,* 34.

DARTNELL, L. R., FALLAIZE, D. R., WHITING, S. J. & WARD, J. M. 2010. Desiccation resistance of Antarctic Dry Valley bacteria isolated from contrasting locations. *Antarctic Science,* 22**,** 171-172.

DASSARMA, S. 2006. Extreme Halophiles Are Models for Astrobiology. *Microbe-American Society for Microbiology,* 1**,** 120-126.

DE CASTRO, A. G., BREDHOLT, H., STROM, A. R. & TUNNACLIFFE, A. 2000. Anhydrobiotic engineering of gram-negative bacteria. *Applied and Environmental Microbiology,* 66**,** 4142-4144.

DE LAAT, W. L., JASPERS, G. J. & HOEIJMAKERS, J. H. J. 1999. Molecular mechanism of nucleotide excision repair. *Genes & development,* 13**,** 768.

DECKERT, G., WARREN, P. V., GAASTERLAND, T., YOUNG, W. G., LENOX, A. L., GRAHAM, D. E., OVERBEEK, R., SNEAD, M. A., KELLER, M., AUJAY, M., HUBER, R., FELDMAN, R. A., SHORT, J. M., OLSEN, G. J. & SWANSON, R. V. 1998. The complete genome of the hyperthermophilic bacterium Aquifex aeolicus. *Nature,* 392**,** 353-358.

DEL MORAL, A., SEVERIN, J., RAMOSCORMENZANA, A., TRUPER, H. G. & GALINSKI, E. A. 1994. Compatible solutes in new moderately halophilic isolates. *FEMS microbiology letters,* 122**,** 165-172.

DELONG, C. F. 1992. Archaea in coastal marine environments. *Proceedings of the National Academy of Sciences of the United States of America,* 89**,** 5685.

DELONG, E. F. 2009. The microbial ocean from genomes to biomes. *Nature,* 459**,** 200-206.

DESANTIS, T. Z., HUGENHOLTZ, P., KELLER, K., BRODIE, E. L., LARSEN, N., PICENO, Y. M., PHAN, R. & ANDERSEN, G. L. 2006. NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes. *Nucleic Acids Research,* 34**,** 394-399.

DESHMUKH, K., PATHAK, A. & KARUPPAYIL, M. 2011. Bacterial Diversity of Lonar Soda Lake of India. *Indian Journal of Microbiology,* 51**,** 107-111.

DIAZ, B. & SCHULZE-MAKUCH, D. 2006. Microbial Survival Rates of Escherichia coli and Deinococcus radiodurans Under Low Temperature, Low Pressure, and UV-Irradiation Conditions, and Their Relevance to Possible Martian Life. *Astrobiology,* 6**,** 332-347.

DIRUGGIERO, J., BROWN, J. R., BOGERT, A. P. & ROBB, F. T. 1999. DNA Repair Systems in Archaea: Mementos from the Last Universal Common Ancestor? *Journal of molecular evolution,* 49**,** 474-484.

DONACHIE, S. P., HOU, S., GREGORY, T. S., MALAHOFF, A. & ALAM, M. 2003. *Idiomarina* loihiensis sp. nov., a halophilic {gamma}-Proteobacterium from the Lo'ihi submarine volcano, Hawai'i. *International Journal of Systematic and Evolutionary Microbiology,* 53**,** 1873-1879.

DOWNS, R. T. 2006. The RRUFF Project: an integrated study of the chemistry, crystallography, Raman and infrared spectroscopy of minerals. *Program and Abstracts of the 19th General Meeting of the International Mineralogical Association.* Kobe, Japan.

DRAKE, N. 1995. Refelctance spectra of evaportie minerals (400-2500nm) - Applications for remote-sensing. *International Journal of remote sensing,* 16**,** 2555-2571.

DUBININ, A., GRASIMENKOL., ZAVARZIN, G., 1995. Ecophysiology and species diversity of cyanobacteria from Lake Magadi. *Microbiology,* 64**,** 717-721.

DUCKWORTH, A. W., GRANT, W. D., JONES, B. E. & STEENBERGEN, R. 1996. Phylogenetic diversity of soda lake alkaliphiles. *FEMS Microbiology Ecology,* 19**,** 181-191.

DUMMER, A. M., BONSALL, J. C., CIHLA, J. B., LAWRY, S. M., JOHNSON, G. C. & PECK, R. F. 2011. Bacterioopsin-Mediated Regulation of Bacterioruberin Biosynthesis in Halobacterium salinarum. *Journal of Bacteriology,* 193**,** 5658-5667.

DUTRIZAC, J. E. & JAMBOR, J. L. 2000. Jarosites and their application in hydrometallurgy. *In:* ALPERS, C. N., JAMBOR, J. L. & NORDSTROM, D. K. (eds.) *Sulfate Minerals: Crystallography, Geochemistry and Environmental Significance.* Washington D.C.: Mineralogical society of America.

DYAR, M. D., CARMOSINO, M. L., TUCKER, J. M., BROWN, E. A., CLEGG, S. M., WIENS, R. C., BAREFIELD, J. E., DELANEY, J. S., ASHLEY, G. M. & DRIESE, S. G. 2012. Remote laser-induced breakdown spectroscopy analysis of East African Rift sedimentary samples under Mars conditions. *Chemical Geology,* 294–295**,** 135-151.

EDWARDS, C. D., BARBIERI, A., BROWER, E., ESTABROOK, P., GIBBS, R., HORTTOR, R., LUDWINSKI, J., MASE, R., THEISINGER, P., THORPE, T., WAGGONER, B., MCCARTHY, C. & SCHMIDT, R. Year. A Martian Telecommunications Network: UHF Relay Support of the Mars Exploration Rovers by the Mars Global Surveyor, Mars Odyssey, and Mars Express Orbiters. *In:* International Astronautical Congress, 2004 Vancouver, British Columbia.

EDWARDS, H. G. M. 2006. Life in the sabkha: Raman spectroscopy of halotrophic extremophiles of relevance to planetary exploration. *Analytical and Bioanalytical Chemistry,* 385**,** 46.

EDWARDS, H. G. M., SADOONI, F., VITEK, P. & JEHLICKA, J. 2010. Raman spectroscopy of the Dukhan sabkha: identification of geological and biogeological molecules in an extreme environment. *Philosophical transactions - Royal Society. Mathematical, Physical and engineering sciences,* 368**,** 3099-3107.

EHLING-SCHULZ, M., BILGER, W. & SCHERER, S. 1997. UV-B-induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium Nostoc commune. *Journal of Bacteriology,* 179**,** 1940-5.

EHLMANN, B., MUSTARD, J. F., SAWYZE, G. A., CLARK, R. N., BISHOP, J. L., POULET, F., DES MARAIS, D. J., ROACH, L. H., MILLIKEN, R. E., WRAY, J. J., BARNOUIN-JHA, O. & MURCHIE., S. L. 2009. Identification of hydrated silicate minerals on Mars using MRO-CRISM: Geologic context near Nili Fossae and implications for aqueous alteration. *Journal of geophysical research,* 114.

EHLMANN, B. L., MUSTARD, J. F. & BISH, D. L. 2010a. Weathering and hydrothermal alteration of basalts in iceland: mineralogy from vnir, tir, xrd, and implications for linking mars orbital and surface datasets. *41st Lunar and Planetary Science Conference* The Woodlands Texas.

EHLMANN, B. L., MUSTARD, J. F. & BISH, D. L. 2011a. Aqueous alteration of basaltic lavas in Iceland: an analogue for Noachian Mars. *Analogue sites for Mars Missions: MSL and beyond.* The Woodlands, Texas.

EHLMANN, B. L., MUSTARD, J. F., FASSETT, C. I., SCHON, S. C., HEAD III, J. W., MARAIS, D. J. D., GRANT, J. A. & MURCHIE, S. L. 2008a. Clay minerals in delta deposits and organic preservation potential on Mars. *Nature Geoscience,* 1**,** 355-358.

EHLMANN, B. L., MUSTARD, J. F., FASSETT, C. I., SCHON, S.C,, HEAD III, J. W., MARAIS, D. J., GRANT, J. A. & MURCHIE, S. L. 2008b. Orbital Identification of Carbonate-Bearing Rocks on Mars. *Science,* 322**,** 1828.

EHLMANN, B. L., MUSTARD, J. F. & MURCHIE, S. L. 2010b. Geological setting of serpentine deposits on Mars. *41st Lunar and Planetary Science COnference.* Woodlands Texas.

EHLMANN, B. L., MUSTARD, J. F., MURCHIE, S. L., BIBRING, J.-P., MEUNIER, A., FRAEMAN, A. A. & LANGEVIN, Y. 2011b. Subsurface water and clay mineral formation during the early history of Mars. *Nature,* 479**,** 53-60.

EHLMANN, B. L., MUSTARD, J. F., SWAYZE, G. A., WRAY, R. N., BARNOUIN-JHA, J. J., BISHOP, J. J., POULET, F., DES MARAIS, D. J., ROACH, L. H., MILLIKEN, R. E., CLARK, J. L. & MURCHIE. S.L. 2008c. Phyllosilicates, zeolites, and carbonate Near Nili Foassae, Mars: Evidence for distinct environments of aqueous alteration *Martian Phyllosilicates: Recorders of Aqueous Processes*

ELLERY, A., WYNN-WILLIAMS, D., PARNELL, J., EDWARDS, H. G. M. & DICKENSHEETS, D. 2004. The role of Raman spectroscopy as an astrobiological tool in the exploration of Mars. *Journal of Raman Spectroscopy,* 35**,** 441-457.

ELWOOD MADDEN, M. E., BODNAR, R. J. & RIMSTIDT, J. D. 2004. Jarosite as an indicator of water-limited chemical weathering on Mars. *Nature,* 431**,** 821-823.

ENGELHARDT, M. A., DALY, K., SWANNELL, R. P. J. & HEAD, I. M. 2001. Isolation and characterization of a novel hydrocarbon-degrading, Gram-positive bacterium, isolated from intertidal beach sediment, and description of *Planococcus* alkanoclasticus sp. nov. *Journal of Applied Microbiology,* 90**,** 237-247.

ENGLISH, P. 2001. Formation of analcime and moganite at Lake Lewis, central Australia: significance of groundwater evolution in diagenesis. *Sedimentary Geology,* 143**,** 219-244.

EUGSTER, H. 1980. Geochemistry of evaporitic lacustrine deposists. *Annual review of Earth and Planetary Science,* 8**,** 35-63.

EUGSTER, H. P. 1967. Hydrous Sodium Silicates from Lake Magadi, Kenya: Precursors of Bedded Chert. *Science,* 157**,** 1177-1180.

EUGSTER, H. P. 1986. Lake Magadi, Kenya; a model for rift valley hydrochemistry and sedimentation? *Geological Society Special Publications,* 25**,** 177-189.

EUGSTER, H. P. & JONES, B. F. 1968. Gels Composed of Sodium-Aluminum Silicate, Lake Magadi, Kenya. *Science,* 161**,** 160-163.

FAIREN, A., CHEVRIER, V., ABRAMOV, O., MARZO, G. & GAVIN, P. 2010. Noachian and more recent phyllosilicates in impact craters on Mars. *Proceedings of the National Academy of Sciences of the United States of America,* 107**,** 12095-12100.

FAIRÉN, A. G., DAVILA, A. F., LIM, D., BRAMALL, N., BONACCORSI, R., ZAVALETA, J., UCEDA, E. R., STOKER, C., WIERZCHOS, J., DOHM, J. M., AMILS, R., ANDERSEN, D. & MCKAY, C. P. 2010. Astrobiology through the Ages of Mars: The Study of Terrestrial Analogues to Understand the Habitability of Mars. *Astrobiology,* 10**,** 821-843.

FALL, A. 2005. *Fluid evolution in the nepheline syenites of the DitrÄƒu Alkaline Massif Transylvania Romania.* Master of Science, Virginia polytechnic institute and state Univeristy.

FARRAND, W., GLOTCH, T., RICE, J., HUROWITZ, J. & SWAYZE, G. 2009. Discovery of jarosite within the Mawrth Vallis region of Mars: Implications for the geologic history of the region. *Icarus,* 204**,** 478-488.

FARRAND, W. H. 2008. Rock spectral classes observed by the Spirit Rover's Pancam on the Gusev Crater Plains and in the Columbia Hills. *Journal of Geophysical Research,* 113**,** E12S38.

FASSETT, C. I. & HEAD, J. W. 2005. Fluvial sedimentary deposits on Mars: Ancient deltas in a crater lake in the Nili Fossae region. *Geophysical Research Letters,* 32.

FASSETT, C. I. & HEAD, J. W. 2008. Valley network-fed, open-basin lakes on Mars: Distribution and implications for Noachian surface and subsurface hydrology. *Icarus,* 198**,** 37.

FELDMAN, W. C., BOYNTON, W. V., TOKAR, R. L., PRETTYMAN, T. H., GASNAULT, O., SQUYRES, S. W., ELPHIC, R. C., LAWRENCE, D. J., LAWSON, S. L., MAURICE, S., MCKINNEY, G. W., MOORE, K. R. & REEDY, R. C. 2002. Global Distribution of Neutrons from Mars: Results from Mars Odyssey. *Science,* 297**,** 75-78.

FENDRIHAN, S., BERCES, A., LAMMER, H., MUSSO, M., RONTO, G. & POLACSEK, T. 2009. Investigating the Effects of Simulated Martian Ultraviolet Radiation on Halococcus dombrowskii and Other Extremely Halophilic Archaebacteria. *Astrobiology,* 9**,** 104-112.

FERNÁNDEZ-REMOLAR, D. C., MORRIS, R. V., GRUENER, J. E., AMILS, R. & KNOLL, A. H. 2005. The Río Tinto Basin, Spain: Mineralogy, sedimentary geobiology, and implications for interpretation of outcrop rocks at Meridiani Planum, Mars. *Earth and Planetary Science Letters,* 240**,** 149-167.

FERNANDEZ REMOLAR, D. & KNOLL, A. 2008. Fossilization potential of iron-bearing minerals in acidic environments of Rio Tinto, Spain: Implications for Mars exploration. *Icarus,* 194**,** 72-85.

FETZER, S., BAK, F. & CONRAD, R. 1993. Sensitivity of methanogenic bacteria from paddy soil to oxygen and desiccation. *FEMS Microbiology Ecology,* 12**,** 107-115.

FIALIPS, C. I. 2005. Hydration state of zeolites, clays, and hydrated salts under present-day martian surface conditions: Can hydrous minerals account for Mars Odyssey observations of near-equatorial water-equivalent hydrogen? *Icarus,* 178**,** 74.

FISH, S., SHEPHERD, T., MCGENITY, T. & GRANT, W. 2002. Recovery of 16S ribosomal RNA gene fragments from ancient halite. *Nature,* 417**,** 432-436.

FLEMING, E. D. & CASTENHOLZ, R. W. 2007. Effects of periodic desiccation on the synthesis of the UV-screening compound, scytonemin, in cyanobacteria. *Environmental Microbiology,* 9**,** 1448-1455.

FORSYTHE, R. D. & ZIMBELMAN, J. R. 1995. A case for ancient evaporite basins on Mars. *Journal of Geophysical Research,* 100**,** 5553-5563.

FOX, G. E. 1992. How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. *International journal of systematic bacteriology,* 42**,** 166.

FRANZMANN, P. D., BURTON, H. R. & MCMEEKIN, T. A. 1987. Halomonas subglaciescola, a New Species of Halotolerant Bacteria Isolated from Antarctica. *International journal of systematic bacteriology,* 37**,** 27-34.

FREDRICKSON, J. K. 2008. Protein oxidation: key to bacterial desiccation resistance? *The ISME journal,* 2**,** 393.

FYNAN, E. F., HUNT, M. R., DOWLING, P., AUGUSTE, J., SMYTH, A. P. & BRADLEY, P. M. 2009. Natural Antibiotic Resistance of Bacteria Isolated from a Peat Bog at Poutwater Pond, Holden, Massachusetts. *BIOS,* 80**,** 9-13.

GAFFEY, S. 1985. Reflectance spectroscopy in the visible and near-infrared (0.35-2.55 mu-m) - applications in carbonate petrology. *Geology,* 13**,** 270-273.

GARCIA-VALLVÉ, S., ROMEU, A. & PALAU, J. 2000. Horizontal Gene Transfer in Bacterial and Archaeal Complete Genomes. *Genome Research,* 10**,** 1719-1725.

GARLAND, J. 1997. Analysis and interpretation of community-level physiological profiles in microbial ecology. *FEMS Microbiology Ecology,* 24**,** 289-300.

GARLAND, J. L. & MILLS, A. L. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization

*Applied and Environmental Microbiology,* 57**,** 2351-2359.

GENDRIN, A., MANGOLD, N., BIBRING, J.-P., LANGEVIN, Y., GONDET, B., POULET, F. O., BONELLO, G., QUANTIN, C., MUSTARD, J., ARVIDSON, R. & LEMOUÃ©LIC, S. P. 2005. Sulfates in Martian Layered Terrains: The OMEGA/Mars Express View. *Science,* 307**,** 1587-1591.

GÉRARD, E., JOLIVET, E., PRIEUR, D. & FORTERRE, P. 2001. DNA protection mechanisms are not involved in the radioresistance of the hyperthermophilic archaea Pyrococcus abyssi and P. furiosus. *Molecular Genetics and Genomics,* 266**,** 72-78.

GETACHEW, M., AMBELU, A., TIKU, S., LEGESSE, W., ADUGNA, A. & KLOOS, H. 2012. Ecological assessment of Cheffa Wetland in the Borkena Valley, northeast Ethiopia: Macroinvertebrate and bird communities. *Ecological Indicators,* 15**,** 63-71.

GHOSH, S., OSMAN, S., VAISHAMPAYAN, P. & VENKATESWARAN, K. 2010. Recurrent Isolation of Extremotolerant Bacteria from the Clean Room Where Phoenix Spacecraft Components Were Assembled. *Astrobiology,* 10**,** 325-335.

GILBERT, J. A., HILL, P. J., DODD, C. E. R. & LAYBOURN-PARRY, J. 2004. Demonstration of antifreeze protein activity in Antarctic lake bacteria. *Microbiology,* 150**,** 171-180.

GOLOMBEK, M. P. 1997. Overview of the Mars Pathfinder mission and assessment of landing site predictions. *Science,* 278**,** 1743.

GOMEZ, E., FERRERAS, L. & TORESANI, S. 2006. Soil bacterial functional diversity as influenced by organic amendment application. *Bioresource technology,* 97**,** 1484-1489.

GONTANG, E. A., FENICAL, W. & JENSEN, P. R. 2007. Phylogenetic Diversity of Gram-Positive Bacteria Cultured from Marine Sediments. *Applied and environmental microbiology,* 73**,** 3272-3282.

GOOGLE-EARTH. 2011. *Lake Magadi 1deg 54'17.28"S, 36deg 16'42.46"E*.

GOTTARDI, G. 1985. *Natural zeolites*.

GRANT, J. A., SOROKIN, D.Y., 2010. Distribution and diveristy of soda Lake alkaliphiles. *In:* HORIKOSHI, K. (ed.) *Extremophiles handbook.* Tokyo; New York: Springer.

GRANT, S., GRANT, W. D., JONES, B. E., KATO, C. & LI, L. 1999. Novel archaeal phylotypes from an East African alkaline saltern. *Extremophiles,* 3**,** 139-145.

GRANT, W. D. 2006. Alkaline environments and biodiversity. *In:* GERDAY, C., AND GLANSDORFF, N., (ed.) *Encyclopedia of Life support systems.* Oxford: Eoloss publisher.

GRANT, W. D., ROSS, H.N.M. 1986. The ecology and taxonomy of halobacteria. *FEMS Microbiology Letters,* 39**,** 9-15.

GRASBY, S. E. & LONDRY, K. L. Year. Biogeochemistry of hypersaline springs supporting a mid-continent marine ecosystem: An analogue for martian springs? *In:* Special Session on Extreme Environments held at the Geological-Society-of-America Annual Meeting, 2005 2007 Salt Lake City, UT. 662-683.

GREENBERG, R. 2002. Tidal-tectonic processes and their implications for the character of Europa's icy crust. *Reviews of geophysics,* 40**,** 1004.

GREENBERG, R. 2010. Transport rates of radiolytic substances into Europa's ocean: implications for the potential origin and maintenance of life. *Astrobiology,* 10**,** 275.

GRIFFITHS, A. D. 2005. The Beagle 2 stereo camera system. *Planetary and space science,* 53**,** 1466.

GRIFFITHS, A. D., COATES, A. J., JAUMANN, R., MICHAELIS, H., PAAR, G., BARNES, D. & JOSSET, J. 2006. Context for the ESA ExoMars rover: the Panoramic Camera (PanCam) instrument. *International Journal of Astrobiology,* 5**,** 269.

GRINSTEAD, J. & LACEY, R. W. 1973. Ecological and Genetic Implications of Pigmentation in Staphylococcus aureus. *Journal of General Microbiology,* 75**,** 259-267.

GUTMAN, P. D., FUCHS, P. & MINTON, K. W. 1994. Restoration of the DNA damage resistance of Deinococcus radiodurans DNA polymerase mutants by Escherichia coli DNA polymerase I and Klenow fragment. *Mutation Research/DNA Repair,* 314**,** 87-97.

GYGLI, P., PRAJAPATI, S., DEVEAUX, L., DASSARMA, S. & DASSARMA, P. 2009. Resistance of the Extreme Halophile Halobacterium sp NRC-1 to Multiple Stresses

APPLICATION OF ACCELERATORS IN RESEARCH AND INDUSTRY. *AIP conference proceedings,* 1099**,** 993-996.

HAGEN, C. A. 1967. Survival of microorganisms in a simulated martian environment: II. Moisture and oxygen requirements for germination of *Bacillus* cereus and *Bacillus* subtilis var. niger spores. *Applied and Environmental Microbiology,* 15**,** 285.

HAGERTY, J. J. & NEWSOM, H. E. 2003. Hydrothermal alteration at the Lonar Lake impact structure, India: Implications for impact cratering on Mars. *Meteoritics & planetary science,* 38**,** 365-381.

HALE, M. 2000. *Geochemical Remote Sensing of the Sub-Surface*.

HALL, A. 1998. Zeolitization of volcaniclastic sediments; the role of temperature and pH *ournal of sedimentary research,* 68**,** 739-745.

HALL, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic acid symposium series,* 41**,** 95-98.

HAND, K. & CHYBA, C. 2007. Empirical constraints on the salinity of the europan ocean and implications for a thin ice shell. *Icarus,* 189**,** 424-438.

HANSEN, A. A., JENSEN, L. L., KRISTOFFERSEN, T., MIKKELSEN, K., MERRISON, J., FINSTER, K. W. & LOMSTEIN, B. A. 2009. Effects of long-term simulated Martian conditions on a freeze-dried and homogenized bacterial permafrost community. *Astrobiology,* 9**,** 229-240.

HANSEN, A. A., MERRISON, J., NORNBERG, P., LOMSTEIN, A. & FINSTER, K. 2005. Activity and stability of a complex bacterial soil community under simulated martian conditions. *International journal of astrobiology,* 4**,** 135-144.

HANSEN, C. J. 2006. Enceladus' water vapor plume. *Science,* 311**,** 1422.

HARADA, Y. 2006. The dependence of surface tidal stress on the internal structure of Europa: The possibility of cracking of the icy shell. *Planetary and space science,* 54**,** 170.

HARM, W. & HARM 1968. DARK REPAIR OF PHOTOREPAIRABLE UV LESIONS IN ESCHERICHIA COLI. *Mutation research,* 6**,** 25-&.

HARRISON, A. P. 1984. The Acidophilic Thiobacilli and other Acidophilic Bacteria that Share their Habitat. *Annual Review of Microbiology,* 38**,** 265-292.

HAY, R. L. 1986. Geologcial occurence of zeolties and soem associated minerals. *Pure and applied chemsitry,* 58**,** 1339-1342.

HE, Z., GENTRY, T. J., SCHADT, C. W., WU, L., LIEBICH, J., CHONG, S. C., HUANG, Z., WU, W., GU, B., JARDINE, P., CRIDDLE, C. & ZHOU, J. 2007. GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. *ISME J,* 1**,** 67-77.

HE, Z. L., VAN-NOSTRAND, J. D., WU, L. Y. & ZHOU, J. Z. 2008. Development and application of functional gene arrays for microbial community analysis. *Transactions of Nonferrous Metals Society of China,* 18**,** 1319-1327.

HEBRAUD, M. & POTIER, P. 1999. Cold shock response and low temperature adaptation in psychrotrophic bacteria. *Journal of molecular microbiology and biotechnology,* 1**,** 211-9.

HECHT, M. H., KOUNAVES, S. P., QUINN, R. C., WEST, S. J., YOUNG, S. M. M., MING, D. W., CATLING, D. C., CLARK, B. C., BOYNTON, W. V., HOFFMAN, J., DEFLORES, L. P., GOSPODINOVA, K., KAPIT, J. & SMITH, P. H. 2009. Detection of Perchlorate and the Soluble Chemistry of Martian Soil at the Phoenix Lander Site. *Science,* 325**,** 64-67.

HELDMANN, J. L., CONLEY, C. A., BROWN, A. J., FLETCHER, L. & BISHOP, J. L. 2010. Possible liquid water origin for Atacama Desert mudflow and recent gully deposits on Mars. *Icarus,* 206**,** 685-690.

HILL, D. R., HLADUN, S. L., SCHERER, S. & POTTS, M. 1994. Water stress proteins of Nostoc commune (Cyanobacteria) are secreted with UV-A/B-absorbing pigments and associate with 1,4-beta-D-xylanxylanohydrolase activity. *Journal of Biological Chemistry,* 269**,** 7726-7734.

HOBEL, C. V. F., MARTEINSSON, V. T., HREGGVIDSSON, Ó. & KRISTJÁNSSON, J. K. 2005. Investigation of the Microbial Ecology of Intertidal Hot Springs by Using Diversity Analysis of 16S rRNA and Chitinase Genes. *Applied and Environmental Microbiology,* 71**,** 2771–2776.

HORIKOSHI, K. 1999. Alkaliphiles: Some Applications of their products for biotechnology. *Microbial and molecular biology reviews,* 63**,** 735-750,.

HORIKOSHI, K. 2006. Physiology. *Alkaliphiles.* Springer Berlin Heidelberg.

HORNECK, G. 2000. The microbial world and the case for Mars. *Planetary and space science,* 48**,** 1053-1063.

HOVORKA, S. D., HOLT, R., M. & POWERS, D. W. 2007. Permian basin evaporties. *In:* SCHREIBER, B. C., LUGLI, S., BABEL, M. & GEOLOGICAL SOCIETY OF, L. (eds.) *Evaporites through space and time.* London: Geological Society.

HUBBARD, G. S., NADERI, F. M. & GARVIN, J. B. 2003. Following the water, the new program for Mars exploration. *Acta Astronautica,* 51**,** 337-350.

HUDSON, K. D. 2001. Localization of GerAA and GerAC germination proteins in the *Bacillus* subtilis spore. *Journal of bacteriology,* 183**,** 4317.

IIJIMA, A. & UTADA, M. 1966. Zeolites in sedimentary rocks, with reference to the depositional environments and zonal distribution. *Sedimentology,* 7**,** 327.

IMSHENETSKY, A. A., KOUZYURINA, L. A. & JAKSHINA, V. M. 1973. On the multiplication of xerophilic micro-organisms under simulated Martian conditions. *Institute of Microbiology,* 11**,** 63-66.

ITOH, T., YAMAGUCHI, T., ZHOU, P. & TAKASHINA, T. 2005. *Natronolimnobius baerhuensis* gen. nov., sp. nov. and *Natronolimnobius innermongolicus* sp. nov., novel haloalkaliphilic archaea isolated from soda lakes in Inner Mongolia, China. *Extremophiles,* 9**,** 111-116.

IVANOVA, E. P. 2000. *Idiomarina* gen. nov., comprising novel indigenous deep-sea bacteria from the Pacific Ocean, including descriptions of two species, *Idiomarina* abyssalis sp. nov. and *Idiomarina* zobellii sp. nov. *International Journal of Systematic and Evolutionary Microbiology,* 50**,** 901.

JACKSON, J. A. & BATES, R. L. 1997. *Glossary of Geology,* Alexandria Virginia, American Geologic Institute.

JAGNIECKI, E. A. & BENISON, K. C. 2010. Criteria for the recognition of acid-precipitated halite. *Sedimentology,* 57**,** 273.

JAIN, R., RIVERA, M. C., MOORE, J. E. & LAKE, J. A. 2003. Horizontal Gene Transfer Accelerates Genome Innovation and Evolution. *Molecular Biology and Evolution,* 20**,** 1598-1602.

JANSSEN, P. H. 2006. Identifying the Dominant Soil Bacterial Taxa in Libraries of 16S rRNA and 16S rRNA Genes. *Applied and Environmental Microbiology,* 72**,** 1719-1728.

JENKIN, P. M. 1932. Reports of the Percy Sladen expeditions to some Rift Valley Lakes in Kenya in 1929: VII Summary of the ecological results with special reference to the alkaline lakes. *Annual Magazine of Natural Histiry SerX X,* 8**,** 133-181.

JOHNSEN, U., DAMBECK, M., ZAISS, H., FUHRER, T., SOPPA, J., SAUER, U. & SCHÖNHEIT, P. 2009. d-Xylose Degradation Pathway in the Halophilic Archaeon Haloferax volcanii. *Journal of Biological Chemistry,* 284**,** 27290-27303.

JOHNSON, A. P., PRATT, L. M., VISHNIVETSKAYA, T., PFIFFNER, S., BRYAN, R. A., DADACHOVA, E., WHYTE, L., RADTKE, K., CHAN, E., TRONICK, S., BORGONIE, G., MANCINELLI, R. L., ROTHSCHILD, L. J., ROGOFF, D. A., HORIKAWA, D. D. & ONSTOTT, T. C. 2011. Extended survival of several organisms and amino acids under simulated martian surface conditions. *Icarus,* 211**,** 1162-1178.

JONES, B. E., GRANT, W. D., DUCKWORTH, A. W. & OWENSON, G. G. 1998. Microbial diversity of soda lakes. *Extremophiles,* 2**,** 191-200.

JONES, B. E. J., GRANT, W. D., COLINS, N. C. & MWARTHA, W. E. 1994. Alkaliphiles: Diversity and identification. *In:* PRIEST, F. G., RAMOS-CORMENZANA, A., AND TINDALL, B.J., (ed.) *Bacterial diversity and systematics.* New York: Plenum Publishing Corporation.

JOSHI, A., KANEKAR, P., KELKAR, A., SHOUCHE, Y., VANI, A., BORGAVE, S. & SARNAIK, S. 2008. Cultivable Bacterial Diversity of Alkaline Lonar Lake, India. *Microbial ecology,* 55**,** 163-172.

JOSHI, A. A., KANEKAR, P. P., KELKAR, A. S., SARNAIK, S. S., SHOUCHE, Y. & WANI, A. 2007. Moderately halophilic, alkalitolerant Halomonas campisalis MCM B-365 from Lonar Lake, India. *Journal of Basic Microbiology,* 47**,** 213-221.

JUNI, E. & HWYM, G. A. 1986. Psychrobacter immobilis gen. nov., sp. nov.: Genospecies Composed of Gram-Negative, Aerobic, Oxidase-Positive Coccobacilli. *International journal of systematic bacteriology,* 36**,** 388-391.

KALLÓ, D., SHERRY, H.S., 1988. *Occurrence Properties and Utilization of Natural Zeolites*, Akademiai Kiado.

KAMEKURA, M., DYALL-SMITH, M. L., UPASANI, V., VENTOSA, A. & KATES, M. 1997. Diversity of Alkaliphilic Halobacteria: Proposals for Transfer of Natronobacterium vacuolatum, Natronobacterium magadii, and Natronobacterium pharaonis to Halorubrum, Natrialba, and Natronomonas gen. nov., Respectively, as Halorubrum vacuolatum comb. nov., Natrialba magadii comb. nov., and Natronomonas pharaonis comb. nov., Respectively. *International journal of systematic bacteriology,* 47**,** 853-857.

KANAI, H. & KANAI 1995. Natronococcus amylolyticus sp. nov., a haloalkaliphilic archaeon. *International journal of systematic bacteriology,* 45**,** 762.

KANEKAR, P. P., JOSHI, A. A., KELKAR, A. S., BORGAVE, S. B. & SARNAIK, S. S. 2008. Alkaline Lonar lake, India- A treasure of alkaliphilic and halophilic bacteria. *In:* SENGUPTA, M., AND DALWANI, R., (ed.) *Proceedings of Taal007: The 12th World Lakes Conference.* Jaipur, INDIA

KANG, Y., CHENG, J., MEI, L., HU, J., PIAO, Z. & YIN, S. 2010. Multiple copies of 16S rRNA gene affect the restriction patterns and DGGE profile revealed by analysis of genome database. *Microbiology,* 79**,** 655-662.

KARGEL, J. S., KAYE, J. Z., HEAD, J. W., MARION, G. M., SASSEN, R., CROWLEY, J. K., BALLESTEROS, O. P., GRANT, S. A. & HOGENBOOM, D. L. 2000. Europa's Crust and Ocean: Origin, Composition, and the Prospects for Life. *Icarus,* 148**,** 226-265.

KATO, C., LI, L., NOGI, Y., NAKAMURA, Y., TAMAOKA, J. & HORIKOSHI, K. 1998. Extremely Barophilic Bacteria Isolated from the Mariana Trench, Challenger Deep, at a Depth of 11,000 Meters. *Applied and Environmental Microbiology,* 64**,** 1510-1513.

KEELING, P. J. & PALMER, J. D. 2008. Horizontal gene transfer in eukaryotic evolution. *Nat Rev Genet,* 9**,** 605-618.

KEMPE, S., AND KAZMIERCZAK, J., 1997. **A terrestrial model for an alkaline martian hydrosphere**. *Planetary Space Science,* 45**,** 1493-1499.

KEMPE, S. & KAZMIERCZAK, J. 2002. Biogenesis and early life on Earth and Europa: Favored by an alkaline ocean? *Astrobiology,* 2**,** 123-130.

KENDRICK, M. G. & KRAL, T. A. 2006. Survival of Methanogens During Desiccation: Implications for Life on Mars. *Astrobiology,* 6**,** 546-551.

KIESEL, B., MÜLLER, R. H. & KLEINSTEUBER, R. 2007. Adaptative Potential of Alkaliphilic Bacteria towards Chloroaromatic Substrates Assessed by a gfp-tagged 2,4-D Degradation Plasmid. *Engineering in Life Sciences,* 7**,** 361-372.

KISAND, V. & WIKNER, J. 2003. Combining Culture-Dependent and -Independent Methodologies for Estimation of Richness of Estuarine Bacterioplankton Consuming Riverine Dissolved Organic Matter. *Applied and Environmental Microbiology,* 69**,** 3607-3616.

KIVELSON, M. G. 2000. Galileo magnetometer measurements: A stronger case for a subsurface ocean at Europa. *Science,* 289**,** 1340.

KLEIN, H. P. 1978. The Viking biological experiments on Mars. *Icarus,* 34**,** 666-674.

KLEIN, H. P., HOROWITZ, N. H., LEVIN, G. V., OYAMA, V. I., LEDERBERG, J., RICH, A., HUBBARD, J. S., HOBBY, G. L., STRAAT, P. A., BERDAHL, B. J., CARLE, G. C., BROWN, F. S., JOHNSON, R. D. & . 1976. The Viking Biological Investigation: Preliminary Results. *Science,* 194**,** 99-105.

KLOPROGGE, J. T., KOMARNENI, S. & AMONETTE, J. E. 1999. Synthesis of smectite clay minerals: A critical review. *Clays and Clay Minerals,* 47**,** 529-554.

KMINEK, G. & BADA, J. L. 2006. The effect of ionizing radiation on the preservation of amino acids on Mars. *Earth and Planetary Science Letters,* 245**,** 1-5.

KNOWLES, E. J. & CASTENHOLZ, R. W. 2008. Effect of exogenous extracellular polysaccharides on the desiccation and freezing tolerance of rock-inhabiting phototrophic microorganisms. *FEMS Microbiology Ecology,* 66**,** 261-270.

KOBAYASHI, I. & HANDA, N. 2001. DNA Double-strand Breaks and Their Consequences in Bacteria. *eLS.* John Wiley & Sons, Ltd.

KOIKE, J., HORI, T., KATAHIRA, Y., KOIKE, K. A., TANAKA, K., KOBAYASHI, K. & KAWASAKI, Y. 1996. Fundamental studies concerning planetary quarantine in space. *Advances in Space Research,* 18**,** 339-344.

KOIKE, J., OSHIMA, T., KOIKE, K. A., TAGUCHI, H., TANAKA, R., NISHIMURA, K. & MIYAJI, M. 1992. Survival rates of some terrestrial microorganisms under simulated space conditions. *Advances in Space Research,* 12**,** 271-274.

KOOISTRA, J., MITCHELL, R. & STRUGHOLD, H. 1958. The behavior of microorganisms under simulated martian environmental conditions. *Publications of the Astronomical Society of the Pacific,* 70**,** 64-69.

KOTTEMANN, M., KISH, A., ILOANUSI, C., BJORK, S. & DIRUGGIERO, J. 2005a. Physiological responses of the halophilic archaeon Halobacterium sp. strain NRC1 to desiccation and gamma irradiation. *Extremophiles,* 9**,** 219-227.

KOTTEMANN, M., KISH, A., ILOANUSI, C., BJORK, S. & DIRUGGIERO, J. 2005b. Physiological responses of the halophilic archaeon; *Halobacterium* sp. strain NRC1 to desiccation and gamma irradiation. *Extremophiles,* 9**,** 219-227.

KOWALSKI, W. J. 2009. Mathematical modeling of UV disinfection. *In:* KOWALSKI, W. J. (ed.) *Ultraviolet germicidal irradiation handbook UVGI for air and surface disinfection.* Heidelberg; New York: Springer-Verlag.

KRINSKY, N. I. 1979. Carotenoid protection against oxidation. *Pure and Applied Chemistry,* 51**,** 649-660.

KRISKO, A. & RADMAN, M. 2010. Protein damage and death by radiation in Escherichia coli and Deinococcus radiodurans. *Proceedings of the National Academy of Sciences,* 107**,** 14373-14377.

KRISTJANSSON, J. K., HREGGVIDSSON, G. O. & ALFREDSSON, G. A. 1986. Isolation of Halotolerant Thermus spp. from Submarine Hot Springs in Iceland. *Applied and Environmental Microbiology,* 52**,** 1313-1316.

LAETSCH, T. & DOWNS, R. T. Year. Software For Identification and Refinement of Cell Parameters From Powder Diffraction Data of Minerals Using the RRUFF Project and American Mineralogist Crystal Structure Databases. *In:* 19th General Meeting of the International Mineralogical Association, 2006 Kobe Japan.

LAGALY, G., BENEKE, K., 1975. Magadiite and H-Magadiite: I. Sodium Magadiite and some of its derivatives. *American Mineraologist,* 60**,** 642-649.

LAISK, A. 2000. Chlorophyll a fluorescence and 800 nm absorbance changes as tools for probing leaf photosynthesis. *Bulletin de la Societe Royale des Sciences de Liege* 69.

LANE, N. 2010. Chance or Necessity? Bioenergetics and the Probability of Life. *Journal of Cosmology,* 10**,** 3286-3304.

LANGEVIN, Y., POULET, F., BIBRING, J.-P., SCHMITT, B., DOUTÃ©, S. & GONDET, B. 2005. Summer Evolution of the North Polar Cap of Mars as Observed by OMEGA/Mars Express. *Science,* 307**,** 1581-1584.

LANZA, N. L., MEYER, G. A., OKUBO, C. H., NEWSOM, H. E. & WIENS, R. C. 2010. Evidence for debris flow gully formation initiated by shallow subsurface water on Mars. *Icarus,* 205**,** 103-112.

LEFÈVRE, C. T., FRANKEL, R. B., PÓSFAI, M., PROZOROV, T. & BAZYLINSKI, D. A. 2011. Isolation of obligately alkaliphilic magnetotactic bacteria from extremely alkaline environments. *Environmental Microbiology,* 13**,** 2342-2350.

LEHMANN, K., CROMBIE, A. & SINGER, A. C. 2008. Reproducibility of a microbial river water community to self-organize upon perturbation with the natural chemical enantiomers, R-and S-carvone. *FEMS Microbiology Ecology,* 66**,** 208.

LEONE, A. P. & SOMMER, S. 2000. Multivariate Analysis of Laboratory Spectra for the Assessment of Soil Development and Soil Degradation in the Southern Apennines (Italy). *Remote Sensing of Environment,* 72**,** 346-359.

LEUKO, S., NEILAN, B. A., BURNS, B. P., WALTER, M. R. & ROTHSCHILD, L. J. 2011. Molecular assessment of UVC radiation-induced DNA damage repair in the stromatolitic halophilic archaeon, Halococcus hamelinensis. *Journal of Photochemistry and Photobiology B: Biology,* 102**,** 140-145.

LEVEILLE, R. J. Year. Formation of jarosite and Mars-like minerals in a polar desert: Implications for Mars aqueous geochemistry. *In:* Geological Society of America, 2007.

LI, Y., ENGLE, M., WEISS, N., MANDELCO, L. & WIEGEL, J. 1994. Clostridium thermoalcaliphilum sp. nov., an Anaerobic and Thermotolerant Facultative Alkaliphile. *International journal of systematic bacteriology,* 44**,** 111-118.

LICHTENTHALER, H. K. & BUSCHMANN, C. 2001. Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy. *Current Protocols in Food Analytical Chemistry.* John Wiley & Sons, Inc.

LIN, J., RADAJEWSKI, S., ESHINIMAEV, B. T., TROTSENKO, Y. A., MCDONALD, I. R. & MURRELL, J. C. 2004. Molecular diversity of methanotrophs in Transbaikal soda lake sediments and identification of potentially active populations by stable isotope probing. *Environmental Microbiology,* 6**,** 1049-1060.

LOIZEAU, D., MANGOLD, N., POULET, F., BIBRING, J. P., GENDRIN, A., ANSAN, V., GOMEZ, C., GONDET, B., LANGEVIN, Y., MASSON, P. & NEUKUM, G. 2007. Phyllosilicates in the Mawrth Vallis region of Mars as Seen by OMEGA and HRSC/Mars Express

*Journal of Geophysical Research-Planets,* 112**,** -.

LORENZ, M. G. & WACKERNAGEL, W. 1994. Bacterial gene transfer by natural genetic transformation in the environment. *Microbiological Reviews,* 58**,** 563-602.

LU, C., SCHEUERMANN, R. H. & ECHOLS, H. 1986. Capacity of RecA protein to bind preferentially to UV lesions and inhibit the editing subunit (epsilon) of DNA polymerase III: a possible mechanism for SOS-induced targeted mutagenesis. *Proceedings of the National Academy of Sciences,* 83**,** 619-623.

LUTTMANN, W., BRATKE, K. & KÜPPER, M. 2006. The Cell: Live, Eat, Die. *Immunology.* Academic Press.

MA, Y., XUE, Y., GRANT, W. D., COLLINS, N. C., DUCKWORTH, A. W., STEENBERGEN, R. P. & JONES, B. E. 2004a. *Alkalimonas amylolytica* gen. nov., sp. nov., and *Alkalimonas delamerensis* gen. nov., sp. nov., novel alkaliphilic bacteria from soda lakes in China and East Africa. *Extremophiles,* 8**,** 193-200.

MA, Y., ZHANG, W., XUE, Y., ZHOU, P., VENTOSA, A. & GRANT, W. D. 2004b. Bacterial diversity of the Inner Mongolian Baer Soda Lake as revealed by 16S rRNA gene sequence analyses. *Extremophiles,* 8**,** 45-51.

MACCLUNE, K. L., FOUNTAIN, A. G., KARGEL, J. S. & MACAYEAL, D. R. 2003. Glaciers of the McMurdo dry valleys: Terrestrial analog for Martian polar sublimation. *Journal of Geophysical Research,* 108**,** 5031.

MAHAFFY, P., BOTTA, O., BADA, J. L., GOMEZ-ELVIRA, J., JAVAUX, E., SELSIS, F. & SUMMONS, R. 2008. Exploration of the Habitability of Mars: Development of Analytical Protocols for Measurement of Organic Carbon on the 2009 Mars Science Laboratory

Strategies of Life Detection. Springer US.

MAIDAK, B. L., LARSEN, N., MCCAUGHEY, M. J., OVERBEEK, R., OLSEN, G. J., FOGEL, K., BLANDY, J. & WOESE, C. R. 1994. The Ribosomal Database project. *Nucleic Acids Research,* 22**,** 3485-3487.

MAKAROVA, K. S., ARAVIND, L., WOLF, Y. I., TATUSOV, R. L., MINTON, K. W., KOONIN, E. V. & DALY, M. J. 2001. Genome of the Extremely Radiation-Resistant Bacterium Deinococcus radiodurans Viewed from the Perspective of Comparative Genomics. *Microbiology and molecular biology reviews,* 65**,** 44-79.

MALIN, M. C., BELL, J. F., CAMERON, J., DIETRICH, W. E., EDGETT, K. S., HALLET, B., HERKENHOFF, K. E., LEMMON, M. T., PARKER, T. J., SULLIVAN, R. J., SUMNER, D. Y., THOMAS, P. C., WOHL, E. E., RAVINE, M. A., CAPLINGER, M. A. & MAKI, J. N. 2005. The Mast Cameras And Mars Descent Imager (Mardi) For The 2009 Mars Science Laboratory *irty-Sixth Lunar and Planetary Science Conference.* Houston.

MALIN, M. C., CAPLINGER, M. A., EDGETT, K. S., GHAEMI, F. T., RAVINE, M. A., SCHAFFNER, J. A., BAKER, J. M., BARDIS, J. D., DIBIASE, D. R., MAKI, J. N., WILLSON, R. G., BELL, J. F., DIETRICH, W. E., EDWARDS, L. J., HALLET, B., HERKENHOFF, K. E., HEYDARI, E., KAH, L. C., LEMMON, M. T., MINITTI, M. E., OLSON, T. S., PARKER, T. J., ROWLAND, S. K., SCHIEBER, J., SULLIVAN, R. J., SUMNER, D. Y., THOMAS, P. C. & YINGST, R. A. 2010. The Mars Science Laboratory (MSL) Mast-mounted Cameras (Mastcams) Flight Instruments. *1st Lunar and Planetary Science Conference.* The Woodlands, Texas.

MALIN, M. C. & EDGETT, K. S. 2000. Evidence for Recent Groundwater Seepage and Surface Runoff on Mars. *Science,* 288**,** 2330-2335.

MALOY, S. R. 1994. *Microbial Genetics*.

MANCINELLI, R. 2005a. Halophiles: A terrestrial analog for life in brines on Mars - Halophiles on Mars

Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya. 9**,** 137.

MANCINELLI, R. L. 2000. Martian soil and UV radiation: microbial viability assessment on spacecraft surfaces. *Planetary and space science,* 48**,** 1093.

MANCINELLI, R. L. 2005b. Microbial Life in Brines, Evaporites and Saline Sediments: the Search for Life on Mars. *In:* TOKANO, T. (ed.) *Water in Mars and Life.* Heidleberg: Springerlink.

MANCINELLI, R. L., FAHLEN, T. F., LANDHEIM, R. & KLOVSTAD, M. R. 2004. Brines and evaporites: analogs for Martian life. *Advances in Space Research,* 33**,** 1244-1246.

MARINANGELI, L., HUTCHINSON, I., BALIVA, A., STEVOLI, A., AMBROSI, R., CRITANI, F., DELHEZ, R., SCANDELLI, L., HOLLAND, A., NELMS, N. & THE MARS-XRD TEAM. Year. An European XRD/XRF instrument for the ExoMars Mission. *In:* Proceedings of the 38th Lunar and Planetary Science Conference, 2007 South Shore Harbour Resort and Conference Center, League City, Texas, USA

MARION, G. M., FRITSEN, C. H., EICKEN, H. & PAYNE, M. C. 2003. The search for life on Europa: Limiting environmental factors, potential habitats, and Earth analogues. *Astrobiology,* 3**,** 785-811.

MARTIN, W. 2008. Hydrothermal vents and the origin of life. *Nature review, Microbiology,* 6**,** 805.

MARTINEZ-CANOVAS, M. J. 2004. *Idiomarina* fontislapidosi sp. nov. and *Idiomarina* ramblicola sp. nov., isolated from inland hypersaline habitats in Spain. *International Journal of Systematic and Evolutionary Microbiology,* 54**,** 1793.

MARTINS, Z. 2011. In situ biomarkers and the Life Marker Chip. *Astronomy & Geophysics,* 52**,** 1.34-1.35.

MATTIMORE, V. 1996. Radioresistance of Deinococcus radiodurans: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. *Journal of bacteriology,* 178**,** 633.

MAYILRAJ, S., PRASAD, G., SURESH, K., SAINI, H., SHIVAJI, S. & CHAKRABARTI, T. 2005. *Planococcus* stackebrandtii sp nov., isolated from a cold desert of the Himalayas, India. *International Journal of Systematic and Evolutionary Microbiology,* 55**,** 91-94.

MCDONALD, G. D., TSAPIN, A. I., STORRIE-LOMARDI, K. H., NEALSON, K. H., NBRINTON, K. L. F., SUN, H., VENKATESWAREN K., TSAPIN, I., MELACK, J. & JELLISON, R. 1999. Alkaline hypersaline lakes as analogs for ancient microbial habitats on Mars. *The Fifth International Conference on Mars.* Pasadena.

MCEWEN, A., OJHA, L., DUNDAS, C., MATTSON, S. & BYRNE, S. 2011. Seasonal Flows on Warm Martian Slopes. *Science,* 333**,** 740-743.

MCHENRY, L., CHEVRIER, V. & SCHROEDER, C. 2011. Jarosite in a Pleistocene East African saline-alkaline paleolacustrine deposit: Implications for Mars aqueous geochemistry. *Journal of geophysical research,* 116.

MCHENRY, L. J., CHERVIER, V. F. & SCHRODER, C. 2009. Jarosite in an East African saline-alkaline paleolake deposit: Implications for Mars. *40th Lunar and Planetary Science Conference.* The Woodlands, Texas.

MCKAY, D. S., GIBSON, E. K., THOMASKEPRTA, K. L., VALI, H. & ROMANEK, C. S. 1996. Search for past life on Mars: Possible relic biogenic activity in Martian meteorite ALH84001. *Science,* 273**,** 924-930.

MCKEOWN, N. K., BISHOP, J. L., NOE DOBREA, E. Z., EHLMANN, B. L., PARENTE, M., MUSTARD, J. F., MURCHIE, S. L., SWAYZE, G. A., BIBRING, J.-P. & SILVER, E. A. 2009. Characterization of phyllosilicates observed in the central Mawrth Vallis region, Mars, their potential formational processes, and implications for past climate. *J. Geophys. Res.,* 114**,** E00D10.

MCLAGGAN, D., STEPHEN, J. & BOOTH, I. R. 1998. Chapter 5 Regulation of cytoplasmic pH in bacteria. *In:* BITTAR, E. E. & NEVILLE, B. (eds.) *Principles of Medical Biology.* Elsevier.

MELLERSH, A. M. & SMITH, P. M. 2010. The Alkaline World and the Origin of Life *Journal of Cosmology,* 10**,** 3230-3242.

MILLIKEN, R. E. & BISH, D. L. 2010. Sources and sinks of clay minerals on Mars. *Philosophical Magazine,* 90**,** 2293-2308.

MILLOT, G. 1970. *Geology of clays,* Paris, Masson et C$^{ie}$.

MINTON, K. W. 1996. Repair of ionizing-radiation damage in the radiation resistant bacterium Deinococcus radiodurans. *Mutation research. DNA repair,* 363**,** 1-7.

MINTON, K. W. & DALY, M. J. 1995. A model for repair of radiation-induced DNA double-strand breaks in the extreme radiophile Deinococcus radiodurans. *BioEssays,* 17**,** 457-464.

MOHAPATRA , P. K. 2008. *Textbook of Environmental Microbiology,* New Dehli, I K International.

MONGODIN, E. F., NELSON, K. E., DAUGHERTY, S., DEBOY, R. T., WISTER, J., KHOURI, H., WEIDMAN, J., WALSH, D. A., PAPKE, R. T., SANCHEZ PEREZ, G., SHARMA, A. K.,

NESBÃ¸, C. L., MACLEOD, D., BAPTESTE, E., DOOLITTLE, W. F., CHARLEBOIS, R. L., LEGAULT, B. & RODRIGUEZ-VALERA, F. 2005. The genome of Salinibacter ruber: Convergence and gene exchange among hyperhalophilic bacteria and archaea. *Proceedings of the National Academy of Sciences of the United States of America,* 102**,** 18147-18152.

MORMILE, M., BIESEN, M., GUTIERREZ, M., VENTOSA, A., PAVLOVICH, J. & ONSTOTT, T. 2003. Isolation of Halobacterium salinarum retrieved directly from halite brine inclusions. *Environmental Microbiology,* 5**,** 1094-1102.

MOROZOVA, D., MÖHLMANN, D. & WAGNER, D. 2007. Survival of Methanogenic Archaea from Siberian Permafrost under Simulated Martian Thermal Conditions. *Origins of Life and Evolution of Biospheres,* 37**,** 189-200.

MOROZOVA, D. & WAGNER, D. 2007. Stress response of methanogenic archaea from Siberian permafrost compared with methanogens from nonpermafrost habitats. *FEMS Microbiology Ecology,* 61**,** 16-25.

MORRIS, R. V. 2010. Identification of Carbonate-Rich Outcrops on Mars by the Spirit Rover. *Science,* 329**,** 421.

MURCHIE, S., MUSTARD, J. F., EHLMANN, B. L., MILLIKEN, R. E., BISHOP, J. L., MCKEOWN, N. K., NOE DOBREA, E. Z., SEELOS, F. P., BUCZKOWSKI, D. L., WISEMAN, S. M., ARVIDSON, R. E., WRAY, J. J., SWAYZE, G., CLARK, R. N., DES MARAIS, D. J., MCEWEN, A. S. & BIBRING, J. 2009. A synthesis of Martian aqueous mineralogy after 1 Mars year of observations from the Mars Reconnaissance Orbiter. *Journal of geophysical research,* 114**,** E00D06.

MUSTARD, C. F. 2008. Hydrated silicate minerals on Mars observed by the Mars Reconnaissance Orbiter CRISM instrument. *Nature,* 454**,** 305.

MUTCH, T. A. & MUTCH 1976a. The surface of Mars-The view from the Viking 1 lander. *Science,* 193**,** 791.

MUTCH, T. A. & MUTCH 1976b. The surface of Mars: the view from the Viking 2 lander. *Science,* 194**,** 1277.

MUYZER, G. 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology,* 73**,** 127.

MUYZER, G., DE WAAL, E.C., UITTERLINDEN, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology,* 59**,** 695.

MWATHA, W. E. 1993. Natronobacterium vacuolata sp. nov., a haloalkaliphilic archaeon isolated from Lake Magadi, Kenya. *International journal of systematic bacteriology,* 43**,** 401.

MWIRICHIA, R., COUSIN, S., MUIGAI, A., BOGA, H. & STACKEBRANDT, E. 2010a. Archaeal Diversity in the Haloalkaline Lake Elmenteita in Kenya. *Current microbiology,* 60**,** 47-52.

MWIRICHIA, R., MUIGAI, A., TINDALL, B., BOGA, H. & STACKEBRANDT, E. 2010b. Isolation and characterisation of bacteria from the haloalkaline Lake Elmenteita, Kenya. *Extremophiles,* 14**,** 339-348.

NAGANUMA, T., HUA, P., OKAMOTO, T., BAN, S., IMURA, S. & KANDA, H. 2005. Depth distribution of euryhaline halophilic bacteria in Suribati Ike, a meromictic lake in East Antarctica. *Polar Biology,* 28**,** 964-970.

NAUSER, T., KOPPENOL, W. H. & GEBICKI, J. M. 2005. The kinetics of oxidation of GSH by protein radicals. *Biochemical journal,* 392**,** 693–701.

NEEFS, J., VAN DE PEER, Y., HENDRIKS, L. & DE WACHTER, R. 1990. Compilation of small ribosomal subunit RNA sequences. *Nucleic Acid Research,* 18**,** 2237-2317.

NIELSEN, P., FRITZE, D. & PRIEST, F. G. 1995. Phenetic diversity of alkaliphilic *Bacillus* strains: proposal for nine new species. *Microbiology,* 141**,** 1745-1761.

NIEMETZ, R., KÄRCHER, U., KANDLER, O., TINDALL, B. J. & KÖNIG, H. 1997. The Cell Wall Polymer of the Extremely Halophilic Archaeon Natronococcus occultus. *European Journal of Biochemistry,* 249**,** 905-911.

NOGI, Y. & KATO, C. 1999. Taxonomic studies of extremely barophilic bacteria isolated from the Mariana Trench and description of <i>Moritella yayanosii</i> sp. nov., a new barophilic bacterial isolate. *Extremophiles,* 3**,** 71-77.

NOGI, Y., TAKAMI, H. & HORIKOSHI, K. 2005. Characterization of alkaliphilic *Bacillus* strains used in industry: proposal of five novel species. *International Journal of Systematic and Evolutionary Microbiology,* 55**,** 2309-2315.

NORTON, C. & GRANT, W. 1988. Survival of halobacteria within fluid inclusions in salt crystals

*The Journal of general microbiology,* 134**,** 1365-1373.

NOWLAN, B., DODIA, M., SINGH, S. & PATEL, B. 2006. *Bacillus* okhensis sp nov., a halotolerant and alkalitolerant bacterium from an Indian saltpan. *International Journal of Systematic and Evolutionary Microbiology,* 56**,** 1073-1077.

OLAKA, L., ODADA, E., TRAUTH, M. & OLAGO, D. 2010. The sensitivity of East African rift lakes to climate fluctuations. *Journal of paleolimnology,* 44**,** 629-644.

OLSSON-FRANCIS, K. & COCKELL, C. S. 2009. Experimental methods for studying microbial survival in extraterrestrial environments. *Journal of microbiological methods,* 80**,** 1-13.

OLSSON-FRANCIS, K., DE LA TORRE, R. & COCKELL, C. S. 2010. Isolation of Novel Extreme-Tolerant Cyanobacteria from a Rock-Dwelling Microbial Community by Using Exposure to Low Earth Orbit. *Applied and Environmental Microbiology,* 76**,** 2115-2121.

OPHIR, T. & GUTNICK, D. L. 1994. A Role for Exopolysaccharides in the Protection of Microorganisms from Desiccation. *Applied and Environmental Microbiology,* 60**,** 740-745.

OREN, A. 2001. The bioenergetic basis for the decrease in metabolic diversity at increasing salt concentrations: implications for the functioning of salt lake ecosystems. *Hydrobiologia,* 466**,** 61.

OREN, A. 2002a. Alkaline hypersaline lakes in Africa and Asia. *In:* OREN, A. (ed.) *Halophilic Microorganisms and their Environments.* Springer.

OREN, A. 2002b. Diversity of halophilic microorganisms: Environments, phylogeny, physiology, and applications. *Journal of Industrial Microbiology & Biotechnology,* 28**,** 56-63.

OREN, A. 2002c. *Halophilic Microorganisms and their Environments (Cellular Origin, Life in Extreme Habitats and Astrobiology)* Springer.

OREN, A. 2008. Microbial life at high salt concentrations: phylogenetic and metabolic diversity. *Saline systems,* 4**,** 1-13.

OREN, A. 2009. Microbial Metabolism:Importance for Environmental Biotechnology. *In:* WANG, L. K., IVANOV, V., TAY, J. & HUNG, Y. (eds.) *Environmental Biotechnology.* Springer.

ORENBERG, J. 1992. Reflectance spectroscopy of palagonite and iron-rich montmorillonite clay mixtures: Implications for the surface composition of Mars. *Icarus,* 96**,** 219.

OROFINO, V., GOLDSPIEL, J., CAROFALO, I., BLANCO, A. & FONTI, S. 2009. Evaluation of carbonate abundance in putative martian paleolake basins. *Icarus,* 200**,** 426-435.

OSMAN, S., PEETERS, Z., LA DUC, M., MANCINELLI, R., EHRENFREUND, P. & VENKATESWARAN, K. 2008. Effect of shadowing on survival of bacteria under conditions simulating the Martian atmosphere and UV radiation. *Applied and Environmental Microbiology,* 74**,** 959-970.

OSTERLOO, M., HAMILTON, V., BANDFIELD, J., GLOTCH, T., BALDRIDGE, A. & CHRISTENSEN, P. 2008. Chloride-bearing materials in the southern highlands of Mars. *Science,* 319**,** 1651-1654.

PAIDHUNGAT, M. & SETLOW, P. 2000. Role of Ger Proteins in Nutrient and Nonnutrient Triggering of Spore Germination in *Bacillus* subtilis. *Journal of Bacteriology,* 182**,** 2513-2519.

PALLERONI, N. J. 1997. Prokaryotic diversity and the importance of culturing. *Antonie van Leeuwenhoek,* 72**,** 3-19.

PARK, J. S., VREELAND, R. H., CHO, B. C., LOWENSTEIN, T. K., TIMOFEEFF, M. N. & ROSENZWEIG, W. D. 2009. Haloarchaeal diversity in 23, 121 and 419 MYA salts. *Geobiology,* 7**,** 515-523.

PATEL, M. R., ZARNECKI, J. C. & CATLING, D. C. 2002. Ultraviolet radiation on the surface of Mars and the Beagle 2 UV sensor. *Planetary and space science,* 50**,** 915-927.

PIRAJNO, F., VAN KRANENDONK, M. J. 2005. Review of hydrothermal processes and systems on Earth and implications for Martian analogues. *Australian Journal of Earth Sciences,* 52**,** 329-351.

POGODA DE LA VEGA, U., RETTBERG, P. & REITZ, G. 2007. Simulation of the environmental climate conditions on martian surface and its effect on Deinococcus radiodurans. *Advances in space research,* 40**,** 1672-1677.

POLLACK, J. B., KASTING, J. F., RICHARDSON, S. M. & POLIAKOFF, K. 1987. The case for a wet, warm climate on early Mars. *Icarus,* 71**,** 203-224.

PORCO, C. C. 2006. Cassini observes the active south pole of Enceladus. *Science,* 311**,** 1393.

POSTBERG, F. 2011. A salt-water reservoir as the source of a compositionally stratified plume on Enceladus. *Nature,* 474**,** 620.

POSTBERG, F., KEMPF, S., SCHMIDT, J., BRILLIANTOV, N., BEINSEN, A. & ABEL, B. 2009. Sodium salts in E-ring ice grains from an ocean below the surface of Enceladus. *Nature,* 459**,** 1098-1101.

POSTBERG, F., SCHMIDT, J., HILLIER, J., KEMPF, S. & SRAMA, R. 2011. A salt-water reservoir as the source of a compositionally stratified plume on Enceladus. *Nature,* 474**,** 620-622.

POTTS, M. 1986. The protein index of *Nostoc commune* UTEX 584 (cyanobacteria): changes induced in immobilized cells by water stress. *Archives of Microbiology,* 146**,** 87-95.

POTTS, M. 1994. Desiccation tolerance of prokaryotes. *Microbiol. Mol. Biol. Rev.,* 58**,** 755-805.

POULET, F., ARVIDSON, R. E., GOMEZ, C., MORRIS, R. V., BIBRING, J. P., LANGEVIN, Y., GONDET, B. & GRIFFES, J. 2008. Mineralogy of Terra Meridiani and western Arabia Terra from OMEGA/MEx and implications for their formation. *Icarus,* 195**,** 106-130.

POULET, F., ARVIDSON, R.E., BIBRING, J-P., GONDET, B., JOUGLET, D., LANGEVIN, Y., AND MORRIS, R.V., 2010. Mineralogy of the Phoenix landing site from OMEGA observations and how that relates to in situ Phoenix measurements. *Icarus,* 205**,** 712-715.

POULET, F., J.-P. BIBRING, J. F. MUSTARD, A. GENDRIN, N. MANGOLD, Y. LANGEVIN, R. E. ARVIDSON, B. GONDET, AND C. GOMEZ & THE OMEGA TEAM 2005. Phyllosilicates on Mars and implications for early Martian climate. *Nature,* 438**,** 5.

PRIETO-BALLESTEROS, O., VOROBYOVA, E., PARRO, V., RODRIGUEZ MANFREDI, J. A. & GÓMEZ, F. 2011. Strategies for detection of putative life on Europa. *Advances in Space Research,* 48**,** 678-688.

RADMER, R. 1971. A unified procedure for the detection of life on Mars. *Science,* 174**,** 233.

RAMOS, J. L. 2001. Responses of Gram-negative bacteria to certain environmental stressors. *Current opinion in microbiology,* 4**,** 166.

RAMPELOTTO , P. H. 2011. A Mission to Titan, Europa, or Enceladus? *Journal of Cosmology*.

RASTOGI, S. C. 2006. *Cell and molecular biology*.

RAYMOND, J. C. & SISTROM, W. R. 1967. The isolation and preliminary characterization of a halophilic photosynthetic bacterium. *Archives of Microbiology,* 59**,** 255-268.

REDDY, G., PRAKASH, J., VAIRAMANI, M., PRABHAKAR, S., MATSUMOTO, G. & SHIVAJI, S. 2002. *Planococcus* antarcticus and *Planococcus* psychrophilus spp. nov isolated from cyanobacterial mat samples collected from ponds in Antarctica. *Extremophiles,* 6**,** 253-261.

REES, C., GRANT,W.D., JONES, B.E., HEAPHY, S., 2004. Diversity of Kenyan soda lake alkaliphiles assessed by molecular methods. *Extremophiles,* 8**,** 63-71.

RELAB-SPECTRAL-DATABASE 2008. RELAB Spectral Database *In:* UNIVERSITY, B. (ed.). Providence.

RENNO, N., BOS, B., CATLING, D., CLARK, B. & DRUBE, L. 2009. Possible physical and thermodynamical evidence for liquid water at the Phoenix landing site. *Journal of geophysical research,* 114.

RENNO, N. O. & MEHTA2, M. 2011. Spectral evidence for liquid water on Mars. *Lunar and Planetary Science Conference.* Texas.

RHODES, O. E., CHESSER, R. K. & SMITH, M. H. 1996. *Population dynamics in ecological space and time,* Chicago, Univ. of Chicago Press.

RICE, M. S., BELL, J. F., CLOUTIS, E. A., WANG, A. & RUFF, S. W. 2010. Silica-rich deposits and hydrated minerals at Gusev Crater, Mars: Vis-NIR spectral characterization and regional mapping. *Icarus,* 205**,** 375-395.

RICHMOND, R. C., SRIDHAR, R., ZHOU, Y. & DALY, M. J. Year. Physico-chemical survival pattern for the radiophile D. radiodurans - A polyextremophile model for life on Mars. *In:*

Instruments, methods, and missions for astrobiology II, 1999 Denver, CO; UNITED STATES. 210-222.

RIJKENBERG, M., KORT, R. & HELLINGWERF, K. 2001. Alkalispirillum mobile gen. nov., spec. nov., an alkaliphilic non-phototrophic member of the Ectothiorhodospiraceae. *Archives of Microbiology,* 175**,** 369-375.

ROBERSON, E. B. & FIRESTONE, M. K. 1992. Relationship between Desiccation and Exopolysaccharide Production in a Soil Pseudomonas sp. *Applied and environmental microbiology,* 58**,** 1284-1291.

ROCHELLE, P. A., CAMPER, A., K,, NOCKER, A. & BURR, M. 2011. Are they alive? Detection of viable organisms and functional gene expression using molecular techniques. *In:* SEN, K. (ed.) *Environmental microbiology : current technology and water applications.* Norfolk: Caister Academic Press.

ROELFSEMA, J. H., PETERS, D. J. M., WALKER, J. M. & RAPLEY, R. 2005. Denaturing Gradient Gel Electrophoresis (DGGE). *In:* WALKER, J. M. & RAPLEY, R. (eds.) *Medical Biomethods Handbook.* Humana Press.

ROGERS, A. D. 2008. Mineralogical composition of sands in Meridiani Planum determined from Mars Exploration Rover data and comparison to orbital measurements. *Journal of geophysical research,* 113**,** E06S14.

ROH, S. W., NAM, Y.-D., CHANG, H.-W., SUNG, Y., KIM, K.-H., OH, H.-M. & BAE, J.-W. 2007. Halalkalicoccus jeotgali sp. nov., a halophilic archaeon from shrimp jeotgal, a traditional Korean fermented seafood. *International Journal of Systematic and Evolutionary Microbiology,* 57**,** 2296-2298.

ROMANO, I., GIORDANO, A., LAMA, L., NICOLAUS, B. & GAMBACORTA, A. 2003. *Planococcus* rifietensis sp nov, isolated from algal mat collected from a sulfurous spring in Campania (Italy). *Systematic and Applied Microbiology,* 26**,** 357-366.

ROMANO, I., LAMA, L., NICOLAUS, B., GAMBACORTA, A. & GIORDANO, A. 2005. *Bacillus* saliphilus sp. nov., isolated from a mineral pool in Campania, Italy. *International Journal of Systematic and Evolutionary Microbiology,* 55**,** 159-163.

RONCOLI, R. B. & LUDWINSKI, J. M. 2002. Mission design overview for the Mars Exploration Rover mission. *AIAA/AAS Astrodynamics Specialist Conference and Exhibit.* Wahington DC.

ROSENZWEIG, W. D. & D. ROSENZWEIG, J. P. J. 2000. Development of a protocol to retrieve microorganisms from ancient salt crystals. *Geomicrobiology journal,* 17**,** 185.

ROTHSCHILD, L., GIVER, L., WHITE, M. & MANCINELLI, R. 1994. METABOLIC-ACTIVITY OF MICROORGANISMS IN EVAPORITES. *Journal of phycology,* 30**,** 431-438.

ROTHSCHILD, L. J. & MANCINELLI, R. L. 2001. Life in extreme environments. *Nature,* 409**,** 1092-1101.

RUFF, C. W. 2004a. Spectral evidence for zeolite in the dust on Mars. *Icarus,* 168**,** 131.

RUFF, S. W. 2004b. Spectral evidence for zeolite in the dust on Mars. *Icarus,* 168**,** 131.

SADFI ZOUAOUI, N., ESSGHAIER, B., HAJLAOUI, M., FARDEAU, M., CAYAOL, J. & OLLIVIER, B. 2007. Ability of moderately halophilic bacteria to control grey mould disease on tomato fruits. *Journal of phytopathology,* 156**,** 42-52.

SAHA, I. & PAUL, B. 2010. Correlation. *In:* SAHA, I. & PAUL, B. (eds.) *Biostatistics; MCQ and essentials.* Academic publishers.

SALLES, J. F., VAN VEEN, J. A. & VAN ELSAS, J. D. 2004. Multivariate Analyses of Burkholderia Species in Soil: Effect of Crop and Land Use History. *Applied and environmental microbiology,* 70**,** 4012-4020.

SANCAR, A. 1994. Structure and function of DNA photolyase. *Biochemistry,* 33**,** 2-9.

SANCAR, A. & TANG, M. S. 1993. Nucleotide excision repair. *Photochemistry and Photobiology,* 57**,** 905-921.

SATTERFIELD, C., LOWENSTEIN, T., VREELAND, R., ROSENZWEIG, W. & POWERS, D. 2005. New evidence for 250 Ma age of halotolerant bacterium from a Permian salt crystal. *Geology,* 33**,** 265-268.

SAUNDERS, R. S., ARVIDSON, R. E., BADHWAR, G. D., BOYNTON, W. V. & CHRISTENSEN, P. R. 2004. 2001 Mars Odyssey mission summary. *Space science reviews,* 110**,** 1-36.

SCHUBERT, B., LOWENSTEIN, T., TIMOFEEFF, M. & PARKER, M. 2009. How do prokaryotes survive in fluid inclusions in halite for 30 k.y.? *Geology,* 37**,** 1059-1062.

SCHUBERT, B. A., LOWENSTEIN, T. K., TIMOFEEFF, M. N. & PARKER, M. A. 2010. Halophilic Archaea cultured from ancient halite, Death Valley, California. *Environmental Microbiology,* 12**,** 440-454.

SCHUERGER, A., RICHARDS, J., NEWCOMBE, D. & VENKATESWARAN, K. 2006. Rapid inactivation of seven *Bacillus* spp. under simulated Mars UV irradiation. *Icarus,* 181**,** 52-62.

SCHUMANN, W. 2006. Stress genes and their regulation. *Dynamics of the Bacterial Chromosome Structure and Function.* Weinheim: Wiley-VCH.

SECKBACH, J. 1999. *Enigmatic microorganisms and life in extreme environments*.

SECKBACH, J. 2000. *Journey to diverse microbial worlds : adaptation to exotic environments,* Dordrecht [u.a.], Kluwer Acad. Publ.

SETLOW, B. & SETLOW, P. 1996. Role of DNA repair in *Bacillus* subtilis spore resistance. *J. Bacteriol.,* 178**,** 3486-3495.

SETLOW, P. 2001. Resistance of spores of *Bacillus* species to ultraviolet light. *Environmental and Molecular Mutagenesis,* 38**,** 97-104.

SETLOW, P. 2006. Spores of *Bacillus* subtilis: their resistance to and killing by radiation, heat and chemicals. *Journal of applied microbiology,* 101**,** 514-25.

SGHAIER, H., MITOMO, H. & NARUMI, I. 2005. Genomic confession of Deinococcus radiodurans: it started out as a radiation-resistant organism. *Viva Origino,* 33.

SGHAIER, H., NARUMI, I., SATOH, K., OHBA, H. & MITOMO, H. 2007. Problems with the current deinococcal hypothesis: an alternative theory. *Theory in biosciences,* 126**,** 43-45.

SHAH, V. & BELOZEROVA, I. 2009. Influence of Metal Nanoparticles on the Soil Microbial Community and Germination of Lettuce Seeds. *Water, air and soil pollution,* 197**,** 143-148.

SHAHMOHAMMADI, H. R., ASGARANI, E., TERATO, H., SAITO, T., OHYAMA, Y., GEKKO, K., YAMAMOTO, O. & HIROSHI, I. 1998. Protective Roles of Bacterioruberin and Intracellular KCl in the Resistance of Halobacterium salinarium against DNA-damaging Agents. *Journal of Radiation Research,* 39**,** 251-262.

SHEPPARD, R. & HAY, R. 2001. Formation of zeolites in open hydrologic systems. *Reviews in mineralogy and geochemistry,* 45**,** 261-275.

SHINDO, K., ENDO, M., MIYAKE, Y., WAKASUGI, K., MORRITT, D., BRAMLEY, P.M., FRASER, P.D., KASAI, H., MISAWA, N., 2008. Methyl Glucosyl-3,4-dehydro-apo-8' -lycopenoate (I), a Novel Antioxidative Glyco-C-30-carotenoic Acid Produced by a Marine Bacterium *Planococcus* maritimus. *The Journal of Antibiotics,* 61**,** 729-735.

SHIRAI, T., KOBAYASHI, T., ITO, S., AND HORIKOSHI, K., 2008. Alkaline Adaptation of Proteins. *In:* SIDDIQUI, K. S., THOMAS, T., (ed.) *Protein adaptations in extremophiles.* Nova Science Publishers Inc.

SHOCK, E. L. 2009. Minerals as Energy Sources for Microorganisms. *Economic Geology,* 104**,** 1235-1248.

SIDDIQUI, K. S. & THOMAS, T. 2008. *Protein Adaptation in Extremophiles,* New york, Nova Science Publishers.

SKELLEY, A. M., SCHERER, J. R., AUBREY, A. D., GROVER, W. H., IVESTER, R. H. C., EHRENFREUND, P., GRUNTHANER, F. J., BADA, J. L. & MATHIES, R. A. 2005. Development and evaluation of a microdevice for amino acid biomarker detection and analysis on Mars. *Proceedings of the National Academy of Sciences of the United States of America,* 102**,** 1041-1046.

SLADE, D. 2011. Oxidative Stress Resistance in Deinococcus radiodurans. *Microbiology and molecular biology reviews,* 75**,** 133.

SLEEP, N. H. 2001. Initiation of clement surface conditions on the earliest Earth. *Proceedings of the National Academy of Sciences of the United States of America,* 98**,** 3666.

SLEEP, N. H., MEIBOM, A., FRIDRIKSSON, T., COLEMAN, R. G. & BIRD, D. K. 2004. H2-rich fluids from serpentinization: Geochemical and biotic implications. *Proceedings of the National Academy of Sciences of the United States of America,* 101**,** 12818-12823.

SLIEMAN, T. A. & NICHOLSON, W. L. 2000. Artificial and Solar UV Radiation Induces Strand Breaks and Cyclobutane Pyrimidine Dimers in *Bacillus* subtilis Spore DNA. *Applied and Environmental Microbiology,* 66**,** 199-205.

SLONCZEWSKI, J. L., FUJISAWA, M., DOPSON, M., KRULWICH, T. A. & ROBERT, K. P. 2009. Cytoplasmic pH Measurement and Homeostasis in Bacteria and Archaea. *Advances in Microbial Physiology.* Academic Press.

SMALLA, K., WACHTENDORF, U., HEUER, H., LIU, W.-T. & FORNEY, L. 1998. Analysis of BIOLOG GN Substrate Utilization Patterns by Microbial Communities. *Applied and Environmental Microbiology,* 64**,** 1220-1225.

SMITH, D., SCHUERGER, A., DAVIDSON, M., PACALA, S. & BAKERMANS, C. 2009a. Survivability of Psychrobacter cryohalolentis K5 Under Simulated Martian Surface Conditions. *Astrobiology,* 9**,** 221-228.

SMITH, P. H., TAMPPARI, L., ARVIDSON, R. E., BASS, D. & BLANEY, D. 2008. Introduction to special section on the Phoenix Mission: Landing Site Characterization Experiments, Mission Overviews, and Expected Science. *Journal of geophysical research,* 113.

SMITH, P. H., TAMPPARI, L. K., ARVIDSON, R. E., BASS, D., BLANEY, D., BOYNTON, W. V., CARSWELL, A., CATLING, D. C., CLARK, B. C., DUCK, T., DEJONG, E., FISHER, D., GOETZ, W., GUNNLAUGSSON, H. P., HECHT, M. H., HIPKIN, V., HOFFMAN, J., HVIID, S. F., KELLER, H. U., KOUNAVES, S. P., LANGE, C. F., LEMMON, M. T., MADSEN, M. B., MARKIEWICZ, W. J., MARSHALL, J., MCKAY, C. P., MELLON, M. T., MING, D. W., MORRIS, R. V., PIKE, W. T., RENNO, N., STAUFER, U., STOKER, C., TAYLOR, P.,

WHITEWAY, J. A. & ZENT, A. P. 2009b. H2O at the Phoenix Landing Site. *Science,* 325**,** 58-61.

SMITH, P. H., TOMASKO, M. G., BRITT, D., CROWE, D. G., REID, R., KELLER, H. U., THOMAS, N., GLIEM, F., RUEFFER, P., SULLIVAN, R., GREELEY, R., KNUDSEN, J. M., MADSEN, M. B., GUNNLAUGSSON, H. P., HVIID, S. F., GOETZ, W., SODERBLOM, L. A., GADDIS, L. & KIRK, R. 1997. The imager for Mars Pathfinder experiment. *Journal of geophysical research,* 102**,** 4003.

SOFFEN, G. A. & SNYDER, C. W. 1976. The First Viking Mission to Mars. *Science,* 193**,** 759-766.

SPAHN, F. 2006. Cassini dust measurements at Enceladus and implications for the origin of the E ring. *Science,* 311**,** 1416.

SPARKS, W. B., HOUGH, J., GERMER, T. A., CHEN, F., DASSARMA, S., DASSARMA, P., ROBB, F. T., MANSET, N., KOLOKOLOVA, L., REID, N., MACCHETTO, F. D. & MARTIN, W. 2009. Detection of circular polarization in light scattered from photosynthetic microbes. *Proceedings of the National Academy of Sciences,* 106**,** 7816-7821.

SPECK, M. L. 1976. *Compendium of methods for the microbiological examination of foods,* Washington, American Public Health Association.

SPENCER, J. R. 2006. Cassini encounters Enceladus: Background and the discovery of a south polar hot spot. *Science,* 311**,** 1401.

SPINK, P. C. & STEVENS, J. A. 1946. Notes on the Magadi section of the Eastern rift valley. *The Geographical Journal*.

SQUYRES, C. W. 2004. In situ evidence for an ancient aqueous environment at Meridiani Planum, Mars. *Science,* 306**,** 1709.

SQUYRES, S., KNOLL, A., ARVIDSON, R., CLARK, B., GROTZINGER, J. & JOLLIFF, B. 2006. Two years at Meridiani Planum: Results from the Opportunity Rover. *Science,* 313**,** 1403-1407.

SQUYRES, S. W. 1984. The History of Water on Mars. *Annual review of Earth and planetary sciences,* 12**,** 83-106.

SQUYRES, S. W. 2005. Sedimentary rocks at Meridiani Planum: Origin, diagenesis, and implications for life on Mars. *Earth and Planetary Science Letters,* 240**,** 1.

STAN-LOTTER, H., LEGAT, A., FENDRIHAN, S., LEUKA, S., GRUEBER, C., RADAX, C., PFAFFENHUEMER, M., WEIDLER, G. & RITTMANN, S. Year. Haloarchaeal survival over geological times and the detection of extraterrestrial halite-implications for the search for life on Mars. *In:* HARRIS. R. A., O., L.,, ed. Proceedings of the Third European Workshop on Exo-Astrobiology, 2003 Madrid, Spain. ESA Publications Division, 63 - 66.

STEELE, A., FRIES, M. D., AMUNDSEN, H. E. F., MYSEN, B. O., FOGEL, M. L., SCHWEIZER, M. & BOCTOR, N. Z. 2007. Comprehensive imaging and Raman spectroscopy of carbonate globules from Martian meteorite ALH 84001 and a terrestrial analogue from Svalbard. *Meteoritics & planetary science,* 42**,** 1549-1566.

STEELE, A., SCHWEIZER, M., AMUNDSEN, H. E. F. & WAINWRIGHT, N. 2004. In-Field Testing of Life Detection Instruments and Protocols in a Mars Analogue Arctic Environment. *Lunar and Planetary Science Conference.* Houston, Texas.

STEWART, L., CLOUTIS, E., BISHOP, J. L., CRAIG, M., KALETZKE, L. & MCCORMACK, K. 2006. Classification of Iron Bearing Phyllosilicates Based on Ferric and Ferrous Iron

Absorption Bands in the 400-1300 nm Region. *37th Annual Lunar and Planetary Science Conference.* League City Texas.

STORZ, G., HENGGE, R. & AMERICAN SOCIETY FOR, M. 2000. *Bacterial stress responses,* Washington, DC, ASM Press.

SUMMONS, R., AMEND, J., BISH, D., BUICK, R., CODY, G. & DES MARAIS, D. 2011. Preservation of Martian Organic and Environmental Records: Final Report of the Mars Biosignature Working Group. *Astrobiology,* 11**,** 157-181.

SURDAM, R. C., EUGSTER, H.P., 1976. Mineral reactions in the sedimentary deposits of the Lake Magadi region, Kenya. *Geological Society of America* 87**,** 1739-1752.

SURDAM, R. C. & PARKER, R. D. 1972. Authigenic Aluminosilicate Minerals in the Tuffaceous Rocks of the Green River Formation, Wyoming. *Geological Society of America bulletin,* 83**,** 689-700.

SURESH, K., MAYILRAJ, S., BHATTACHARYA, A. & CHAKRABARTI, T. 2007. *Planococcus* columbae sp. nov., isolated from pigeon faeces. *Int J Syst Evol Microbiol,* 57**,** 1266-1271.

SWITZER BLUM, J., BURNS BINDI, A., BUZZELLI, J., STOLZ, J. F. & OREMLAND, R. S. 1998. *Bacillus* arsenicoselenatis, sp. nov., and *Bacillus* selenitireducens, sp. nov.: two haloalkaliphiles from Mono Lake, California that respire oxyanions of selenium and arsenic. *Archives of Microbiology,* 171**,** 19-30.

TABORDA, M. 2009. Description of *Idiomarina* insulisalsae sp. nov., isolated from the soil of a sea salt evaporation pond, proposal to transfer the species of the genus Pseudidiomarina to the genus *Idiomarina* and emended description of the genus *Idiomarina*. *Systematic and Applied Microbiology,* 32**,** 371.

TALEVI, M. & BROWN, D. 2009. *NASA and ESA Prioritize Outer Planet Missions* [Online]. [Accessed].

TAMARU, Y., TAKANI, Y., YOSHIDA, T. & SAKAMOTO, T. 2005. Crucial Role of Extracellular Polysaccharides in Desiccation and Freezing Tolerance in the Terrestrial Cyanobacterium Nostoc commune. *Applied and environmental microbiology,* 71**,** 7327-7333.

TAMURA, K., PETERSON, D., PETERSON, N., STECHER, G., NEI, M. & KUMAR, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*

TER BRAAK, C. J. F. 1987. The analysis of vegetation-environment relationships by canonical correspondence analysis. *Plant Ecology,* 69**,** 69-77.

THIRKELL, D. & SUMMERFIELD, M. 1980. Variation in pigment production by *Planococcus citreus* Migula with cultural age and with sea salt concentration in the medium. *Antonie van Leeuwenhoek,* 46**,** 51-57.

TIAN, B. & HUA, Y. 2010. Carotenoid biosynthesis in extremophilic Deinococcus–Thermus bacteria. *Trends in Microbiology,* 18**,** 512-520.

TIAN, B., SUN, Z., SHEN, S., WANG, H., JIAO, J., WANG, L., HU, Y. & HUA, Y. 2009. Effects of carotenoids from Deinococcus radiodurans on protein oxidation. *Letters in Applied Microbiology,* 49**,** 689-694.

TILLMAN, J. E., JOHNSON, N. C., GUTTORP, P. & PERCIVAL, D. B. 1993. The Martian Annual Atmospheric Pressure Cycle: Years Without Great Dust Storms. *J. Geophys. Res.,* 98**,** 10963-10971.

TINDALL, B., J, MILLS, A.A, GRANT, W.D, 1980. Alkalophilic red halophilic bacterium with a low magnesium requirement from a Kenyan soda lake

*The Journal of general microbiology,* 116**,** 257-260.

TINDALL, B. J., ROSS, H. N. M. & GRANT, W. D. 1984. Natronobacterium gen. nov. and Natronococcus gen. nov., two new genera of haloalkaliphilic archaebacteria. *Systematic and Applied Microbiology,* 5**,** 41-57.

TOBLER , D. J. & BENNING, L. G. 2011. Bacterial diversity in five Icelandic geothermal waters: temperature and sinter growth rate effects. *Extremophiles,* 15**,** 473-485.

TOSCA, N. J., KNOLL, A. H. & MCLENNAN, S. M. 2008. Water Activity and the Challenge for Life on Early Mars. *Science,* 320**,** 1204-1207.

TOSELLI, F. 1992. *Imaging spectroscopy: fundamentals and prospective applications*.

TRIGUI, H., MASMOUDI, S., BROCHIER-ARMANET, C., MAALEJ, S. & DUKAN, S. 2011. Survival of extremely and moderately halophilic isolates of Tunisian solar salterns after UV-B or oxidative stress. *Canadian Journal of Microbiology,* 57**,** 923-933.

TRUN, N. J. & TREMPY, J. E. 2004. *Fundamental bacterial genetics,* Malden, MA, Blackwell.

TYRRELL, R. M. 1973. INDUCTION OF PYRIMIDINE DIMERS IN BACTERIAL DNA BY NM-365 RADIATION. *Photochemistry and Photobiology,* 17**,** 69-73.

UDUPA, K. S., O'CAIN, P. A., MATTIMORE, V. & BATTISTA, J. R. 1994. Novel ionizing radiation-sensitive mutants of Deinococcus radiodurans. *Journal of bacteriology,* 176**,** 7439-7446.

UKU, J. N. & MAVUTI, K. M. 1994. Comparative limnology, species diversity and biomass relationship of zooplankton and phytoplankton in five freshwater lakes in Kenya. *Hydrobiologia,* 272**,** 251-258.

VAISHAMPAYAN, P., PROBST, A., KRISHNAMURTHI, S., GHOSH, S. & OSMAN, S. 2010. *Bacillus* horneckiae sp nov., isolated from a spacecraft-assembly clean room. *International Journal of Systematic and Evolutionary Microbiology,* 60**,** 1031-1037.

VANDEWIELE, D., BORDEN, A., O'GRADY, P. I., WOODGATE, R. & LAWRENCE, C. W. 1998. Efficient translesion replication in the absence of Escherichia coli Umu proteins and 3′–5′ exonuclease proofreading function. *Proceedings of the National Academy of Sciences,* 95**,** 15519-15524.

VANHOUTTE, T., HUYS, G. & CRANENBROUCK, I. S. 2005. Exploring microbial ecosystems with Denaturing Gradient Gel Electrophoresis (DGGE). *Belgian co-ordinated collections of micro-organisms,* 17.

VAZ-MOREIRA, I., EGAS, C., NUNES, O. & MANAIA, C. 2011. Culture-dependent and culture-independent diversity surveys target different bacteria: a case study in a freshwater sample. *Antonie van Leeuwenhoek,* 100**,** 245-257.

VITEK, P., EDWARDS, H., JEHLICKA, J., ASCASO, C., DE LOS RIOS, A. & VALEA, S. 2010. Microbial colonization of halite from the hyper-arid Atacama Desert studied by Raman spectroscopy. *Philosophical transactions - Royal Society. Mathematical, Physical and engineering sciences,* 368**,** 3205-3221.

VÍTEK, P., JEHLIČKA, J., EDWARDS, H. G. M. & WIERZCHOS, J. 2010. Identification of microbial pigments in evaporitic matrices using Raman spectroscopy. *EGU General Assembly.* Vienna, Austria.

VREELAND, R., JONES, J., MONSON, A., ROSENZWEIG, W., LOWENSTEIN, T. & TIMOFEEFF, M. 2007. Isolation of live Cretaceous (121-112 million years old) halophilic Archaea from primary salt crystals. *Geomicrobiology journal,* 24**,** 275-282.

VREELAND, R., ROSENZWEIG, W. & POWERS, D. 2000. Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. *Nature,* 407**,** 897-900.

WAITE JR, J. H., LEWIS, W. S., MAGEE, B. A., LUNINE, J. I., MCKINNON, W. B., GLEIN, C. R., MOUSIS, O., YOUNG, D. T., BROCKWELL, T., WESTLAKE, J., NGUYEN, M. J., TEOLIS, B. D., NIEMANN, H. B., MCNUTT JR, R. L., PERRY, M. & IP, W. H. 2009. Liquid water on Enceladus from observations of ammonia and 40Ar in the plume. *Nature,* 460**,** 487-490.

WANG, L., LAI, Q., FU, Y., CHEN, H., WANG, W., WANG, J., SUN, F. & SHAO, Z. 2011. *Idiomarina* xiamenensis sp. nov., isolated from surface seawater, and proposal to transfer Pseudidiomarina aestuarii to the genus *Idiomarina* as *Idiomarina* aestuarii comb. nov. *Int J Syst Evol Microbiol,* 61**,** 969-973.

WANG, Z., WANG, Y., LIU, J., CHEN, Y. & ZHANG, X. 2009. Fodinibacter luteus gen. nov., sp nov., an actinobacterium isolated from a salt mine. *International Journal of Systematic and Evolutionary Microbiology,* 59**,** 2185-2190.

WANI, A. A., SURAKASI, V. P., SIDDHARTH, J., RAGHAVAN, R. G., PATOLE, M. S., RANADE, D. & SHOUCHE, Y. S. 2006. Molecular analyses of microbial diversity associated with the Lonar soda lake in India: An impact crater in a basalt area. *Research in Microbiology,* 157**,** 928-937.

WARNECKE, F., SOMMARUGA, R., SEKAR, R., HOFER, J. S. & PERNTHALER, J. 2005. Abundances, Identity, and Growth State of Actinobacteria in Mountain Lakes of Different UV Transparency. *Applied and Environmental Microbiology,* 71**,** 5551-5559.

WARNICK, T. A., METHÂˆŠÂ©, B. A. & LESCHINE, S. B. 2002. Clostridium phytofermentans sp. nov., a cellulolytic mesophile from forest soil. *International Journal of Systematic and Evolutionary Microbiology,* 52**,** 1155-60.

WARREN, J. K. 2006. Subaqueous salts: salinas and perennial lakes. *Evaporites sediments, resources and hydrocarbons.* Springer.

WEAVER, C. E. 1989. *Clays, Muds and Shales*, Elsevier Science Publishers.

WEBER, K. P., GEHDER, M. & LEGGE, R. L. 2008. Assessment of changes in the microbial community of constructed wetland mesocosms in response to acid mine drainage exposure. *Water Research,* 42**,** 180-188.

WEINBAUER, M. G. & HOFLE, M. G. 2001. Quanitifivtaion of nucleic acids from aquatic environments by using green fluorensent dyes and microtiter plates. *In:* AKKERMANS, A. D. L. & VAN ELSAS, J. D. (eds.) *Molecular Microbial Ecology Manual.* Netherlands: Kluwer Accademic Publishers.

WEISENBERGER, T., AND SELBEKK, R.S., 2009. Multi-stage zeolite facies mineralization in the Hvalfjordur area, Iceland. *International journal of earth sciences,* 98**,** 985.

WELSH, D. T. & HERBERT, R. A. 1999. Osmotically induced intracellular trehalose, but not glycine betaine accumulation promotes desiccation tolerance in Escherichia coli. *FEMS Microbiology Letters,* 174**,** 57-63.

WENTWORTH, S. J., GIBSON, E. K., VELBEL, M. A. & MCKAY, D. S. 2005. Antarctic Dry Valleys and indigenous weathering in Mars meteorites: Implications for water and life on Mars. *Icarus,* 174**,** 383-395.

WESTALL, F. 2005. Early life on Earth and analogies to Mars. *In:* TOKANO, T. (ed.) *Water on Mars and life.* Berlin Heidelberg: Springer.

WETTERGREEN, D., CABROL, N., BASKARAN, V., CALDERÓN, F., HEYS, S., JONAK, D., LÜDERS, A., PANE, D., SMITH, T., TEZA, J., TOMPKINS, P., VILLA, P., WILLIAMS, C. & WAGNER, M. Year. Second experiments in the robotic investigation of life in the Atacama desert of Chile

*In:* ISAIRAS 2005 Conference, 2005 Munich Germany.

WIEGEL, J. 2011. Anaerobic Alkaliphiles and Alkaliphilic Poly-Extremophiles *In:* HORIKOSHI, H., ANTRANIKIAN, G., BULL, A. T., ROBB, F. T. & STETTER, K. O. (eds.) *Extremophiles Handbook.* Springer.

WITKIN, E. M. 1976. Ultraviolet mutagenesis and inducible DNA-repair in Escherichia-coli. *Bacteriological reviews,* 40**,** 869-907.

WOHLFARTH, A., SEVERIN, J. & GALINSKI, E. A. 1990. The spectrum of compatible solutes in heterotrophic halophilic eubacteria of the family Halomonadaceae. *Journal of General Microbiology,* 136**,** 705-712.

WRAY, J. J., EHLMANN, B. L., SQUYRES, S. W., MUSTARD, J. F. & KIRK, R. L. 2008. Compositional stratigraphy of clay-bearing layered deposits at Mawrth Vallis, Mars. *Geophysical Research Letters,* 35**,** -.

WRAY, J. J., MURCHIE, S. L., SQUYRES, S. W., SEELOS, F. P. & TORNABENE, L. L. 2009. Diverse aqueous environments on ancient Mars revealed in the southern highlands. *Geology,* 37**,** 1043-1046.

WU, L. C., CHOW, K. C. & MARK, K. K. 1983. The role of pigments in Halobacterium cutirubrum against UV irradiation. *Microbios Letters,* 24**,** 85-90.

XIN, H., ITOH, T., ZHOU, P., SUZUKI, K. & NAKASE, T. 2001. Natronobacterium nitratireducens sp. nov., a haloalkaliphilic archaeon isolated from a soda lake in China. *International Journal of Systematic and Evolutionary Microbiology,* 51**,** 1825-9.

XU, Y. 2001. Natrialba hulunbeirensis sp. nov. and Natrialba chahannaoensis sp. nov., novel haloalkaliphilic archaea from soda lakes in Inner Mongolia Autonomous Region, China. *International Journal of Systematic and Evolutionary Microbiology,* 51**,** 1693.

XUE, Y., ZHANG, X., ZHOU, C., ZHAO, Y., COWAN, D. A., HEAPHY, S., GRANT, W. D., JONES, B. E., VENTOSA, A. & MA, Y. 2006. *Caldalkalibacillus* thermarum gen. nov., sp. nov., a novel alkalithermophilic bacterium from a hot spring in China. *Int J Syst Evol Microbiol,* 56**,** 1217-1221.

YERGEAU, E., KANG, S., HE, Z., ZHOU, J. & KOWALCHUK, G. A. 2007. Functional microarray analysis of nitrogen and carbon cycling genes across an Antarctic latitudinal transect. *ISME J,* 1**,** 163-179.

YERGEAU, E. & KOWALCHUK, G. A. 2008. Responses of Antarctic soil microbial communities and associated functions to temperature and freeze–thaw cycle frequency. *Environmental Microbiology,* 10**,** 2223-2235.

YERGEAU, E., SCHOONDERMARK-STOLK, S. A., BRODIE, E. L., DEJEAN, S., DESANTIS, T. Z., GONCALVES, O., PICENO, Y. M., ANDERSEN, G. L. & KOWALCHUK, G. A. 2008. Environmental microarray analyses of Antarctic soil microbial communities. *ISME J,* 3**,** 340-351.

YEUNG, A. T., MATTES, W. B. & GROSSMAN, L. 1986. Protein complexes formed during the incision reaction catalyzed by the Escherichia coli UvrABC endonuclease. *Nucleic Acids Research,* 14**,** 2567-2582.

YOON, J.-H., JUNG, S.-Y., JUNG, Y.-T. & OH, T.-K. 2007. *Idiomarina* salinarum sp. nov., isolated from a marine solar saltern in Korea. *Int J Syst Evol Microbiol,* 57**,** 2503-2506.

YOON, J., WEISS, N., YANG, K., OH, T., AND PARK, Y., 2003. *Planococcus* maritimus sp. nov., isolated from sea water of a tidal flat in Korea *International Journal of Systematic and Evolutionary Microbiology* 53**,** 2013-2017.

YUMOTO, I., HIROTA, K., NODASAKA, Y., TOKIWA, Y. & NAKAJIMA, K. 2008. Alkalibacterium indicireducens sp. nov., an obligate alkaliphile that reduces indigo dye. *International Journal of Systematic and Evolutionary Microbiology,* 58**,** 901-905.

YUMOTO, I., HIROTA, K., NODASAKA, Y., YOKOTA, Y., HOSHINO, T. & NAKAJIMA, K. 2004. Alkalibacterium psychrotolerans sp. nov., a psychrotolerant obligate alkaliphile that reduces an indigo dye. *Int J Syst Evol Microbiol,* 54**,** 2379-2383.

YUMOTO, I., HIROTA, K. & YOSHIMUNE, K. 2010. Environmental distribution and taxonomic diversity of alkaliphiles. *In:* HORIKOSHI, K. (ed.) *Extremophile Handbook.* Tokyo: Springer

YUMOTO, I., HIROTA, K. & YOSHIMUNE, K. 2011. Environmental distribution and taxonomic diversity of alkaliphiles. *In:* HORIKOSHI, K., ANTRANIKIAN, G., BULL, A. T., ROBB, F. T. & STETTER, K. O. (eds.) *Extremophile Handbook.* Tokyo: Springer.

ZAVARZIN, G. A., ZHILINA, T.N., 2000. Anaerobic chemotrophic alkaliphiles. *In:* SECKBACH, J. (ed.) *Journey to diverse microbial worlds: adaptation to exotic environment.* Kluwer Academic Publishers.

ZAVARZINA, D., TOUROVA, T., KOLGANOVA, T., BOULYGINA, E. & ZHILINA, T. 2009. Description of Anaerobacillus alkalilacustre gen. nov., sp nov.-Strictly anaerobic diazotrophic bacillus isolated from soda lake and transfer of *Bacillus* arseniciselenatis, *Bacillus* macyae, and *Bacillus* alkalidiazotrophicus to Anaerobacillus as the new combinations A. arseniciselenatis comb. nov., A. macyae comb. nov., and A. alkalidiazotrophicus comb. nov. *Microbiology,* 78**,** 723-731.

ZENG, J., YANG, L., LI, J., LIANG, Y. & XIAO, L. 2009. Vertical distribution of bacterial community structure in the sediments of two eutrophic lakes revealed by denaturing gradient gel electrophoresis (DGGE) and multivariate analysis techniques. *World journal of microbiology & biotechnology,* 25**,** 225-233.

ZHANG, W. 2002. Salinicoccus alkaliphilus sp. nov., a novel alkaliphile and moderate halophile from Baer Soda Lake in Inner Mongolia Autonomous Region, China. *International Journal of Systematic and Evolutionary Microbiology,* 52**,** 789.

ZHAO, W., ZHANG, C., ROMANEK, C. & WIEGEL, J. 2008. Description of *Caldalkalibacillus uzonensis* sp. nov. and embeded desription of the genus *Caldalkalibacillus*. *Internattional Journal of Systematic and Evolutionary Microbiology,* 58**,** 1106-1108.

ZOLOTOV, M. Y. 2004. *A model for low-temperature biogeochemistry of sulfur, carbon, and iron on Europa*

*Journal of geophysical research*.

ZOLOTOV, M. Y. 2007. An oceanic composition on early and today's Enceladus. *Geophysical Research Letters,* 34**,** 5.

ZOLOTOV, M. Y. & SHOCK, E. L. 2001. Composition and stability of salts on the surface of Europa and their oceanic origin. *Journal of geophysical research. Planets,* 106**,** 815.

ZUREK, R. W. 2007. An overview of the Mars Reconnaissance Orbiter (MRO) science mission. *Journal of geophysical research,* 112**,** E05S01.

# Appendices

## Appendix A

*Table A.1. Isolates from the northern end of Little Magadi, samples 106, 108, 109, 110, 113, 114 and 115 from Chapter 3, isolates are given with closest database match and closest strain match. Only those with a query coverage of >95 were included.*

| Isolate name | No. nucleotide | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| 6.8_106A | 784 | - | - | *Caldalkalibacillus* uzonensis strain JW/WZ-YB58 (NR_043653.1) | 93 |
| 6.8_106B | 785 | - | - | *Caldalkalibacillus* uzonensis strain JW/WZ-YB58 (NR_043653.1) | 93 |
| 6.8_106C | 774 | *Bacillus* sp. 9-3AIA (FN397519.1) | 76 | *Caldalkalibacillus* uzonensis strain JW/WZ-YB58 (NR_043653.1) | 97 |
| 0.7_108A | 761 | Halomonas sp. 4AB3 (HM587243.1) | 88 | Halomonas campisalis strain HB10.1) (GU228480.1) | 88 |
| 0.7_108C | 759 | Gamma proteobacterium E-113 FJ764789.1 | 86 | *Idiomarina* baltica strain YCSD64 (GQ169047.1) | 81 |
| 0.7_108D | 755 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_108DA | 756 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_108E | 755 | Gamma proteobacterium. E-034 (FJ764787.1) | 99 | *Idiomarina* seosinensis strain 2PR51-17 (EU440983.1) | 92 |
| 0.7_108GA | 755 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_115AA | 790 | Halomonas sp. 4AB3 (HM587243.1) | 98 | Halomonas campisalis strain HB10.1) (GU228480.1) | 98 |
| 15_108DB | 765 | Alkalibacterium sp. E-119. (FJ764767.1) | 99 | Alkalibacterium pelagium strain T143-1-1 (NR_041574.1) | 99 |
| 15_108DC | 764 | *Bacillus* sp. T41 (AB111934.1) | 98 | *Bacillus* okhensis strain Kh10-101 (NR_043484.1) | 99 |
| 6.8_108A | 754 | Halomonas sp. 4AB3 (HM587243.1) | 100 | Halomonas campisalis strain HB10.1) (GU228480.1) | 100 |
| 6.8_108AB | 754 | Halomonas sp. 4AB3 (HM587243.1) | 100 | Halomonas campisalis strain HB10.1) (GU228480.1) | 100 |
| 6.8_108AC | 754 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8_108B | 754 | Halomonas sp. 4AB3 (HM587243.1) | 97 | Halomonas campisalis strain HB10.1) (GU228480.1) | 97 |
| 6.8_108B2.A | 766 | Uncultured *Bacillus* sp. clone (EU676874.1) | 99 | *Bacillus* pseudofirmus strain NT10I3.2AB (GQ365198.1) | 99 |
| 6.8_108BA | 754 | Halomonas sp. 4AB3 (HM587243.1) | 100 | Halomonas campisalis strain HB10.1) (GU228480.1) | 100 |
| 6.8_108BB | 754 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8_108C | 752 | Halomonadaceae bacterium GFAJ-1 (HQ449183.1) | 95 | Halomonas meridiana strain SL145 (JN645873.1) | 94 |
| 6.8_108D | 757 | Uncultured bacterium clone 101-159 (EF157253.1) | 92 | Halomonas pantelleriensis strain AAP(NR_026298.1 | 91 |
| 6.8_108DA | 765 | Alkalibacterium sp. E-119 ((FJ764767.1)) | 99 | Alkalibacterium pelagium strain T143-1-1 (NR_041574.1) | 99 |
| 6.8_108DB | 766 | Alkalibacterium sp. 10011 (EU432578.1) | 98 | Alkalibacterium psychrotolerans strain IDR2-2 (NR_024830.1) | 98 |
| 6.8_108E | 749 | Halomonas sp. N1(2011) JF937425.1 | 98 | Halomonas variabilis strain HTG7 (AY204638.1) | 97 |

*Table A.1 continued.*

| Isolate name | No. nucleotide | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| 6.8_108F | 754 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8_108G | 757 | Halomonas sp. 4AB3 (HM587243.1) | 96 | Halomonas campisalis strain HB10.1) (GU228480.1) | 96 |
| 6.8_108GA | 719 | *Planococcus* sp. MTCC 8491 JF775504.1 | 98 | *Planococcus* maritimus strain SS-06 (EU624446.1) | 98 |
| 0.7_109A | 721 | Yaniella sp. G5 (FJ871122.1) | 99 | Actinomycetales bacterium SSCS15 (AB211026.1) | 97 |
| 0.7_109AA | 721 | Halomonas sp. 4AB3 (HM587243.1) | 100 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_109AAA | 729 | Halomonas sp. 4AB3 (HM587243.1) | 88 | Halomonas campisalis strain HB10.1) (GU228480.1) | 88 |
| 0.7_109AB | 741 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 98 |
| 0.7_109B | 732 | Gamma proteobacterium E-410 ((FJ764791.1)) | 97 | *Idiomarina* seosinensis strain CL-SP19 (NR_025826.1) | 91 |
| 0.7_109D | 787 | *Planococcus* sp. MC01 (JN566126.1) | 76 | *Planococcus* maritimus strain KP8 (EU594443.1) | 76 |
| 0.7_109EB | 729 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 15_109B | 731 | Halomonas sp. 4AB3 (HM587243.1) | 95 | Halomonas campisalis strain HB10.1) (GU228480.1) | 95 |
| 15_109D | 740 | Alkalibacterium sp. E-119 (FJ764767.1) | 99 | Alkalibacterium pelagium strain T143-1-1(NR_041574.1) | 99 |
| 6.8_109AA | 740 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 99 |
| 6.8_109AB | 741 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 99 |
| 6.8_109ABC | 741 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 99 |
| 6.8_109AC | 745 | Alkalibacterium sp. E-119 (FJ764767.1) | 99 | Alkalibacterium pelagium strain T143-1-1 (NR_041574.1) | 99 |
| 6.8_109BB | 728 | Gamma proteobacterium E-410 (FJ764791.1) | 99 | *Idiomarina* sp. (JK16 EF554894.1) | 99 |
| 6.8_109C | 729 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8_109CB | 739 | Alkalibacterium sp. E-119 (FJ764767.1) | 99 | Alkalibacterium pelagium strain T143-1-1 (NR_041574.1) | 99 |
| 6.8_109E | 740 | Alkalibacterium sp. E-119 (FJ764767.1) | 100 | Alkalibacterium pelagium strain T143-1-1 (NR_041574.1) | 100 |
| 6.8_109EA | 752 | Alkalibacterium sp. E-119 (FJ764767.1) | 92 | Alkalibacterium pelagium strain T143-1-1 (NR_041574.1) | 92 |
| 6.8_109GB | 741 | Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 98 |
| 6.8_109H | 739 | Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 98 |
| 0.7_110A | 778 | Alkalibacterium sp. E-119 (FJ764767.1) | 99 | Alkalibacterium pelagium strain T143-1-1 (NR_041574.1) | 99 |
| 0.7_110AA | 772 | Hailaer soda lake bacterium T10 AF275707.1 | 99 | *Bacillus* aurantiacus, strain K1-10 (AJ605772.2) | 96 |
| 0.7_110B | 778 | *Bacillus* sp. AMnr1 FJ788526.1 | 97 | *Bacillus* arseniciselenatis, type strain DSM 15340T (AJ865469.1) | 97 |
| 0.7_110D | 764 | Halomonas sp. 4AB3 (HM587243.1) | 100 | Halomonas campisalis strain HB10.1) (GU228480.1) | 100 |

*Table A.1 continued.*

| Isolate name | No. nucleotide | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| 0.7_110DA | 765 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_110E | 777 | Alkalibacterium sp. E-119 (FJ764767.1) | 99 | Alkalibacterium pelagium strain T143-1-1 (NR_041574.1) | 99 |
| 0.7_110FA | 743 | Halomonas sp. 10022(EU432574.1 | 84 | Halomonas nitritophilus strain 5-5-12 (GU113002.1) | 84 |
| 0.7_110FB | 767 | Gamma proteobacterium E-410 (FJ764791.1) | 99 | *Idiomarina* seosinensis strain CL-SP19 (NR_025826.1) | 93 |
| 0.7_110FC | 769 | Gamma proteobacterium E-410 (FJ764791.1) | 97 | *Idiomarina* fontislapidosi strain F23 (NR_029115.1) | 92 |
| 15_110A | 709 | Halomonas sp. 15-7 HM598402.1 | 95 | Halomonas pantelleriensis strain (GQ505338.1) | 95 |
| 15_110AA | 764 | Halomonas sp. 4AB3 (HM587243.1) | 100 | Halomonas campisalis strain HB10.1) (GU228480.1) | 100 |
| 15_110AB | 763 | Halomonas sp. 4AB3 (HM587243.1) | 100 | Halomonas campisalis strain HB10.1) (GU228480.1) | 100 |
| 6.8_110A | 780 | Hailaer soda lake bacterium T10 (AF275707.1) | 99 | *Bacillus* aurantiacus strain K1-10 (AJ605772.2) | 97 |
| 6.8_110AB | 761 | Halomonas sp. E-069 (FJ764763.1) | 98 | Halomonas venusta strain NBSL25 (FJ973522.1) | 98 |
| 6.8_110B | 768 | Uncultured bacterium clone 101-159 (EF157253.1) | 96 | Halomonas pantelleriensis strain 4-5-9 (GQ505338.1) | 96 |
| 6.8_110C | 764 | Halomonas sp. 4AB3 (HM587243.1) | 100 | Halomonas campisalis strain HB10.1) (GU228480.1) | 100 |
| 6.8_110AB | 761 | Halomonas sp. E-069 (FJ764763.1) | 98 | Halomonas venusta strain NBSL25 (FJ973522.1) | 98 |
| 6.8_110B | 768 | Uncultured bacterium clone 101-159 (EF157253.1) | 96 | Halomonas pantelleriensis strain 4-5-9 (GQ505338.1) | 96 |
| 6.8_110C | 764 | Halomonas sp. 4AB3 (HM587243.1) | 100 | Halomonas campisalis strain HB10.1) (GU228480.1) | 100 |
| 6.8_110CA | 766 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8_110CB | 767 | Gamma proteobacterium E-410 (FJ764791.1) | 86 | Pseudidiomarina salinarum strain ISL-52 (NR_044246.1) | 81 |
| 6.8_110CC | 780 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 98 |
| 6.8_110D | 769 | Gamma proteobacterium E-410 (FJ764791.1) | 90 | *Idiomarina* seosinensis strain 2PR51-17 (EU440983.1) | 85 |
| 6.8_110DA | 763 | Gamma proteobacterium E-410 (FJ764791.1) | 98 | *Idiomarina* seosinensis strain 2PR51-17 (EU440983.1) | 93 |
| 6.8_110DB | 761 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8_110E | 763 | Gamma proteobacterium E-410(FJ764791.1) | 99 | *Idiomarina* seosinensis strain 2PR51-17 (EU440983.1) | 93 |
| 6.8_110EA | 769 | Pseudidiomarina sp. QA8 GQ202579.1 | 99 | *Idiomarina* seosinensis strain CL-SP19 (NR_025826.1) | 92 |
| 0.7_113B | 755 | Halomonas sp. AMP-12 HM104378.1 | 95 | Halomonas nitritophilus strain 5-5-12 (GU113002.1) | 95 |
| 15_113A | 773 | Bacillaceae bacterium halo-2(EU581836.1 | 99 | *Bacillus* agaradhaerens strain Mi10-62 (GQ121032.1) | 93 |
| 15_113B | 749 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 15_113BA | 747 | Halomonas sp. AGD 3(EU447166.1 | 98 | Halomonas campisalis strain 4A (NR_028702.1) | 97 |
| 15_113C | 748 | Halomonas sp. 4AB3 (HM587243.1) | 100 | Halomonas campisalis strain HB10.1) (GU228480.1) | 100 |

*Table A.1 continued.*

| Isolate name | No. nucleotide | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| 15_113D | 777 | Bacterial sp. Lake Natron isolate 105NT4 X92166.1 | 98 | Salsuginibacillus halophilus strain halo-1 (EU581835.1) | 98 |
| 15_113E | 741 | Gamma proteobacterium E-410 (FJ764791.1) | 94 | *Idiomarina* zobellii strain M8B (GU397405.1) | 91 |
| 6.8_113A | 758 | Alkalibacterium sp. E-119 16S (FJ764767.1) | 99 | Alkalibacterium pelagium strain T143-1-1 (NR_041574.1) | 99 |
| 6.8_113AA | 741 | Halomonas sp. W1025 (AM941396.2) | 99 | Halomonas sp. TM1S2 (EU430697.1) | 99 |
| 6.8_113B | 748 | Alkalibacterium sp. E-119 (FJ764767.1) | 99 | Alkalibacterium pelagium strain T143-1-1 (NR_041574.1) | 99 |
| 6.8_113BC | 763 | Alkalibacterium sp. 10011 (EU432578.1) | 88 | Alkalibacterium psychrotolerans strain IDR2-2(NR_024830.1 | 88 |
| 6.8_113C | 757 | Alkalibacterium sp. E-119 (FJ764767.1) | 99 | Alkalibacterium pelagium strain T143-1-1 (NR_041574.1) | 99 |
| 6.8_113DB | 742 | Uncultured bacterium clone Luq_GN490_030 (HQ445815.1) | 99 | Halomonas sp. W1025 (AM941396.2) | 99 |
| 6.8_113G | 772 | Piscibacillus sp. 401C1-1 (HM222702.1) | 94 | Piscibacillus halophilus HS224 (FM864227.1) | 94 |
| 15_114B | 810 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium sp. 10011 (EU432578.1) | 98 |
| 6.8_114A | 740 | Uncultured alpha proteobacterium clone WN-HSB-198 (DQ432305.1) | 98 | Rhodobacter sp. EL-50 strain EL-50 (NR_042212.1) | 97 |
| 6.8_114B | 807 | *Bacillus* sp. E-127 (FJ764772.1) | 98 | *Bacillus* pseudofirmus strain MC02 ((JN566125.1)) | 96 |
| 6.8_114D | 801 | Alkalibacterium sp. E-119 (FJ764767.1) | 99 | Alkalibacterium pelagium strain T143-1-1 (NR_041574.1) | 99 |
| 0.7_115A | 782 | Halomonas sp. 4AB3 (HM587243.1) | 98 | Halomonas campisalis strain HB10.1) (GU228480.1) | 98 |
| 15_115A | 801 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium sp. 10011 (EU432578.1) | 98 |
| 15_115AB | 786 | *Bacillus* sp. ANL-isoa2 (EF422410.1) | 97 | *Bacillus* arseniciselenatis 16S rRNA gene, type strain DSM 15340T (AJ865469.1) | 93 |
| 15_115B | 790 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 15_115D | 805 | *Bacillus* cellulosilyticus DSM 2522 (CP002394.1) | 96 | *Bacillus* vedderi strain JaH (NR_026526.1) | 95 |
| 15_115E | 804 | *Bacillus* cellulosilyticus DSM 2522 (CP002394.1) | 94 | *Bacillus* vedderi strain JaH (NR_026526.1) | 94 |
| 15_115EA | 806 | *Bacillus* cellulosilyticus DSM 2522 (CP002394.1) | 95 | *Bacillus* vedderi strain JaH (NR_026526.1) | 95 |

*Table A.2 Isolates from the northern end of Little Magadi, samples 106, 108, 109, 110, 113, 114 and 115 from Chapter 3, isolates are given with closest database match and closest strain match. Only those with a query coverage of >95 were included.*

| Isolate name | No. nucleotide | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| 6.8_81PC | 778 | Halomonas sp. 4AB3 (HM587243.1) | 92 | Halomonas campisalis strain HB10.1) (GU228480.1) | 92 |
| 6.8_81PA | 785 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 100 |
| 6.8_81P | 786 | Halomonas sp. 4AB3 (HM587243.1) | 98 | Halomonas campisalis strain HB10.1) (GU228480.1) | 98 |
| 6.8_81GCBB | 786 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8_81GC | 785 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8_81GB | 787 | Halomonas sp. 4AB3 (HM587243.1) | 97 | Halomonas campisalis strain HB10.1) (GU228480.1) | 97 |
| 6.8_81GA | 785 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8_81F | 801 | *Bacillus* sp. ISO_02_Chiprana (EU676882.1) | 99 | *Bacillus* clarkii strain DSM 8720 (NR_026141.1) | 99 |
| 6.8_81E | 787 | Alkalibacterium sp. 10011 (EU432578.1) | 99 | Alkalibacterium psychrotolerans strain IDR2-2 (NR_024830.1) | 99 |
| 6.8_81C | 799 | Alkalibacterium sp. 10011 (EU432578.1) | 99 | Alkalibacterium psychrotolerans strain IDR2-2 (NR_024830.1) | 99 |
| 6.8_81B | 797 | *Bacillus* sp. B-3(2011) (N128828.1) | 85 | *Bacillus* cohnii strain D7055 (FJ161352.1) | 85 |
| 6.8_81A | 800 | *Bacillus* sp. ISO_02_Chiprana (EU676882.1) | 99 | *Bacillus* polygoni strain YN-1 (NR_041571.1) | 99 |
| 15_81PC | 785 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 15_81GB | 785 | Halomonas sp. HI.br (GU228482.1) | 99 | Halomonas nitritophilus strain 5-5-12 (GU113002.1) | 99 |
| 0.7_81PE | 785 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_81PC | 786 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_81PB | 785 | Halomonas sp. 4AB3 (HM587243.1) | 98 | Halomonas campisalis strain HB10.1) (GU228480.1) | 98 |
| 0.7_81PA | 788 | Alkalibacterium sp. E-119 (FJ764767.1) | 99 | Alkalibacterium pelagium strain T143-1-1 (NR_04 1574.1 | 99 |
| 0.7_81GBA | 788 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_81GB | 782 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_81GAA | 790 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_81GA | 793 | Alkalibacterium sp. 10011 (EU432578.1 | 98 | Alkalibacterium psychrotolerans strain IDR2-2 | 98 |
| 6.8_82D | 795 | Unidentified Hailaer soda lake bacterium | 99 | Alkalibacterium psychrotolerans strain JCM 12281 | 99 |
| 6.8_82C | 777 | Bacterial sp. 16S rRNA gene (Lake Bogoria isolate | 98 | Halomonas kenyensis strain AIR-2 (NR_043299.1 | 98 |
| 6.8_82BB | 778 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8_82BA | 775 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |

| Isolate name | No. nucleotide | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| 6.8_82B | 769 | Uncultured bacterium clone MB-A2-149 (AY093468.1) | 95 | Pseudidiomarina salinarum strain ISL-52 (NR_044246.1) | 92 |
| 6.8_82AB | 798 | Uncultured Sporolactobacillaceae bacterium clone Cat004C_E03 (EU572326.1) | 97 | *Bacillus* pseudofirmus strain MC02 (JN566125.1) | 97 |
| 6.8_82A | 770 | Uncultured bacterium clone MB-A2-149 | 96 | Pseudidiomarina salinarum strain ISL-52 (NR_044246.1) | 92 |
| 15_82B | 775 | Halomonas sp. 4AB3 (HM587243.1) | 96 | Halomonas campisalis strain HB10.1) (GU228480.1) | 96 |
| 15_82A | 767 | *Bacillus* sp. GSP69 (AY553128.1) | 96 | *Bacillus* hemicellulosilyticus strain C-11 (NR_040848.1) | 96 |
| 15_82AB | 835 | *Bacillus* sp. AMnr1 (FJ788526.1) | 98 | *Bacillus* arseniciselenatis, type strain DSM 15340T | 98 |
| 0.7_82H | 775 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_82G | 775 | Halomonas sp. 4AB3 (HM587243.1) | 100 | Halomonas campisalis strain HB10.1) (GU228480.1) | 100 |
| 0.7_82F | 798 | Uncultured bacterium clone LCKS000B19 Uncultured bacterium clone (LCKS000B19) | 97 | *Bacillus* vedderi strain JaH (NR_026526.1) | 97 |
| 0.7_82E | 775 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_82D | 786 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_82BA | 776 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_82B | 777 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_82AC | 788 | Halomonas sp. 4AB3 (HM587243.1) | 98 | Halomonas campisalis strain HB10.1) (GU228480.1) | 98 |
| 0.7_82AB | 782 | Halomonas sp. 4AB3 (HM587243.1) | 97 | Halomonas campisalis strain HB10.1) (GU228480.1) | 97 |
| 0.7_82A | 783 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8_83BB | 687 | Hailaer soda lake bacterium F24 (AF275702.1) | 84 | Alkalibacterium psychrotolerans strain JCM 12281 | 84 |
| 0.7_83H | 747 | Bacterial sp. 16S rRNA gene (Lake Nakuru isolate | 94 | Pseudidiomarina salinarum strain ISL-52 (NR_044246.1) | 92 |
| 0.7_83G | 752 | Alkalibacterium sp. E-119 (FJ764767.1) | 99 | Alkalibacterium pelagium strain T143-1-1 (NR_04 1574.1) | 99 |
| 0.7_83E | 756 | Marine sponge bacterium PLATErafhis+(1)- | 96 | *Bacillus* arseniciselenatis DSM 15340T (AJ865469.1) | 95 |
| 0.7_83DAB | 747 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_83D | 741 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_83CB | 752 | Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 98 |
| 0.7_83CA | 754 | *Bacillus* sp. E-127 (FJ764772.1) | 98 | *Bacillus* pseudofirmus strain MC02 (JN566125.1) | 96 |
| 0.7_83C | 754 | *Bacillus* sp. ISO_06_Kulunda (EU676884.1) | 99 | *Bacillus* alkalinitrilicus strain ANL-iso4 (NR_044204.1) | 98 |
| 0.7_83B | 744 | Halomonas sp. 4AB3 (HM587243.1) | 100 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_83AD | 745 | Halomonas sp. 4AB3 (HM587243.1) | 100 | Halomonas campisalis strain HB10.1) (GU228480.1) | 100 |

| Isolate name | No. nucleotide | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| 0.7_83A | 754 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 98 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 98 |
| 6.8_85E | 808 | *Bacillus* sp. A-11 (AY347312.1) | 98 | *Bacillus* sp. LW6 (FM956481.1) | 98 |
| 6.8_85DB | 807 | *Bacillus* sp. E-127 (FJ764772.1) | 98 | *Bacillus* pseudofirmus strain MC02 (JN566125.1) | 96 |
| 6.8_85D | 676 | *Bacillus* sp. E-127 (FJ764772.1) | 98 | *Bacillus* pseudofirmus strain MC02 (JN566125.1) | 95 |
| 6.8_83C | 755 | Halolactibacillus miurensis, NRIC 0637 | 94 | Halolactibacillus xiariensis strain H-5 16S (NR_044282.1) | 96 |
| 0.7_85A | 833 | *Bacillus* sp. ISO_06_Kulunda (EU676884.1) | 99 | *Bacillus* alkalinitrilicus strain ANL-iso4 (NR_044204.1) | 97 |
| 6.8 _86C | 775 | Gamma proteobacterium E-034. (FJ764787.1) | 99 | *Idiomarina seosinensis* CL-SP19. (NR_025826.1) | 92 |
| 6.8_86DB | 800 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 98 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 97 |
| 6.8_86BA | 786 | Halomonas sp. 4AB3 (HM587243.1) | 95 | Halomonas campisalis strain HB10.1) (GU228480.1) | 95 |
| 6.8_86B | 787 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8_86A | 792 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 99 |
| 15_86B | 785 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_86H | 797 | *Bacillus* sp. B-3(2011) JN128828.1 | 85 | *Bacillus* cohnii strain D7055 (FJ161352.1) | 85 |
| 0.7_86G | 798 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 98 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 98 |
| 0.7_86F | 797 | *Bacillus* sp. B-3(2011) (JN128828.1) | 92 | *Bacillus* cohnii strain D7055 (FJ161352.1) | 92 |
| 0.7_86E | 787 | Halomonas sp. HB.br (GU228481.1) | 97 | Halomonas campisalis strain 4-5-8 (GU112958.1) | 97 |
| 0.7_86D | 796 | *Bacillus* sp. DV9-38 (GQ407183.1) | 99 | *Bacillus* cohnii strain T-46 (HQ202864.1) | 99 |
| 0.7_86CB | 798 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 100 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 100 |
| 0.7_86C | 801 | Uncultured bacterium clone LCKS000B19 EF201718.1 | 97 | *Bacillus* vedderi strain JaH (NR_026526.1) | 97 |
| 0.7_86B | 799 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 98 |
| 0.7_86A | 798 | *Bacillus* sp. ISO_06_Kulunda (EU676884.1) | 94 | *Bacillus* alkalinitrilicus strain ANL-iso4 (NR_044204.1) | 93 |
| 6.8_94E | 796 | Halomonas sp. 4AB3 (HM587243.1) | 98 | Halomonas campisalis strain HB10.1) (GU228480.1) | 98 |
| 6.8_94D | 801 | Uncultured bacterium clone 101- 159 EF 157253.1) | 94 | Halomonas pantelleriensis strain AAP (NR_026298.1 | 94 |

| Isolate name | No. nucleotide | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| 6.8_94CB | 785 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 98 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 98 |
| 6.8_94CA | 798 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 98 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 98 |
| 6.8_94C2B | 809 | Bacillaceae bacterium halo-2(EU581836.1) | 99 | *Bacillus* sp. CG2 16S rRNA gene, strain CG2 FR714931.1 | 99 |
| 6.8_94C | 787 | Alkalibacterium sp. 10011 (EU432578.1) | 99 | Alkalibacterium psychrotolerans strain IDR2-2 | 99 |
| 6.8_94BD | 800 | *Bacillus* sp. ISO_06_Kulunda(EU676884.1) | 99 | *Bacillus* alkalinitrilicus strain ANL-iso4 (NR_044204.1) | 99 |
| 6.8_94BC | 792 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8_94B | 793 | *Idiomarina* sp. JK38_EF554896.1) | 98 | Pseudidiomarina salinarum strain ISL-52 (NR_044246.1) | 92 |
| 6.8_94AD | 793 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8_94A | 785 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 99 |
| 0.7_94H | 795 | Gamma proteobacterium E-034 (FJ764787.1) | 99 | *Idiomarina* seosinensis strain 2PR51-17(EU440983.1) | 92 |
| 0.7_94G | 792 | Gamma proteobacterium E-410 (FJ764791.1) | 99 | *Idiomarina* seosinensis strain CL-SP19 (NR_025826.1) | 93 |
| 0.7_94F | 794 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_94D | 809 | Bacillaceae bacterium halo-2 (EU581836.1) | 99 | *Bacillus* locisalis. CG2 16S rRNA gene, strain CG2 FR714931.1 | 98 |
| 0.7_94CC | 792 | Halomonas sp. 4AB3 (HM587243.1) | 97 | Halomonas campisalis strain HB10.1) (GU228480.1) | 97 |
| 0.7_94CB | 799 | Halomonas sp. 4AB3 (HM587243.1) | 97 | Halomonas campisalis strain HB10.1) (GU228480.1) | 97 |
| 0.7_94C | 792 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 0.7_94BC | 807 | *Bacillus* sp. ISO_06_Kulunda (EU676884.1) | 98 | *Bacillus* alkalinitrilicus strain ANL-iso4 (NR_044204.1 | 97 |
| 0.7_94BBA | 807 | *Bacillus* cellulosilyticus DSM 2522 CP002394.1) | 97 | *Bacillus* vedderi strain JaH (NR_026526.1) | 96 |
| 0.7_94BB | 777 | Uncultured bacterium clone | 99 | Dietzia cinnamea strain :IMMIB RIV-399 (NR_042390.1) | 99 |
| 0.7_94B | 804 | Uncultured bacterium clone ncd2190g02c1_JF190672.1 | 99 | *Bacillus* okhensis strain Kh10-101 (NR_043484.1) | 100 |
| 0.7_94AB | 707 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 94 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 94 |
| 0.7_94A | 810 | Bacillaceae bacterium halo-2(EU581836.1 | 99 | *Bacillus* sp. CG2 16S rRNA gene, strain CG2 (FR714931.1) | 99 |
| | | | | | |

*Table A.3 Isolates from Western Lagoon, samples 70 and 72.2 from Chapter 3, isolates are given with closest database match and closest strain match. Only those with a query coverage of >95 were included.*

| Isolate name | No. nucleotide | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| LS_70A | 842 | Gamma proteobacterium E-410 FJ764791.1 | 99 | *Idiomarina* seosinensis strain CL-SP19 (NR_025826.1 | *94* |
| LS_70A | 808 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | *98* |
| LS_70B | 841 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | *99* |
| LS_70D | 842 | Uncultured bacterium clone 46-SI5 HQ116731.1 | 95 | Dietzia maris strain: SSCS4 AB211032.1 | *95* |
| 6.8 _70A | 838 | Alkalibacterium sp. 10011(EU432578.1 | 99 | Alkalibacterium psychrotolerans strain IDR2- (NR_024830.1) | *99* |
| 6.8 _70B | 808 | Alkalibacterium sp. 10011(EU432578.1 | 99 | Alkalibacterium psychrotolerans strain IDR2- (NR_024830.1) | *98* |
| 6.8 _70C | 840 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | *99* |
| 6.8 70D | 831 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | *99* |
| 15 _70A | 807 | Halomonas sp. 4AB3 (HM587243.1) | 97 | Halomonas campisalis strain HB10.1) (GU228480.1) | *97* |
| LS_72.2H | 816 | Halomonas sp. 4AB3 (HM587243.1) | 93 | Halomonas campisalis strain HB10.1) (GU228480.1) | *93* |
| LS_72.2B | 831 | Gamma proteobacterium E-034 FJ764787.1 | 99 | Pseudidiomarina sp. AK5 strain FN995239.1 | *93* |
| LS_72.2AB | 809 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | *99* |
| 6.8 _72.2D | 807 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | *99* |
| 6.8 _72.2B | 809 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | *99* |
| 15 _72.2A | 840 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | *99* |
| 15 _72.2D | 840 | - | - | *Caldalkalibacillus* uzonensis strain JW/WZ-YB58 | *92* |
| 15 _72.2B | 839 | Uncultured bacterium clone 101-159 EF157253.1 | 95 | Halomonas pantelleriensis strain AAP (NR_026298.1 | *94* |

*Table A.4 Isolates from Western Lagoon, samples 1, 2, 58, 60, 61, 63, 74, 76, 123, 124, 125. 133 from Chapter 3, isolates are given with closest database match and closest strain match. Only those with a query coverage of >95 were included.*

| Isolate name | No. nucleotide | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| LS_1-4O | 828 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_1-4L | 841 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_1-4H | 826 | Uncultured *Bacillus* sp. clone ENR_before_Kulunda(EU676874.1 | 99 | *Bacillus* pseudofirmus strain Mn6(EU315248.1 | 99 |
| LS_1-4G | 826 | Uncultured bacterium clone 101-159 EF157253.1 | 94 | Halomonas pantelleriensis strain AAP (NR_026298.1 | 94 |
| LS_1-4E | 834 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_1-4BC | 834 | Uncultured bacterium clone HS-LN4B FJ536456.1 | 98 | *Bacillus* hemicellulosilyticus strain C-11 (NR_040848.1 | 95 |
| LS_1-4BA | 809 | Halomonas sp. 4AB3 (HM587243.1) | 98 | Halomonas campisalis strain HB10.1) (GU228480.1) | 98 |
| LS_1-4B | 839 | Bacterial sp. gene (Lake Bogoria isolate WB4) | 98 | Halomonas kenyensis strain AIR-2 (NR_043299.1 | 99 |
| LS_1-4A | 833 | Halomonas sp. HB.br GU228481.1 | 99 | Halomonas sp. IB-G4 partial 16S rRNA gene, strain IB-G4 AM490139.1 | 99 |
| LS_1-1E | 836 | Halomonas sp. 4AB3 (HM587243.1) | 98 | Halomonas campisalis strain HB10.1) (GU228480.1) | 98 |
| 6.8 _1-4L | 816 | Alkalispirillum sp. ACO1 FJ976677.1 | 99 | Alkalispirillum mobile strain DSM 12769 (NR_024961.1 | 99 |
| 6.8 _1-4K | 809 | *Bacillus* sp. ISO_02_Chiprana(EU676882.1 | 97 | *Bacillus* clarkii strain DSM 8720 (NR_026141.1 | 97 |
| 6.8 _1-4J | 840 | *Bacillus* sp. ISO_02_Chiprana(EU676882.1 | 96 | *Bacillus* polygoni strain YN-1 (NR_041571.1 | 96 |
| 6.8 _1-4F | 810 | Halomonas sp. HB.br GU228481.1 | 96 | Halomonas sp. IB-G4 partial 16S rRNA gene, strain IB-G4 AM490139.1 | 96 |
| 6.8 _1-4E | 809 | Halomonas sp. AGD 3(EU447166.1 | 98 | Halomonas campisalis strain 4A (NR_028702.1 | 97 |
| 6.8 _1-4A | 839 | Bacillaceae bacterium halo-2(EU581836.1 | 95 | *Bacillus* sp. CG2 16S rRNA gene, strain CG2 FR714931.1 | 94 |
| 6.8 _1-3H | 818 | Uncultured *Bacillus* sp. clone ENR_before_Kulunda(EU676874.1 | 99 | *Bacillus* pseudofirmus strain Mn6(EU315248.1 | 99 |
| 6.8 _1-3G | 831 | Uncultured bacterium clone ncd1610f11c1 JF138980.1 | 99 | Halomonas sp. F22171 AM941395.3 | 99 |
| 6.8 _1-3F | 806 | Halomonas sp. 4AB3 (HM587243.1) | 98 | Halomonas campisalis strain HB10.1) (GU228480.1) | 98 |
| 6.8 _1-3C | 822 | Uncultured bacterium clone MB-A2-149 AY093468.1 | 96 | Pseudidiomarina sp. AK5 FN995239.1 | 95 |
| 6.8 _1-3A | 840 | Uncultured bacterium clone 101-159 EF157253.1 | 93 | Halomonas pantelleriensis strain AAP (NR_026298.1 | 92 |
| 6.8 1-4I | 836 | *Idiomarina* sp. JK38 EF554896.1 | 99 | Pseudidiomarina sp. AK5 FN995239.1 | 99 |

*Table A.4 Continued.*

| Isolate name | No. nucleotide | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| 6.8 1-4D | 826 | Halomonas sp. HB.br GU228481.1 | 99 | Halomonas sp. IB-G4, strain IB-G4 AM490139.1 | 99 |
| 6.8 1-4A | 815 | *Bacillus* sp. ISO_02_Chiprana(EU676882.1 | 97 | *Bacillus* polygoni strain YN-1 (NR_041571.1 | 97 |
| 6.8 1-3B | 811 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 15 _1-3U | 805 | *Bacillus* sp. Zby6 GU583650.1 | 91 | *Bacillus* vedderi strain JaH (NR_026526.1 | 91 |
| 15 _1-3T | 807 | *Bacillus* clarkii strain DSM 8720 (NR_026141.1 | 88 | *Bacillus* polygoni strain YN-1 (NR_041571.1 | 88 |
| 15 _1-3N | 840 | Halomonas sp. 4AB3 (HM587243.1) | 96 | Halomonas campisalis strain HB10.1) (GU228480.1) | 96 |
| 15 _1-3M | 838 | Uncultured bacterium clone nbw287f02c1 GQ086265.1 | 95 | *Bacillus* agaradhaerens strain Mi10-62 GQ121032.1 | 95 |
| 15 _1-3L | 819 | *Bacillus* cellulosilyticus DSM 2522 CP002394.1 | 93 | *Bacillus* vedderi strain JaH (NR_026526.1 | 93 |
| 15 _1-3I | 818 | Alkalispirillum sp. ACO1 FJ976677.1 | 99 | Alkalispirillum mobile strain DSM 12769 (NR_024961.1 | 99 |
| 15 _1-3H | 811 | *Bacillus* sp. X10-7 HM598405.1 | 99 | *Bacillus* saliphilus strain 6AG (NR_025554.1 | 96 |
| 15 _1-3GA | 819 | Halomonas sp. 4AB3 (HM587243.1) | 94 | Halomonas campisalis strain HB10.1) (GU228480.1) | 94 |
| 15 _1-3G | 839 | Halomonas sp. AGD 3(EU447166.1 | 93 | Halomonas campisalis strain 4A (NR_028702.1 | 93 |
| 15 _1-3F | 807 | *Bacillus* cellulosilyticus DSM 2522 CP002394.1 | 96 | *Bacillus* vedderi strain JaH (NR_026526.1 | 95 |
| 15 _1-3D | 806 | *Bacillus* cellulosilyticus DSM 2522 CP002394.1 | 96 | *Bacillus* vedderi strain JaH (NR_026526.1 | 95 |
| 15 _1-3A | 808 | Bacillaceae bacterium halo-2(EU581836.1 | 99 | Bacterial sp. 16S rRNA gene (Lake Elementaita isolate WE1) X92164.1 | 99 |
| LS_2-3P | 809 | Uncultured bacterium gene for 16S rRNA, partial sequence, clone: SSmAB09-18 AB176371.1 | | *Bacillus* alkalinitrilicus strain ANL-iso4 (NR_044204.1 | 98 |
| LS_2-3N | 832 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_2-3L | 831 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_2-3K | 831 | Bacterial sp. 16S rRNA gene (Lake Nakuru isolate 20N1 X92128.1 | 96 | Pseudidiomarina sp. AK5 FN995239.1 | 94 |
| LS_2-3F | 842 | Bacillaceae bacterium halo-2(EU581836.1 | 99 | Bacterial sp. 16S rRNA gene (Lake Elementaita isolate WE1) X92164.1 | 99 |
| LS_2-3C | 833 | Gamma proteobacterium E-410 FJ764791.1 | 99 | *Idiomarina* seosinensis strain CL-SP19 (NR_025826.1 | 93 |
| LS_2-3B | 800 | *Bacillus* cellulosilyticus DSM 2522, complete genome CP002394.1 | 97 | *Bacillus* cellulosilyticus DSM 2522 strain (NR_040850.1 | 97 |

*Table A.4 Continued.*

| Isolate name | No. nucleotide | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| LS_2-3A | 940 | *Bacillus* sp. ISO_02_Chiprana(EU676882.1 | 96 | *Bacillus* polygoni strain YN-1 (NR_041571.1 | 96 |
| LS 2-3M | 843 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS 1-4A | 819 | *Bacillus* selenitireducens MLS10 CP001791.1 | 99 | *Bacillus* selenitireducens strain M1S4-7 GU112950.1 | 97 |
| 6.8 _2-3C | 843 | Uncultured bacterium clone LCKS000B19 EF201718.1 | 97 | *Bacillus* vedderi strain JaH (NR_026526.1 | 98 |
| 6.8 _2-3B | 810 | *Bacillus* sp. Zby6 GU583650.1 | 96 | *Bacillus* vedderi strain JaH (NR_026526.1 | 94 |
| 6.8 2-3A | 824 | Uncultured bacterium clone HS-LN4B FJ536456.1 | 98 | *Bacillus* hemicellulosilyticus strain C-11 (NR_040848.1 | 95 |
| 15 2-3D | 840 | Halomonas sp. HB.br GU228481.1 | 94 | Halomonas campisalis strain HB10.1) (GU228480.1) | 94 |
| 15 _2-3C | 835 | Halomonas sp. HB.br GU228481.1 | 99 | Halomonas sp. IB-G4 partial 16S rRNA gene, strain IB-G4 AM490139.1 | 99 |
| 15 2-3D | 830 | *Bacillus* sp. AMnr1 FJ788526.1 | 98 | *Bacillus* arseniciselenatis type strain DSM 15340T AJ865469.1 | 98 |
| 15 2-3B | 827 | *Bacillus* sp. AMnr1 FJ788526.1 | 98 | *Bacillus* arseniciselenatis 16S rRNA gene, type strain DSM 15340T AJ865469.1 | 98 |
| LS_58H | 839 | Uncultured bacterium clone LCKS000B19 EF201718.1 | 97 | *Bacillus* vedderi strain JaH (NR_026526.1 | 99 |
| LS_58GB | 800 | Halomonas sp. AGD 3(EU447166.1 | 99 | Halomonas campisalis strain 4A (NR_028702.1 | 99 |
| LS_58D | 842 | Halomonas sp. AGD 3(EU447166.1 | 99 | Halomonas campisalis strain 4A (NR_028702.1 | 99 |
| LS_58C | 841 | *Bacillus* sp. DV9-38 GQ407183.1 | 99 | *Bacillus* cohnii strain T-46 HQ202864.1 | 99 |
| LS_58B | 839 | Pseudidiomarina sp. QA8 GQ202579.1 | 99 | Pseudidiomarina sp. AK5 FN995239.1 | 95 |
| 6.8 _58F | 850 | Gamma proteobacterium E-034 FJ764787.1 | 99 | Pseudidiomarina sp. AK5 FN995239.1 | 96 |
| 6.8 _58E | 844 | Uncultured bacterium clone 101-159 EF157253.1 | 98 | Halomonas pantelleriensis strain AAP (NR_026298.1 | 97 |
| 6.8 _58D | 841 | Pseudidiomarina sp. QA8 GQ202579.1 | 99 | Pseudidiomarina sp. AK5 FN995239.1 | 93 |
| 6.8 _58C | 834 | Halomonas sp. AGD 3(EU447166.1 | 91 | Halomonas campisalis strain 4A (NR_028702.1 | 91 |
| 6.8 _58B | 829 | Halomonadaceae bacterium GFAJ-1 HQ449183.1 | 89 | Halomonas taeheungii strain M112P1-7 GU112966.1 | 89 |
| 6.8 _58A | 840 | Uncultured bacterium clone nbw287f02c1 GQ086265.1 | 94 | *Bacillus* clarkii strain DSM 8720 (NR_026141.1 | 94 |
| LS_60H | 840 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_60D | 809 | Halomonas sp. AGD 3(EU447166.1 | 99 | Halomonas campisalis strain 4A (NR_028702.1 | 99 |

*Table A.4 Continued.*

| Isolate name | Sequence length | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| LS_60B | 839 | 9 Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans (AB294176.1) | 99 |
| LS_60A | 810 | 9 Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans (AB294176.1) | 99 |
| 6.8 _60L | 809 | 9Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans (AB294176.1) | 99 |
| 6.8 _60F | 836 | 9Hailaer soda lake bacterium F24 (AF275702.1) | 95 | Alkalibacterium psychrotolerans (AB294176.1) | 95 |
| 6.8 _60E | 834 | 9Hailaer soda lake bacterium F24 (AF275702.1) | 98 | Alkalibacterium psychrotolerans (AB294176.1) | 98 |
| 6.8 _60D | 836 | Uncultured bacterium clone 101-159 EF157253.1 | 95 | Halomonas pantelleriensis strain AAP (NR_026298.1 | 94 |
| 6.8 _60C | 839 | Piscibacillus sp. W9B(2010) HQ433445.2 | 95 | Piscibacillus sp. HS224 partial 16S rRNA gene, strain HS224 FM864227.1 | 95 |
| 6.8 _60B | 808 | 9Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans (AB294176.1) | 99 |
| 6.8 _60AB | 840 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8 _60A | 838 | Halomonas sp. AGD 3(EU447166.1 | 99 | Halomonas campisalis strain 4A (NR_028702.1 | 99 |
| 15 _60B | 828 | Halomonas sp. AGD 3(EU447166.1 | 99 | Halomonas campisalis strain 4A (NR_028702.1 | 99 |
| LS_61PD | 777 | Halomonas sp. 4AB3 (HM587243.1) | 100 | Halomonas campisalis strain HB10.1) (GU228480.1) | 100 |
| LS_61PB | 776 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_61PAB | 779 | Halomonas sp. HB.br 16S GU228481.1 | 99 | Halomonas campisalis strain 4-5-8 GU112958.1 | 99 |
| LS_61PA | 789 | *Bacillus* selenitireducens MLS10 CP001791.1 | 99 | *Bacillus* selenitireducens strain M1S6-17 GU112951.1 | 98 |
| LS_61P | 778 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_61GCC | 776 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_61GCB | 776 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_61GC | 778 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_61GB | 776 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_61GA | 775 | Halomonas sp. 4AB3 (HM587243.1) | 98 | Halomonas campisalis strain HB10.1) (GU228480.1) | 98 |
| LS_61G | 787 | Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans (AB294176.1) | 99 |
| LS_61F | 780 | - | - | Salinicoccus alkaliphilus strain X2B GU397409.2 | 99 |
| LS_61E | 768 | Yaniella sp. G5 FJ871122.1 | 99 | Actinomycetales bacterium SSCS15 AB211026.1 | 98 |
| LS_61D | 778 | Gamma proteobacterium E-034 FJ764787.1 | 99 | Pseudidiomarina sp. AK5 FN995239.1 | 94 |
| LS_61C | 786 | *Bacillus* sp. NBRC 101221 AB681407.1 | 99 | *Bacillus* akibai strain 1139 (NR_028620.1 | 99 |

*Table A.4 Continued.*

| Isolate name | Sequence length | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| LS_61B | 791 | *Bacillus* cellulosilyticus DSM 2522 CP002394.1 | 98 | *Bacillus* vedderi strain JaH (NR_026526.1 | 97 |
| LS_61A | 789 | Uncultured bacterium clone HS-LN4B FJ536456.1 | 98 | *Bacillus* hemicellulosilyticus strain C-11 (NR_040848.1 | 95 |
| 6.8_61PD | 778 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8_61PB | 777 | Uncultured bacterium clone 101-159 EF157253.1 | 99 | Halomonas pantelleriensis strain AAP (NR_026298.1 | 98 |
| 6.8_61GD | 776 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 100 | Alkalibacterium psychrotolerans (AB294176.1) | 99 |
| 6.8_61GC | 788 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans (AB294176.1) | 99 |
| 6.8_61GC | 787 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans (AB294176.1) | 99 |
| 6.8_61GB | 776 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8_61GA | 792 | - | - | Halolactibacillus alkaliphilus NBRC 103919 AB682143.1 | 98 |
| 6.8_61E | 788 | - | - | Salinicoccus alkaliphilus strain X2B GU397409.2 | 99 |
| 6.8_61D | 776 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8_61C | 767 | Halomonadaceae bacterium GFAJ-1 HQ449183.1 | 99 | Halomonas sp. ECS-str.110 partial 16S rRNA gene, strain ECS-str.110 HE586889.1 | 98 |
| 6.8_61B | 777 | Pseudidiomarina sp. QA8 GQ202579.1 | 99 | Pseudidiomarina sp. AK5 FN995239.1 | 94 |
| 15_61B | 780 | Uncultured bacterium clone 101-159 EF157253.1 | 95 | Halomonas pantelleriensis strain 4-5-9 16S GQ505338.1 | 94 |
| LS_63D | 837 | Bacterial sp. 16S rRNA gene (Lake Elementaita isolate 2E1) X92131.1 | 98 | Alkalimonas delamerensis strain :1E1 (NR_044879.1 | 98 |
| LS_63AB | 840 | *Bacillus* sp. E-108 FJ764770.1 | 94 | Alkalimonas amylolytica strain N10 (NR_041797.1 | 93 |
| LS_63A | 829 | Halomonas sp. 4AB3 (HM587243.1) | 97 | Halomonas campisalis strain HB10.1) (GU228480.1) | 97 |
| LS_63A | 836 | *Idiomarina* sp. JK38 EF554896.1 | 99 | Pseudidiomarina sp. AK5 FN995239.1 | 96 |
| 6.8 _63F | 851 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8 _63EA | 841 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8 _63E | 810 | Halomonas sp. HB.br 16S GU228481.1 | 99 | Halomonas campisalis strain 4-5-8 GU112958.1 | 99 |
| 6.8 _63D | 839 | Uncultured clone ncd2190g02c1 JF190672.1 | 99 | *Bacillus* okhensis strain Kh10-101 (NR_043484.1 | 99 |

*Table A.4 Continued.*

| Isolate name | Sequence length | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| 6.8 _63C | 808 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8 _63B | 839 | Halomonas sp. 4AB3 (HM587243.1) | 100 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8 _63AC | 842 | Aquiflexum sp. DL6 JF812063.1 | 96 | Fontibacter sp. AK8 partial 16S rRNA gene, strain FR687204.1 | 95 |
| 6.8 _63A | 810 | Halomonas sp. HB.br 16S GU228481.1 | 99 | Halomonas sp. IB-G4 AM490139.1 | 99 |
| 15 _63A | 841 | *Bacillus* sp. NBRC 101224 AB681410.1 | 99 | *Bacillus* akibai strain 1139 (NR_028620.1 | 99 |
| LS_74D | 810 | *Idiomarina* sp. JK38 EF554896.1 | 99 | Pseudidiomarina sp. AK5 FN995239.1 | 96 |
| LS_74B | 842 | Unidentified Hailaer soda lake bacterium T1 AF275706.1 | 99 | *Bacillus* saliphilus strain 6AG (NR_025554.1 | 99 |
| LS_74A | 840 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans (AB294176.1) | 99 |
| 6.8 _74B | 806 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 87 | Alkalibacterium psychrotolerans (AB294176.1) | 87 |
| 6.8 _74A | 840 | Uncultured bacterium clone HS-LN4B FJ536456.1 | 97 | *Bacillus* hemicellulosilyticus strain C-11 (NR_040848.1 | 94 |
| 15 _74B | 839 | Aquiflexum sp. DL6 JF812063.1 | 97 | Fontibacter sp. AK8 partial 16S rRNA gene, strain FR687204.1 | 97 |
| 15 _74A | 840 | Alkalibacterium sp. 10011(EU432578.1 | 99 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 99 |
| LS_76PF | 789 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_76PE | 795 | Uncultured bacterium clone LCKS30-B24 DQ129946.1 | 82 | Halolactibacillus alkaliphilus gene for 16S rRNA, partial sequence, strain: NBRC 103919 AB682143.1 | 83 |
| LS_76PB | 793 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_76PA | 825 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_76GD | 813 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_76GC | 817 | Halomonas sp. HB.br 16S GU228481.1 | 99 | Halomonas sp. IB-G4 AM490139.1 | 99 |
| 6.8 _76PF | 805 | | | Halolactibacillus alkaliphilus gene for 16S rRNA, partial sequence, strain: NBRC 103919 AB682143.1 | 98 |

*Table A.4 Continued.*

| Isolate name | Sequence length | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| 6.8 _76PE | 811 | | | Halolactibacillus alkaliphilus gene for 16S rRNA, partial sequence, strain: NBRC 103919 AB682143.1 | 99 |
| 6.8 _76PD | 808 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8 _76PC | 808 | | | Halolactibacillus alkaliphilus gene for 16S rRNA, partial sequence, strain: NBRC 103919 AB682143.2 | 99 |
| 6.8 _76PA | 809 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8 _76GD | 808 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 98 | Alkalibacterium psychrotolerans (AB294176.1) | 98 |
| 6.8 _76GC | 809 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans (AB294176.1) | 99 |
| 6.8 _76GA | 807 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans (AB294176.1) | 99 |
| 15 _76PB | 838 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 15 _76B | 844 | Halomonas sp. 15-7 HM598402.1 | 99 | Halomonas pantelleriensis strain AAP (NR_026298.1 | 99 |
| LS_123B | 840 | Gamma proteobacterium E-410 FJ764791.1 | 99 | *Idiomarina* seosinensis strain CL-SP19 (NR_025826.1 | 94 |
| LS_123AC | 840 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_123AB | 808 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 97 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 97 |
| LS 123A | 825 | Uncultured *Bacillus* sp. clone ENR_before_Kulunda(EU676874.1 | 99 | *Bacillus* pseudofirmus strain MC02 JN566125.1 | 99 |
| 6.8 _123E | 809 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8 _123DA | 839 | Alkalibacterium sp. 10011 (EU432578.1 | 99 | Alkalibacterium psychrotolerans strain IDR2-2 (NR_024830.1) | 99 |
| 6.8 _123D | 809 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8 _123C | 839 | *Bacillus* sp. X10-7 HM598405.1 | 99 | *Bacillus* saliphilus strain (NR_025554.1 | 99 |
| 6.8 _123B | 842 | *Bacillus* sp. X10-7 HM598405.1 | 99 | *Bacillus* saliphilus strain (NR_025554.1 | 97 |
| 6.8 _123AB | 841 | *Bacillus* sp. JAEA AB437410.1 | 93 | *Bacillus* macyae strain JMM-4 (NR_025650.1 | 93 |

*Table A.4 Continued.*

| Isolate name | Sequence length | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| 6.8 _123A | 810 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 15 _123B | 809 | Halomonas sp. 4AB3 (HM587243.1) | 95 | Halomonas campisalis strain HB10.1) (GU228480.1) | 95 |
| 15 123D | 833 | Uncultured clone LCKS000B19 EF201718.1 | 98 | *Bacillus* vedderi strain JaH (NR_026526.1 | 97 |
| LS_124WB | 834 | Alkalibacterium sp. 10011 (EU432578.1 | 99 | Alkalibacterium psychrotolerans strain IDR2-2 (NR_024830.1) | 99 |
| LS_124PENA | 830 | Salsuginibacillus halophilus gene AB682229.1 | 99 | Salsuginibacillus halophilus strain halo-1(EU581835.1 | 99 |
| LS_124PEN | 840 | *Bacillus* sp. X10-7 HM598405.1 | 99 | *Bacillus* saliphilus strain (NR_025554.1 | 99 |
| LS_124PEF | 779 | Halomonas sp. AMP-12 HM104378.1 | 95 | Halomonas nitritophilus strain 5-5-12 GU113002.1 | 95 |
| LS_124PEF | 817 | Halomonas sp. AGD 3(EU447166.1 | 98 | Halomonas campisalis strain 4A (NR_028702.1 | 97 |
| LS_124PED | 828 | Alkalibacterium sp. E-119 FJ764767.1 | 99 | Alkalibacterium pelagium AB681986.1 | 99 |
| LS_124PE | 813 | Halomonas sp. 08EPH106 HM566027.1 | 96 | Halomonas nitritophilus isolate WST 3 DQ289066.1 | 9600 |
| LS_124GH | 811 | Halomonas sp. KM-1 AB477015.1 | 99 | Halomonas desiderata strain FB2 (NR_026274.1 | 98 |
| LS_124GG | 839 | Halomonas desiderata AB362300.1 | 99 | Halomonas desiderata strain FB2 (NR_026274.1 | 99 |
| LS_124GF | 808 | *Bacillus* sp. NBRC 101225 AB681411.1 | 95 | *Bacillus* akibai strain 1139 (NR_028620.1 | 94 |
| LS_124GD | 839 | Uncultured Halomonas sp. clone Plot22-2H06(EU665122.1 | 85 | Halomonas desiderata strain FB2 (NR_026274.1 | 85 |
| LS_124GB | 839 | *Bacillus* sp. ISO_06_Kulunda(EU676884.1 | 99 | *Bacillus* alkalinitrilicus strain ANL-iso4 (NR_044204.1 | 98 |
| LS_124F | 808 | *Bacillus* sp. ISO_06_Kulunda(EU676884.1 | 99 | *Bacillus* alkalinitrilicus strain ANL-iso4 (NR_044204.1 | 98 |
| LS_124D | 841 | Uncultured bacterium clone x287 GU083689.1 | 99 | Rhodobaca bogoriensis strain LBB1 (NR_025089.1 | 99 |
| LS_124C | 818 | Micrococcus sp. OS5 clone F12 GU003860.1 | 99 | Micrococcus luteus strain PCSB6 HM449702.1 | 99 |
| LS_124BA | 851 | Uncultured clone LCKS000B19 EF201718.1 | 97 | *Bacillus* vedderi strain JaH (NR_026526.1 | 97 |
| LS_124A | 838 | Halomonas sp. AGD 3(EU447166.1 | 98 | Halomonas campisalis strain 4A (NR_028702.1 | 97 |
| LS 124B | 833 | Hailaer soda lake bacterium F24 (AF275702.1) | 99 | Alkalibacterium psychrotolerans strain JCM 12281 (AB294176.1) | 99 |
| 6.8 _124F | 834 | Gamma proteobacterium E-410 FJ764791.1 | 99 | *Idiomarina* seosinensis strain CL-SP19 (NR_025826.1 | 94 |
| 6.8 _124E | 829 | Gamma proteobacterium E-410 FJ764791.1 | 96 | *Idiomarina* seosinensis strain CL-SP19 (NR_025826.1 | 91 |
| 6.8 _124D | 808 | Halomonas sp. 4AB3 (HM587243.1) | 96 | Halomonas campisalis strain HB10.1) (GU228480.1) | 96 |

*Table A.4 Continued.*

| Isolate name | Sequence length | Closest database match | % match | Closest database strain match | % match |
|---|---|---|---|---|---|
| 6.8 _124C | 845 | Uncultured bacterium clone 101-159 EF157253.1 | 99 | Halomonas pantelleriensis strain AAP (NR_026298.1 | 98 |
| 6.8 _124C | 842 | Gamma proteobacterium E-410 FJ764791.1 | 98 | *Idiomarina* seosinensis strain CL-SP19 (NR_025826.1 | 93 |
| 6.8 _124B | 845 | Halomonas sp. 15-7 HM598402.1 | 95 | Halomonas pantelleriensis strain AAP (NR_026298.1 | 94 |
| 6.8 _124A | 840 | Halomonas sp. AGD 3(EU447166.1 | 99 | Halomonas campisalis strain 4A (NR_028702.1 | 98 |
| 15 _124C | 836 | *Bacillus* selenitireducens MLS10 CP001791.1 | 99 | *Bacillus* selenitireducens strain M1S4-7 GU112950.1 | 98 |
| 15 _124B | 826 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 15 _124A | 837 | Halomonas sp. AGD 3(EU447166.1 | 98 | Halomonas campisalis strain 4A (NR_028702.1 | 97 |
| LS_125GD | 790 | Halomonas sp. 5A22 HM587244.1 | 99 | Halomonas desiderata strain FB2 (NR_026274.1 | 99 |
| LS_125C | 805 | Uncultured bacterium clone TX4CB_93 FJ152962.1 | 99 | *Idiomarina* seosinensis strain CL-SP19 (NR_025826.1 | 94 |
| LS_125A | 790 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 6.8 _125C | 809 | Alkalibacterium sp. 10011 (EU432578.1 | 99 | Alkalibacterium psychrotolerans strain IDR2-2 (NR_024830.1) | 99 |
| 6.8 _125B | 843 | Halomonas sp. AGD 3(EU447166.1 | 99 | Halomonas campisalis strain 4A (NR_028702.1 | 99 |
| 6.8 _125A | 840 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| LS_133E | 800 | Alkalibacterium sp. E-119 FJ764767.1 | 99 | Alkalibacterium pelagium AB681986.1 | 99 |
| LS_133D | 808 | Uncultured low G+C Gram-positive bacterium clone WN-HSB-264 DQ432299.1 | 97 | Amphibacillus fermentum strain Z-7984 (NR_025193.1 | 96 |
| LS_133D | 797 | Alkalibacterium sp. 10011 (EU432578.1 | 98 | Alkalibacterium psychrotolerans strain IDR2-2 (NR_024830.1) | 97 |
| LS_133C | 816 | Unidentified Hailaer soda lake bacterium F24 (AF275702.1) | 98 | Alkalibacterium psychrotolerans (AB294176.1) | 98 |
| LS_133A | 840 | - | - | Desmospora activa strain : IMMIB L-1269 (NR_042692.1 | 96 |
| 6.8 _133D | 809 | Gamma proteobacterium E-410 FJ764791.1 | 99 | *Idiomarina* seosinensis strain CL-SP19 (NR_025826.1 | 94 |
| 6.8 _133A | 808 | Halomonas sp. 4AB3 (HM587243.1) | 99 | Halomonas campisalis strain HB10.1) (GU228480.1) | 99 |
| 15 _133D | 790 | Uncultured low G+C Gram-positive bacterium clone WN-HSB-264 DQ432299.1 | 98 | Amphibacillus fermentum strain Z-7984 (NR_025193.1 | 97 |

## Appendix B

*Table B.1 Statistical analysis of growth rate for Idiomarina sp. LMLD01 at different salt concentrations*

**ANOVA**

Growth rate

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .590 | 9 | .066 | 56.030 | 3.16E-12 |
| Within Groups | .023 | 20 | .001 | | |
| Total | .613 | 29 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

GrowthrateTukey HSD

| | | | | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| Idiomarina | NaCl concentration | Mean Difference (I-J) | Std. Error | Sig. | Lower Bound | Upper Bound |
| 0.007% | 1% | -.19133* | .02792 | 4.19E-05 | -.2902 | -.0925 |
| | 2% | -.18180* | .02792 | 8.48E-05 | -.2807 | -.0829 |
| | 3% | -.19470* | .02792 | 3.28E-05 | -.2936 | -.0958 |
| | 4% | -.15293* | .02792 | .001 | -.2518 | -.0541 |
| | 5% | -.14207* | .02792 | .002 | -.2409 | -.0432 |
| | 6% | -.07837 | .02792 | .199 | -.1772 | .0205 |
| | 7% | -.01503 | .02792 | 1.000 | -.1139 | .0838 |
| | 8% | -.03198 | .02792 | .973 | -.1308 | .0669 |
| | 10% | .29367* | .02792 | 5.20E-08 | .1948 | .3925 |
| 1% | 0.007% | .19133* | .02792 | 4.19E-05 | .0925 | .2902 |
| | 2% | .00953 | .02792 | 1.000 | -.0893 | .1084 |
| | 3% | -.00337 | .02792 | 1.000 | -.1022 | .0955 |
| | 4% | .03840 | .02792 | .921 | -.0605 | .1373 |
| | 5% | .04927 | .02792 | .748 | -.0496 | .1481 |
| | 6% | .11297* | .02792 | .017 | .0141 | .2118 |
| | 7% | .17630* | .02792 | 1.28E-04 | .0774 | .2752 |
| | 8% | .15935* | .02792 | 4.68E-04 | .0605 | .2582 |
| | 10% | .48500* | .02792 | 7.15E-12 | .3861 | .5839 |
| 2% | 0.007% | .18180* | .02792 | 8.48E-05 | .0829 | .2807 |
| | 1% | -.00953 | .02792 | 1.000 | -.1084 | .0893 |
| | 3% | -.01290 | .02792 | 1.000 | -.1118 | .0860 |
| | 4% | .02887 | .02792 | .986 | -.0700 | .1277 |
| | 5% | .03973 | .02792 | .906 | -.0591 | .1386 |
| | 6% | .10343* | .02792 | .036 | .0046 | .2023 |
| | 7% | .16677* | .02792 | 2.64E-04 | .0679 | .2656 |
| | 8% | .14982* | .02792 | .001 | .0510 | .2487 |
| | 10% | .47547* | .02792 | 9.97E-12 | .3766 | .5743 |
| 3% | 0.007% | .19470* | .02792 | 3.28E-05 | .0958 | .2936 |
| | 1% | .00337 | .02792 | 1.000 | -.0955 | .1022 |
| | 2% | .01290 | .02792 | 1.000 | -.0860 | .1118 |
| | 4% | .04177 | .02792 | .879 | -.0571 | .1406 |
| | 5% | .05263 | .02792 | .678 | -.0462 | .1515 |
| | 6% | .11633* | .02792 | .013 | .0175 | .2152 |
| | 7% | .17967* | .02792 | 9.95E-05 | .0808 | .2785 |
| | 8% | .16272* | .02792 | 3.61E-04 | .0639 | .2616 |
| | 10% | .48837* | .02792 | 6.39E-12 | .3895 | .5872 |
| 4% | 0.007% | .15293* | .02792 | .001 | .0541 | .2518 |
| | 1% | -.03840 | .02792 | .921 | -.1373 | .0605 |
| | 2% | -.02887 | .02792 | .986 | -.1277 | .0700 |
| | 3% | -.04177 | .02792 | .879 | -.1406 | .0571 |
| | 5% | .01087 | .02792 | 1.000 | -.0880 | .1097 |
| | 6% | .07457 | .02792 | .249 | -.0243 | .1734 |
| | 7% | .13790* | .02792 | .002 | .0390 | .2368 |
| | 8% | .12095* | .02792 | .009 | .0221 | .2198 |
| | 10% | .44660* | .02792 | 3.01E-11 | .3477 | .5455 |

*Table B.1 Statistical analysis of growth rate and final OD for Idiomarina sp. LMLD01 at different salt concentrations continued.*

| | | | | | | |
|---|---|---|---|---|---|---|
| 5% | 0.007% | .14207* | .02792 | .002 | .0432 | .2409 |
| | 1% | -.04927 | .02792 | .748 | -.1481 | .0496 |
| | 2% | -.03973 | .02792 | .906 | -.1386 | .0591 |
| | 3% | -.05263 | .02792 | .678 | -.1515 | .0462 |
| | 4% | -.01087 | .02792 | 1.000 | -.1097 | .0880 |
| | 6% | .06370 | .02792 | .440 | -.0352 | .1626 |
| | 7% | .12703* | .02792 | .006 | .0282 | .2259 |
| | 8% | .11009* | .02792 | .022 | .0112 | .2090 |
| | 10% | .43573* | .02792 | 4.69E-11 | .3369 | .5346 |
| 6% | 0.007% | .07837 | .02792 | .199 | -.0205 | .1772 |
| | 1% | -.11297* | .02792 | .017 | -.2118 | -.0141 |
| | 2% | -.10343* | .02792 | .036 | -.2023 | -.0046 |
| | 3% | -.11633* | .02792 | .013 | -.2152 | -.0175 |
| | 4% | -.07457 | .02792 | .249 | -.1734 | .0243 |
| | 5% | -.06370 | .02792 | .440 | -.1626 | .0352 |
| | 7% | .06333 | .02792 | .447 | -.0355 | .1622 |
| | 8% | .04639 | .02792 | .803 | -.0525 | .1453 |
| | 10% | .37203* | .02792 | 8.26E-10 | .2732 | .4709 |
| 7% | 0.007% | .01503 | .02792 | 1.000 | -.0838 | .1139 |
| | 1% | -.17630* | .02792 | 1.28E-04 | -.2752 | -.0774 |
| | 2% | -.16677* | .02792 | 2.64E-04 | -.2656 | -.0679 |
| | 3% | -.17967* | .02792 | 9.95E-05 | -.2785 | -.0808 |
| | 4% | -.13790* | .02792 | .002 | -.2368 | -.0390 |
| | 5% | -.12703* | .02792 | .006 | -.2259 | -.0282 |
| | 6% | -.06333 | .02792 | .447 | -.1622 | .0355 |
| | 8% | -.01695 | .02792 | 1.000 | -.1158 | .0819 |
| | 10% | .30870* | .02792 | 2.22E-08 | .2098 | .4076 |
| 8% | 0.007% | .03198 | .02792 | .973 | -.0669 | .1308 |
| | 1% | -.15935* | .02792 | 4.68E-04 | -.2582 | -.0605 |
| | 2% | -.14982* | .02792 | .001 | -.2487 | -.0510 |
| | 3% | -.16272* | .02792 | 3.61E-04 | -.2616 | -.0639 |
| | 4% | -.12095* | .02792 | .009 | -.2198 | -.0221 |
| | 5% | -.11009* | .02792 | .022 | -.2090 | -.0112 |
| | 6% | -.04639 | .02792 | .803 | -.1453 | .0525 |
| | 7% | .01695 | .02792 | 1.000 | -.0819 | .1158 |
| | 10% | .32565* | .02792 | 8.77E-09 | .2268 | .4245 |
| 10% | 0.007% | -.29367* | .02792 | 5.20E-08 | -.3925 | -.1948 |
| | 1% | -.48500* | .02792 | 7.15E-12 | -.5839 | -.3861 |
| | 2% | -.47547* | .02792 | 9.97E-12 | -.5743 | -.3766 |
| | 3% | -.48837* | .02792 | 6.39E-12 | -.5872 | -.3895 |
| | 4% | -.44660* | .02792 | 3.01E-11 | -.5455 | -.3477 |
| | 5% | -.43573* | .02792 | 4.69E-11 | -.5346 | -.3369 |
| | 6% | -.37203* | .02792 | 8.26E-10 | -.4709 | -.2732 |
| | 7% | -.30870* | .02792 | 2.22E-08 | -.4076 | -.2098 |
| | 8% | -.32565* | .02792 | 8.77E-09 | -.4245 | -.2268 |

*. The mean difference is significant at the 0.05 level.

*Table B.2 Statistical analysis of final OD for Idiomarina sp. LMLD01 at different salt concentrations*

**ANOVA**

OD

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 15.929 | 9 | 1.770 | 26.548 | 3.21E-09 |
| Within Groups | 1.333 | 20 | .067 | | |
| Total | 17.262 | 29 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

ODTukey HSD

| | | | | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| Idiomarina sp. LMLD01 | NaCl concentration | Mean Difference (I-J) | Std. Error | Sig. | Lower Bound | Upper Bound |
| 0.007% | 1% | -1.90000* | .21082 | 6.70E-07 | -2.6465 | -1.1535 |
| | 2% | -2.28333* | .21082 | 3.16E-08 | -3.0299 | -1.5368 |
| | 3% | -2.03333* | .21082 | 2.22E-07 | -2.7799 | -1.2868 |
| | 4% | -2.60000* | .21082 | 3.29E-09 | -3.3465 | -1.8535 |
| | 5% | -2.43333* | .21082 | 1.05E-08 | -3.1799 | -1.6868 |
| | 6% | -2.30000* | .21082 | 2.79E-08 | -3.0465 | -1.5535 |
| | 7% | -2.40000* | .21082 | 1.34E-08 | -3.1465 | -1.6535 |
| | 8% | -2.63333* | .21082 | 2.62E-09 | -3.3799 | -1.8868 |
| | 10% | -2.31667* | .21082 | 2.46E-08 | -3.0632 | -1.5701 |
| 1% | 0.007% | 1.90000* | .21082 | 6.70E-07 | 1.1535 | 2.6465 |
| | 2% | -.38333 | .21082 | .718 | -1.1299 | .3632 |
| | 3% | -.13333 | .21082 | 1.000 | -.8799 | .6132 |
| | 4% | -.70000 | .21082 | .078 | -1.4465 | .0465 |
| | 5% | -.53333 | .21082 | .311 | -1.2799 | .2132 |
| | 6% | -.40000 | .21082 | .671 | -1.1465 | .3465 |
| | 7% | -.50000 | .21082 | .390 | -1.2465 | .2465 |
| | 8% | -.73333 | .21082 | .057 | -1.4799 | .0132 |
| | 10% | -.41667 | .21082 | .623 | -1.1632 | .3299 |
| 2% | 0.007% | 2.28333* | .21082 | .000 | 1.5368 | 3.0299 |
| | 1% | .38333 | .21082 | .718 | -.3632 | 1.1299 |
| | 3% | .25000 | .21082 | .966 | -.4965 | .9965 |
| | 4% | -.31667 | .21082 | .876 | -1.0632 | .4299 |
| | 5% | -.15000 | .21082 | .999 | -.8965 | .5965 |
| | 6% | -.01667 | .21082 | 1.000 | -.7632 | .7299 |
| | 7% | -.11667 | .21082 | 1.000 | -.8632 | .6299 |
| | 8% | -.35000 | .21082 | .804 | -1.0965 | .3965 |
| | 10% | -.03333 | .21082 | 1.000 | -.7799 | .7132 |
| 3% | 0.007% | 2.03333* | .21082 | 2.22E-07 | 1.2868 | 2.7799 |
| | 1% | .13333 | .21082 | 1.000 | -.6132 | .8799 |
| | 2% | -.25000 | .21082 | .966 | -.9965 | .4965 |
| | 4% | -.56667 | .21082 | .243 | -1.3132 | .1799 |
| | 5% | -.40000 | .21082 | .671 | -1.1465 | .3465 |
| | 6% | -.26667 | .21082 | .951 | -1.0132 | .4799 |
| | 7% | -.36667 | .21082 | .762 | -1.1132 | .3799 |
| | 8% | -.60000 | .21082 | .186 | -1.3465 | .1465 |
| | 10% | -.28333 | .21082 | .931 | -1.0299 | .4632 |
| 4% | 0.007% | 2.60000* | .21082 | 3.29E-09 | 1.8535 | 3.3465 |
| | 1% | .70000 | .21082 | .078 | -.0465 | 1.4465 |
| | 2% | .31667 | .21082 | .876 | -.4299 | 1.0632 |
| | 3% | .56667 | .21082 | .243 | -.1799 | 1.3132 |
| | 5% | .16667 | .21082 | .998 | -.5799 | .9132 |
| | 6% | .30000 | .21082 | .906 | -.4465 | 1.0465 |
| | 7% | .20000 | .21082 | .992 | -.5465 | .9465 |
| | 8% | -.03333 | .21082 | 1.000 | -.7799 | .7132 |
| | 10% | .28333 | .21082 | .931 | -.4632 | 1.0299 |
| 5% | 0.007% | 2.43333* | .21082 | 1.05E-08 | 1.6868 | 3.1799 |
| | 1% | .53333 | .21082 | .311 | -.2132 | 1.2799 |
| | 2% | .15000 | .21082 | .999 | -.5965 | .8965 |
| | 3% | .40000 | .21082 | .671 | -.3465 | 1.1465 |
| | 4% | -.16667 | .21082 | .998 | -.9132 | .5799 |
| | 6% | .13333 | .21082 | 1.000 | -.6132 | .8799 |
| | 7% | .03333 | .21082 | 1.000 | -.7132 | .7799 |
| | 8% | -.20000 | .21082 | .992 | -.9465 | .5465 |
| | 10% | .11667 | .21082 | 1.000 | -.6299 | .8632 |

*Table B.2 Statistical analysis of final OD for Idiomarina sp. LMLD01 at different salt concentrations*

| | | | | | | |
|---|---|---|---|---|---|---|
| 6% | 0.007% | 2.30000* | .21082 | 2.79E-08 | 1.5535 | 3.0465 |
| | 1% | .40000 | .21082 | .671 | -.3465 | 1.1465 |
| | 2% | .01667 | .21082 | 1.000 | -.7299 | .7632 |
| | 3% | .26667 | .21082 | .951 | -.4799 | 1.0132 |
| | 4% | -.30000 | .21082 | .906 | -1.0465 | .4465 |
| | 5% | -.13333 | .21082 | 1.000 | -.8799 | .6132 |
| | 7% | -.10000 | .21082 | 1.000 | -.8465 | .6465 |
| | 8% | -.33333 | .21082 | .842 | -1.0799 | .4132 |
| | 10% | -.01667 | .21082 | 1.000 | -.7632 | .7299 |
| 7% | 0.007% | 2.40000* | .21082 | 1.34E-08 | 1.6535 | 3.1465 |
| | 1% | .50000 | .21082 | .390 | -.2465 | 1.2465 |
| | 2% | .11667 | .21082 | 1.000 | -.6299 | .8632 |
| | 3% | .36667 | .21082 | .762 | -.3799 | 1.1132 |
| | 4% | -.20000 | .21082 | .992 | -.9465 | .5465 |
| | 5% | -.03333 | .21082 | 1.000 | -.7799 | .7132 |
| | 6% | .10000 | .21082 | 1.000 | -.6465 | .8465 |
| | 8% | -.23333 | .21082 | .978 | -.9799 | .5132 |
| | 10% | .08333 | .21082 | 1.000 | -.6632 | .8299 |
| 8% | 0.007% | 2.63333* | .21082 | 2.62E-09 | 1.8868 | 3.3799 |
| | 1% | .73333 | .21082 | .057 | -.0132 | 1.4799 |
| | 2% | .35000 | .21082 | .804 | -.3965 | 1.0965 |
| | 3% | .60000 | .21082 | .186 | -.1465 | 1.3465 |
| | 4% | .03333 | .21082 | 1.000 | -.7132 | .7799 |
| | 5% | .20000 | .21082 | .992 | -.5465 | .9465 |
| | 6% | .33333 | .21082 | .842 | -.4132 | 1.0799 |
| | 7% | .23333 | .21082 | .978 | -.5132 | .9799 |
| | 10% | .31667 | .21082 | .876 | -.4299 | 1.0632 |
| 10% | 0.007% | 2.31667* | .21082 | 2.46E-08 | 1.5701 | 3.0632 |
| | 1% | .41667 | .21082 | .623 | -.3299 | 1.1632 |
| | 2% | .03333 | .21082 | 1.000 | -.7132 | .7799 |
| | 3% | .28333 | .21082 | .931 | -.4632 | 1.0299 |
| | 4% | -.28333 | .21082 | .931 | -1.0299 | .4632 |
| | 5% | -.11667 | .21082 | 1.000 | -.8632 | .6299 |
| | 6% | .01667 | .21082 | 1.000 | -.7299 | .7632 |
| | 7% | -.08333 | .21082 | 1.000 | -.8299 | .6632 |
| | 8% | -.31667 | .21082 | .876 | -1.0632 | .4299 |

*. The mean difference is significant at the 0.05 level.

## Appendix C

*Table C.1. Statistical analysis of growth rate for Planococcus sp. LMLD02 at different salt concentrations*

**ANOVA**

Growthrate

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1.151 | 8 | .144 | 21.910 | 8.92E-08 |
| Within Groups | .118 | 18 | .007 | | |
| Total | 1.269 | 26 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

GrowthrateTukey HSD

| | | | | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| Planococcus sp. LMLD02 | NaCl concentration | Mean Difference (I-J) | Std. Error | Sig. | Lower Bound | Upper Bound |
| 0.007% | 1% | -.14267 | .06616 | .470 | -.3745 | .0892 |
| | 2% | .00247 | .06616 | 1.000 | -.2294 | .2343 |
| | 3% | .06973 | .06616 | .974 | -.1621 | .3016 |
| | 4% | .05810 | .06616 | .992 | -.1737 | .2899 |
| | 5% | .08097 | .06616 | .940 | -.1509 | .3128 |
| | 6% | .11503 | .06616 | .718 | -.1168 | .3469 |
| | 8% | .33310* | .06616 | 2.18E-03 | .1013 | .5649 |
| | 10% | .60303* | .06616 | 1.09E-06 | .3712 | .8349 |
| 1% | 0.007% | .14267 | .06616 | .470 | -.0892 | .3745 |
| | 2% | .14513 | .06616 | .448 | -.0867 | .3770 |
| | 3% | .21240 | .06616 | .088 | -.0194 | .4442 |
| | 4% | .20077 | .06616 | .121 | -.0311 | .4326 |
| | 5% | .22363 | .06616 | 6.36E-02 | -.0082 | .4555 |
| | 6% | .25770* | .06616 | 2.29E-02 | .0259 | .4895 |
| | 8% | .47577* | .06616 | 3.09E-05 | .2439 | .7076 |
| | 10% | .74570* | .06616 | 4.20E-08 | .5139 | .9775 |
| 2% | 0.007% | -.00247 | .06616 | 1.000 | -.2343 | .2294 |
| | 1% | -.14513 | .06616 | .448 | -.3770 | .0867 |
| | 3% | .06727 | .06616 | .979 | -.1646 | .2991 |
| | 4% | .05563 | .06616 | .994 | -.1762 | .2875 |
| | 5% | .07850 | .06616 | .949 | -.1533 | .3103 |
| | 6% | .11257 | .06616 | .739 | -.1193 | .3444 |
| | 8% | .33063* | .06616 | 2.36E-03 | .0988 | .5625 |
| | 10% | .60057* | .06616 | 1.16E-06 | .3687 | .8324 |
| 3% | 0.007% | -.06973 | .06616 | .974 | -.3016 | .1621 |
| | 1% | -.21240 | .06616 | .088 | -.4442 | .0194 |
| | 2% | -.06727 | .06616 | .979 | -.2991 | .1646 |
| | 4% | -.01163 | .06616 | 1.000 | -.2435 | .2202 |
| | 5% | .01123 | .06616 | 1.000 | -.2206 | .2431 |
| | 6% | .04530 | .06616 | .998 | -.1865 | .2771 |
| | 8% | .26337* | .06616 | 1.93E-02 | .0315 | .4952 |
| | 10% | .53330* | .06616 | 6.45E-06 | .3015 | .7651 |
| 4% | 0.007% | -.05810 | .06616 | .992 | -.2899 | .1737 |
| | 1% | -.20077 | .06616 | .121 | -.4326 | .0311 |
| | 2% | -.05563 | .06616 | .994 | -.2875 | .1762 |
| | 3% | .01163 | .06616 | 1.000 | -.2202 | .2435 |
| | 5% | .02287 | .06616 | 1.000 | -.2090 | .2547 |
| | 6% | .05693 | .06616 | .993 | -.1749 | .2888 |
| | 8% | .27500* | .06616 | 1.34E-02 | .0432 | .5068 |
| | 10% | .54493* | .06616 | 4.75E-06 | .3131 | .7768 |
| 5% | 0.007% | -.08097 | .06616 | .940 | -.3128 | .1509 |
| | 1% | -.22363 | .06616 | .064 | -.4555 | .0082 |
| | 2% | -.07850 | .06616 | .949 | -.3103 | .1533 |
| | 3% | -.01123 | .06616 | 1.000 | -.2431 | .2206 |
| | 4% | -.02287 | .06616 | 1.000 | -.2547 | .2090 |
| | 6% | .03407 | .06616 | 1.000 | -.1978 | .2659 |
| | 8% | .25213* | .06616 | 2.72E-02 | .0203 | .4840 |
| | 10% | .52207* | .06616 | 8.69E-06 | .2902 | .7539 |

*Table C.2. Statistical analysis of growth rate for Planococcus sp. LMLD02 at different salt concentrations continued*

| | | | | | | |
|---|---|---|---|---|---|---|
| 6% | 0.007% | -.11503 | .06616 | .718 | -.3469 | .1168 |
| | 1% | -.25770* | .06616 | .023 | -.4895 | -.0259 |
| | 2% | -.11257 | .06616 | .739 | -.3444 | .1193 |
| | 3% | -.04530 | .06616 | .998 | -.2771 | .1865 |
| | 4% | -.05693 | .06616 | .993 | -.2888 | .1749 |
| | 5% | -.03407 | .06616 | 1.000 | -.2659 | .1978 |
| | 8% | .21807 | .06616 | 7.46E-02 | -.0138 | .4499 |
| | 10% | .48800* | .06616 | 2.20E-05 | .2562 | .7198 |
| 8% | 0.007% | -.33310* | .06616 | 2.18E-03 | -.5649 | -.1013 |
| | 1% | -.47577* | .06616 | 3.09E-05 | -.7076 | -.2439 |
| | 2% | -.33063* | .06616 | 2.36E-03 | -.5625 | -.0988 |
| | 3% | -.26337* | .06616 | 1.93E-02 | -.4952 | -.0315 |
| | 4% | -.27500* | .06616 | 1.34E-02 | -.5068 | -.0432 |
| | 5% | -.25213* | .06616 | 2.72E-02 | -.4840 | -.0203 |
| | 6% | -.21807 | .06616 | .075 | -.4499 | .0138 |
| | 10% | .26993* | .06616 | 1.57E-02 | .0381 | .5018 |
| 10% | 0.007% | -.60303* | .06616 | 1.09E-06 | -.8349 | -.3712 |
| | 1% | -.74570* | .06616 | 4.20E-08 | -.9775 | -.5139 |
| | 2% | -.60057* | .06616 | 1.16E-06 | -.8324 | -.3687 |
| | 3% | -.53330* | .06616 | 6.45E-06 | -.7651 | -.3015 |
| | 4% | -.54493* | .06616 | 4.75E-06 | -.7768 | -.3131 |
| | 5% | -.52207* | .06616 | 8.69E-06 | -.7539 | -.2902 |
| | 6% | -.48800* | .06616 | 2.20E-05 | -.7198 | -.2562 |
| | 8% | -.26993* | .06616 | 1.57E-02 | -.5018 | -.0381 |

*. The mean difference is significant at the 0.05 level.

*Table C.3. Statistical analysis of final OD for Planococcus sp. LMLD02 at different salt concentrations*

**ANOVA**

OD

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 17.890 | 8 | 2.236 | 71.293 | 4.75E-12 |
| Within Groups | .565 | 18 | .031 | | |
| Total | 18.454 | 26 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

ODTukey HSD

| | | | | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| Planococcus sp. LMLD02 | NaCl concentration | Mean Difference (I-J) | Std. Error | Sig. | Lower Bound | Upper Bound |
| 0.007% | 1% | -.13333 | .14461 | .988 | -.6400 | .3733 |
| | 2% | -.18333 | .14461 | .928 | -.6900 | .3233 |
| | 3% | -.13333 | .14461 | .988 | -.6400 | .3733 |
| | 4% | -.25000 | .14461 | .723 | -.7567 | .2567 |
| | 5% | .21667 | .14461 | .842 | -.2900 | .7233 |
| | 6% | .40000 | .14461 | .193 | -.1067 | .9067 |
| | 8% | 1.50000* | .14461 | 1.54E-07 | .9933 | 2.0067 |
| | 10% | 2.19667* | .14461 | 3.26E-10 | 1.6900 | 2.7033 |
| 1% | 0.007% | .13333 | .14461 | .988 | -.3733 | .6400 |
| | 2% | -.05000 | .14461 | 1.000 | -.5567 | .4567 |
| | 3% | .00000 | .14461 | 1.000 | -.5067 | .5067 |
| | 4% | -.11667 | .14461 | .995 | -.6233 | .3900 |
| | 5% | .35000 | .14461 | .330 | -.1567 | .8567 |
| | 6% | .53333* | .14461 | 3.47E-02 | .0267 | 1.0400 |
| | 8% | 1.63333* | .14461 | 4.06E-08 | 1.1267 | 2.1400 |
| | 10% | 2.33000* | .14461 | 1.22E-10 | 1.8233 | 2.8367 |
| 2% | 0.007% | .18333 | .14461 | .928 | -.3233 | .6900 |
| | 1% | .05000 | .14461 | 1.000 | -.4567 | .5567 |
| | 3% | .05000 | .14461 | 1.000 | -.4567 | .5567 |
| | 4% | -.06667 | .14461 | 1.000 | -.5733 | .4400 |
| | 5% | .40000 | .14461 | .193 | -.1067 | .9067 |
| | 6% | .58333* | .14461 | 1.73E-02 | .0767 | 1.0900 |
| | 8% | 1.68333* | .14461 | 2.52E-08 | 1.1767 | 2.1900 |
| | 10% | 2.38000* | .14461 | 8.53E-11 | 1.8733 | 2.8867 |

*Table C.4. Statistical analysis of final OD for Planococcus sp. LMLD02 at different salt concentrations continued*

| | | | | | | |
|---|---|---|---|---|---|---|
| 3% | 0.007% | .13333 | .14461 | .988 | -.3733 | .6400 |
| | 1% | .00000 | .14461 | 1.000 | -.5067 | .5067 |
| | 2% | -.05000 | .14461 | 1.000 | -.5567 | .4567 |
| | 4% | -.11667 | .14461 | .995 | -.6233 | .3900 |
| | 5% | .35000 | .14461 | .330 | -.1567 | .8567 |
| | 6% | .53333* | .14461 | 3.47E-02 | .0267 | 1.0400 |
| | 8% | 1.63333* | .14461 | 4.06E-08 | 1.1267 | 2.1400 |
| | 10% | 2.33000* | .14461 | 1.22E-10 | 1.8233 | 2.8367 |
| 4% | 0.007% | .25000 | .14461 | .723 | -.2567 | .7567 |
| | 1% | .11667 | .14461 | .995 | -.3900 | .6233 |
| | 2% | .06667 | .14461 | 1.000 | -.4400 | .5733 |
| | 3% | .11667 | .14461 | .995 | -.3900 | .6233 |
| | 5% | .46667 | .14461 | .085 | -.0400 | .9733 |
| | 6% | .65000* | .14461 | 6.68E-03 | .1433 | 1.1567 |
| | 8% | 1.75000* | .14461 | 1.35E-08 | 1.2433 | 2.2567 |
| | 10% | 2.44667* | .14461 | 5.38E-11 | 1.9400 | 2.9533 |
| 5% | 0.007% | -.21667 | .14461 | .842 | -.7233 | .2900 |
| | 1% | -.35000 | .14461 | .330 | -.8567 | .1567 |
| | 2% | -.40000 | .14461 | .193 | -.9067 | .1067 |
| | 3% | -.35000 | .14461 | .330 | -.8567 | .1567 |
| | 4% | -.46667 | .14461 | .085 | -.9733 | .0400 |
| | 6% | .18333 | .14461 | .928 | -.3233 | .6900 |
| | 8% | 1.28333* | .14461 | 1.61E-06 | .7767 | 1.7900 |
| | 10% | 1.98000* | .14461 | 1.82E-09 | 1.4733 | 2.4867 |
| 6% | 0.007% | -.40000 | .14461 | .193 | -.9067 | .1067 |
| | 1% | -.53333* | .14461 | 3.47E-02 | -1.0400 | -.0267 |
| | 2% | -.58333* | .14461 | 1.73E-02 | -1.0900 | -.0767 |
| | 3% | -.53333* | .14461 | 3.47E-02 | -1.0400 | -.0267 |
| | 4% | -.65000* | .14461 | 6.68E-03 | -1.1567 | -.1433 |
| | 5% | -.18333 | .14461 | .928 | -.6900 | .3233 |
| | 8% | 1.10000* | .14461 | 1.44E-05 | .5933 | 1.6067 |
| | 10% | 1.79667* | .14461 | 8.86E-09 | 1.2900 | 2.3033 |
| 8% | 0.007% | -1.50000* | .14461 | 1.54E-07 | -2.0067 | -.9933 |
| | 1% | -1.63333* | .14461 | 4.06E-08 | -2.1400 | -1.1267 |
| | 2% | -1.68333* | .14461 | 2.52E-08 | -2.1900 | -1.1767 |
| | 3% | -1.63333* | .14461 | 4.06E-08 | -2.1400 | -1.1267 |
| | 4% | -1.75000* | .14461 | 1.35E-08 | -2.2567 | -1.2433 |
| | 5% | -1.28333* | .14461 | 1.61E-06 | -1.7900 | -.7767 |
| | 6% | -1.10000* | .14461 | 1.44E-05 | -1.6067 | -.5933 |
| | 10% | .69667* | .14461 | 3.42E-03 | .1900 | 1.2033 |
| 10% | 0.007% | -2.19667* | .14461 | 3.26E-10 | -2.7033 | -1.6900 |
| | 1% | -2.33000* | .14461 | 1.22E-10 | -2.8367 | -1.8233 |
| | 2% | -2.38000* | .14461 | 8.53E-11 | -2.8867 | -1.8733 |
| | 3% | -2.33000* | .14461 | 1.22E-10 | -2.8367 | -1.8233 |
| | 4% | -2.44667* | .14461 | 5.38E-11 | -2.9533 | -1.9400 |
| | 5% | -1.98000* | .14461 | 1.82E-09 | -2.4867 | -1.4733 |
| | 6% | -1.79667* | .14461 | 8.86E-09 | -2.3033 | -1.2900 |
| | 8% | -.69667* | .14461 | .003 | -1.2033 | -.1900 |

*. The mean difference is significant at the 0.05 level.

## Appendix D

*Table D.1 Statistical analysis of growth rate for Caldalkalibacillus sp. LMLD03 at different salt concentrations*

**ANOVA**

Growthrate

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .241 | 10 | .024 | 5.790 | 2.96E-04 |
| Within Groups | .092 | 22 | .004 | | |
| Total | .333 | 32 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

GrowthrateTukey HSD

| | | | | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| (I) VAR00001 | (J) VAR00001 | Mean Difference (I-J) | Std. Error | Sig. | Lower Bound | Upper Bound |
| 0.007% | 1% | -.16563 | .05270 | .119 | -.3540 | .0227 |
| | 2% | -.15797 | .05270 | .156 | -.3463 | .0304 |
| | 3% | -.17373 | .05270 | .088 | -.3621 | .0146 |
| | 4% | -.01823 | .05270 | 1.000 | -.2066 | .1701 |
| | 5% | -.10297 | .05270 | .679 | -.2913 | .0854 |
| | 6% | -.03533 | .05270 | 1.000 | -.2237 | .1530 |
| | 7% | -.07560 | .05270 | .926 | -.2640 | .1128 |
| | 8% | -.01817 | .05270 | 1.000 | -.2065 | .1702 |
| | 9% | .03877 | .05270 | .999 | -.1496 | .2271 |
| | 10% | .10567 | .05270 | .648 | -.0827 | .2940 |
| 1% | 0.007% | .16563 | .05270 | .119 | -.0227 | .3540 |
| | 2% | .00767 | .05270 | 1.000 | -.1807 | .1960 |
| | 3% | -.00810 | .05270 | 1.000 | -.1965 | .1803 |
| | 4% | .14740 | .05270 | .222 | -.0410 | .3358 |
| | 5% | .06267 | .05270 | .977 | -.1257 | .2510 |
| | 6% | .13030 | .05270 | .370 | -.0581 | .3187 |
| | 7% | .09003 | .05270 | .816 | -.0983 | .2784 |
| | 8% | .14747 | .05270 | .222 | -.0409 | .3358 |
| | 9% | .20440* | .05270 | .026 | .0160 | .3928 |
| | 10% | .27130* | .05270 | .001 | .0829 | .4597 |
| 2% | 0.007% | .15797 | .05270 | .156 | -.0304 | .3463 |
| | 1% | -.00767 | .05270 | 1.000 | -.1960 | .1807 |
| | 3% | -.01577 | .05270 | 1.000 | -.2041 | .1726 |
| | 4% | .13973 | .05270 | .282 | -.0486 | .3281 |
| | 5% | .05500 | .05270 | .991 | -.1334 | .2434 |
| | 6% | .12263 | .05270 | .451 | -.0657 | .3110 |
| | 7% | .08237 | .05270 | .881 | -.1060 | .2707 |
| | 8% | .13980 | .05270 | .281 | -.0486 | .3282 |
| | 9% | .19673* | .05270 | .036 | .0084 | .3851 |
| | 10% | .26363* | .05270 | .002 | .0753 | .4520 |
| 3% | 0.007% | .17373 | .05270 | .088 | -.0146 | .3621 |
| | 1% | .00810 | .05270 | 1.000 | -.1803 | .1965 |
| | 2% | .01577 | .05270 | 1.000 | -.1726 | .2041 |
| | 4% | .15550 | .05270 | .170 | -.0329 | .3439 |
| | 5% | .07077 | .05270 | .950 | -.1176 | .2591 |
| | 6% | .13840 | .05270 | .293 | -.0500 | .3268 |
| | 7% | .09813 | .05270 | .733 | -.0902 | .2865 |
| | 8% | .15557 | .05270 | .169 | -.0328 | .3439 |
| | 9% | .21250* | .05270 | .019 | .0241 | .4009 |
| | 10% | .27940* | .05270 | .001 | .0910 | .4678 |
| 4% | 0.007% | .01823 | .05270 | 1.000 | -.1701 | .2066 |
| | 1% | -.14740 | .05270 | .222 | -.3358 | .0410 |
| | 2% | -.13973 | .05270 | .282 | -.3281 | .0486 |
| | 3% | -.15550 | .05270 | .170 | -.3439 | .0329 |
| | 5% | -.08473 | .05270 | .863 | -.2731 | .1036 |
| | 6% | -.01710 | .05270 | 1.000 | -.2055 | .1713 |
| | 7% | -.05737 | .05270 | .988 | -.2457 | .1310 |
| | 8% | .00007 | .05270 | 1.000 | -.1883 | .1884 |
| | 9% | .05700 | .05270 | .988 | -.1314 | .2454 |
| | 10% | .12390 | .05270 | .437 | -.0645 | .3123 |

*Table D.2. Statistical analysis of growth rate for Caldalkalibacillus sp. LMLD03 at different salt concentrations continued*

| | | | | | | |
|---|---|---|---|---|---|---|
| 5% | 0.007% | .10297 | .05270 | .679 | -.0854 | .2913 |
| | 1% | -.06267 | .05270 | .977 | -.2510 | .1257 |
| | 2% | -.05500 | .05270 | .991 | -.2434 | .1334 |
| | 3% | -.07077 | .05270 | .950 | -.2591 | .1176 |
| | 4% | .08473 | .05270 | .863 | -.1036 | .2731 |
| | 6% | .06763 | .05270 | .962 | -.1207 | .2560 |
| | 7% | .02737 | .05270 | 1.000 | -.1610 | .2157 |
| | 8% | .08480 | .05270 | .862 | -.1036 | .2732 |
| | 9% | .14173 | .05270 | .265 | -.0466 | .3301 |
| | 10% | .20863* | .05270 | .022 | .0203 | .3970 |
| 6% | 0.007% | .03533 | .05270 | 1.000 | -.1530 | .2237 |
| | 1% | -.13030 | .05270 | .370 | -.3187 | .0581 |
| | 2% | -.12263 | .05270 | .451 | -.3110 | .0657 |
| | 3% | -.13840 | .05270 | .293 | -.3268 | .0500 |
| | 4% | .01710 | .05270 | 1.000 | -.1713 | .2055 |
| | 5% | -.06763 | .05270 | .962 | -.2560 | .1207 |
| | 7% | -.04027 | .05270 | .999 | -.2286 | .1481 |
| | 8% | .01717 | .05270 | 1.000 | -.1712 | .2055 |
| | 9% | .07410 | .05270 | .934 | -.1143 | .2625 |
| | 10% | .14100 | .05270 | .271 | -.0474 | .3294 |
| 7% | 0.007% | .07560 | .05270 | .926 | -.1128 | .2640 |
| | 1% | -.09003 | .05270 | .816 | -.2784 | .0983 |
| | 2% | -.08237 | .05270 | .881 | -.2707 | .1060 |
| | 3% | -.09813 | .05270 | .733 | -.2865 | .0902 |
| | 4% | .05737 | .05270 | .988 | -.1310 | .2457 |
| | 5% | -.02737 | .05270 | 1.000 | -.2157 | .1610 |
| | 6% | .04027 | .05270 | .999 | -.1481 | .2286 |
| | 8% | .05743 | .05270 | .988 | -.1309 | .2458 |
| | 9% | .11437 | .05270 | .545 | -.0740 | .3027 |
| | 10% | .18127 | .05270 | .066 | -.0071 | .3696 |
| 8% | 0.007% | .01817 | .05270 | 1.000 | -.1702 | .2065 |
| | 1% | -.14747 | .05270 | .222 | -.3358 | .0409 |
| | 2% | -.13980 | .05270 | .281 | -.3282 | .0486 |
| | 3% | -.15557 | .05270 | .169 | -.3439 | .0328 |
| | 4% | -.00007 | .05270 | 1.000 | -.1884 | .1883 |
| | 5% | -.08480 | .05270 | .862 | -.2732 | .1036 |
| | 6% | -.01717 | .05270 | 1.000 | -.2055 | .1712 |
| | 7% | -.05743 | .05270 | .988 | -.2458 | .1309 |
| | 9% | .05693 | .05270 | .988 | -.1314 | .2453 |
| | 10% | .12383 | .05270 | .438 | -.0645 | .3122 |
| 9% | 0.007% | -.03877 | .05270 | .999 | -.2271 | .1496 |
| | 1% | -.20440* | .05270 | .026 | -.3928 | -.0160 |
| | 2% | -.19673* | .05270 | .036 | -.3851 | -.0084 |
| | 3% | -.21250* | .05270 | .019 | -.4009 | -.0241 |
| | 4% | -.05700 | .05270 | .988 | -.2454 | .1314 |
| | 5% | -.14173 | .05270 | .265 | -.3301 | .0466 |
| | 6% | -.07410 | .05270 | .934 | -.2625 | .1143 |
| | 7% | -.11437 | .05270 | .545 | -.3027 | .0740 |
| | 8% | -.05693 | .05270 | .988 | -.2453 | .1314 |
| | 10% | .06690 | .05270 | .965 | -.1215 | .2553 |
| 10% | 0.007% | -.10567 | .05270 | .648 | -.2940 | .0827 |
| | 1% | -.27130* | .05270 | .001 | -.4597 | -.0829 |
| | 2% | -.26363* | .05270 | .002 | -.4520 | -.0753 |
| | 3% | -.27940* | .05270 | .001 | -.4678 | -.0910 |
| | 4% | -.12390 | .05270 | .437 | -.3123 | .0645 |
| | 5% | -.20863* | .05270 | .022 | -.3970 | -.0203 |
| | 6% | -.14100 | .05270 | .271 | -.3294 | .0474 |
| | 7% | -.18127 | .05270 | .066 | -.3696 | .0071 |
| | 8% | -.12383 | .05270 | .438 | -.3122 | .0645 |
| | 9% | -.06690 | .05270 | .965 | -.2553 | .1215 |

*. The mean difference is significant at the 0.05 level.

*Table D.3. Statistical analysis of final OD for Caldalkalibacillus sp. LMLD03 at different salt concentrations*

**ANOVA**

OD

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 10.823 | 10 | 1.082 | 10.345 | 3.27E-06 |
| Within Groups | 2.302 | 22 | .105 | | |
| Total | 13.125 | 32 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

ODTukey HSD

| | | | | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| (I) VAR00001 | (J) VAR00001 | Mean Difference (I-J) | Std. Error | Sig. | Lower Bound | Upper Bound |
| 0.007% | 1% | .16667 | .26410 | 1.000 | -.7774 | 1.1108 |
| | 2% | .53333 | .26410 | .639 | -.4108 | 1.4774 |
| | 3% | .88333 | .26410 | .080 | -.0608 | 1.8274 |
| | 4% | 1.26667* | .26410 | .003 | .3226 | 2.2108 |
| | 5% | 1.33333* | .26410 | .002 | .3892 | 2.2774 |
| | 6% | 1.46667* | .26410 | .001 | .5226 | 2.4108 |
| | 7% | 1.56667* | .26410 | 2.43E-04 | .6226 | 2.5108 |
| | 8% | 1.75000* | .26410 | 5.11E-05 | .8059 | 2.6941 |
| | 9% | 1.58333* | .26410 | 2.10E-04 | .6392 | 2.5274 |
| | 10% | 1.36667* | .26410 | .001 | .4226 | 2.3108 |
| 1% | 0.007% | -.16667 | .26410 | 1.000 | -1.1108 | .7774 |
| | 2% | .36667 | .26410 | .939 | -.5774 | 1.3108 |
| | 3% | .71667 | .26410 | .255 | -.2274 | 1.6608 |
| | 4% | 1.10000* | .26410 | .014 | .1559 | 2.0441 |
| | 5% | 1.16667* | .26410 | .008 | .2226 | 2.1108 |
| | 6% | 1.30000* | .26410 | .002 | .3559 | 2.2441 |
| | 7% | 1.40000* | .26410 | .001 | .4559 | 2.3441 |
| | 8% | 1.58333* | .26410 | 2.10E-04 | .6392 | 2.5274 |
| | 9% | 1.41667* | .26410 | .001 | .4726 | 2.3608 |
| | 10% | 1.20000* | .26410 | .006 | .2559 | 2.1441 |
| 2% | 0.007% | -.53333 | .26410 | .639 | -1.4774 | .4108 |
| | 1% | -.36667 | .26410 | .939 | -1.3108 | .5774 |
| | 3% | .35000 | .26410 | .954 | -.5941 | 1.2941 |
| | 4% | .73333 | .26410 | .230 | -.2108 | 1.6774 |
| | 5% | .80000 | .26410 | .147 | -.1441 | 1.7441 |
| | 6% | .93333 | .26410 | .054 | -.0108 | 1.8774 |
| | 7% | 1.03333* | .26410 | .024 | .0892 | 1.9774 |
| | 8% | 1.21667* | .26410 | .005 | .2726 | 2.1608 |
| | 9% | 1.05000* | .26410 | .021 | .1059 | 1.9941 |
| | 10% | .83333 | .26410 | .116 | -.1108 | 1.7774 |
| 3% | 0.007% | -.88333 | .26410 | .080 | -1.8274 | .0608 |
| | 1% | -.71667 | .26410 | .255 | -1.6608 | .2274 |
| | 2% | -.35000 | .26410 | .954 | -1.2941 | .5941 |
| | 4% | .38333 | .26410 | .921 | -.5608 | 1.3274 |
| | 5% | .45000 | .26410 | .818 | -.4941 | 1.3941 |
| | 6% | .58333 | .26410 | .522 | -.3608 | 1.5274 |
| | 7% | .68333 | .26410 | .312 | -.2608 | 1.6274 |
| | 8% | .86667 | .26410 | .091 | -.0774 | 1.8108 |
| | 9% | .70000 | .26410 | .283 | -.2441 | 1.6441 |
| | 10% | .48333 | .26410 | .752 | -.4608 | 1.4274 |
| 4% | 0.007% | -1.26667* | .26410 | .003 | -2.2108 | -.3226 |
| | 1% | -1.10000* | .26410 | .014 | -2.0441 | -.1559 |
| | 2% | -.73333 | .26410 | .230 | -1.6774 | .2108 |
| | 3% | -.38333 | .26410 | .921 | -1.3274 | .5608 |
| | 5% | .06667 | .26410 | 1.000 | -.8774 | 1.0108 |
| | 6% | .20000 | .26410 | .999 | -.7441 | 1.1441 |
| | 7% | .30000 | .26410 | .983 | -.6441 | 1.2441 |
| | 8% | .48333 | .26410 | .752 | -.4608 | 1.4274 |
| | 9% | .31667 | .26410 | .976 | -.6274 | 1.2608 |
| | 10% | .10000 | .26410 | 1.000 | -.8441 | 1.0441 |

*Table D.4. Statistical analysis of final OD for Caldalkalibacillus sp. LMLD03 at different salt concentrations continued*

| | | | | | | |
|---|---|---|---|---|---|---|
| 5% | 0.007% | -1.33333* | .26410 | .002 | -2.2774 | -.3892 |
| | 1% | -1.16667* | .26410 | .008 | -2.1108 | -.2226 |
| | 2% | -.80000 | .26410 | .147 | -1.7441 | .1441 |
| | 3% | -.45000 | .26410 | .818 | -1.3941 | .4941 |
| | 4% | -.06667 | .26410 | 1.000 | -1.0108 | .8774 |
| | 6% | .13333 | .26410 | 1.000 | -.8108 | 1.0774 |
| | 7% | .23333 | .26410 | .998 | -.7108 | 1.1774 |
| | 8% | .41667 | .26410 | .875 | -.5274 | 1.3608 |
| | 9% | .25000 | .26410 | .996 | -.6941 | 1.1941 |
| | 10% | .03333 | .26410 | 1.000 | -.9108 | .9774 |
| 6% | 0.007% | -1.46667* | .26410 | .001 | -2.4108 | -.5226 |
| | 1% | -1.30000* | .26410 | .002 | -2.2441 | -.3559 |
| | 2% | -.93333 | .26410 | .054 | -1.8774 | .0108 |
| | 3% | -.58333 | .26410 | .522 | -1.5274 | .3608 |
| | 4% | -.20000 | .26410 | .999 | -1.1441 | .7441 |
| | 5% | -.13333 | .26410 | 1.000 | -1.0774 | .8108 |
| | 7% | .10000 | .26410 | 1.000 | -.8441 | 1.0441 |
| | 8% | .28333 | .26410 | .989 | -.6608 | 1.2274 |
| | 9% | .11667 | .26410 | 1.000 | -.8274 | 1.0608 |
| | 10% | -.10000 | .26410 | 1.000 | -1.0441 | .8441 |
| 7% | 0.007% | -1.56667* | .26410 | 2.43E-04 | -2.5108 | -.6226 |
| | 1% | -1.40000* | .26410 | .001 | -2.3441 | -.4559 |
| | 2% | -1.03333* | .26410 | .024 | -1.9774 | -.0892 |
| | 3% | -.68333 | .26410 | .312 | -1.6274 | .2608 |
| | 4% | -.30000 | .26410 | .983 | -1.2441 | .6441 |
| | 5% | -.23333 | .26410 | .998 | -1.1774 | .7108 |
| | 6% | -.10000 | .26410 | 1.000 | -1.0441 | .8441 |
| | 8% | .18333 | .26410 | 1.000 | -.7608 | 1.1274 |
| | 9% | .01667 | .26410 | 1.000 | -.9274 | .9608 |
| | 10% | -.20000 | .26410 | .999 | -1.1441 | .7441 |
| 8% | 0.007% | -1.75000* | .26410 | 5.11E-05 | -2.6941 | -.8059 |
| | 1% | -1.58333* | .26410 | 2.10E-04 | -2.5274 | -.6392 |
| | 2% | -1.21667* | .26410 | .005 | -2.1608 | -.2726 |
| | 3% | -.86667 | .26410 | .091 | -1.8108 | .0774 |
| | 4% | -.48333 | .26410 | .752 | -1.4274 | .4608 |
| | 5% | -.41667 | .26410 | .875 | -1.3608 | .5274 |
| | 6% | -.28333 | .26410 | .989 | -1.2274 | .6608 |
| | 7% | -.18333 | .26410 | 1.000 | -1.1274 | .7608 |
| | 9% | -.16667 | .26410 | 1.000 | -1.1108 | .7774 |
| | 10% | -.38333 | .26410 | .921 | -1.3274 | .5608 |
| 9% | 0.007% | -1.58333* | .26410 | 2.10E-04 | -2.5274 | -.6392 |
| | 1% | -1.41667* | .26410 | .001 | -2.3608 | -.4726 |
| | 2% | -1.05000* | .26410 | .021 | -1.9941 | -.1059 |
| | 3% | -.70000 | .26410 | .283 | -1.6441 | .2441 |
| | 4% | -.31667 | .26410 | .976 | -1.2608 | .6274 |
| | 5% | -.25000 | .26410 | .996 | -1.1941 | .6941 |
| | 6% | -.11667 | .26410 | 1.000 | -1.0608 | .8274 |
| | 7% | -.01667 | .26410 | 1.000 | -.9608 | .9274 |
| | 8% | .16667 | .26410 | 1.000 | -.7774 | 1.1108 |
| | 10% | -.21667 | .26410 | .999 | -1.1608 | .7274 |
| 10% | 0.007% | -1.36667* | .26410 | .001 | -2.3108 | -.4226 |
| | 1% | -1.20000* | .26410 | .006 | -2.1441 | -.2559 |
| | 2% | -.83333 | .26410 | .116 | -1.7774 | .1108 |
| | 3% | -.48333 | .26410 | .752 | -1.4274 | .4608 |
| | 4% | -.10000 | .26410 | 1.000 | -1.0441 | .8441 |
| | 5% | -.03333 | .26410 | 1.000 | -.9774 | .9108 |
| | 6% | .10000 | .26410 | 1.000 | -.8441 | 1.0441 |
| | 7% | .20000 | .26410 | .999 | -.7441 | 1.1441 |
| | 8% | .38333 | .26410 | .921 | -.5608 | 1.3274 |
| | 9% | .21667 | .26410 | .999 | -.7274 | 1.1608 |

*. The mean difference is significant at the 0.05 level.

## Appendix E

*Table E.1 Statistical analysis of growth rate for Idiomarina sp. LMLD01 at different temperatures*

**ANOVA**

GR

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 2.580 | 6 | .430 | 40.203 | 4.88E-08 |
| Within Groups | .150 | 14 | .011 | | |
| Total | 2.730 | 20 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

GRTukey HSD

| | | | | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| Idiomarina sp. LMLD01 | Temperature | Mean Difference (I-J) | Std. Error | Sig. | Lower Bound | Upper Bound |
| 20 °C | 25 °C | -.11297 | .08444 | .824 | -.4013 | .1754 |
| | 30 °C | -.23547 | .08444 | .146 | -.5238 | .0529 |
| | 35 °C | -.31023* | .08444 | .031 | -.5986 | -.0219 |
| | 37 °C | -.52463* | .08444 | 3.53E-04 | -.8130 | -.2363 |
| | 40 °C | -.25517 | .08444 | .099 | -.5435 | .0332 |
| | 45 °C | .66403* | .08444 | 2.73E-05 | .3757 | .9524 |
| 25 °C | 20 °C | .11297 | .08444 | .824 | -.1754 | .4013 |
| | 30 °C | -.12250 | .08444 | .767 | -.4108 | .1658 |
| | 35 °C | -.19727 | .08444 | .293 | -.4856 | .0911 |
| | 37 °C | -.41167* | .08444 | .004 | -.7000 | -.1233 |
| | 40 °C | -.14220 | .08444 | .636 | -.4305 | .1461 |
| | 45 °C | .77700* | .08444 | 4.32E-06 | .4887 | 1.0653 |
| 30 °C | 20 °C | .23547 | .08444 | .146 | -.0529 | .5238 |
| | 25 °C | .12250 | .08444 | .767 | -.1658 | .4108 |
| | 35 °C | -.07477 | .08444 | .969 | -.3631 | .2136 |
| | 37 °C | -.28917* | .08444 | .049 | -.5775 | -.0008 |
| | 40 °C | -.01970 | .08444 | 1.000 | -.3080 | .2686 |
| | 45 °C | .89950* | .08444 | 7.19E-07 | .6112 | 1.1878 |
| 35 °C | 20 °C | .31023* | .08444 | .031 | .0219 | .5986 |
| | 25 °C | .19727 | .08444 | .293 | -.0911 | .4856 |
| | 30 °C | .07477 | .08444 | .969 | -.2136 | .3631 |
| | 37 °C | -.21440 | .08444 | .217 | -.5027 | .0739 |
| | 40 °C | .05507 | .08444 | .993 | -.2333 | .3434 |
| | 45 °C | .97427* | .08444 | 2.63E-07 | .6859 | 1.2626 |
| 37 °C | 20 °C | .52463* | .08444 | 3.53E-04 | .2363 | .8130 |
| | 25 °C | .41167* | .08444 | .004 | .1233 | .7000 |
| | 30 °C | .28917* | .08444 | .049 | .0008 | .5775 |
| | 35 °C | .21440 | .08444 | .217 | -.0739 | .5027 |
| | 40 °C | .26947 | .08444 | .074 | -.0189 | .5578 |
| | 45 °C | 1.18867* | .08444 | 2.03E-08 | .9003 | 1.4770 |
| 40 °C | 20 °C | .25517 | .08444 | .099 | -.0332 | .5435 |
| | 25 °C | .14220 | .08444 | .636 | -.1461 | .4305 |
| | 30 °C | .01970 | .08444 | 1.000 | -.2686 | .3080 |
| | 35 °C | -.05507 | .08444 | .993 | -.3434 | .2333 |
| | 37 °C | -.26947 | .08444 | .074 | -.5578 | .0189 |
| | 45 °C | .91920* | .08444 | 5.48E-07 | .6309 | 1.2075 |
| 45 °C | 20 °C | -.66403* | .08444 | 2.73E-05 | -.9524 | -.3757 |
| | 25 °C | -.77700* | .08444 | 4.32E-06 | -1.0653 | -.4887 |
| | 30 °C | -.89950* | .08444 | 7.19E-07 | -1.1878 | -.6112 |
| | 35 °C | -.97427* | .08444 | 2.63E-07 | -1.2626 | -.6859 |
| | 37 °C | -1.18867* | .08444 | 2.03E-08 | -1.4770 | -.9003 |
| | 40 °C | -.91920* | .08444 | 5.48E-07 | -1.2075 | -.6309 |

*. The mean difference is significant at the 0.05 level.

*Table E.2 Statistical analysis of final OD for Idiomarina sp. LMLD01 at different temperatures*

**ANOVA**

OD

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 60.764 | 6 | 10.127 | 521.900 | 1.24E-15 |
| Within Groups | .272 | 14 | .019 | | |
| Total | 61.036 | 20 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

ODTukey HSD

| | | | | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| Plancocccus sp. LMLS02 | Temperature | Mean Difference (I-J) | Std. Error | Sig. | Lower Bound | Upper Bound |
| 20 °C | 25 °C | -.26667 | .11374 | .290 | -.6550 | .1217 |
| | 30 °C | -.13333 | .11374 | .893 | -.5217 | .2550 |
| | 35 °C | .76667* | .11374 | 1.50E-04 | .3783 | 1.1550 |
| | 37 °C | .65000* | .11374 | .001 | .2616 | 1.0384 |
| | 40 °C | 2.18333* | .11374 | 3.27E-10 | 1.7950 | 2.5717 |
| | 45 °C | 4.86667* | .11374 | 1.21E-12 | 4.4783 | 5.2550 |
| 25 °C | 20 °C | .26667 | .11374 | .290 | -.1217 | .6550 |
| | 30 °C | .13333 | .11374 | .893 | -.2550 | .5217 |
| | 35 °C | 1.03333* | .11374 | 5.04E-06 | .6450 | 1.4217 |
| | 37 °C | .91667* | .11374 | 2.06E-05 | .5283 | 1.3050 |
| | 40 °C | 2.45000* | .11374 | 6.93E-11 | 2.0616 | 2.8384 |
| | 45 °C | 5.13333* | .11374 | 1.21E-12 | 4.7450 | 5.5217 |
| 30 °C | 20 °C | .13333 | .11374 | .893 | -.2550 | .5217 |
| | 25 °C | -.13333 | .11374 | .893 | -.5217 | .2550 |
| | 35 °C | .90000* | .11374 | 2.54E-05 | .5116 | 1.2884 |
| | 37 °C | .78333* | .11374 | 1.19E-04 | .3950 | 1.1717 |
| | 40 °C | 2.31667* | .11374 | 1.47E-10 | 1.9283 | 2.7050 |
| | 45 °C | 5.00000* | .11374 | 1.21E-12 | 4.6116 | 5.3884 |
| 35 °C | 20 °C | -.76667* | .11374 | 1.50E-04 | -1.1550 | -.3783 |
| | 25 °C | -1.03333* | .11374 | 5.04E-06 | -1.4217 | -.6450 |
| | 30 °C | -.90000* | .11374 | 2.54E-05 | -1.2884 | -.5116 |
| | 37 °C | -.11667 | .11374 | .939 | -.5050 | .2717 |
| | 40 °C | 1.41667* | .11374 | 9.90E-08 | 1.0283 | 1.8050 |
| | 45 °C | 4.10000* | .11374 | 1.24E-12 | 3.7116 | 4.4884 |
| 37 °C | 20 °C | -.65000* | .11374 | .001 | -1.0384 | -.2616 |
| | 25 °C | -.91667* | .11374 | 2.06E-05 | -1.3050 | -.5283 |
| | 30 °C | -.78333* | .11374 | 1.19E-04 | -1.1717 | -.3950 |
| | 35 °C | .11667 | .11374 | .939 | -.2717 | .5050 |
| | 40 °C | 1.53333* | .11374 | 3.56E-08 | 1.1450 | 1.9217 |
| | 45 °C | 4.21667* | .11374 | 1.23E-12 | 3.8283 | 4.6050 |
| 40 °C | 20 °C | -2.18333* | .11374 | 3.27E-10 | -2.5717 | -1.7950 |
| | 25 °C | -2.45000* | .11374 | 6.93E-11 | -2.8384 | -2.0616 |
| | 30 °C | -2.31667* | .11374 | 1.47E-10 | -2.7050 | -1.9283 |
| | 35 °C | -1.41667* | .11374 | 9.90E-08 | -1.8050 | -1.0283 |
| | 37 °C | -1.53333* | .11374 | 3.56E-08 | -1.9217 | -1.1450 |
| | 45 °C | 2.68333* | .11374 | 2.07E-11 | 2.2950 | 3.0717 |
| 45 °C | 20 °C | -4.86667* | .11374 | 1.21E-12 | -5.2550 | -4.4783 |
| | 25 °C | -5.13333* | .11374 | 1.21E-12 | -5.5217 | -4.7450 |
| | 30 °C | -5.00000* | .11374 | 1.21E-12 | -5.3884 | -4.6116 |
| | 35 °C | -4.10000* | .11374 | 1.24E-12 | -4.4884 | -3.7116 |
| | 37 °C | -4.21667* | .11374 | 1.23E-12 | -4.6050 | -3.8283 |
| | 40 °C | -2.68333* | .11374 | 2.07E-11 | -3.0717 | -2.2950 |

*. The mean difference is significant at the 0.05 level.

## Appendix F

*Table F.1 Statistical analysis of growth rate for Planococcus sp. LMLD01 at different temperatures*

**ANOVA**

GR

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 2.657 | 6 | .443 | 88.295 | 2.58E-10 |
| Within Groups | .070 | 14 | .005 | | |
| Total | 2.728 | 20 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

GR Tukey HSD

| Caldalkalibacillus sp. LMLD03 | Temperature | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 20 °C | 25 °C | -.06017 | .05783 | .935 | -.2576 | .1373 |
| | 30 °C | -.25013* | .05783 | .010 | -.4476 | -.0527 |
| | 35 °C | -.59303* | .05783 | 1.15E-06 | -.7905 | -.3956 |
| | 37 °C | -.52707* | .05783 | 4.85E-06 | -.7245 | -.3296 |
| | 40 °C | -.35300* | .05783 | 4.23E-04 | -.5505 | -.1555 |
| | 45 °C | .53637* | .05783 | 3.93E-06 | .3389 | .7338 |
| 25 °C | 20 °C | .06017 | .05783 | .935 | -.1373 | .2576 |
| | 30 °C | -.18997 | .05783 | .063 | -.3874 | .0075 |
| | 35 °C | -.53287* | .05783 | 4.25E-06 | -.7303 | -.3354 |
| | 37 °C | -.46690* | .05783 | 2.02E-05 | -.6644 | -.2694 |
| | 40 °C | -.29283* | .05783 | .003 | -.4903 | -.0954 |
| | 45 °C | .59653* | .05783 | 1.07E-06 | .3991 | .7940 |
| 30 °C | 20 °C | .25013* | .05783 | .010 | .0527 | .4476 |
| | 25 °C | .18997 | .05783 | .063 | -.0075 | .3874 |
| | 35 °C | -.34290* | .05783 | .001 | -.5404 | -.1454 |
| | 37 °C | -.27693* | .05783 | .004 | -.4744 | -.0795 |
| | 40 °C | -.10287 | .05783 | .581 | -.3003 | .0946 |
| | 45 °C | .78650* | .05783 | 3.17E-08 | .5890 | .9840 |
| 35 °C | 20 °C | .59303* | .05783 | 1.15E-06 | .3956 | .7905 |
| | 25 °C | .53287* | .05783 | 4.25E-06 | .3354 | .7303 |
| | 30 °C | .34290* | .05783 | .001 | .1454 | .5404 |
| | 37 °C | .06597 | .05783 | .905 | -.1315 | .2634 |
| | 40 °C | .24003* | .05783 | .013 | .0426 | .4375 |
| | 45 °C | 1.12940* | .05783 | 2.59E-10 | .9319 | 1.3269 |
| 37 °C | 20 °C | .52707* | .05783 | 4.85E-06 | .3296 | .7245 |
| | 25 °C | .46690* | .05783 | 2.02E-05 | .2694 | .6644 |
| | 30 °C | .27693* | .05783 | .004 | .0795 | .4744 |
| | 35 °C | -.06597 | .05783 | .905 | -.2634 | .1315 |
| | 40 °C | .17407 | .05783 | .101 | -.0234 | .3715 |
| | 45 °C | 1.06343* | .05783 | 5.82E-10 | .8660 | 1.2609 |
| 40 °C | 20 °C | .35300* | .05783 | 4.23E-04 | .1555 | .5505 |
| | 25 °C | .29283* | .05783 | .003 | .0954 | .4903 |
| | 30 °C | .10287 | .05783 | .581 | -.0946 | .3003 |
| | 35 °C | -.24003* | .05783 | .013 | -.4375 | -.0426 |
| | 37 °C | -.17407 | .05783 | .101 | -.3715 | .0234 |
| | 45 °C | .88937* | .05783 | 6.32E-09 | .6919 | 1.0868 |
| 45 °C | 20 °C | -.53637* | .05783 | 3.93E-06 | -.7338 | -.3389 |
| | 25 °C | -.59653* | .05783 | 1.07E-06 | -.7940 | -.3991 |
| | 30 °C | -.78650* | .05783 | 3.17E-08 | -.9840 | -.5890 |
| | 35 °C | -1.12940* | .05783 | 2.59E-10 | -1.3269 | -.9319 |
| | 37 °C | -1.06343* | .05783 | 5.82E-10 | -1.2609 | -.8660 |
| | 40 °C | -.88937* | .05783 | 6.32E-09 | -1.0868 | -.6919 |

*. The mean difference is significant at the 0.05 level.

Table F.2 Statistical analysis of final OD for *Planococcus* sp. LMLD01 at different temperatures

**ANOVA**

OD

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 61.468 | 6 | 10.245 | 368.134 | 1.40E-14 |
| Within Groups | .390 | 14 | .028 | | |
| Total | 61.857 | 20 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

ODTukey HSD

| | | | | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| Plancoccus sp. LMLD02 | Temperature | Mean Difference (I-J) | Std. Error | Sig. | Lower Bound | Upper Bound |
| 20 °C | 25 °C | -1.31667* | .13621 | 2.38E-06 | -1.7818 | -.8516 |
| | 30 °C | -1.18333* | .13621 | 8.58E-06 | -1.6484 | -.7182 |
| | 35 °C | -.86667* | .13621 | 2.76E-04 | -1.3318 | -.4016 |
| | 37 °C | -1.55000* | .13621 | 3.13E-07 | -2.0151 | -1.0849 |
| | 40 °C | -.35000 | .13621 | .207 | -.8151 | .1151 |
| | 45 °C | 3.79333* | .13621 | 3.06E-12 | 3.3282 | 4.2584 |
| 25 °C | 20 °C | 1.31667* | .13621 | 2.38E-06 | .8516 | 1.7818 |
| | 30 °C | .13333 | .13621 | .951 | -.3318 | .5984 |
| | 35 °C | .45000 | .13621 | .061 | -.0151 | .9151 |
| | 37 °C | -.23333 | .13621 | .619 | -.6984 | .2318 |
| | 40 °C | .96667* | .13621 | 8.61E-05 | .5016 | 1.4318 |
| | 45 °C | 5.11000* | .13621 | 1.22E-12 | 4.6449 | 5.5751 |
| 30 °C | 20 °C | 1.18333* | .13621 | 8.58E-06 | .7182 | 1.6484 |
| | 25 °C | -.13333 | .13621 | .951 | -.5984 | .3318 |
| | 35 °C | .31667 | .13621 | .298 | -.1484 | .7818 |
| | 37 °C | -.36667 | .13621 | .171 | -.8318 | .0984 |
| | 40 °C | .83333* | .13621 | 4.13E-04 | .3682 | 1.2984 |
| | 45 °C | 4.97667* | .13621 | 1.23E-12 | 4.5116 | 5.4418 |
| 35 °C | 20 °C | .86667* | .13621 | 2.76E-04 | .4016 | 1.3318 |
| | 25 °C | -.45000 | .13621 | .061 | -.9151 | .0151 |
| | 30 °C | -.31667 | .13621 | .298 | -.7818 | .1484 |
| | 37 °C | -.68333* | .13621 | .003 | -1.1484 | -.2182 |
| | 40 °C | .51667* | .13621 | .025 | .0516 | .9818 |
| | 45 °C | 4.66000* | .13621 | 1.28E-12 | 4.1949 | 5.1251 |
| 37 °C | 20 °C | 1.55000* | .13621 | 3.13E-07 | 1.0849 | 2.0151 |
| | 25 °C | .23333 | .13621 | .619 | -.2318 | .6984 |
| | 30 °C | .36667 | .13621 | .171 | -.0984 | .8318 |
| | 35 °C | .68333* | .13621 | .003 | .2182 | 1.1484 |
| | 40 °C | 1.20000* | .13621 | 7.27E-06 | .7349 | 1.6651 |
| | 45 °C | 5.34333* | .13621 | 1.22E-12 | 4.8782 | 5.8084 |
| 40 °C | 20 °C | .35000 | .13621 | .207 | -.1151 | .8151 |
| | 25 °C | -.96667* | .13621 | 8.61E-05 | -1.4318 | -.5016 |
| | 30 °C | -.83333* | .13621 | 4.13E-04 | -1.2984 | -.3682 |
| | 35 °C | -.51667* | .13621 | .025 | -.9818 | -.0516 |
| | 37 °C | -1.20000* | .13621 | 7.27E-06 | -1.6651 | -.7349 |
| | 45 °C | 4.14333* | .13621 | 1.71E-12 | 3.6782 | 4.6084 |
| 45 °C | 20 °C | -3.79333* | .13621 | 3.06E-12 | -4.2584 | -3.3282 |
| | 25 °C | -5.11000* | .13621 | 1.22E-12 | -5.5751 | -4.6449 |
| | 30 °C | -4.97667* | .13621 | 1.23E-12 | -5.4418 | -4.5116 |
| | 35 °C | -4.66000* | .13621 | 1.28E-12 | -5.1251 | -4.1949 |
| | 37 °C | -5.34333* | .13621 | 1.22E-12 | -5.8084 | -4.8782 |
| | 40 °C | -4.14333* | .13621 | 1.71E-12 | -4.6084 | -3.6782 |

*. The mean difference is significant at the 0.05 level.

## Appendix G

*Table G.1 Statistical analysis of growth rate for Caldalkalibacillus sp. LMLD03 at different temperatures*

**ANOVA**

GR

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .913 | 4 | .228 | 4.524 | .024 |
| Within Groups | .505 | 10 | .050 | | |
| Total | 1.418 | 14 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

GRTukey HSD

| Caldalkalibacillus sp. LMLD03 | Temperature | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 37 °C | 40 °C | -.32963 | .18343 | .425 | -.9333 | .2741 |
| | 45 °C | -.51920 | .18343 | .102 | -1.1229 | .0845 |
| | 50 °C | -.46323 | .18343 | .160 | -1.0669 | .1405 |
| | 55 °C | .09333 | .18343 | .985 | -.5104 | .6970 |
| 40 °C | 37 °C | .32963 | .18343 | .425 | -.2741 | .9333 |
| | 45 °C | -.18957 | .18343 | .835 | -.7933 | .4141 |
| | 50 °C | -.13360 | .18343 | .945 | -.7373 | .4701 |
| | 55 °C | .42297 | .18343 | .220 | -.1807 | 1.0267 |
| 45 °C | 37 °C | .51920 | .18343 | .102 | -.0845 | 1.1229 |
| | 40 °C | .18957 | .18343 | .835 | -.4141 | .7933 |
| | 50 °C | .05597 | .18343 | .998 | -.5477 | .6597 |
| | 55 °C | .61253* | .18343 | .046 | .0088 | 1.2162 |
| 50 °C | 37 °C | .46323 | .18343 | .160 | -.1405 | 1.0669 |
| | 40 °C | .13360 | .18343 | .945 | -.4701 | .7373 |
| | 45 °C | -.05597 | .18343 | .998 | -.6597 | .5477 |
| | 55 °C | .55657 | .18343 | .074 | -.0471 | 1.1603 |
| 55 °C | 37 °C | -.09333 | .18343 | .985 | -.6970 | .5104 |
| | 40 °C | -.42297 | .18343 | .220 | -1.0267 | .1807 |
| | 45 °C | -.61253* | .18343 | .046 | -1.2162 | -.0088 |
| | 50 °C | -.55657 | .18343 | .074 | -1.1603 | .0471 |

*. The mean difference is significant at the 0.05 level.

*Table G.2 Statistical analysis of final OD for Caldalkalibacillus sp. LMLD03 at different temperatures*

**ANOVA**

OD

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 18.599 | 4 | 4.650 | 24.424 | 3.82E-05 |
| Within Groups | 1.904 | 10 | .190 | | |
| Total | 20.503 | 14 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

ODTukey HSD

| Caldalkalibacillus sp. LMLD03 | Temperature | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 37 °C | 40 °C | -1.55667* | .35626 | .010 | -2.7291 | -.3842 |
| | 45 °C | -1.55667* | .35626 | .010 | -2.7291 | -.3842 |
| | 50 °C | -1.37333* | .35626 | .021 | -2.5458 | -.2009 |
| | 55 °C | 1.25000* | .35626 | .036 | .0775 | 2.4225 |
| 40 °C | 37 °C | 1.55667* | .35626 | .010 | .3842 | 2.7291 |
| | 45 °C | .00000 | .35626 | 1.000 | -1.1725 | 1.1725 |
| | 50 °C | .18333 | .35626 | .984 | -.9891 | 1.3558 |
| | 55 °C | 2.80667* | .35626 | 1.02E-04 | 1.6342 | 3.9791 |
| 45 °C | 37 °C | 1.55667* | .35626 | .010 | .3842 | 2.7291 |
| | 40 °C | .00000 | .35626 | 1.000 | -1.1725 | 1.1725 |
| | 50 °C | .18333 | .35626 | .984 | -.9891 | 1.3558 |
| | 55 °C | 2.80667* | .35626 | 1.02E-04 | 1.6342 | 3.9791 |
| 50 °C | 37 °C | 1.37333* | .35626 | .021 | .2009 | 2.5458 |
| | 40 °C | -.18333 | .35626 | .984 | -1.3558 | .9891 |
| | 45 °C | -.18333 | .35626 | .984 | -1.3558 | .9891 |
| | 55 °C | 2.62333* | .35626 | 1.82E-04 | 1.4509 | 3.7958 |
| 55 °C | 37 °C | -1.25000* | .35626 | .036 | -2.4225 | -.0775 |
| | 40 °C | -2.80667* | .35626 | 1.02E-04 | -3.9791 | -1.6342 |
| | 45 °C | -2.80667* | .35626 | 1.02E-04 | -3.9791 | -1.6342 |
| | 50 °C | -2.62333* | .35626 | 1.82E-04 | -3.7958 | -1.4509 |

*. The mean difference is significant at the 0.05 level.

## Appendix H

*Table H.1 Statistical analysis of growth rate for Planococcus sp. LMLD02 at different pH*

**ANOVA**

GR

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1.570 | 6 | .262 | 98.943 | 1.19E-10 |
| Within Groups | .037 | 14 | .003 | | |
| Total | 1.607 | 20 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

GRTukey HSD

| | | | | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| (I) VAR00001 | (J) VAR00001 | Mean Difference (I-J) | Std. Error | Sig. | Lower Bound | Upper Bound |
| pH 7 | pH 7.5 | -.33947* | .04198 | 1.98E-05 | -.4828 | -.1961 |
| | pH 9 | -.24897* | .04198 | .001 | -.3923 | -.1056 |
| | pH 9.6 | -.17463* | .04198 | .013 | -.3180 | -.0313 |
| | pH 10 | -.09303 | .04198 | .346 | -.2364 | .0503 |
| | pH 10.3 | .00977 | .04198 | 1.000 | -.1336 | .1531 |
| | pH 10.4 | .56399* | .04198 | 3.72E-08 | .4206 | .7073 |
| pH 7.5 | pH 7 | .33947* | .04198 | 1.98E-05 | .1961 | .4828 |
| | pH 9 | .09050 | .04198 | .375 | -.0529 | .2339 |
| | pH 9.6 | .16483* | .04198 | .020 | .0215 | .3082 |
| | pH 10 | .24643* | .04198 | .001 | .1031 | .3898 |
| | pH 10.3 | .34923* | .04198 | 1.43E-05 | .2059 | .4926 |
| | pH 10.4 | .90346* | .04198 | 7.02E-11 | .7601 | 1.0468 |
| pH 9 | pH 7 | .24897* | .04198 | .001 | .1056 | .3923 |
| | pH 7.5 | -.09050 | .04198 | .375 | -.2339 | .0529 |
| | pH 9.6 | .07433 | .04198 | .585 | -.0690 | .2177 |
| | pH 10 | .15593* | .04198 | .029 | .0126 | .2993 |
| | pH 10.3 | .25873* | .04198 | 3.84E-04 | .1154 | .4021 |
| | pH 10.4 | .81296* | .04198 | 2.91E-10 | .6696 | .9563 |
| pH 9.6 | pH 7 | .17463* | .04198 | .013 | .0313 | .3180 |
| | pH 7.5 | -.16483* | .04198 | .020 | -.3082 | -.0215 |
| | pH 9 | -.07433 | .04198 | .585 | -.2177 | .0690 |
| | pH 10 | .08160 | .04198 | .486 | -.0618 | .2250 |
| | pH 10.3 | .18440* | .04198 | .008 | .0410 | .3278 |
| | pH 10.4 | .73863* | .04198 | 1.05E-09 | .5953 | .8820 |
| pH 10 | pH 7 | .09303 | .04198 | .346 | -.0503 | .2364 |
| | pH 7.5 | -.24643* | .04198 | .001 | -.3898 | -.1031 |
| | pH 9 | -.15593* | .04198 | .029 | -.2993 | -.0126 |
| | pH 9.6 | -.08160 | .04198 | .486 | -.2250 | .0618 |
| | pH 10.3 | .10280 | .04198 | .249 | -.0406 | .2462 |
| | pH 10.4 | .65703* | .04198 | 5.02E-09 | .5137 | .8004 |
| pH 10.3 | pH 7 | -.00977 | .04198 | 1.000 | -.1531 | .1336 |
| | pH 7.5 | -.34923* | .04198 | 1.43E-05 | -.4926 | -.2059 |
| | pH 9 | -.25873* | .04198 | 3.84E-04 | -.4021 | -.1154 |
| | pH 9.6 | -.18440* | .04198 | .008 | -.3278 | -.0410 |
| | pH 10 | -.10280 | .04198 | .249 | -.2462 | .0406 |
| | pH 10.4 | .55423* | .04198 | 4.67E-08 | .4109 | .6976 |
| pH 10.4 | pH 7 | -.56399* | .04198 | 3.72E-08 | -.7073 | -.4206 |
| | pH 7.5 | -.90346* | .04198 | 7.02E-11 | -1.0468 | -.7601 |
| | pH 9 | -.81296* | .04198 | 2.91E-10 | -.9563 | -.6696 |
| | pH 9.6 | -.73863* | .04198 | 1.05E-09 | -.8820 | -.5953 |
| | pH 10 | -.65703* | .04198 | 5.02E-09 | -.8004 | -.5137 |
| | pH 10.3 | -.55423* | .04198 | 4.67E-08 | -.6976 | -.4109 |

*. The mean difference is significant at the 0.05 level.

*Table H.2 Statistical analysis of growth rate for Planococcus sp. LMLD02 at different pH*

**ANOVA**

GR

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1.570 | 6 | .262 | 98.943 | 1.19E-10 |
| Within Groups | .037 | 14 | .003 | | |
| Total | 1.607 | 20 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

GR Tukey HSD

| | | | | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| Planococcus sp. LMLD02 | pH | Mean Difference (I-J) | Std. Error | Sig. | Lower Bound | Upper Bound |
| pH 7 | pH 7.5 | -.33947* | .04198 | 1.98E-05 | -.4828 | -.1961 |
| | pH 9 | -.24897* | .04198 | .001 | -.3923 | -.1056 |
| | pH 9.6 | -.17463* | .04198 | .013 | -.3180 | -.0313 |
| | pH 10 | -.09303 | .04198 | .346 | -.2364 | .0503 |
| | pH 10.3 | .00977 | .04198 | 1.000 | -.1336 | .1531 |
| | pH 10.4 | .56399* | .04198 | 3.72E-08 | .4206 | .7073 |
| pH 7.5 | pH 7 | .33947* | .04198 | 1.98E-05 | .1961 | .4828 |
| | pH 9 | .09050 | .04198 | .375 | -.0529 | .2339 |
| | pH 9.6 | .16483* | .04198 | .020 | .0215 | .3082 |
| | pH 10 | .24643* | .04198 | .001 | .1031 | .3898 |
| | pH 10.3 | .34923* | .04198 | 1.43E-05 | .2059 | .4926 |
| | pH 10.4 | .90346* | .04198 | 7.02E-11 | .7601 | 1.0468 |
| pH 9 | pH 7 | .24897* | .04198 | .001 | .1056 | .3923 |
| | pH 7.5 | -.09050 | .04198 | .375 | -.2339 | .0529 |
| | pH 9.6 | .07433 | .04198 | .585 | -.0690 | .2177 |
| | pH 10 | .15593* | .04198 | .029 | .0126 | .2993 |
| | pH 10.3 | .25873* | .04198 | 3.84E-04 | .1154 | .4021 |
| | pH 10.4 | .81296* | .04198 | 2.91E-10 | .6696 | .9563 |
| pH 9.6 | pH 7 | .17463* | .04198 | .013 | .0313 | .3180 |
| | pH 7.5 | -.16483* | .04198 | .020 | -.3082 | -.0215 |
| | pH 9 | -.07433 | .04198 | .585 | -.2177 | .0690 |
| | pH 10 | .08160 | .04198 | .486 | -.0618 | .2250 |
| | pH 10.3 | .18440* | .04198 | .008 | .0410 | .3278 |
| | pH 10.4 | .73863* | .04198 | 1.05E-09 | .5953 | .8820 |
| pH 10 | pH 7 | .09303 | .04198 | .346 | -.0503 | .2364 |
| | pH 7.5 | -.24643* | .04198 | .001 | -.3898 | -.1031 |
| | pH 9 | -.15593* | .04198 | .029 | -.2993 | -.0126 |
| | pH 9.6 | -.08160 | .04198 | .486 | -.2250 | .0618 |
| | pH 10.3 | .10280 | .04198 | .249 | -.0406 | .2462 |
| | pH 10.4 | .65703* | .04198 | 5.02E-09 | .5137 | .8004 |
| pH 10.3 | pH 7 | -.00977 | .04198 | 1.000 | -.1531 | .1336 |
| | pH 7.5 | -.34923* | .04198 | 1.43E-05 | -.4926 | -.2059 |
| | pH 9 | -.25873* | .04198 | 3.84E-04 | -.4021 | -.1154 |
| | pH 9.6 | -.18440* | .04198 | .008 | -.3278 | -.0410 |
| | pH 10 | -.10280 | .04198 | .249 | -.2462 | .0406 |
| | pH 10.4 | .55423* | .04198 | 4.67E-08 | .4109 | .6976 |
| pH 10.4 | pH 7 | -.56399* | .04198 | 3.72E-08 | -.7073 | -.4206 |
| | pH 7.5 | -.90346* | .04198 | 7.02E-11 | -1.0468 | -.7601 |
| | pH 9 | -.81296* | .04198 | 2.91E-10 | -.9563 | -.6696 |
| | pH 9.6 | -.73863* | .04198 | 1.05E-09 | -.8820 | -.5953 |
| | pH 10 | -.65703* | .04198 | 5.02E-09 | -.8004 | -.5137 |
| | pH 10.3 | -.55423* | .04198 | 4.67E-08 | -.6976 | -.4109 |

*. The mean difference is significant at the 0.05 level.

## Appendix I

*Table I.1 Statistical analysis of growth rate for Caldalkalibacillus sp. LMLD03 at different pH*

**ANOVA**

GR

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .006 | 2 | .003 | 6.127 | .036 |
| Within Groups | .003 | 6 | .000 | | |
| Total | .008 | 8 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

GRTukey HSD

| | | | | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| Caldalkalibacillus sp. LMLD03 | pH | Mean Difference (I-J) | Std. Error | Sig. | Lower Bound | Upper Bound |
| pH 9 | pH 9.6 | -.02133 | .01758 | .489 | -.0753 | .0326 |
| | pH 10 | -.06067* | .01758 | .032 | -.1146 | -.0067 |
| pH 9.6 | pH 9 | .02133 | .01758 | .489 | -.0326 | .0753 |
| | pH 10 | -.03933 | .01758 | .143 | -.0933 | .0146 |
| pH 10 | pH 9 | .06067* | .01758 | .032 | .0067 | .1146 |
| | pH 9.6 | .03933 | .01758 | .143 | -.0146 | .0933 |

*. The mean difference is significant at the 0.05 level.

*Table I.2 Statistical analysis of growth rate for Caldalkalibacillus sp. LMLD03 at different pH*

**ANOVA**

OD

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .012 | 2 | .006 | .135 | .876 |
| Within Groups | .258 | 6 | .043 | | |
| Total | .270 | 8 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

ODTukey HSD

| | | | | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| Caldalkalibacillus sp. LMD01 | pH | Mean Difference (I-J) | Std. Error | Sig. | Lower Bound | Upper Bound |
| pH 9 | pH 9.6 | .01667 | .16942 | .995 | -.5032 | .5365 |
| | pH 10 | -.06667 | .16942 | .919 | -.5865 | .4532 |
| pH 9.6 | pH 9 | -.01667 | .16942 | .995 | -.5365 | .5032 |
| | pH 10 | -.08333 | .16942 | .878 | -.6032 | .4365 |
| pH 10 | pH 9 | .06667 | .16942 | .919 | -.4532 | .5865 |
| | pH 9.6 | .08333 | .16942 | .878 | -.4365 | .6032 |

## Appendix J

Table J.1 Mars chamber t-Test, and ANOVA- Tukey Post Hoc test for colony count on AP+0.7% medium

| 0.7% NaCl | **Students t-Test** |
|---|---|
| One tailed, Paired | Sig. |
| Wet control | |
| Bench control | 0.0027 |

**ANOVA**

0.7% NaCl

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1.688 | 5 | .338 | 14.183 | 1.105E-04 |
| Within Groups | .286 | 12 | .024 | | |
| Total | 1.973 | 17 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

0.7% NaCl
Tukey HSD

| | | | | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| (I) low_salt | (J) low_salt | Mean Difference (I-J) | Std. Error | Sig. | Lower Bound | Upper Bound |
| Dried control | Bench control | .85380* | .12596 | 2.2E-04 | .4307 | 1.2769 |
| | Horizon 1 | .85867* | .12596 | 2.1E-04 | .4356 | 1.2818 |
| | Horizon 2 | .71000* | .12596 | .001 | .2869 | 1.1331 |
| | Horizon 3 | .74233* | .12596 | .001 | .3192 | 1.1654 |
| | Horizon 4 | .40233 | .12596 | .066 | -.0208 | .8254 |
| Bench control | Dried control | -.85380* | .12596 | 2.2E-04 | -1.2769 | -.4307 |
| | Horizon 1 | .00487 | .12596 | 1.000 | -.4182 | .4280 |
| | Horizon 2 | -.14380 | .12596 | .855 | -.5669 | .2793 |
| | Horizon 3 | -.11147 | .12596 | .943 | -.5346 | .3116 |
| | Horizon 4 | -.45147* | .12596 | .034 | -.8746 | -.0284 |
| Horizon 1 | Dried control | -.85867* | .12596 | 2.1E-04 | -1.2818 | -.4356 |
| | Bench control | -.00487 | .12596 | 1.000 | -.4280 | .4182 |
| | Horizon 2 | -.14867 | .12596 | .838 | -.5718 | .2744 |
| | Horizon 3 | -.11633 | .12596 | .933 | -.5394 | .3068 |
| | Horizon 4 | -.45633* | .12596 | .032 | -.8794 | -.0332 |
| Horizon 2 | Dried control | -.71000* | .12596 | .001 | -1.1331 | -.2869 |
| | Bench control | .14380 | .12596 | .855 | -.2793 | .5669 |
| | Horizon 1 | .14867 | .12596 | .838 | -.2744 | .5718 |
| | Horizon 3 | .03233 | .12596 | 1.000 | -.3908 | .4554 |
| | Horizon 4 | -.30767 | .12596 | .216 | -.7308 | .1154 |
| Horizon 3 | Dried control | -.74233* | .12596 | .001 | -1.1654 | -.3192 |
| | Bench control | .11147 | .12596 | .943 | -.3116 | .5346 |
| | Horizon 1 | .11633 | .12596 | .933 | -.3068 | .5394 |
| | Horizon 2 | -.03233 | .12596 | 1.000 | -.4554 | .3908 |
| | Horizon 4 | -.34000 | .12596 | .146 | -.7631 | .0831 |
| Horizon 4 | Dried control | -.40233 | .12596 | .066 | -.8254 | .0208 |
| | Bench control | .45147* | .12596 | .034 | .0284 | .8746 |
| | Horizon 1 | .45633* | .12596 | .032 | .0332 | .8794 |
| | Horizon 2 | .30767 | .12596 | .216 | -.1154 | .7308 |
| | Horizon 3 | .34000 | .12596 | .146 | -.0831 | .7631 |

*. The mean difference is significant at the 0.05 level.

Table J.2. Mars chamber t-Test, and ANOVA- Tukey Post Hoc test for colony count on AP+6.8% medium

| 6.8% NaCl | **Students t-Test** |
|---|---|
| One tailed, Paired | Sig. |
| Wet control | |
| Bench control | 0.002 |

**ANOVA**

6.8% NaCl

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1.532 | 5 | .306 | 6.488 | .004 |
| Within Groups | .567 | 12 | .047 | | |
| Total | 2.099 | 17 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

6.8% NaCl
Tukey HSD

| (I) low_salt | (J) low_salt | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Dried control | Bench control | .54300 | .17743 | .082 | -.0530 | 1.1390 |
| | Horizon 1 | .89553* | .17743 | .003 | .2996 | 1.4915 |
| | Horizon 2 | .74633* | .17743 | .012 | .1504 | 1.3423 |
| | Horizon 3 | .38067 | .17743 | .328 | -.2153 | .9766 |
| | Horizon 4 | .69733* | .17743 | .019 | .1014 | 1.2933 |
| Bench control | Dried control | -.54300 | .17743 | .082 | -1.1390 | .0530 |
| | Horizon 1 | .35253 | .17743 | .402 | -.2434 | .9485 |
| | Horizon 2 | .20333 | .17743 | .853 | -.3926 | .7993 |
| | Horizon 3 | -.16233 | .17743 | .935 | -.7583 | .4336 |
| | Horizon 4 | .15433 | .17743 | .947 | -.4416 | .7503 |
| Horizon 1 | Dried control | -.89553* | .17743 | .003 | -1.4915 | -.2996 |
| | Bench control | -.35253 | .17743 | .402 | -.9485 | .2434 |
| | Horizon 2 | -.14920 | .17743 | .954 | -.7452 | .4468 |
| | Horizon 3 | -.51487 | .17743 | .106 | -1.1108 | .0811 |
| | Horizon 4 | -.19820 | .17743 | .865 | -.7942 | .3978 |
| Horizon 2 | Dried control | -.74633* | .17743 | .012 | -1.3423 | -.1504 |
| | Bench control | -.20333 | .17743 | .853 | -.7993 | .3926 |
| | Horizon 1 | .14920 | .17743 | .954 | -.4468 | .7452 |
| | Horizon 3 | -.36567 | .17743 | .366 | -.9616 | .2303 |
| | Horizon 4 | -.04900 | .17743 | 1.000 | -.6450 | .5470 |
| Horizon 3 | Dried control | -.38067 | .17743 | .328 | -.9766 | .2153 |
| | Bench control | .16233 | .17743 | .935 | -.4336 | .7583 |
| | Horizon 1 | .51487 | .17743 | .106 | -.0811 | 1.1108 |
| | Horizon 2 | .36567 | .17743 | .366 | -.2303 | .9616 |
| | Horizon 4 | .31667 | .17743 | .509 | -.2793 | .9126 |
| Horizon 4 | Dried control | -.69733* | .17743 | .019 | -1.2933 | -.1014 |
| | Bench control | -.15433 | .17743 | .947 | -.7503 | .4416 |
| | Horizon 1 | .19820 | .17743 | .865 | -.3978 | .7942 |
| | Horizon 2 | .04900 | .17743 | 1.000 | -.5470 | .6450 |
| | Horizon 3 | -.31667 | .17743 | .509 | -.9126 | .2793 |

*. The mean difference is significant at the 0.05 level.

Table J.3.Mars chamber t-Test, and ANOVA- Tukey Post Hoc test for colony count on AP+6.8% medium

| 15% NaCl | **Students t-Test** |
|---|---|
| One tailed, Paired | Sig. |
| Wet control | |
| Bench control | 0.0006 |

**ANOVA**

15% NaCl

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 2.575 | 5 | .515 | 2.600 | .081 |
| Within Groups | 2.377 | 12 | .198 | | |
| Total | 4.952 | 17 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

15% NaCl
Tukey HSD

| (I) low_salt | (J) low_salt | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Dried control | Bench control | -.33333 | .36339 | .935 | -1.5539 | .8873 |
| | Horizon 1 | .66700 | .36339 | .481 | -.5536 | 1.8876 |
| | Horizon 2 | .77800 | .36339 | .330 | -.4426 | 1.9986 |
| | Horizon 3 | .33233 | .36339 | .935 | -.8883 | 1.5529 |
| | Horizon 4 | .22200 | .36339 | .988 | -.9986 | 1.4426 |
| Bench control | Dried control | .33333 | .36339 | .935 | -.8873 | 1.5539 |
| | Horizon 1 | 1.00033 | .36339 | .135 | -.2203 | 2.2209 |
| | Horizon 2 | 1.11133 | .36339 | .082 | -.1093 | 2.3319 |
| | Horizon 3 | .66567 | .36339 | .483 | -.5549 | 1.8863 |
| | Horizon 4 | .55533 | .36339 | .655 | -.6653 | 1.7759 |
| Horizon 1 | Dried control | -.66700 | .36339 | .481 | -1.8876 | .5536 |
| | Bench control | -1.00033 | .36339 | .135 | -2.2209 | .2203 |
| | Horizon 2 | .11100 | .36339 | 1.000 | -1.1096 | 1.3316 |
| | Horizon 3 | -.33467 | .36339 | .934 | -1.5553 | .8859 |
| | Horizon 4 | -.44500 | .36339 | .817 | -1.6656 | .7756 |
| Horizon 2 | Dried control | -.77800 | .36339 | .330 | -1.9986 | .4426 |
| | Bench control | -1.11133 | .36339 | .082 | -2.3319 | .1093 |
| | Horizon 1 | -.11100 | .36339 | 1.000 | -1.3316 | 1.1096 |
| | Horizon 3 | -.44567 | .36339 | .816 | -1.6663 | .7749 |
| | Horizon 4 | -.55600 | .36339 | .654 | -1.7766 | .6646 |
| Horizon 3 | Dried control | -.33233 | .36339 | .935 | -1.5529 | .8883 |
| | Bench control | -.66567 | .36339 | .483 | -1.8863 | .5549 |
| | Horizon 1 | .33467 | .36339 | .934 | -.8859 | 1.5553 |
| | Horizon 2 | .44567 | .36339 | .816 | -.7749 | 1.6663 |
| | Horizon 4 | -.11033 | .36339 | 1.000 | -1.3309 | 1.1103 |
| Horizon 4 | Dried control | -.22200 | .36339 | .988 | -1.4426 | .9986 |
| | Bench control | -.55533 | .36339 | .655 | -1.7759 | .6653 |
| | Horizon 1 | .44500 | .36339 | .817 | -.7756 | 1.6656 |
| | Horizon 2 | .55600 | .36339 | .654 | -.6646 | 1.7766 |
| | Horizon 3 | .11033 | .36339 | 1.000 | -1.1103 | 1.3309 |