Bacteriophage diversity in haloalkaline environments

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Abstract

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There are limited reports on virus population in haloalkaline environments; therefore the aim of this study was to investigate the genetic diversity and biology of bacteriophage communities in these environments. Bacteria were isolated to be used as phage hosts. One bacterium from Lake Magadi and four bacteria from Lake Shala were successfully isolated from sediment samples. A further two Lake Shala bacterial hosts from the IMBM culture collection were also used to isolate bacteriophages. Bacterial isolates were identified to be most closely related to *Bacillius halodurans, Halomonas axialensis, Virgibacillus salarius, Bacillus licheniformis, Halomonas venusta, Bacillus pseudofirmus* and *Paracoccus aminovorans*. Bacteriophages were screened using all bacteria against sediment samples from both Lake Shala and Lake Magadi. One phage was identified from Lake Magadi sediments (MGBH1) and two phages from Lake Shala sediments (SHBH1 and SHPA). TEM analysis showed that these phages belong to three different dsDNA phage families; *Siphoviridae* (MGBH1)*, Myoviridae* (SHBH1) and *Podoviridae* (SHPA). All phages showed different genome sizes on agarose gel. Due to the small genome size, phage SHPA was chosen for further investigation. Partial, genome sequence analysis showed homology to both bacterial and phage proteins. A further investigation of phage diversity in this environment is essential using metagenomic approaches to understand these unique communities. **July 2013**

Declaration

I declare that **Bacteriophage diversity in haloalkaline environments** is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Shonisani Nemavhulani July 2013

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My family, thank you for your support and everlasting love and for always believing in me when at times I didn't believe in my self, you truly are a gift from God.

This thesis is dedicated to my late grandmother

Vho-Vele Mamphwe

"If you can't fly then run, if you can't run then walk, if you can't walk then crawl, but whatever you do you have to keep moving forward."

- Martin Luther King, Jr.

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List of symbols and abbreviations

BLAST	Basic local alignment sequencing tool
bp	Base pair
BSA	Bovine Serum Albumen
cfu	Colony forming unit
ºC	Degrees Celsius
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTPs	Deoxynucleotide tri-phosphate
ds	Double stranded
EDTA	Ethylenediaminetetra-acetic acid
x g	Centrifugal force
g	Gram
GeneBank	Nucleotide sequence database
ICTV	International Committee for Taxonomy of Viruses
kb	Kilobase
keV	Kilo electron volt
kV	Kilo volt
L	Litre
LB	Luria Bertani
µF	Micro Farad
µl	Microlitre
µg	Microgram
µm	Micromolar
M	Molar
mg	Milligram

ml	Millilitre
min	Minutes
mM	Millimolar
MOI	Multiplicity of infection
NCBI	National Centre for Biotechnology Information
ng	Nanogram
nm	Nanometre
Ω	Ohm
OD	Optical density
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PEG	Polyethylene glycol
PFU	Plaque forming unit
RNA	Ribonucleic acid
RNase	Ribonuclease
rRNA	Ribosomal ribonucleic acid
rpm	Revolutions per minute
sec	Seconds
SDS	Sodium Dodecyl Sulfate
sp.	Species
ss	Single-stranded
TAE	Tris acetic acid EDTA
TE	Tris EDTA
TEM	Transmission electron microscopy
U	Units
v/v	Volume per volume
w/v	Weight per volume

Chapter 1: Literature review

1.1. Bacteriophage introduction and background

1.1.1. Phage history

Bacteriophages were first suspected to exist in 1896 when Ernest Hankin, a British bacteriologist, discovered that agents in the waters of the Ganges River could destroy *Vibrio cholera* (Hankin, 1896). In 1915, the British pathologist Frederick William Twort discovered the glassy transformation of *Micrococcus* colonies by a 'transmissible agent'. His conclusion was that the transformation was caused by agents small enough to pass through a fine-pored filter and that these agents killed bacteria and multiplied in the process (Twort, 1915). Although Twort did not pursue his discovery another report of phage activity was published in 1917 by the French-Canadian microbiologist Felix d'Herelle. Upon discovering lysis of *Shigella* cultures in broth, he coined the name bacteriophage which simply means 'the bacteria eater' and described the clear zones formed in a bacterial lawn as 'plaques' (d'Hérelle, 1917).

d'Herelle played a large role in phage history as he was the first to introduce the use of phage as a therapeutic agent to kill pathogenic bacteria in humans. He also co-founded the Phage Research Institute in 1933. The institute continued to supply phage for therapeutic use until the 1940s, when penicillin and other highly efficient and broad range antibiotics were discovered and phage therapy was abandoned in Western Europe (Summers, 1999; McAuliffe *et al.,* 2007; Clokie *et al.,* 2011).

Interestingly the use of phage therapy has recently been revived due to the appearance of multidrug resistant pathogens (Weinbauer, 2004; Dublanchet & Bourne, 2007). d'Herelle was first to begin the research on the nature of bacterial viruses and discovered that the chemical composition of phage virions was proteins and deoxyribonucleic acid (DNA) (McAuliffe *et al.,* 2007).

Scientists at that time did not know how hereditary information was carried between protein and DNA for viral replication but in 1952, Alfred Hershey and Martha Chase at the Carnegie Laboratory of Genetics discovered that DNA was the component which carries genetic information (Hershey & Chase, 1952). In 1967 when phage morphologies were visualized using a transmission electron microscope (TEM) a wide variety of phage were discovered (Eisenstark, 1967). Phage research played an important role in the elucidation of the role of DNA as genetic material and in understanding the genetic code, information which led to a clearer understanding of science and molecular biology.

1.1.2. Phage taxonomy and classification

Felix d'Herelle classified bacteriophage as a single species *Bacteriophagum intestinale*, but recognised that there were different types (d'Herelle, 1918). In 1943, Ruska proved that phages have different morphologies, and proposed a system of virus classification based on morphological identification using electron microscopy (Ruska, 1943). Viruses were first classified according to their morphology and nucleic acid type by Lwoff, Horne, and Tournier in 1962

(Lwoff *et al.*, 1962). They suggested the order *Urovirales* for tailed phage, the family *Inoviridae* for filamentous phage and the family *Microviridae* for ϕX-type phage, respectively. This was followed by Bradley's classification system in 1967; he discovered six basic types of phage: tailed phage, filamentous phage and cubic phage with ssDNA, dsDNA, ssRNA or dsRNA (Bradley, 1967). This simple scheme still forms the basis of bacteriophage classification. In 1971, the International Committee for Taxonomy of Viruses (ICTV) published their first paper which classified phage into six genera corresponding to Bradley's basic types: T4, λ, ϕX174, MS2, fd phage groups and PM2 were included (Wildy, 1971). ICTV today classifies all known viruses, of which bacteriophages are included, into three orders, 61 families and 241 genera (Ackermann, 2003).

Phages alone are comprised of one order, 13 families and 30 genera. They are classified into the order *Caudovirales*, which are tailed phages, and constitute three tailed families (Ackermann, 2003). Among the characterized tailed phage, 61% are *Siphoviridae* with long, noncontractile tails, 24.5% are *Myoviridae* with contractile tails and 14% are *Podoviridae* with very short, noncontractile tails. Only 3.7% are polyhedral, filamentous and pleumorphic phage (PFP) (Ackermann, 2007). PFP phages are very simple to classify as they have small families which can comprise as few as one member. Bacteriophage classification and their properties are shown in Table 1. New morphotypes and phage families were added as they were discovered over the years (Ackermann, 2003, 2007, 2011). The most important properties used to classify bacteriophage are nucleic acid type (ssDNA, dsDNA, ssRNA and dsRNA), morphology and physicochemical properties (Ackermann, 2011).

Table 1: Overview of phage classification and properties (adapted from Ackermann, 2007)

Shape	Nucleic acid	Virus group	Particulars	Example
Tailed	DNA, 2, L	*Myoviridae*	tail contractile	T4
		Siphoviridae	tail long, noncontractile	λ
		Podoviridae	tail short	T7
Polyhedral	DNA, 1, C	*Microviridae*	conspicuous capsomers	ϕX174
	2, C, S	*Corticoviridae*	complex capsid, lipids	PM2
	2, L	*Tectiviridae*	inner lipid vesicle, pseudotail	PRD1
	2, L	SHI, group*	inner lipid vesicle	SH1
	2, C	STV1 group*	turret-shaped protrusions	STIV
	RNA, 1, L	*Leviviridae*	poliovirus-like	MS2
	2, L, seg	*Cystoviridae*	envelope, lipids	ϕ6
Filamentous	DNA, 1, C	*Inoviridae*	a. long filaments	fd
			b. short rods	MVL1
	2, L	*Lipothrixviridae*	envelope, lipids	TTV1
	2, L	*Rudiviridae*	TMV-like	SIRV-1
Pleomorphic	DNA, 2, C, S	*Plasmaviridae*	envelope, lipids, no capsid	L2
	2, C, S	*Fuselloviridae*	same, lemon-shaped	SSV1
	2, L, S	*Salterprovirus*	same, lemon-shaped	His1
	2, C, S	*Guttaviridae*	droplet-shaped	SNDV
	2, L	*Ampullaviridae**	bottle-shaped	ABV
	2, C	*Bicaudaviridae**	two-tailed, growth cycle	ATV
	2, L	*Globuloviridae**	paramyxovirus-like	PSV

C Circular; *L* linear; *S* superhelical; *seg* segmented; *1* single-stranded; *2* double-stranded
*Awaiting classification

1.1.3. Definitions

Phages are viruses that infect bacterial hosts and are considered to be the most numerous and diverse biological entities in the biosphere (Hatfull *et al*., 2006; Shapiro & Kushmaro, 2011). Phage genomes contain either ss/dsDNA or ss/dsRNA, depending on the bacteriophage in question (Trun & Trempy, 2004). Lytic (virulent) phage have the ability to lyse host cells after replication whereas

lysogenic (temperate) phage DNA integrates into the host chromosome forming a prophage (Heilmann *et al.,* 2010), a genome state of temperate phage replicating in synchrony with that of the host (Madigan *et al.,* 2009).

1.1.4. Phage structure and sizes

Since the introduction of negative staining in 1959, more than 6000 phages have been examined by electron microscopy: of these 96% are tailed and 3.7% are PFP phages. Tailed phages are described according to their various tail morphologies (Ackermann, 2007, 2011). A schematic representation of phage morphologies is shown in Figure 1.

Phage capsids have icosahedral heads and the vertices are made of protein pentamers, with each side made up of hexamers of the same or similar protein (Guttman *et al.,* 2005). Each phage head and tail are linked together by a connector, however phages with various shapes including filamentous, pleomorphic, cubic, spindle and lemon-shaped viruses, and other phage structures such as head appendages, collar and tail fibers or spikes, are also known (Frank & Moebus, 1987; Demuth *et al*., 1993; Proctor, 1997).

In 1933, Sir Macfarlane Burnet discovered that the size of phages varies greatly (Burnet, 1933). Most phages have a head diameter ranging from 30nm to 60nm, and this is generally determined by the genome size (Wommack & Colwell, 2000). The highly condensed chromosome within the dsDNA phage capsid represents 20 to 50% of its mass (Earnshaw & Harrison, 1977).

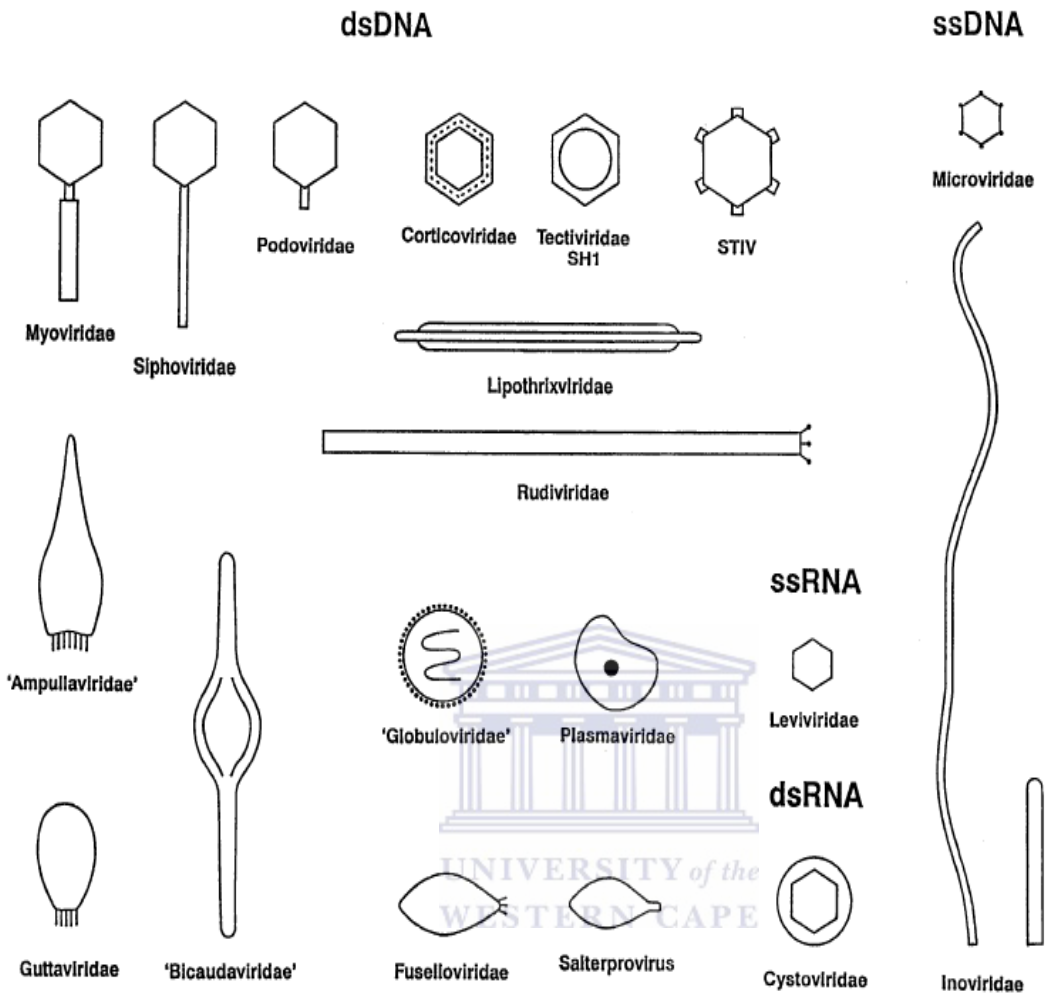

Figure 1: Schematic representation of phage morphologies (adapted from Ackermann, 2007).

1.1.5. Phage life cycles

Viruses, including phage, are not technically classed as living organisms as they have no intrinsic metabolism, parasitizing the host by using their cellular machinery to replicate (Trun & Trempy, 2004).

There are four types of phage life cycles: lytic, lysogenic, chronic and pseudolysogenic, with lytic and lysogenic being the most common (Figure 2). Lytic infection is the pathway where phage infection and replication causes the bacterial cell to burst at the end of the cycle, releasing progeny. Initial adsorption of a phage onto the bacterial cell is reversible, but once phage structures attach to bacterial receptors, the process becomes irreversible. Phage enzymes, present in the head or tail, facilitate bacterial cell wall penetration, and once inside the cell, phage genetic material is released. Phage protein coat is left outside of the cell while the DNA enters the bacterium. After injection, phage nucleic acid either stays in the cytoplasm or integrates with the host genome while simultaneously being protected from bacterial exonuclease degradation. At this stage gene expression, genome replication and development of genomes, capsids and tails, if present, occur. This is followed by phage genome packaging into capsids. The last stage of the phage life cycle is the lysis of the host cell with the release of mature phages (Gani & Yeo, 1965; Weinbauer, 2004).

In lysogenic infection, the phage genetic material integrates with the host cell genome and forms a prophage. This can trigger a host stress response which causes the phage genome to enter the lytic pathway. Chronic infection is non-

lethal; the new phage particles are released from the host cell by budding or extrusion. In pseudolysogenic infection the phage genomic material remains in the host cell before lysis of the host cell occurs. This process is thought to be related to host starvation, where the virus adopts an inactivated state and is unable to initiate viral gene expression owing to the low energy of the host cell (Fuhrman, 1999; Pringsulaka *et al*., 2003; Weinbauer, 2004; Clokie *et al*., 2011).

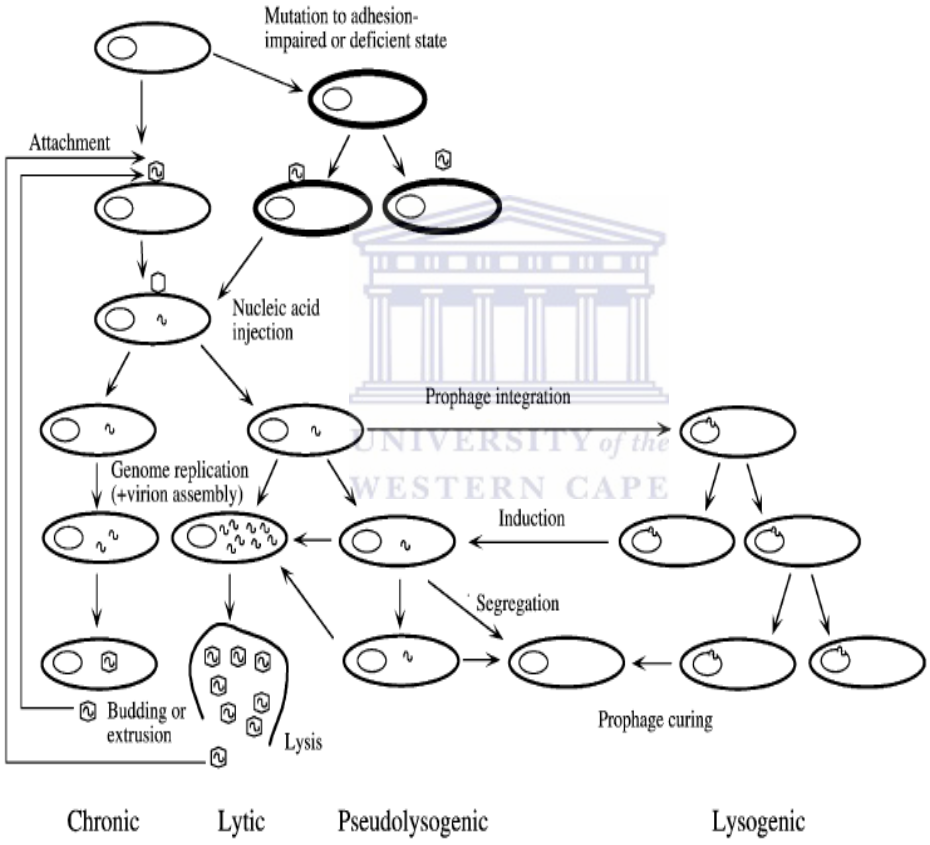

Figure 2: The four types of virus life cycles (taken from Weinbauer, 2004)

1.1.6. Phage genomics

1.1.6.1. Phage genomes

In 1976, the first phage genome of phage MS2 was sequenced (Fiers *et al.,* 1976), followed by the sequencing of the ΦX174 phage genome in 1977 (Sanger *et al.,* 1977) and phage λ in 1982 (Sanger *et al.,* 1982). By 2010 the NCBI phage genome database contained 579 completely sequenced genomes (Zhu *et al.*, 2010). The distribution of completely sequenced phage genomes retrieved from the Genbank Phage Database is shown in Figure 3. Bacteriophages have genome sizes which range from 2 kb to over 700 kb (Brüssow & Hendrix, 2002; Edwards & Rohwer, 2005; Deschavanne *et al.*, 2010). The size of the completely sequenced phage genomes ranges from 2 kb (*Leuconostoc* phage L5) to 498 kb (*Bacillus* phage G) (Deschavanne *et al.*, 2010). Phage morphologies and genome sizes based on the NCBI phage genome database are shown in Figure 4. All the phages isolated in this study were *Caudovirales* (*Siphoviridae*, *Myoviridae* and *Podoviridae*), and only the *Podoviridae* was sequenced. The list of sequenced *Podoviridae* genomes is shown in Table 2. Most phages contain dsDNA with very few having ssDNA, ssRNA, or dsRNA (Ackermann, 2007). Very few reports on genetic studies of ssDNA, ss/dsRNA have been reported as they are not commonly found in the environment (Beck *et al*., 1978; Russel, 1991; Frilander *et al*., 1995; Klovins *et al*., 2002).

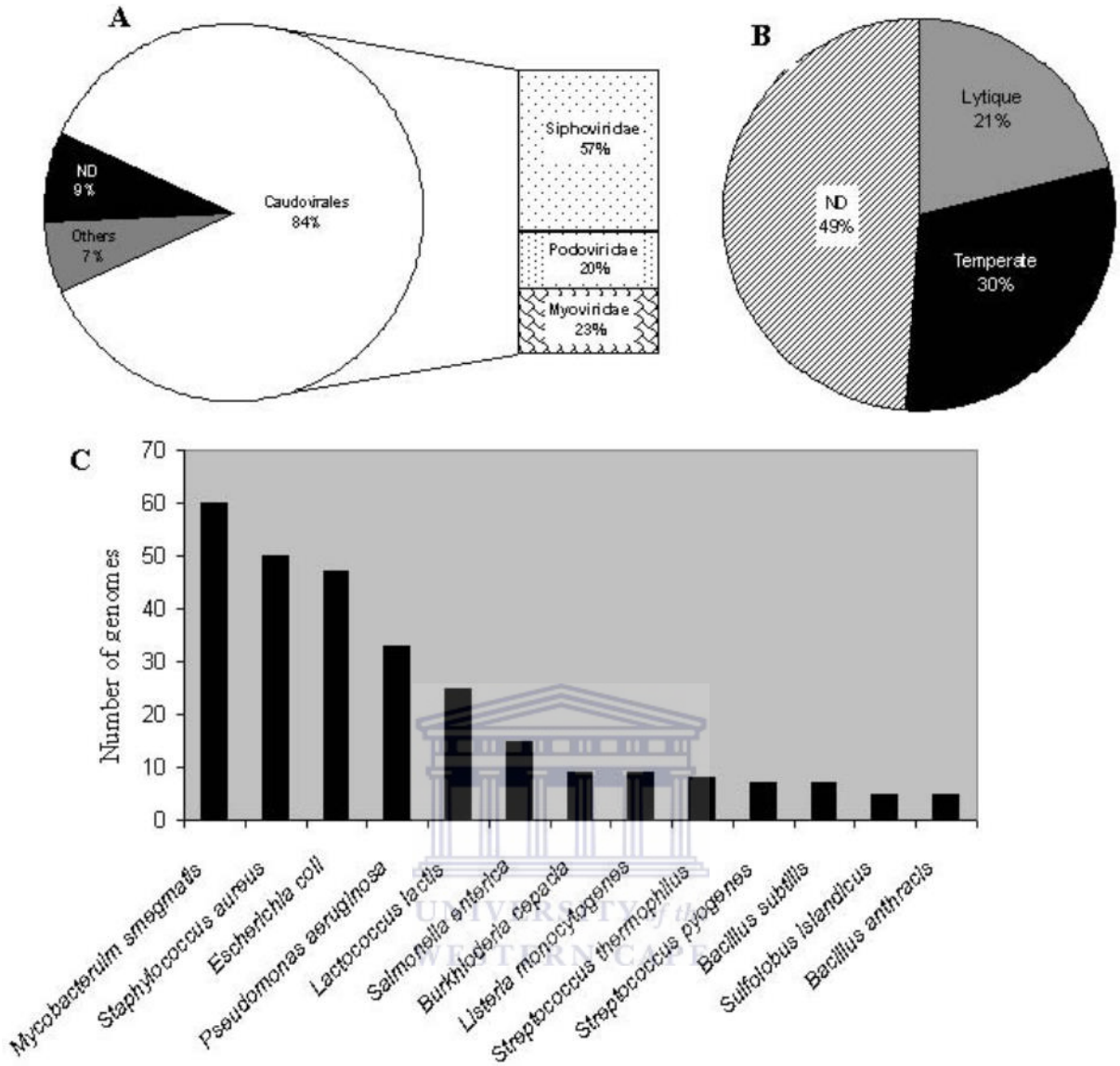

Figure 3: Distribution of completely sequenced phage genomes retrieved from Genbank phage Database. A, Proportion of bacteriophage genomes that belong to the different phage families. B, Proportion of genomes from temperate or lytic phages. C, Number of completely sequenced genomes of phages infecting the same host. Only hosts infected by at least 5 different phages are shown. ND, not indicated in the database (taken from Deschavanne *et al*., 2010).

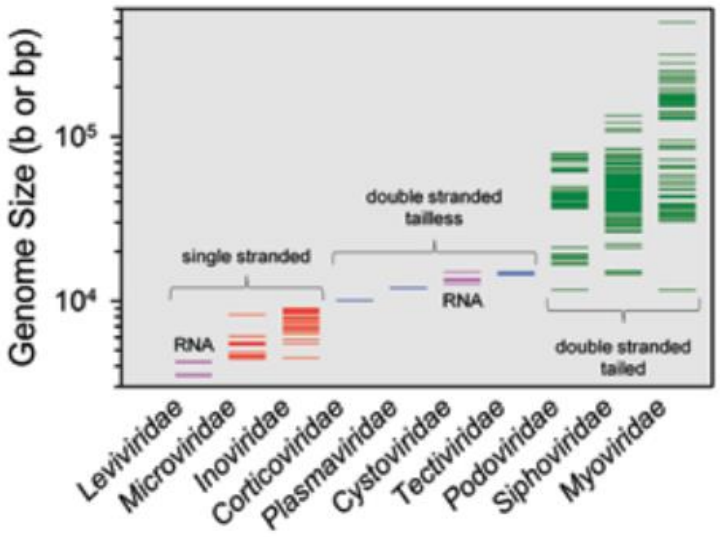

Figure 4: Phage morphologies and genome sizes based on the NCBI phage genome database (taken from Abedon, 2011).

Table 2: List of completely sequenced *Podoviridae* genomes retrieved from Genbank phage Database

Phage	Host genus	Source	Size (kb)	Accession number	Reference
KP32	*Klebsiella pneumoniae*	Sewage samples, Poland	41.1	NC_013647.1	Kęsik-Szeloch *et al*., 2013
KP34	*Klebsiella pneumoniae*	Sewage sample, Poland	43.8	NC_013649.2	Drulis-Kawa *et al*., 2011
N4	*Vibrio cholerae*	Sewage water	38.5	NC_013651.1	Das *et al*., 2012
933W	*Escherichia coli* O157:H7	USA	61.7	NC_000924.1	Plunkett *et al*., 1999
Phi-S1	*Pseudomonas fluorescens*	Sewage Processing plant, Greater Vancouver	40.2	NC_021062.1	Sillankorva *et al.*, 2012
S-RIP1	*Synechococcus* sp. WH8101	Narragansett Bay, Rhode Island	44.9	NC_020867.1	Henn,M.R., Marston,M., unpublished data
S-RIP2	*Synechococcus* sp. WH7803	Rhode Island Sound, Rhode Island	45.7	NC_020838.1	Henn,M.R., Marston,M., unpublished data
VvAW1	*Vibrio vulnificus*	Surface waters, Ala Wai Canal	38.7	NC_020488.1	Nigro *et al*., 2012
HTVC019P	*Candidatus Pelagibacter*	Sea water, Oregon coast	42.1	NC_020483.1	Zhao *et al.,* 2013
HTVC011P	*Candidatus Pelagibacter*	Sea water, Oregon coast	39.9	NC_020482.1	Zhao *et al.,* 2013
HTVC010P	*Candidatus Pelagibacter*	Sea water, Hydrostation S, Bermuda	34.9	NC_020481.1	Zhao *et al*., 2013
UAB_Phi78	*Salmonella enterica* subsp. *enterica* serovar	Spain	43.9	NC_020414.1	Spricigo D.A., Bardina C., unpublished data
vB_CskP_GAP227	*Cronobacter sakazakii*	Sewage samples	41.8	NC_020078.1	Abbasifar *et al*., 2013
KHP30	*Helicobacter pylori*	Japanese patients	26.2	NC_019928.1	Uchiyama *et al.,* 2012
KHP40	*Helicobacter pylori*	Japanese patients	26.5	NC_019931.1	Uchiyama *et al.,* 2012
phiEa100	*Erwinia tasmaniensis*	N/A	45.6	NC_019926.1	Müller *et al*., 2011
AF	*Pseudomonas putida*	Soil, Ilinois, Chicago, USA	42.7	NC_019923.1	Cornelissen *et al*., 2012
BcepMigl	*Burkholderia cenocepacia*	Soil	63.0	NC_019917.1	Gill J.J., Migl D.M., unpublished data

Table 2 continued

CW02	*Salinivibrio costicola*	Great Salt Lake, Utah, USA	49.4	NC_019540.1	Shen *et al*., 2012
SC2	*Ca*. *Liberibacter asiaticus*	Citrus trees, Delray Beach, Florida, U.S.A.	39.4	NC_019550.1	Zhang *et al*., 2011
SC1	*Ca*. *Liberibacter asiaticus*	Citrus trees, Delray Beach, Florida, U.S.A.	40.1	HQ377372.1	Zhang *et al*., 2011
PP1	*Pectobacterium carotovorum*	N/A	44.4	NC_019542.1	Lee *et al.,* 2012
1961P	*Helicobacter pylori*	Clinical strain of *H. pylori*, Taiwan	26.8	NC_019512.1	Luo *et al.,* 2012
IME10	*Escherichia coli*	Sewage, China	39.7	JF974339.1	Tong Y.G., Huang Y., unpublished data
vB_EamP-S6	*Erwinia amylovora*	Soil, Switzerland	74.7	NC_019514.1	Born *et al*., 2011
vB_EamP-L1	*Erwinia amylovora*	Soil, Switzerland	39.3	NC_019510.1	Born *et al.,* 2011
phiKT	*Escherichia* sp.	Horse feces	42.6	NC_019520.1	Tarasyan K.K., Kulikov E.E., unpublished data
phiAS7	*Aeromonas salmonicida* subsp. *salmonicida*	Sediment samples from a fish farm	41.6	NC_019528.1	Kim *et al*., 2012
phi24R	*Clostridium perfringens*	Raw sewage from a waste treatment plant	18.9	NC_019523.1	Morales *et al*., 2012
LIMElight	*Pantoea agglomerans*	Potato fields, Merelbeke, Belgium	44.6	NC_019454.1	Adriaenssens *et al*., 2011
TL-2011c	*Escherichia coli* O103:H25 strain	Patient with HUS during an EHEC outbreak, Norway	60.5	NC_019442.1	L'Abée-Lund *et al*., 2012
TL-2011b	*Escherichia coli* O103:H25 strain	Patient with HUS during an EHEC FT outbreak, Norway	44.8	NC_019445.1	L'Abée-Lund *et al*., 2012
EcP1	*Enterobacter cloacae*	N/A	59.1	NC_019485.1	Zhu J., Rao X., unpublished data
Pr	*Brucella abortus*	Perote, Mexico	38.3	NC_019447.1	Flores *et al*., 2012
Tb	*Brucella abortus*	Manure, Tbilisi, Georgia	41.2	NC_019446.1	Flores *et al*., 2012

Table 2 continued

vB_PaeP_C1-14_Or	*Pseudomonas aeruginosa*	Sewer water sample, Orsay, France	45.5	HE983844.1	Thien *et al.,* 2013
phi80-18	*Yersinia enterocolitica*	N/A	42.1	NC_019911.1	Skurnik M., Nawaz A., unpublished data
phiR8-01	*Yersinia enterocolitica*	N/A	41.7	HE956707.1	Skurnik M., Nawaz A., unpublished data
IME15	*Stenotrophomonas maltophilia*	Hospital sewage	38.5	NC_019416.1	Huang *et al*., 2012
IME11	*Escherichia coli*	The sewage of the no. 307 hospital, Beijing, China	72.6	NC_019423.1	Fan *et al.,* 2012
vB_EcoP_ACG-C91	*Escherichia coli*	Sewage, Canada	43.7	NC_019403.1	Chibeu A., Kropinski A.M., unpublished data
vB_CsaP_GAP52	*Cronobacter sakazaki*	Canada	76.6	NC_019402.1	Abbasifar R., Griffiths M.W., unpublished data
UFV-P2	*Pseudomonas fluorescens*	Wastewater, dairy industry, Brazil	45.5	NC_018850.2	Eller *et al*., 2013
KBNP21	*Escherichia coli*	Chicken farms, Yesan, South Korea	69.9	NC_018854.1	Nho *et al.,* 2012
DC1	*Burkholderia cepacia*	An extract of soil used to cultivate a *Dracaena* sp. in Edmonton, Canada	61.9	NC_018452.1	Lynch *et al*., 2012
phiYS61	*Weissella cibaria*	Kimchi, a Korean fermented vegetable dish	33.6	NC_018270.1	Kleppen *et al*., 2012
vB_SemP_Emek	*Salmonella* Serovar *Haardt*	Sewage, Canada	39.8	NC_018275.1	Ho *et al*., 2012
tf	*Pseudomonas putida* PpG1	Aeration tank, sewage plant, East Lansing, Mich.	46.3	NC_017971.2	Glukhov *et al*., 2012
SPN9CC	*Salmonella* sp.	N/A	40.1	NC_017985.1	Shin H., Lee J.-H., Ryu S., unpublished data
phiCP7R	*Clostridium perfringens*	Raw sewage	18.4	NC_017980.1	Volozhantsev *et al*., 2012

Table 2 continued

vB_Pae-TbilisiM32	*Pseudomonas aeruginosa*	Sewage water, Tbilisi	43.0	NC_017865.1	Karumidze *et al.,* 2012
PEp14	*Erwinia pyrifoliae*	N/A	60.7	NC_016767.1	Kim I.-G., Rhim S.-L., unpublished data
Bf7	*Pseudomonas tolaasii* LMG 2342T	Smashed sporocarps of oyster mushroom	40.1	NC_016764.1	Sajben-Nagy *et al.,* 2012
SPN1S	*Salmonella enterica* Serovar *Typhimurium*	Environmental water	38.7	NC_016761.1	Shin *et al*., 2012
NATL2A-133	*Prochlorococcus* sp. NATL2A	N/A	47.5	NC_016659.1	Henn M.R., Sullivan M.S., unpublished data
NATL1A-7	*Prochlorococcus* sp. NATL1A	Red sea	47.7	NC_016658.1	Henn M.R., Sullivan M.S., unpublished data
9515-10a	*Prochlorococcus* sp. MIT9515	N/A	47.1	NC_016657.1	Henn M.R., Sullivan M.S., unpublished data
S24-1	*Staphylococcus aureus strain* SA27	Sewage plant, Kochi, Japan	18.2	NC_016565.1	Uchiyama J., Takemura I., unpublished data
PaV-LD	*Planktothrix agardhii*	Lake Donghu, a shallow freshwater lake, China.	95.3	NC_016564.1	Gao *et al*., 2011
vB_EcoP_G7C	*Escherichia coli*	Horse feces, Russia	72.9	NC_015933.1	Kulikov *et al*., 2012
285P	*Escherichia coli*	N/A	39.3	NC_015249.1	Ma X., Xiong H., Y., unpublished data
Yepe2	*Yersinia pestis* A1122	N/A	38.7	NC_011038.1	Savalia D., Severinov K., unpublished data
K11	*Klebsiella* sp. 390	N/A	41.2	NC_011043.1	Savalia D., Severinov K., unpublished data
EcoDS1	*Escherichia coli*	N/A	39.3	NC_011042.1	Savalia D., Severinov K., unpublished data
BA14	*Escherichia coli* B208	Sewage	39.8	NC_011040.1	Mertens & Hausmann, 1982
13a	*Escherichia coli*	N/A	38.8	NC_011045.1	Savalia D., Severinov K., unpublished data
P-SSP7	*Prochlorococcus* MED4	N/A	45.2	NC_006882	Sabehi & Lindell, 2012

Table 2 continued

T7	*Escherichia coli*	N/A	39.9	NC_001604.1	Dunn & Studier, 1983
K30	*Eschericha coli*	N/A	40.9	NC_015719.1	Bouwman C.W., Kropinski A.M., unpublished data
LIMEzero	*Pantoea agglomerans*	Soil samples from potato fields in Merelbeke, Belgium	43.0	NC_015585.1	Adriaenssens *et al*., 2011
BcepIL02	*Burkholderia cenocepacia*	A corn field soil sample, Champaign County, IL	62.7	NC_012743.2	Gill *et al*., 2011
DSS3phi2	*Silicibacter pomeroyi* DSS-3	Baltimore Inner Harbor water.	74.6	NC_012697.1	Zhao *et al.,* 2009
EE36phi1	*Sulfitobacter* sp. EE-36	Baltimore Inner Harbor water	73.3	NC_012696.1	Zhao *et al.,* 2009
VP93	*Vibrio parahaemolyticus*	N/A	43.9	NC_012662.1	Bastías *et al*., 2010
phikF77	*Pseudomonas aeruginosa*	Water sample, Russia	43.2	NC_012418.1	Kulakov *et al*., 1985
epsilon34	*Salmonella enterica* serovar Anatum	N/A	43.0	NC_011976.1	Villafane *et al*., 2008
APSE-2	*Candidatus* Hamiltonella defensa	Madison, WI, USA	39.7	NC_011551.1	Degnan & Moran, 2008
Kvp1	*Kluyvera cryocrescens*	N/A	39.5	NC_011534.1	Lingohr *et al*., 2008
RSB1	*Ralstonia solanacearum*	Soil sample from a tomato crop field	43.1	NC_011201.1	Kawasaki *et al.,* 2009
PT2	*Pseudomonas aeruginosa*	N/A	43.0	NC_011107.1	Glonti T., Lingohr E.J., unpublished data
PT5	*Pseudomonas aeruginosa*	N/A	43.0	NC_011105.1	Glonti T., Lingohr E.J., unpublished data
MmP1	*Morganella morganii*	Sewage	38.2	NC_011085.2	Zhu *et al.,* 2010

Table 2 continued

phi29	*Bacillus subtilis*	N/A	19.3	NC_011048.1	Villegas A.P., Lingohr E.J., unpublished data
phiSG-JL2	*Salmonella enterica* serovar Gallinarum	Sewage water collected in Seoul, Korea	38.8	NC_010807.1	Kwon *et al.,* 2008
Min27	*Escherichia coli* O157:H7 str. Min27	China	63.4	NC_010237.1	Su *et al*., 2010
asccphi28	*Lactococcus lactis*	cheese factory whey sample, Australia	18.8	NC_010363.1	Kotsonis *et al.,* 2008
LUZ19	*Pseudomonas aeruginosa* PAO1	Hospital sewage sample, Belgium	43.6	NC_010326.1	Lammens *et al*., 2009
LUZ24	*Pseudomonas aeruginosa* Li010	Hospital sewage sample, Belgium	45.6	NC_010325.1	Ceyssens P.J., Demeke M., unpublished data
Phieco32	*Escherichia coli*	Kura river, Tbilisi, Georgia.	77.6	NC_010324.1	Savalia *et al.,* 2008
BA3	*Thalassomonas loyana* LMG 22536	Israel	37.3	NC_009990.1	Efrony *et al*., 2007
LKA1	*Pseudomonas aeruginosa*	Diljie river water , Kasteelpark Arenberg, Leuven, Belgium	41.6	NC_009936.1	Ceyssens *et al.,* 2006
LKD16	*Pseudomonas aeruginosa*	Pond water, Kortrijk-Dutsel, Leuven, Belgium	43.2	NC_009935.1	Ceyssens *et al*., 2006
SAP-2	*Staphylococcus aureus*	Sewage, South Korea	17.9	NC_009875.1	Son *et al.,* 2010
KSY1	*Lactococcus lactis* IE-16	N/A	79.2	NC_009817.1	Chopin *et al.,* 2007
Av-1	*Actinomyces naeslundii*	N/A	17.2	NC_009643.1	Delisle *et al*., 2006
Pf-WMP3	*Phormidium foveolarum*	Fresh water	43.3	NC_009551.1	Liu *et al.,* 2008
Syn5	*Synechococcus* sp. WH 8109	Sargasso Sea	46.2	NC_009531.1	Pope *et al.,* 2007
Era103	*Erwinia amylovora*	Infected plant tissue	45.5	NC_009014.1	Vandenbergh & Cole, 1986
N4	*Escherichia coli* K-12	N/A	70.2	NC_008720.1	Willis *et al.,* 2002

Table 2 continued

Berlin	*Yersinia pestis*	Bulgaria	38.6	NC_008694.1	Rakin A., unpublished data
Pf-WMP4	*Phormidium foveolarum*	Fresh water	40.9	NC_008367.1	Liu *et al.,* 2007
K1-5	*Escherichia coli* serotypes K1 *and* K5	N/A	44.4	NC_008152.1	Scholl *et al*., 2004
119X	*Pseudomonas aeruginosa*	N/A	43.4	NC_007807.1	Kwan *et al*., 2006
phiV10	*Escherichia coli* O157:H7	N/A	39.1	NC_007804.2	Perry L.L., Applegate B.M.Sr., unpublished data
K1E	*Escherichia coli* K1	Sewage samples	45.3	AM084415.1	Stummeyer *et al*., 2006
K1F	*Escherichia coli* K1	Sewage samples	39.7	NC_007456.1	Scholl & Merril, 2005
VP4	*Vibrio* sp.	N/A	39.5	NC_007149.1	Wang D., Zhao Y., Unpublished data
66	*Staphylococcus aureus*	N/A	18.2	NC_007046.1	Kwan *et al*., 2005
phiKMV	*Pseudomonas aeruginosa*	N/A	42.5	NC_005045.1	Lavigne *et al*., 2003
GA-1	*Bacillus* sp. G1R	United Kingdom: Scotland	21.1	NC_002649.1	Freire *et al.,* 1996
F116	*Pseudomonas aeruginosa*	N/A	65.2	NC_006552.1	Byrne & Kropinski, 2005
P60	*Synechococcus* WH7803	Georgia's coastal rivers, the Satilla River.	47.9	AF338467.2	Chen & Lu, 2002
phiYeO3-12	*Yersinia enterocolitica* serotype *O:3.*	Raw incoming sewage of the Turku, Finland, city sewage treatment plant	39.6	NC_001271.1	Pajunen *et al.,* 2001
Cp-1	*Streptococcus pneumoniae*	N/A	19.3	NC_001825.1	Martin *et al.,* 1996
ST104	*Salmonella enterica* serotype Typhimurium	N/A	41.4	NC_005841.1	Tanaka *et al*., 2004

Table 2 continued

Sf6	*Shigella flexneri*	N/A	39.0	NC_005344.1	Casjens *et al.,* 2004
P22	*Salmonella enterica* serovar Typhimurium	N/A	41.7	NC_002371.2	Pedulla *et al.,* 2003
Mx8	*Myxococcus xanthus* strain DK883	N/A	49.5	NC_003085.1	Youderian P., Walthers D., unpublished data
VP5	*Vibrio cholerae*	N/A	39.8	NC_005891.1	Wang D.Jr., Kan B., unpublished data
BcepC6B	*Burkholderia cepacia*	N/A	42.4	NC_005887.1	Summer E.J., Christian B.N., unpublished data
PaP2	*Pseudomonas aeruginosa*	N/A	43.8	NC_005884.1	Hu F., Huang J., unpublished data
BIP-1	*Bordetella bronchiseptica*	N/A	42.6	NC_005809.1	Liu *et al.,* 2004
BMP-1	*Bordetella bronchiseptica*	N/A	42.7	NC_005808.1	Liu *et al*., 2004
BPP-1	*Bordetella bronchiseptica*	Clinical isolate of B. *bronchiseptica*	42.5	NC_005357.1	Liu *et al.,* 2004
T3	*Escherichia coli*	N/A	38.2	NC_003298.1	Pajunen *et al.,* 2002
Bcep22	*Burkholderia cepacia*	Soil, Orange County, NY	63.9	NC_005262.2	Gill *et al*., 2011
phiA1122	*Yersinia pestis*	Blood of a patient with a clinical case of bubonic plague.	37.6	NC_004777.1	Garcia *et al*., 2003
PaP3	*Pseudomonas aeruginosa*	Hospital sewage	45.5	NC_004466.2	Hardies *et al*., 2007
VpV262	*Vibrio parahaemolyticus*	Natural marine viral community concentrated from seawater	46.0	NC_003907.2	Hardies *et al*., 2003
SIO1	*Rosebacter* SIO67	N/A	39.9	NC_002519.1	Rohwer *et al.,* 2000
SP6	*Salmonella enterica*	N/A	43.8	NC_004831.2	Dobbins *et al*., 2004

Table 2 continued

C1	*Streptococcus* sp. 'group C'	N/A	16.7	NC_004814.1	Nelson *et al.,* 2006
epsilon15	*Salmonella enterica*	N/A	39.7	NC_004775.1	Kropinski *et al*., 2007
P68	*Staphylococcus aureus*	N/A	18.2	NC_004679.1	Vybiral *et al*., 2003
44AHJD	*Staphylococcus aureus*	N/A	16.8	NC_004678.1	Vybiral *et al.,* 2003
gh-1	*Pseudomonas putida*	Sewage samples	37.4	NC_004665.1	Kovalyova & Kropinski, 2003
ST64T	*Salmonella enterica* serovar Typhimurium DT 64	N/A	40.7	NC_004348.1	Mmolawa *et al*., 2003
B103	*Bacillus subtilis*	N/A	18.6	NC_004165.1	Pečenková *et al.,* 1997
P1	*Mycoplasma pulmonis*	N/A	11.7	NC_002515.1	Tu *et al*., 2001
HK620	*Escherichia coli* H, strain 2158	N/A	38.3	NC_002730.1	Clark *et al.,* 2001
Vi06	*Salmonella enterica* serovar Typhimurium	Clinical samples of stools from patients with typhoid fever, Toronto, Canada	38.4	NC_015271.1	Pickard *et al.,* 2010
philBB-PF7A	*Pseudomonas fluorescens*	Raw sewage	41.0	NC_015264.1	Sillankorva *et al.,* 2011
ICP3	*Vibrio cholerae*	Stool sample	39.2	NC_015159.1	Seed *et al.,* 2011
ICP2	*Vibrio cholerae*	Stool sample	49.7	NC_015158.1	Seed *et al*., 2011
ST160	*Salmonella enterica* serotype Typhimurium	N/A	41.0	NC_014900.1	Price-Carter *et al*., 2011
LUZ7	*Pseudomonas aeruginosa*	Hospital sewage samples, Belgium	74.9	NC_013691.1	Ceyssens *et al*., 2010
LIT1	*Pseudomonas aeruginosa*	Hospital sewage samples, Belgium	72.5	NC_013692.1	Ceyssens *et al.,* 2010
Xfas53	*Xylella fastidiosa*	N/A	36.7	NC_013599.1	Summer *et al.*, 2010
phi-2	*Pseudomonas fluorescens* SBW25	N/A	43.14	NC_013638.1	Paterson *et al.,* 2010

1.1.6.2. Phage genomic structure

The genetic structures of the phage population from all environments have been studied (Juhala *et al.*, 2000; Ravin *et al.*, 2000; Clark *et al.*, 2001; Hendrix, 2003). Although a limited number of phage genomic sequences have been reported, sequence comparisons together with environmental studies provide an indication of the size, genetic structure and dynamics of phage populations as well as the mechanisms through which their genomes have evolved (Hendrix, 2003; Casjens, 2005).

The average size of phage gene is approximately 0.6 kb. Many virus structure and assembly genes are relatively large e.g. tape measure genes are >6 kb long, but those genes located on the nonstructural genomic segments are small and often shorter than 100 codons (Pedulla *et al*., 2003; Comeau *et al*., 2007).

The minimal genome of tailed phages encodes DNA packaging, head, tail and tail fibers, DNA replication, transcription regulation, and lysis genes (Brüssow & Hendrix, 2002). The head and tail assembly genes are arranged together with the head genes 5' to the tail genes. The syntenic relationship of the phage structure and assembly genes is conserved in genomes that have no nucleotide sequence similarity and also in phages in which no predicted protein sequences are related (Casjens *et al*., 1992).

Many phages contain a cassette of integration functions (*int*, *attP*). The integration cassette is generally found close to the genome center but in one

instance it was found within a tail gene (Hatfull, 2006; Morris *et al*., 2008). Phages with larger genomes of more than 125 kb have much less well conserved arrangements of gene functions. The gene order of the structure and assembly genes of phage Omega, for example, is conserved and also contains additional genes of unknown functions (Pedulla *et al*., 2003; Comeau *et al*., 2007).

Phages have extra genes which have no function for the virus but are essential for the host bacterium. These genetic elements are called ‘morons’. These genes were initially found in the head and tail regions of lambdoid genomes, inserted between two adjacent phage genes (Juhala *et al*., 2000). The nucleotide composition of the adjacent phage genes, compared to the moron genes, are different causing some to think that morons originate from an outside source after horizontal gene transfer (Campbell & Bostein, 1983; Hendrix *et al.,* 2000).

1.1.6.3. Phage genome during horizontal gene transfer

Phage horizontal gene transfer occurs when substantial fragments of bacterial DNA are transferred from one bacterium to another by a phage or from one phage to another (Hambly & Suttle, 2005). Tailed phages are believed to be the most effective gene transfer vectors known. Temperate phages cause lysogeny in the bacterial genome. Some temperate phages contain ‘lysogenic conversion genes’ that change the bacterial host phenotype. The discovery of a cyanophage containing photosynthetic genes is one of the more interesting

examples of phage lateral gene transfer (Hambly & Suttle, 2005). Bacteriophage also exchange virulence genes amongst themselves by horizontal gene transfer, causing phage mosaicism (Canchaya *et al*., 2003; Hatfull *et al*., 2006).

1.1.6.4. Phage genetic mosaicism

The mosaicism of phages is caused by lateral gene transfer and contributes significantly to phage evolution (Balding *et al.,* 2005; Morgan, 2008). Tailed phages especially share a high degree of mosaicism. These phages may have a high degree of similarity in gene organization with some sequence regions being separated by non-homologous regions. The existence of genetic exchange was initially discovered in the 1960's in the lambdoid phage of *E.coli* as a result of electron microscopic studies and DNA-DNA heteroduplex experiments. The comparison of ST64T and P22 lambdoid phage genomic mosaicism is shown in Figure 5. These investigations demonstrated that the phages are related and also showed the presence of the mosaic boundaries at specific sites in the phage genome sequence (Figure 5) (Hendrix *et al.,* 1999; Juhala *et al.,* 2000; Hendrix, 2002, 2003; Casjen, 2003).

These early heteroduplex studies confirmed that the mosaic boundaries are located between genes and led to the suggestion that recombinant linkers might be present between the genes. It was assumed that the phage community benefited from the linkers by facilitating the reassortment of genes in the population and producing new gene combinations (Hendrix, 2003).

Another example of phage mosaicism was found in a number of dairy phages that infect lactic acid bacteria. Some phage display less mosaicism e.g. the large lytic phage of *E.coli* , phage T4 (Lawrence *et al.,* 2002).

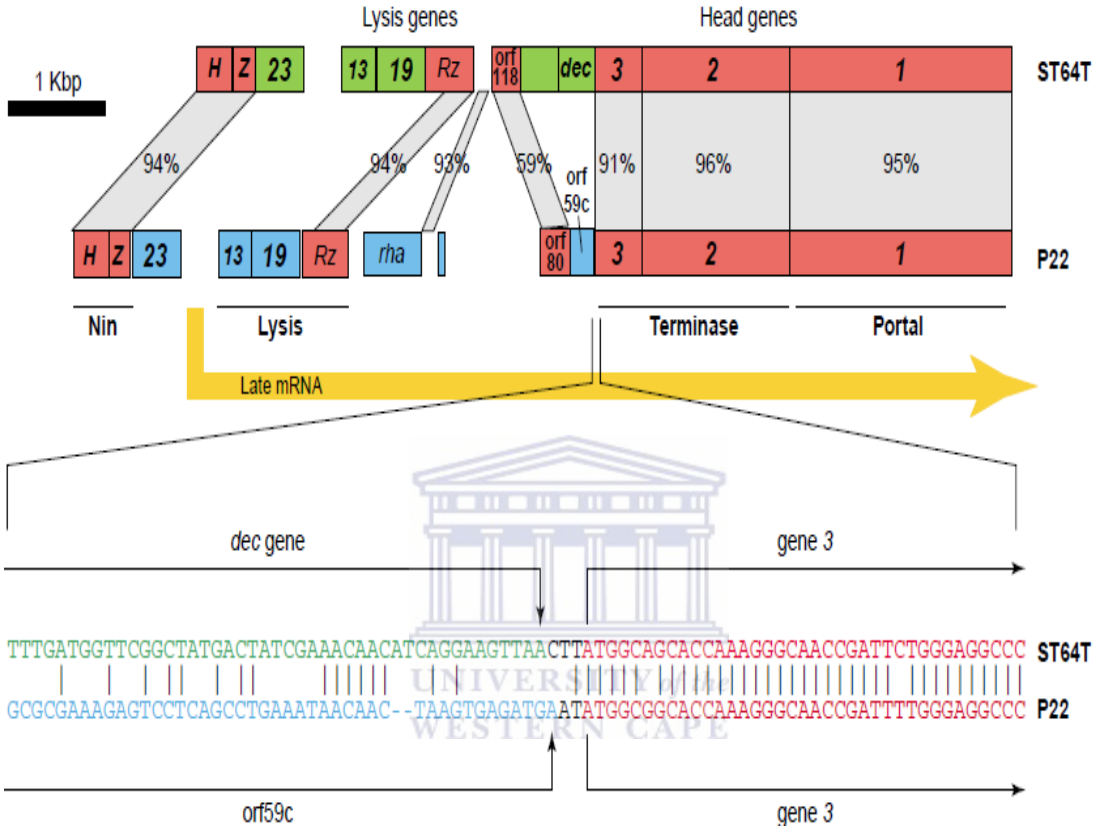

Figure 5: The comparison of ST64T and P22 lambdoid phages genomic mosaicism, illustrated by the late promoter regions (adapted from Casjens, 2005).

1.1.7. Phage impact on the biosphere

1.1.7.1. Bacteriophage abundance and diversity in the biosphere

Bacteriophages occur everywhere in the biosphere, including extreme habitats such as volcanic hot springs (Breitbart & Rohwer, 2005). They play an important role in bacterial evolution by increasing bacterial mutation rates (Weinbauer, 2004). There are three parameters used to assess phage diversity: (i) species evenness, which is the significance of a single species in terms of abundance, activity or biomass (ii) species richness, referring to the species number and (iii) species difference, meaning the taxonomic relatedness of different species within a system (Weinbauer, 2004).

Phages are believed to be the most abundant biological entities in the biosphere, numbering an estimated 10^{31} virus particles (Hendrix, 2002; Weinbauer, 2004; Deresinski, 2009; Ackermann, 2011). Phage abundance is currently estimated to be 10^{8} particles/g of soil and 10^{6} particles/drop of sea water (McNair *et al.,* 2012). Phage hosts are found in feces, sewage, natural bodies of water, in soil and deep thermal vents (Guttman *et al.,* 2005). Approximately 10^{25} bacteriophage infections are estimated to occur globally per second, a process supposedly ongoing since the beginning of cellular life (Casjens, 2003). Phages, particularly the dsDNA phages are more diverse in certain environments such as the oceans and topsoil (Suttle, 2005).

1.1.7.2. Mechanisms influencing phage diversity

The mechanisms controlling phage diversity are not well documented (Weinbauer, 2004). The high level of specificity, long-term survivability, and ability to reproduce rapidly in appropriate hosts all contribute to the ability of phages to mainten a dynamic balance among the wide variety of bacterial species in any natural ecosystem. When no appropriate hosts are present, many phages can maintain the ability to infect for decades unless they are damaged by external agents (Guttman *et al.,* 2005).

The quality and availability of bacterial hosts is the major factor influencing viral diversity. Removal of viruses by grazing by heterotrophic nanoflagellates has been well documented (González & Suttle, 1993; Weinbauer & Höfle, 1998; Bettarel *et al.*, 2005). The grazing of heterotrophic nanoflagellates on viruses is highest when viruses are abundant and the rate of grazing is dependent on the virus type and the grazer type present in the environment. Grazing can only influence viral diversity when viral types are grazed at different rates (González & Suttle, 1993).

Gene exchange between phages and their hosts during transduction and co-infection is another factor influencing viral diversity (Moineau, 1994; 1995). The diversity of lysogenic phages should be higher than that of lytic phages since the genetic exchange rate in lysogenic phages is higher than in lytic phages (Chen & Lu, 2002). Different physiological factors that describe the niches of

phages, such as adsorption to particles, UV light or temperature can also influence phage diversity (Weinbauer, 2004).

1.1.7.3. Relationship between phage and bacterial hosts

In the biosphere bacteria and viruses are estimated to exist in a population consisting of 10^{30} bacterial cells and 10^{31} virus particles, respectively, with numbers varying with the seasons and the geographical environment. Phages are involved in the evolution of their bacterial hosts. Large numbers of phage particles will be generated by the fastest growing hosts within a given environment. Bacteriophages with high replication rates can decrease rapidly in number under host limiting conditions. The survival strategy for both phages and bacteria in any environment is lysogeny, where the bacteriophage nucleic acid becomes incorporated into the host genome (Chibani-Chennoufi *et al.,* 2004; Deschavanne *et al.,* 2010; Winter *et al.,* 2010). High phage concentrations mostly result in bacterial host resistance or lysogeny, which may reduce the rate of phage infection or prolong the phage and bacterial existence in the environment. These aspects show that the relationship between phages and their hosts are complex, especially in natural mixed communities with regime factors such as predation and starvation playing a role (Wolf *et al.,* 2004).

1.1.8. Current applications in biotechnology

1.1.8.1. Phage therapy

In 1919, shortly after phages were discovered, the potential use of phage as therapeutic agents was realised (Summers, 1999). The unreliable and inconsistent results of early attempts at phage therapy, poor understanding of phage biology, the discovery of antibiotics and poor quality control when preparing phage therapeutic stocks caused a reluctance to further develop phage therapy in many western countries (Haq *et al*., 2012). Although phage therapy was abandoned in western countries in the 1940s, it continued in the former Soviet Union and is still in practice there today (Haq *et al*., 2012).

Since its conception, phage therapy has been tried on humans, animals and plants with varying success. It was also proposed that phage therapy could be used to decontaminate carcasses and the local environment. There is a potential for phage therapy to replace conventional antibiotic treatments or to become a complementary addition to antibiotic treatment (Lorch, 1999; Sulakvelidze *et al.,* 2001; Clark & March, 2006; Petty *et al.,* 2006). Phage therapy has advantages and disadvantages over antibiotic therapy. A significant advantage is that phages are very specific to the target bacteria, reducing the chance of disruption of the surrounding bacteria (Haq *et al.,* 2012). The specificity of phage is a disadvantage when the target bacterium is unknown or if the problem is caused by infection by more than one bacterial species (Häusler, 2006).

1.1.8.2. Phage display

In 1985, phage display was used for the first time (Smith, 1985). In phage display, the DNA that encodes a polypeptide is fused with a phage coat-protein gene to display heterologous peptides or proteins on the surface of the phage (Sidhu, 2000). This produces novel phage particles with a wide variety of potential uses. Phage display has been verified as an effective technique to obtain libraries containing billions of different displayed peptides. M13 and related filamentous phages of *E. coli* are the most widely used, but other *E. coli* phages (lambda and T7) have also been used (Benhar, 2001; Willats, 2002). There are several ways of screening phage display libraries to isolate specific displayed proteins e.g. the isolation of those which bind proteins with similar affinities to those of antibodies (Griffiths & Duncan, 1998; Benhar, 2001; Clark & March, 2006). These peptides can be used as therapeutics through inhibition of receptor–ligand interactions, or as antagonists in the detection of pathogens that can cause a threat to the environment, to enhance enzymatic activity or binding properties and in nanowire engineering of nanoparticles (Willats, 2002; Clark & March, 2006; Petty *et al.,* 2006).

1.1.8.3. Phage as vaccine delivery vehicles

There are two approaches to achieve vaccine delivery using phage (i) entire phage particles can be used with the vaccine antigens expressed on the surface (ii) eukaryotic promoter-driven vaccine genes can be incorporated into the phage genome (Gao *et al*., 2010; Haq *et al.,* 2012). Superior antibody

response results have been described in mice and rabbits after phage vaccination (Jepson & March, 2004).

Using the whole phage as a vehicle to deliver vaccine has been described as highly efficient (Jepson & March, 2004). For example, phage genetic vaccination uses expression cassettes with the DNA vaccine under the control of an appropriate eukaryotic promoter (e.g. cytomegalovirus CMV promoter), which is cloned into the lambda phage and purified. The whole phage particle is then injected into the *E. coli* host to replicate. During the process of phage vaccination, phage coat protein protects the DNA from degradation (Jepson & March, 2004; Gao *et al*., 2010).

Phage genome libraries are similar to phage display libraries: expression in *E. coli* and screening with convalescent serum results in the identification of potential vaccines which can be used directly for vaccination (Jepson & March, 2004).

1.1.8.4. Phage typing

Classical bacterial detection methods include phage typing, enrichment on selective media and biochemical testing. These are time consuming procedures which can be problematic in clinical situations. However, the current knowledge of bacterial and phage biology allows the development of quicker, cheaper and more sensitive assays than before. Phage-based detection reduces false

positive results in comparison to other approaches e.g. PCR, which also detect living bacteria (Petty *et al.,* 2006).

Phage typing is the use of phage for bacterial detection. Due to the specificity of phage they have been used to observe low numbers of pathogenic bacteria and can lead to bacterial typing (Clark & March, 2006; Haq *et al.,* 2012). The use of phage in bacterial detection is a highly developed art and many methods are currently in use to identify specific bacteria in samples. Due to the specificity of phage to a target bacterium, and the low-cost and ease-of large-scale production of phages, phages are the perfect tools for bacterial identification when other methods fail. Phage typing has been used in a variety of environments such as identifying food-borne and water-borne pathogens, gauging the effect of disinfection in agriculture and hospitals and in analysing the agents used in bioterrorism incidents (Petty *et al.,* 2006).

1.1.8.5. Phage in recombinant DNA technology

Recombinant DNA techniques have been essential for the isolation and propagation of specific DNA fragments which can be easily sequenced or used as highly specific probes. Several cloning vectors have been developed to meet specific requirements. These cloning vectors have been classified as plasmid or phage vectors. Phages are a potential source of genetic elements for vector construction (Chauthaiwale *et al.,* 1992). The choice of a vector is primarily based on (i) the size of the fragment to be cloned (ii) the restriction enzymes to be used, (iii) the necessity for expression of the cloned fragment

and (iv) the screening method to be used to select the desired clones (Chauthaiwale, 1992).

Phages as cloning vectors

Both single-stranded (filamentous phage such as f1 and M13) and double-stranded *E. coli* phages have been used as cloning vectors. Filamentous phages are lysogenic and can live inside infected cell for decades. They are suitable for cloning genes that produce toxic products. The double-stranded phage, phage lambda, is a suitable cloning vector for several reasons (i) the capsid of phage lambda can package a large foreign DNA fragment (ii) refined techniques aimed at minimizing the problems of background due to nonrecombinants have been developed (iii) thousands of clones can be screened simultaneously on one petri plate and (iv) phage lambda can be stored at 4°C as a clear lysate for months without significant loss in plaque-forming activity (Murray, 1983a & b).

Cosmids

Cosmids such as pWE15 and pWE16 (Wahl *et al.,* 1987) are vectors that are hybrids of plasmids and lambda phages and have sizes of 4 to 6 kb. They are plasmids that contain the phage *cos* sites and can be packaged into phage capsids. Their DNA can replicate in the host cell like a plasmid or may be packaged into capsids like a phage. Cosmids are specifically designed for the cloning of large DNA fragments of between 40 and 50 kb. They contain a drug resistance marker, a plasmid origin of replication, a fragment containing the

ligated cohesive ends (*cos*) of phage lambda and one or more unique restriction sites used for cloning (Collins & Hohn, 1978).

Phagemids

Phagemids, including pUC118 and pUC119 (Vieira & Messing, 1987) and pBS vectors (Short *et al.,* 1988), are the combination of desirable properties of both plasmids and filamentous phages. They contain the ColE1 origin of replication, a selectable marker such as an antibiotic resistance gene and the major intergenic region of a filamentous phage (Dotto & Zinder, 1983, 1984; Dotto *et al.,* 1984). In addition, the major regulatory elements such as promoters, operators, repressor protein encoding genes and terminators of the phage genome that are essential in gene expression technology are present. Promoters that direct the expression of phage structural genes have been used in gene expression technology. The p_L and p_R promoters of phage lambda (Blattner & Dahlberg 1972, Remaut *et al*. 1981, Elvin *et al*. 1990) and the promoter that is situated in front of phage T7 major capsid protein encoding gene10 (Dunn & Studier 1983, Mead *et al*. 1986, Studier *et al.* 1990) are mostly used in recombinant studies using *E. coli*.

1.2. Haloalkaline environments

Haloalkaline environments are environments characterised by high sodium carbonate and sodium chloride levels and high pH values. Examples are the soda lakes and the soda deserts, the desiccated remains of soda lakes (Jones & Grant, 2000). Soda lakes and soda deserts are found worldwide (Jones *et al.,* 1998) in places such as North, Central and South America, Europe, Asia, Africa and Australia (Grant, 2003). This literature review will only cover soda lakes, since the samples used in this work originated from soda lakes.

1.2.1. Introduction to soda lakes

Soda lakes are sodium carbonate (Na_2CO_3)-dominated environments with varying salinity and high pH values, usually between 10 and 11, but occasionally greater than pH 12 (Rainey & Oren, 2006). Soda lakes are found in arid and semi-arid areas where there are high evaporation rates which facilitate the accumulation of salts in local depressions. Due to the high buffering capacity of sodium carbonate, soda lakes are the only habitats that maintain stable high alkaline conditions (Ulukanli & Diúrak, 2002; Sorokin & Kuenen, 2005; Foti *et al.,* 2006). The best studied lakes are those of the East African Rift Valley which have been scientifically documented for many decades (Beadle, 1932; Jenkin, 1932; Rich, 1933; Grant *et al.,* 1990; Jones *et al.,* 1994). The worldwide distribution of soda lakes and soda deserts is shown in Table 3.

The East Africa Rift Valley lakes are situated in an environment of active vulcanism and differ from other soda lakes as surrounding hot springs supply water to the lake depressions, whereas others are supplied by the leaching of rainfall through the surface into the lake basins (Rainey & Oren, 2006).

Table 3: Worldwide distribution of soda lakes and soda deserts (adapted from Grant, 2003)

Continent	Country	Location
Africa	Libya	Lake Fezzan
	Egypt	Wadi Natrun
	Ethiopia	Lake Aranguadi, Lake Kilotes, Lake Abiata, Lake Shala, Lake Chilu, Lake Hertale, Lake Metahara
	Sudan	Dariba Lakes
	Kenya	Lake Bogoria, Lake Nakuru, Lake Elmenteita, Lake Magadi, Lake Simbi, Crater Lake (Lake Sonachi), Lake Oloidien
	Tanzania	Lake Natron, Lake Eyasi, Lake Magad, Lake Manyara, Lake Balangida, Bosotu Crater Lake, Lake Kusare, Lake Tulusia, El Kekhooito, Momela Lakes, Lake Lekandiro, Lake Reshitani, Lake Lgarya, Lake Ndutu
	Uganda	Lake Rukwa North, Lake Katwe, Lake Mahenga, Lake Kikorongo, Lake Nyamunuka
	Chad	Lake Munyanyange, Lake Murumuli, Lake Nunyampaka, Lake Bodu, Lake Rombou, Lake Dijikare, Lake Monboio, Lake Yoan
North America	Canada	Manito
	USA	Alkali Valley, Albert Lake Lenore, Soap Lake, Big Soda Lake, Owens Lake, Borax Lake, Mono Lake, Searles Lake, Deep Springs, Rhodes Marsh, Harney Lake, Summer Lake, Surprise Valley, Pyramid Lake, Walker Lake, Union Pacific Lakes (Green River), Ragtown Soda Lakes
Central America	Mexico	Lake Texcoco
South America	Venezuela	Langunilla Valley
	Chile	Antofagasta
Asia	Siberia	Kulunda Steppe, Tanatar Lakes, Karakul, Chita, Barnaul, Slavgerod, Lake Baikal region, Lake Khatyn
	Armenia	Araxes Plain Lakes
	Turkey	Lake Van, Lake Salda
	India	Lake Looner, Lake Sambhar
	China	Outer Mongolia, various “nors”; Sui-Yuan, Cha-Han-Nor and Na-Lin-Nor, Heilungkiang, Hailar and Tsitsihar; Kirin, Fu-U-Hsein and Taboos-Nor, Liao-Ning, Tao-Nan Hsein, Jehol, various soda lakes; Tibet, alkaline deserts; Chahar, Lang-Chai, Shansi, U-Tsu-Hsein, Shensi, Shen-Hsia-Hsein, Kansu, Ning-Hsia-Hsein, Qinhgai Hu
Australia		Lake Corangamite, Red Rock Lake, Lake Werowrap, Lake Chidnup
Europe	Hungary	Lake Feher
	Former Yugoslavia	Pecena Slatina

1.2.2. Soda lake formation

Soda lakes are comprised of high concentrations of sodium carbonate (usually as sodium carbonate decahydrate or sodium sesquicarbonate) (Ulukanli & Diúrak, 2002). The alkaline conditions of soda lakes are known to be derived from the presence of high levels of sodium carbonate. A number of theories about the source of this sodium carbonate have been proposed (Baker, 1958; Abd-el-Malek & Rizk, 1963). A schematic representation of soda lake formation is shown in Figure 6. There are three conditions leading to the formation of a soda lake: firstly, climatic conditions control the amount of water entering the basin as surface runoff or rainfall, and the amount of water leaving by evaporation leading to the formation of a saline environment. Secondly, geochemical conditions influence alkaline water formation. Groundwaters containing bicarbonate ion (HCO_3^-) are produced, with the molar concentrations of HCO_3^-/CO_3^- being higher than that of Ca^{2+} and Mg^{2+}. Such water quickly reaches the saturation state through evaporation due to the precipitation of Ca^{2+} and Mg^{2+} earth cations as insoluble carbonates, resulting in Na^+, Cl^- and HCO_3^-/CO_3^- being the major ions in solution (Grant & Tindall, 1986; Grant, 1992). The shift in the $CO_2/ HCO_3^-/CO_3^{2-}$ equilibrium towards CO_3^{2-} results in the development of an alkaline environment. This evaporative concentration accumulates other ions, particularly Cl^-, making the lake water saline or hypersaline. Lastly, the influence of topography allows the salt to concentrate in a shallow depression forming a closed drainage basin with high marginal relief. Rainfall sustains the streams entering the basin, producing a standing body of water. Due to the topography the system is closed which prevents water from

flowing out of the basin. Water loss therefore only occurs by evaporation (Eugster & Hardie, 1978; Tindall, 1988).

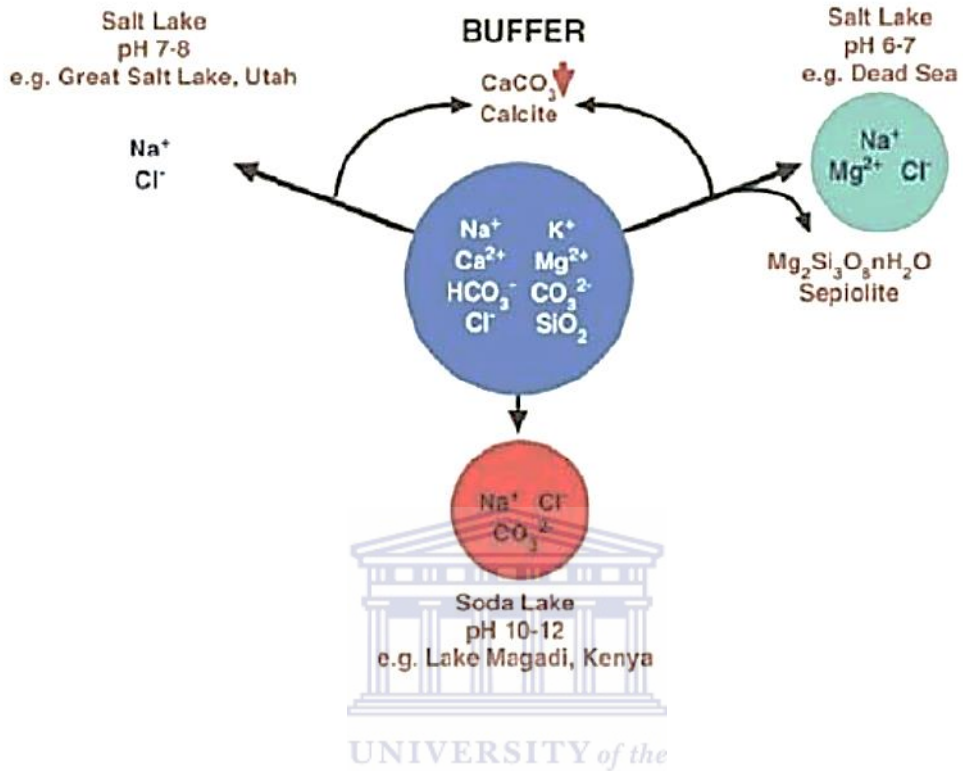

Figure 6: Schematic representation of soda lake formation (taken from Grant, 2003)

1.2.3. Lake Magadi

Lake Magadi in Kenya is an alkaline saline lake with a surface area of approximately 100 sq km. The NaCl concentration of this lake is greater than 30% with a pH of up to 11 (Jones *et al*., 1998; Denson *et al*., 2010). Lake Magadi is the southernmost lake within the Kenyan Rift Valley, situated about 130 km southwest of Nairobi (Baker, 1958; Denson *et al*., 2010). A map of Kenya showing the location of the lake is shown in Figure 7. The lake is comprised of concentrated brines, and precipitated sodium carbonate, trona (a double salt mineral containing sodium carbonate and sodium hydrogen

carbonate) and hydrated sodium bicarbonate. The lake water is supplied by a number of hot springs situated in the area surrounding the lake and by periodic rainfall. There are no perennial rivers flowing into Lake Magadi. Microbial diversity of this lake has been well characterized (Denson *et al.,* 2010). Lake Magadi is hypersaline with volcanic bedrocks, and is the only lake in which bedded evaporites have accumulated (Nissenbaum, 1980).

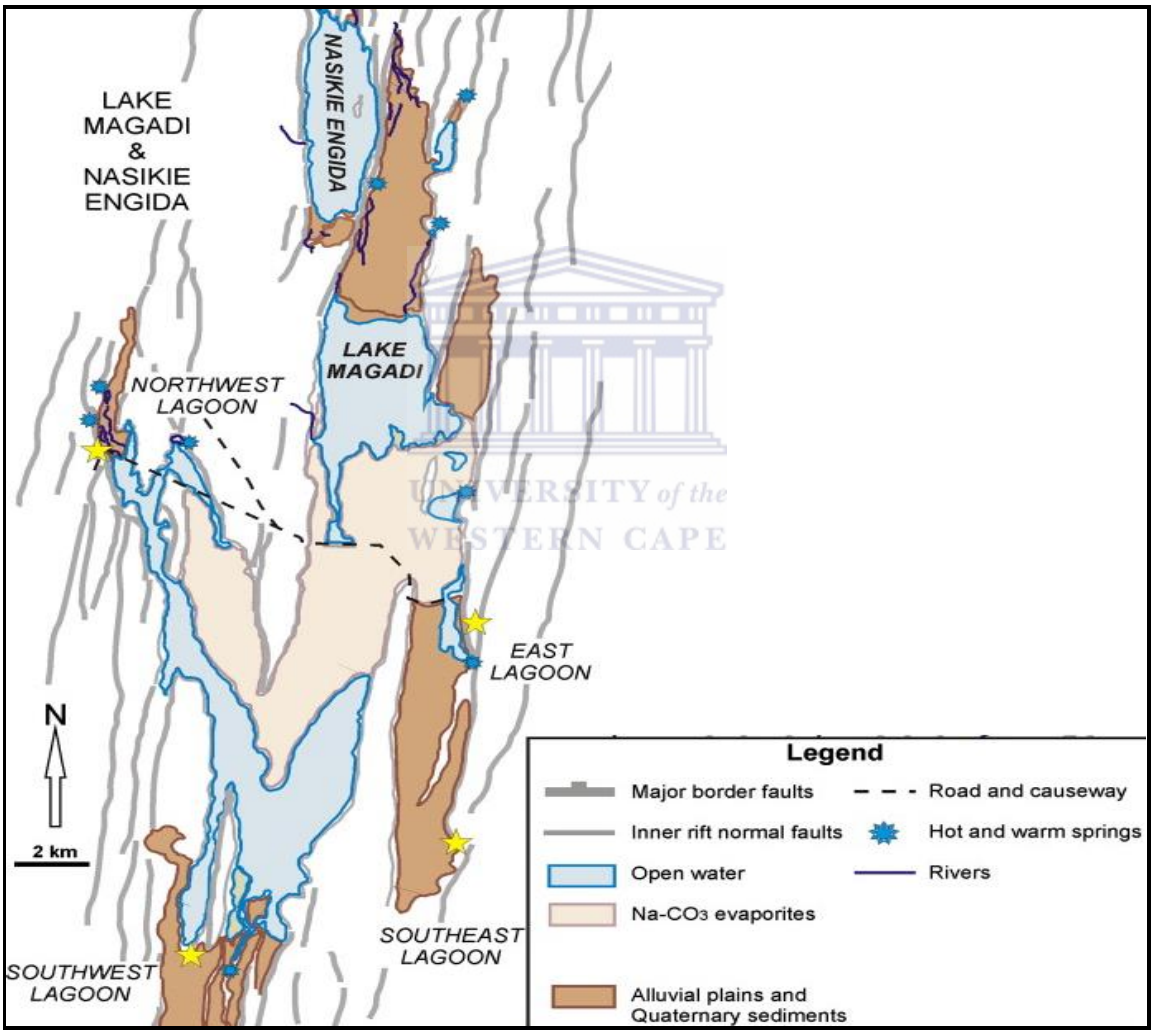

Figure 7: Map showing Lake Magadi, Kenya (taken from Scott *et al*., 2012).

1.2.4. Lake Shala

Lake Shala is a classic soda lake and its waters have an 18% (w/v) NaCl concentration and a pH of 9 (Zinabu, 2002; Legesse *et al.,* 2004). It is 26 km in length lies in a deep caldera with a maximum depth of 260 m. A map showing the locations of Ethiopian soda lakes including Lake Shala is shown in Figure 8. Lake Shala is the deepest of the Ethiopian Rift Valley lakes (Von Damm & Edmond, 1984) and is almost completely surrounded by high hills of volcanic rocks. These are comprised mainly of basalt, rhyolith, pumice, pyroclastics and ignimbrites with some obsidian flows. The lake has sparse phytoplankton, fish and bird communities and a very low calcium concentration. Lake Shala lies between 7°45 'N and 38°45 'W at approximately 1540 m altitude (Gasse & Street, 1978) and is supplied with water by the Digo River from the West and by mountain streams from the East Rift Valley. Around the shore, and probably under the surface, there are a number of hot springs. Lake Shala lacks a high fish population because of the extreme alkaline conditions. The temporal change of the lake alkalinity to highly alkaline further reduces the fish population, leading to the death of existing fish-eating birds. In contrast to Lake Magadi the microbial diversity of this lake has not been well studied (Baumann *et al.,* 1975; Tudorancea & Harrison, 1988; Legesse *et al.,* 2004).

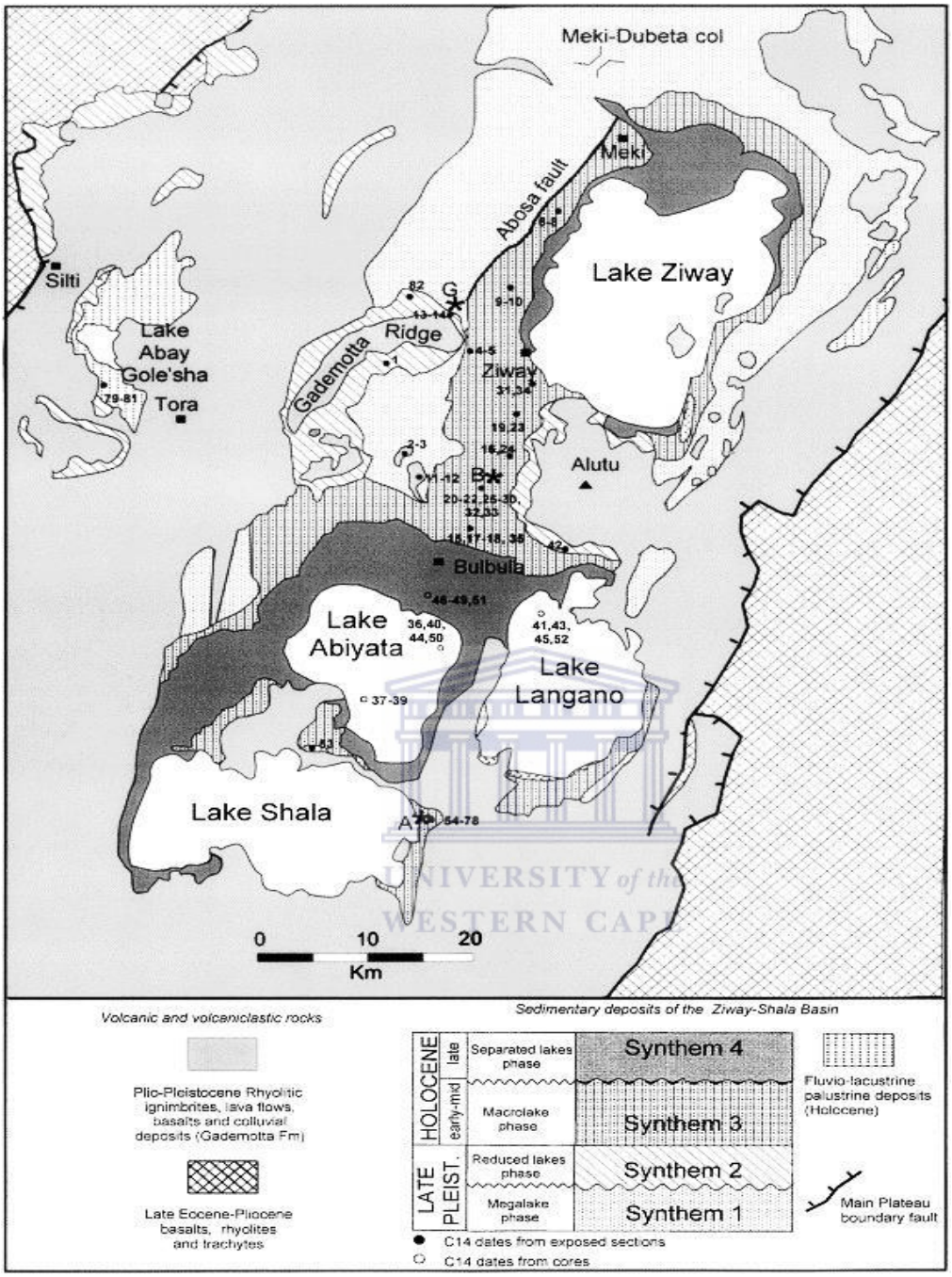

Figure 8: Map showing Lake Shala, Ethiopia (taken from Benvenutia *et al.,* 2002)

1.2.5. Microbial diversity of soda lakes

The colour of soda lakes is their most noticeable feature; depending on the conditions of the water chemistry, the water may appear green, orange, purple or red. The colouration is caused by the bloom of microorganisms in the water. This is reflected in the very high primary productivities associated with these lakes (Melack & Kilham, 1974; Grant *et al.,* 1990). Because of high light intensities, high ambient temperatures, phosphate availability and unlimited supply of CO_2 present in soda lakes, they are despite their extreme conditions considered to be the most productive aquatic environments on earth. Their productivity rates exceed 10g cm^{-2} per day (Grant *et al.,* 1990).

Due to the lack of grazing pressure soda lakes are populated almost entirely by prokaryotes (Sorokin & Kuenen, 2005). Taxonomic groups containing prokaryotes isolated from soda lakes are shown in Table 4. In rare instances, eukaryotic algae and protozoans make up a significant fraction of the population, usually in more dilute and less alkaline soda lakes (Grant, 2003). Diverse populations of aerobic, anaerobic, organotrophic, alkaliphilic and halophilic members of bacteria and archaeal phyla have been found in soda lakes of the East African Rift Valley (Duckworth *et al.,* 1996; Grant *et al.,* 1999; Zavarzin *et al.,* 1999). Phototrophic productivity that results in the production of the dense population of cyanobacteria plays an important role in supporting the rest of the microbial community (Jones *et al.,* 1994; Grant *et al.,* 1999).

Table 4: Taxonomic groups containing prokaryotes isolated from soda lakes.

Eubacteria	
Cyanobacteria	Dubinin *et al.,* 1995
Firmicutes	
(High G+C gram positive bacteria)	
Actinobacteria	
Actinomycetales	
Dietziaceae	
Dietzia	Duckworth *et al*., 1998
Micrococcaceae	
Arthrobacter	Duckworth *et al*., 1996
Intrasporangiaceae	
Terrabacter	Duckworth *et al*., 1996
(Low G+C gram positive bacteria)	
Bacilli	
*Bacillale*s	
Bacillaceae	
Amphibacillus	
Amphibacillus fermentum	Zhilina *et al*., 2001a
Bacillus (group 6)	Duckworth *et al*., 1996
Bacillus (group 7)	Duckworth *et al*., 1996
Clostridia	
Clostridiales	
Clostridiaceae	
Clostridium (clusture XI)	Jones *et al*., 1998
Natronincola	
Natronincola ferrireducens	Zhilina *et al.,* 2009
Tindallia	
Tindallia magadiensis	Kevbrin *et al*., 1998
Heliodacteriaceae	
Heliorestis	
Heliorestis acidaminivorans	Asao *et al*., 2012
Halanaerobiales	
Halobacteroidaceae	
Halonatronum	
Halonatronum saccharophilum	Zhilina *et al*., 2001b
Halanaerobiaceae	
Natroniella	
Natroniella acetigena	Zhilina *et al.,* 1996a
Proteobacteria	
Alpha subdivision	
Rhizobiales	
Bradyrhizobiaceae	
Nitrobacter	Sorokin *et al*., 1998
Rhodobacterals	
Rhodobacteraceae	
Paracoccus	Zavarzin *et al*., 1999
Rhodobaca	Boldareva *et al*., 2008
Gamma subdivision	
Chromatiales	
Chromatiaceae	

Thioalkalicoccus	Bryantseva *et al*., 2000
Ectothiorhodospiraceae	
Alkalilimnicola	
Alkalilimnicola ehrlichii	Hoeft *et al*., 2007
Ectothiorhodospira	
Ectothiorhodospira variabilis	Gorlenko *et al.,* 2009
Halorhodospira	
Halorhodospira abdelmalekii	Imhoff *et al*., 1979
Halorhodospira halophila	Grant & Tindall, 1986
Thiorhodospira	
Thioalkalivibrio	
Thioalkalivibrio paradoxus	Sorokin *et al*., 2002
Thiotrichales	
Piscirickettsiaceae	
Thioalkalimicrobium	
Thioalkalimicrobium aerophilum	Sorokin *et al*., 2001
Thioalkalimicrobium sibericum	Sorokin *et al*., 2001
Methylococcales	
Methylococcaceae	
Methylobacter	Khmelenina *et al*., 1997
Methylomicrobium	
Methylomicrobium buryatense	Kaluzhnaya *et al*., 2001
Oceanspirillales	
Halomonadacea	
Halomonas	
Halomonas mongoliensis	Boltianskaia *et al.,* 2007
Pseudomonadaceae	
Pseudomonas	Duckworth *et al*., 1996
Xanthomonadaceae	
Stenotrophomonas	Duckworth *et al*., 1996
Aeromonadaceae	
Aeromonas	Duckworth *et al*., 1996
Alteromonadaceae	
Alteromonas	Duckworth *et al*., 1996
Vibrionales	
Vibrionaceae	
Vibrio	Duckworth *et al*., 1996
Delta subdivision	
Desulfonatronovibrionales	
Desulfohalobiaceae	
Desulfonatronovibrio	
Desulfonatronovibrio hydrogenovorans	Zhilina *et al*., 1997
Desulfonatronumaceae	
Desulfonatronum	Sorokin *et al*., 2011
Spirochaetes	
Spirochaetales	
Spirochaetaceae	
Spirochaeta	
Spirochaeta alkalica	Zhilina *et al*., 1996b
Archaea	
Euryarchaeota	
Halobacteria	
Halobacteriales	
Halobacteriaceae	

Halorubrum	
Halorubrum alkaliphilum	Feng *et al*., 2005
Natrialba	
Natrialba Magadii	Tindall *et al*., 1984
Natronobacterium	
Natronobacterium vacuolata	Mwatha & Grant 1993
Natronococcus	
Natronococcus amylolyticus	Kanal *et al*., 1995
Natronomonas	
Natronomonas pharaonis	Tindall *et al.,* 1984
Natronorubrum	
Natronorubrum bangense	Xu *et al.,* 1999
Natronorubrum tibetense	Xu *et al.,* 1999
Methanococci	
Methanosarcinales	
Methanosarcinaceae	
Methanohalophilus	
Methanohalophilus oregonense	Liu *et al*., 1990
Methanohalophilus zhilinae	Boone *et al.,* 1986

1.2.6. Survival strategy of microbes in soda lakes

The question of how haloalkaliphiles adapt to harsh environments is a challenging and interesting topic. Success in these high salt and high pH environments requires strong microbial adaptive mechanisms. The cell wall of haloalkaliphiles acts as a defence barrier, protecting the cell from extreme conditions (Horikoshi, 2006). The cell wall is comprised of two membranes which have the ability to reduce the pH at the cell surface. At an alkaline pH the plasma membrane is very unstable and therefore the pH must be maintained below 9. Homeostasis of the plasma membrane can also be maintained by the Na^+/H^+ antiporter, the K^+/H^+ antiporter system and ATPase-driven H^+ expulsion (Horikoshi, 1999).

The internal cytoplasmic pH of alkaliphilic microbes is suggested to be similar to the optimal pH of intracellular enzymes (Horikoshi, 1999). For example a galactosidase enzyme found in the alkaliphilic *Micrococcus* sp. strain 31-2, has an optimal pH of 7.5. This suggests that the internal cytoplasmic pH is around neutral. The presence of Na^+ in soda lakes is essential for the transport of solutes through the membranes of the organisms (Horikoshi, 1999). Because of the low concentration of hydrogen ions, haloalkaliphilic microbes have difficulty using ATP-synthase to produce energy. Other metabolically important ions such as Ca^{2+} and Mg^{2+} are difficult to metabolise as they precipitate out of the solution in the form of salts (Krulwich, 1998). Alkaliphilic microorganisms overcome these problems by pumping in some ions and by exporting others to keep the inner pH near neutral (Horikoshi, 2006).

1.3. Phage diversity in soda lakes

The bacterial diversity of soda lakes has been studied but no viruses were recorded until 2004, when Jiang and co-workers surveyed the population of viruses in Mono Lake, California, USA (Jiang *et al.,* 2004). Their findings placed viral abundance between 10^8 to 10^9 ml^{-1}, among the most numerous biological entities in natural aquatic environments.

ϕMono1 phage is a lytic phage isolated from a haloalkaline environment (Mono Lake) that is still not fully characterized. ϕMono1 is a dsDNA phage that infects bacterial strains closely related to *Idiomarina baltica* which was previously isolated from central Baltic Sea surface waters (Jiang *et al.,* 2004; Romancer *et al.,* 2007). Another phage Ø1M3-16, was isolated from Lake Magadi. This is a lytic phage found to infect *Vibrio metschnikovii*. This tailed phage belongs to the family *Siphoviridae* and contains a genome size of less than 30 kb (Moulton *et al*., 2011).

The seasonal presence of cultured and uncultured phage in Mono Lake was studied by Sabet and colleagues (Sabet *et al.,* 2006). Phages that infect *Halomonas boliviensis*, *Marinospirillum alkaliphilum* and *Vibrio* spp. were identified. The cloning of the entire viral DNA metagenome was attempted to characterize the uncultured phage population. The resulting open reading frames (ORFs) showed homology to several different phage proteins, however some unique and some very low homologies were recorded. The most similar match was to the capsid protein of the haloalkaliphilic phage, ϕCh1 (Sabet *et*

al., 2006). ϕCh1 phage is a unique phage that infects *Natrialba magadii,* a bacterium isolated from Lake Magadi (Tindall *et al.,* 1984). This phage contains both a linear dsDNA genome of about 58 kb and some RNA species (Witte *et al*., 1997; Rössler *et al.,* 2004; Ventosa, 2006). Morphological characterization of viruses isolated from the waters of Mono Lake was undertaken to describe different viral assemblages present in the waters. A variety of morphologies were found. The capsids of viruses were larger on average when compared to those found in other aquatic environments (Brum & Steward, 2010).

1.4. Aims of the study

The main aim of this project was to understand the diversity and biology of bacteriophage communities in particular haloalkaline environments. Culture dependent studies were chosen to investigate the diversity of bacteriophage in Lake Shala and Lake Magadi sediments.

The specific objectives are:

- To isolate and characterize bacterial hosts from haloalkaline lake soil sediment samples using morpological and 16S rRNA sequence analysis
- To isolate bacteriophages from haloalkaline lake soil sediment samples
- To investigate haloalkaliphilic phage morphologies using TEM
- To conduct one step growth curves of haloalkaliphilic phages identified in the study
- To determine/analyse the genome of selected novel phages

Chapter 2: Materials and methods

2.1. Chemicals and reagents

Culture media were supplied by Oxoid Ltd and Biolabs. Chemicals were purchased from Kimix Chemical and Laboratory Supplies, Sigma-Aldrich Chemical Company and Merck Chemical and Laboratory Supplies. All chemicals used in this work were of analytical grade. Whitehead Scientific supplied oligonucleotide primers for polymerase chain reactions (PCR). Other materials such as DNA size markers and restriction endonucleases were supplied by Fermentas Life Sciences Ltd.

2.2. Growth media, buffers and primers

2.2.1. Media

Medium A

Sato *et al.* (1983) and Horikoshi (1999) used this medium to isolate haloalkaliphilic organisms. Medium A (broth, agar and soft agar) was used to culture both bacteria and phage in this study. Medium A broth contained 1% of glucose, 0.5% of peptone, 0.5% of yeast extract, 1% of NaCl, 0.1% of K_2HPO_4 and 0.02% of $MgSO_4.7H_2O$. Medium A agar was prepared from Medium A broth with the addition of 1.5% of bacteriological agar. Soft agar used in plaque assay required 0.75 % of bacteriological agar. All components were dissolved in water and the pH was adjusted to 9 using 32% HCl. The medium was autoclaved at 121^{o}C for 20 min.

Luria-Bertani Medium (LB)

LB medium was used to grow *Escherichia coli* GeneHog strain containing phage clones. Luria-Bertani broth (LB) contained 1% of tryptone, 0.5% of yeast extract and 1% of NaCl. Luria-Bertani agar (LBA) was prepared from LB broth with the addition of 1.5% bacteriological agar. All components were dissolved in water and the pH was adjusted to 7 using 1M NaOH then autoclaved at 121^{o}C for 20 min.

2.2.2. Buffers

SM buffer

SM buffer was prepared using 20 ml of 5M NaCl, 8.5 ml of 1M $MgSO_4$, 50 ml of Tris-HCl (pH 7.5) and 10 ml of 1 % Gelatin Solution. Distilled water was added to make a 1L volume. The pH was adjusted to 7 with 10M NaOH before autoclaving. SM buffer was used for storage of purified phage preparations.

Tris-EDTA (TE)

1x TE contained 10 ml of 1M Tris-Cl (pH 7.5) and 2 ml of 0.5M EDTA (pH 8.0). The volume was adjusted to 1L with dH_2O. The solution was autoclaved and stored at room temperature.

TENS buffer

TENS buffer contained: 5g of SDS, 5.84 of NaCl, 10 ml of 1M Tris, pH 7.4 and 10 ml of 0.5 EDTA. The solution was adjusted to 1L using dH_2O. This buffer was autoclaved at 121^{o}C for 20 min. TENS buffer was used for plasmid extractions.

2.2.3. Primers

Primers sets used in this study for amplification of 16S rRNA gene and sequencing the inserts are shown in Table 5.

Table 5: Primers sets used in this study

Primer	Sequence	Target	References
E9F	GAGTTTGATCCTGGCTCAG	16s rRNA	Hansen *et al.,* 1998
U1510R	GGTTACCTTGTTACGACTT	16s rRNA	Baker *et al.,* 2003
pJET1.2Fwd	CGACTCACTATAGGGAGAGCGGC	pJet1.2 Cloning Vector	Fermentas
pJET1.2Rev	AAGAACATCGATTTTCCATGGCAG	pJet1.2 Cloning Vector	Fermentas

2.3. Sampling sites and collection

Soda lakes soil sediment samples were collected by Professor M.Tuffin from Lake Magadi, Kenya and Lake Shala, Ethiopia in 2009. Samples were collected in 50 ml sterile Falcon tubes and stored at -80^{o}C.

2.4. Bacterial hosts, phage isolation and phage growth curve determination

2.4.1. Bacterial host isolation

Bacteria were isolated from Lake Magadi and Lake Shala soil sediments, which had been stored at -80^{o}C. Ten grams of sediment from each sample were suspended in 100 ml of medium A broth and diluted 10 fold with water. 0.1 ml

volumes of each dilution were spread on medium A agar plates, which were incubated for 24 hours at 37^{o}C. After visual inspection bacterial strains of varying morphologies, were picked from each plate. Strains were purified by three cycles of streaking onto fresh plates. Bacterial strains were stored in medium A broth containing 50% glycerol at -80^{o}C until required.

2.4.2. Bacterial host growth curve

Bacterial hosts were grown in 5 ml of Medium A broth and incubated at 37^{o}C overnight on a shaking platform at 120 rpm. Two millilitres of each overnight culture was inoculated into 200 ml of Medium A broth. One millilitre of each sample was transferred into microfuge tubes and the cultures were incubated at 37^{o}C at 120 rpm shaking platform. The spectrophotometer was blanked using Medium A. Samples were taken every hour for 16 hours and the optical density (OD_{600}) of cultures at each time interval was recorded.

2.4.3. Phage isolation

Fifty grams of each soil sediment was mixed with 100 ml of Medium A broth and incubated at 37^{o}C on a shaking platform at 120 rpm for 24 hours. Fifty millilitre aliquots were removed and centrifuged at 5000 x *g* for 15 min. The suspensions were filter-sterilized using 0.45 followed by 0.22 µm syringe filters. The filtered lysates were used for phage-host infection test plaque assays (Adams, 1959) using two-layer agar plates containing 10 ml agar as the bottom layer and 3 ml of soft agar as the upper layer. The soft agar layer contained

100 μl of both bacterial culture and phage lysate. Plates were incubated at 37 ^{o}C for 24 hours. A single plaque was picked using a sterile 1 ml pipette tip and sub-cultured again using the same host strain. This phage purification process was repeated 3 times. After phage purification, phage stocks were stored in medium A containing 50% glycerol at -80 ^{o}C for long term storage.

2.4.4. Plaque assay

Bacterial hosts were grown in 5 ml of Medium A broth overnight at 37^{o}C on a shaking platform at 120 rpm. A 100 μl volume of both bacterial culture and phage lysate was mixed with 3 ml of Medium A soft agar and poured onto Medium A agar plates. The agar plates were allowed to dry for 20 min at room temperature and incubated overnight at 37^{o}C. Phage infection was indicated by clear or turbid plaques on the bacterial lawn.

2.4.5. Phage lysate preparation and purification

Phage purification was conducted by several re-infections from single plaques until homogeneity was observed. This was performed by picking out a distinct plaque from the overlay agar using a p1000 pipette with a widened tip and transferring the plaque into 10 ml of Medium A broth containing 2 ml of an overnight culture of bacterium. The culture was incubated overnight at 37^{o}C at 120 rpm on a shaking platform. Bacteria were removed by centrifugation at 5000 x *g* for 30 min and the filtrate was filter sterilized using 0.45 followed by 0.22 μm syringe filters.

2.4.6. Agar spot assay

Each bacterial host was grown overnight in 5 ml of Medium A broth at 37^{o}C at 120 rpm on a shaking platform. A 100 ml volume of each bacterial culture was mixed with 3 ml of Medium A soft agar and poured onto a Medium A agar containing plate. The plates were allowed to dry at room temperature for 20 min. A 2 μl volume of each phage lysate was spotted onto the solidified plates and the plates were incubated overnight at 37^{o}C. Clear lysis zones indicated phage infection.

2.4.7. One-step growth curve

A one step growth curve was determined as described by Middelboe *et al.* (2010). Bacterial host strains were cultured overnight in 5 ml of Medium A broth at 37^{o}C at 120 rpm on a shaking platform. Two hundred microliters of each overnight culture was inoculated in 50 ml of Medium A broth and incubated at 37^{o}C at 120 rpm on a shaking platform until the cell density of the cultures reached approximately 1x10^{8} CFU/ml. One millilitre aliquots of each bacterial culture were mixed in microfuge tubes with 0.1 multiplicity of infection (MOI) (MOI = Plaque forming units (pfu) of virus used for infection / number of cells). of phage, in triplicate, and incubated at 37^{o}C at 120 rpm on a shaking platform for 10 min allowing the phage to adsorb to the bacterial host. Cells were centrifuged at 6000 x *g* for 10 min to remove the unadsorbed phage. Supernatants were removed and the pellets were resuspended in 1 ml of medium A broth. Fifty microliters of the resuspended cultures were transferred

to 50 ml of Medium A and mixed well. One millilitre of each culture was transferred into a microfuge tube (time was noted as T=0) and the triplicate cultures were incubated at 37^{o}C at 120 rpm on a shaking platform. Samples were taken every 30 min for 6 and half hours. Plaque forming units (PFU) were determined by the plaque assay (section 2.4.4).

2.5. Phage preparation and visualisation by transmission electron microscopy (TEM)

2.5.1. Phage lysate purification

A method described by Fortier & Moineau (2007) was used. Phage lysates (100 ml) were centrifuged at 13000 x *g* for 1 hour. Supernatants were discarded and the pellets were resuspended in 1 ml of 0.1M ammonium acetate solution, and incubated at 37^{o}C at 120 rpm on a shaking platform for 16 hours to allow resuspension of the phage pellets. Centrifugation and resuspension steps were repeated at least twice to purify phage. Each phage suspension was resuspended in 20 µl of 0.1M ammonium acetate after the last washing step.

2.5.2. Transmission electron microscopy

TEM images were taken with an FEI Tecnai F20 Field Emission Gun operated at 200 keV at the University of Cape Town's Electron Microscopy Unit. Two microliters of each phage suspension were placed onto a carbon coated copper

grid, stained with 2% uranyl acetate and washed with distilled water. The samples were observed with TEM at 50 000 X magnification.

2.6. DNA isolation, quantification and visualization

2.6.1. Phage DNA isolation

2.6.1.1. Precipitation of phage by PEG centrifugation

Approximately 100 ml of phage lysates (section 2.4.5) were filter sterilized using 0.45 μm followed by 0.22 μm syringe filters. This was followed by the addition of 7.5 ml of 20% PEG8000 to every 30 ml of phage lysate and overnight storage at 4^{o}C. Phage lysates were centrifuged at 13000 x *g* for 30 min. The supernatants were discarded and the pellets resuspended in 1 ml SM buffer.

2.6.1.2. Phage DNA extraction

Genomic DNA extractions were performed using an adaptation of the method of Sambrook *et al.* (1989). A 5 μl volume of DNAse I at 1 mg/ml and 5 μl of RNAse A at 12.5 mg/ml concentration were added to 1 ml volume of phage suspensions in SM buffer to remove bacterial DNA and RNA. The reactions were incubated at 37^{o}C for 30 min. Following the addition of 10 μl of Proteinase K at 10 mg/ml and 20μl of 20 % SDS, the reactions were incubated at 55^{o}C for 1 hour to allow for the disruption of the phage capsids. Phenol/chloroform DNA extraction was used to extract phage DNA. An equal volume of

phenol:chloroform:isoamyl alcohol (25:24:1) was added to the supernatants, and the reactions were centrifuged at 13000 x *g* for 5 min. The upper layer was transferred to new tubes, and the process of adding phenol:chloroform:isoamyl alcohol, centrifugation and removal of the upper layer was repeated. An equal volume of chloroform:isoamyl alcohol (24:1) was mixed with the upper layer containing the sample and centrifuged. The upper layer was transferred into 1.5 ml eppendorf tubes. A 45 μl volume of 3M sodium acetate (pH5.2) and a 500 μl of 100% isopropanol were added, and the solution was incubated overnight at -20^{o}C to precipitate the DNA. The pellet was collected by centrifugation at 14000 rpm for 20 min. The DNA pellet was washed twice using 1ml of 70% ethanol. The DNA pellet was air dried at room temperature, resuspended in 30 μl of 1XTE buffer and stored at -20^{o}C.

2.6.2. Bacterial host DNA extraction

Bacterial strains were inoculated in 5 ml of medium A and incubated overnight at 37^{o}C on a shaking platform at 120 rpm. One millilitre of each cultured host was centrifuged at 13000 x *g* for 5 min. The bacterial pellets were resuspended in 1ml of 1 X TE buffer. The host cells were disrupted using proteinase K, 20% SDS and phenol/chloroform as described in section 2.6.1. The DNA pellets were resuspended in 50 μl of 1 X TE buffer and stored at -20^{o}C.

2.6.3. Determination of DNA concentration

Genomic DNA of both phage and bacterial hosts was quantified using a Nanodrop ND-1000 spectrophotometer (Delaware-USA) at 260 nm. The instrument was blanked using 1 µl of distilled water. Aliquots of 1 µl of resuspended DNA were loaded onto the scanning platform and DNA concentrations and purity were recorded in triplicate.

2.6.4. Agarose gel electrophoresis

Analysis of DNA was performed by agarose gel electrophoresis (Sambrook *et al*., 1982). Horizontal 1% (w/v) TAE agarose gels were cast and run at 100V in 0.5 X TAE buffer (40mM Tris-HCl, 1mM EDTA, 10mM glacial acetic acid, pH 8.5). The gels were supplemented with 0.5 µg/ml of ethidium bromide to allow visualization of the DNA on a UV transilluminator. Samples were loaded into the wells of the cast gels. The DNA fragments were sized according to the migration in gels in comparison to standard DNA molecular markers (Lambda DNA cut with *Pst*I restriction endonuclease).

2.7. DNA amplification using 16S rRNA primers

2.7.1. Bacterial 16S rRNA gene amplification

The 16S rRNA gene amplification was carried out using universal bacterial primers E9F 5'GAGTTTGATCCTGGCTCAG3' (Hansen *et al.,* 1998) and

U1510R 5'GGTTACCTTGTTACGACTT3' (Baker et *al.,* 2003) to identify bacterial isolates. The PCR mix included 5 μl of 10X DreamTaq buffer, 5 μl of 2mM dNTPs, 2 μl of both 1mM forward and reverse primers, 1 μg of DNA and 1.25 U DreamTaq Polymerase. Each reaction was adjusted to a final volume of 50 μl with nuclease free water and amplified in an automated thermal cycler (Thermo Hybaid). The PCR conditions were: initial denaturation at 95^{o}C for 3 min, followed by 35 cycles of denaturation at 95^{o}C for 30s, annealing at 55^{o}C for 30s and extension at 72^{o}C for 1 min, with a the final extension at 72^{o}C for 10 min. Positive and negative controls were included in all PCR experiments. DNA fragments of approximately 1556 bp were generated and visualised by electrophoresis on a 1% agarose gel.

2.7.2. PCR product purification

PCR product purification was performed using a Nucleospin® Gel and PCR Clean-up kit (Macherey-Nagel) according to the manufacturer's instructions. Each 100 mg gel slice was mixed with 200 μl of buffer NTI and incubated at 50^{o}C for 20 min. Columns were placed into the collection tubes and the samples were loaded and centrifuged for 30s at 11000 x *g*. The flow-through was discarded and the columns were replaced into the collection tubes. Seven hundred microliters of buffer NT3 was added into each column and the columns were centrifuged. The flow-through was discarded and the columns were placed back into the tube. The washing step with buffer NT3 was repeated twice. To remove buffer NT3 completely columns were centrifuged for 1 min at 11000 x *g*. The columns were transferred into new 1.5 ml tubes. To elute the

purified DNA, 30 μl volume of NE buffer was added to each column followed by incubation at room temperature for 1 min and centrifugation for 1 min at 11000 x *g*.

2.8. Cloning of phage genomic DNA

2.8.1. Restriction endonuclease digestion

Restriction endonuclease digestion of DNA was performed according to the manufacturer's instructions (Fermentas). The reaction mixture contained 1μg of DNA, 2 μl of 2X reaction buffer and 1U of restriction endonuclease. The reaction was adjusted to a final volume of 20 μl and incubated for 6 hours in a water bath at 37^{o}C. The digestion product was then analysed by electrophoresis on a 0.8% agarose gel.

2.8.2. Preparation of electrocompetent cells

Electrocompetent *E. coli* GeneHog cells were prepared as described by Sambrook & Russel (2001). A single colony was used to inoculate 10 ml of LB broth and cultured overnight at 37^{o}C with shaking at 250 rpm. This culture was used to inoculate 1L of LB medium which was incubated at 37^{o}C until the culture reached an OD_{600} of 0.6. Cultures were cooled on ice and 250ml aliquots were transferred to chilled Corning bottles. The bottles were centrifuged at 4000 x *g* for 25 min at 4^{o}C. The supernatant was discarded and the pellet was washed twice in 200 ml of ice-cold water and centrifuged at 4000

x *g* for 25 min at 4^{o}C. The supernatant was removed and the pellet was resuspended in 100 ml ice-cold water and centrifuged at 4000 x *g* for 25 min at 4^{o}C. The cells were then resuspended in 20 ml ice-cold 10% (v/v) glycerol and centrifuged at 4000 x *g* for 25 min at 4^{o}C. After discarding the supernatant, each cell pellet was resuspended in 1 ml 15% (v/v) glycerol and 2% (w/v) sorbitol. Fifty microliters of cells were aliquoted into 500 μl Eppendorf tubes and stored at -80^{o}C.

2.8.3. Blunt-end cloning

Blunt-end cloning was performed using gel purified DNA that was digested with *Alu*I restriction enzyme. Cloning of the blunt-ended fragments was achieved using a CloneJet^{TM} PCR Cloning Kit (Fermentas). The following ligation reaction was prepared in a total volume of 10 μl on ice: 5 μl of 2X reaction buffer, 50-100 ng of purified DNA, 1μl of pJET 1.2/blunt cloning vector (50 ng/μl), 1U of T4 DNA ligase and 2 μl of nuclease-free water. The ligation mixture was incubated at room temperature for 20 min and used directly for transformation.

2.8.4. Transformation of competent cells

Aliquots (50 μl) of electrocompetent *E. coli* GeneHog cells were thawed on ice for 5 min prior to electroporation. For each ligation reaction, an aliquot of electrocompetent cells was mixed with 1 μl of ligation mixture. The mixtures were transferred into pre-cooled electroporation cuvettes followed by

electroporation using a Genepulse™ electroporator (Bio-Rad Laboratories, Hercules, CA, USA) at 25 μF capacitance, 1.8 kV and 200 Ω resistance. One millilitre of LB was added to each cuvette immediately after electroporation. The transformed cells were transferred into sterile tubes and incubated at 37°C for 1 hour with shaking to allow recovery. Aliquots (50 μl) of the transformation mixture were plated out onto LB agar supplemented with ampicillin (100 μg/ml) and grown overnight at 37°C.

2.8.5. Plasmid extraction using TENS buffer

Recombinant *E. coli* GeneHog colonies were grown overnight in 5 ml of LB supplemented with an appropriate antibiotic at 37°C with shaking at 120 rpm. The overnight culture was centrifuged at 13000 x *g* for 5 min. The pellet was resuspended in 300 μl of TENS buffer. A 150 μl volume of 3M sodium acetate was added to the mixture and the mixture was centrifuged at 17000 x *g* for 5 min. The supernatant was transferred to a new 1.5 ml tube and centrifuged at 17000 x *g* for 5 min. The supernatant was transferred in a new tube and 900 μl of ice cold 100% absolute ethanol was added. The mixture was incubated at -20°C for 30 min to precipitate plasmid DNA. This was followed by centrifugation at 17000 x *g* at 4°C for 20 min. The pellet was washed twice by adding 70% ethanol and centrifuged at 17000 x *g* for 5 min. The pellet was air dried at room temperature and resuspended in 30 μl of TE buffer.

2.8.6. Plasmid extraction using the Qiagen® Spin Miniprep Kit

Transformed bacterial cells carrying recombinant plasmids were grown in 5 ml LB broth supplemented with an appropriate antibiotic and incubated overnight at 37^{o}C on a shaking platform at 120 rpm. Plasmid isolations were carried out according to manufacturer's instructions using a Qiagen® Spin Miniprep Kit. Five millilitres of bacterial culture was centrifuged at 7000 x g for 3 min at room temperature (15-25^{o}C). Bacterial cell pellets were resuspended in 250 μl Buffer P1 and lysis was achieved by the addition of 250 μl of Buffer P2 and incubated for 5 min at room temperature. A 350 μl volume of buffer N3 was added, mixed by inverting the tube six times and centrifuged for 10 min at 17000 x *g*. The supernatant was transferred into the column and centrifuged for 30s at 17000 x *g*. The flow-through was discarded and the column was washed using 750 μl of Buffer PE and centrifuged at 17000 x *g* for 30s. The column was centrifuged for 1 min to remove the residual wash buffer and placed into clean tubes. 30 μl volume of Buffer EB was added and the column was incubated at room temperature for 1 min and centrifuged at 17000 x *g* for 1 min to elute the purified plasmid. Samples were analysed by 0.8% agarose gel electrophoresis.

2.9. DNA sequencing

Sequencing was performed using an ABI PRISM® 377 automated DNA sequencer at the Central Analytical Facility of the University of Stellenbosch (South Africa). For bacteria, the sequencing reactions were performed using

16S rRNA primers: E9F forward and U1510R reverse primers. For the phage clone library, pJET forward and pJET reverse primers were used (Table 4).

2.10. Sequence analysis

Sequences were analysed using BioEdit Version 7.0 software (Hall, 1999) and DNAMAN Version 4.13. The NCBI database was used for analysis of DNA sequences and homology searches. The Basic Local Alignment Search Tools (BLAST) programme was used to determine sequence similarity and identity to known sequences in the GenBank database using software from the National Centre for Biotechnology Information (www.ncbi.nlm.nih.gov/).

2.11. Phylogenetic analysis

The phylogenetic tree for all isolated bacteria was constructed with the 16S rRNA sequences of other isolates from different soda lakes. The *Helibacteriaceae* bacterium 16S rRNA region was used as the outlier for the tree. Multiple sequence alignments of the sequences were performed using MEGA 5 software (Tamura *et al*., 2011) and the ClustalW alignment tool, using the neighbour-joining method.

Chapter 3: Results and discussion

3.1. Bacterial isolation

An understanding of environmental factors is needed in order to isolate microbes from environmental samples. Salt concentration and pH are the essential factors to consider when isolating organisms from soda lakes. Lake Magadi and Lake Shala isolates require growth conditions with high pH and NaCl concentrations as the sediments of both lakes have a high pH (11 and 9, respectively) and high salt concentration (30 and 18%, respectively) (Jones *et al*., 1998; Zinabu, 2002; Legesse *et al*., 2004; Denson *et al*., 2010). Therefore Medium A (Sato *et al*., 1983) with a pH of 9 and 5% (w/v) NaCl concentration was used for the isolations from both locations. A total of five bacterial strains were isolated from soil sediment samples (section 2.4.1): one bacterium (MGK1) from Lake Magadi and four bacterial isolates (Shala1-4) from Lake Shala. Bacterial strains were characterized according to colony colour and appearance (Table 6). The pH and NaCl growth optima of the isolates were determined (Table 6).

In addition, two bacterial isolates from Lake Shala obtained from the IMBM culture collection (ERV9 and HS3) were included in this study to screen for phages. These bacterial species isolated from soda lakes survived high salt concentrations, but grew optimally at lower salt concentrations. Although the NaCl concentrations of Lake Magadi and Lake Shala are 30% and 18% respectively, bacterial isolates from both lakes have been found to grow well at

lower salinities (Spanka and Fritze, 1993; Jones *et al.,* 1998; Martins *et al.,* 2001).

Isolates from both lakes were found to be alkalitolerant, growing well at pH 8-9 with an estimated optimum of 10. The bacterial isolates from both soda lakes were moderately halophilic and alkalitolerant, tolerating salt concentrations of up to 10% and having an optimum growth pH of 10. Moderate halophiles grow optimally in media containing 3% to 15% (w/v) NaCl (Ventosa, 2006), and alkalitolerant microbes grow optimally at pH 7-10 (Nowlan *et al.,* 2006) so the host bacteria from this study fit well into these ranges.

Table 6: Morphological characterization of bacterial strains

Sample source	Bacterial strains	Colony colour	pH optimum	NaCl concentration optimum (%)
Lake Magadi	MGK1	Grey	9	6
Lake Shala	Shala1	Cream	9	8
	Shala2	White	9	7
	Shala3	White	9	7
	Shala4	Brown	9	8
IMBM culture collection (from Lake Shala)	ERV9	Grey	9	6
	HS3	Orange	10	6

3.2. 16S rRNA gene amplification

Genomic DNA of each bacterial isolate was extracted using the phenol chloroform protocol. 16S rRNA genes were amplified using the universal primers E9F and U1510R (Hansen *et al.,* 1998; Baker et *al.,* 2003). DNA fragments of approximately 1556bp were obtained (Figure 9). The amplified DNA fragments were then sequenced to identify the bacterial hosts.

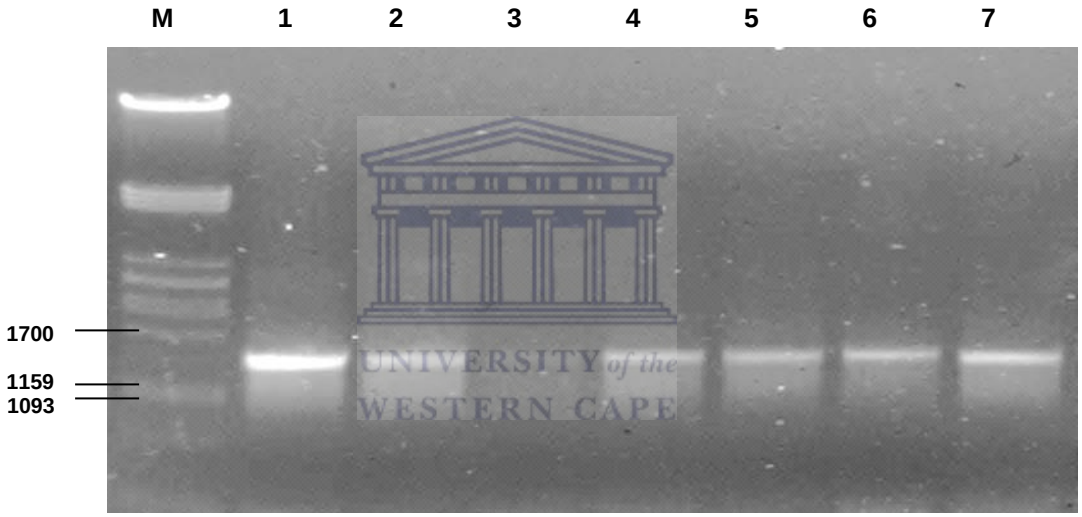

Figure 9: Agarose gel (1%) showing the 16S rRNA gene PCR amplification product of seven bacterial isolates using E9F and U1510R primers. Lane M, Lambda phage DNA marker cut with *Pst*I; Lane 1, MGK1 (Lake Magadi bacterial isolate); Lane 2-7, Shala1-4, ERV9 and HS3 (Lake Shala bacterial isolates).

The 16S rRNA DNA sequences showed very high similarity to existing bacteria in the GenBank database. The top BLASTn matches to all seven isolates shared 96-99% identity to existing sequences in the database (Table 7).

The Lake Magadi MGK1 isolate was described as *Bacillus halodurans*, a rod-shaped Gram-positive bacterium. *B. halodurans* produces white circular colonies with a slightly filamentous margin. Most of *B. halodurans* strains grow at pH 7 with an optimal growth at pH 9 and 10 at temperature between 15 to 55^{o}C. The strains are moderately halotolerant, growing at salt concentrations up to 12% (w/v) NaCl (Nielsen, 1995). *Bacillus* spp. are generally found in soil and are more abundant in alkaline soils than in neutral soils (Horikoshi, 1999). They are among the most commonly isolated bacteria in soda lakes (Mwirichia *et al*., 2010). *Bacillus* species have been extensively studied because of their ability to produce biotechnologically important enzymes such as proteases (Horikoshi, 1971).

The Shala 1- 4 sequences were most similar to *Halomonas axialensis, Bacillus licheniformis, Virgibacillus salaries* and *Halomonas venusta*, respectively. *H. axialensis* is a halophilic Gram-negative bacterium originally isolated from a low-temperature hydrothermal fluid at 1530 m depth on the Axial Seamount in the north-east Pacific Ocean (Kaye *et al.,* 2004). They are rod-shaped cells with rounded ends and found primarily as single cells and doublets. They are psychrotolerant and moderately halophilic because they do not grow at temperatures of 25^{o}C or above (with 18% salts). They do not grow at pH of below 4 or greater than 13 and no growth occurs without salt (Kaye *et al*., 2004).

Table 7: BLASTn results of 16S rRNA sequences for all bacterial host strains showing three closest related sequences (June 2013).

Sample source	Bacterial strains	Highest BLAST matches	% identity	Accession number	Sequence length
Lake Magadi	MGK1	1) *Bacillus halodurans* C-125 strain C-125	99	NR_074984.1	1398
		2) *Bacillus halodurans* strain DSM 497	99	NR 025446.1	
		3) *Bacillus okuhidensis* strain GTC 854	98	NR 024766.1	
Lake Shala	Shala1	1) *Halomonas axialensis* strain Althf1	98	NR_044880.1	1395
		2) *Halomonas meridiana* strain DSM 5425	98	NR_027219.1	
		3) *Halomonas aquamarina* strain DSM 30161	98	NR_042066.1	
	Shala2	1) *Bacillus licheniformis* DSM 13 = ATCC 1458	99	NR_074923.1	1422
		2) *Bacillus aerius* strain :24K	98	NR_042338.1	
		3) *Bacillus subtilis* subsp. *subtilis* strain DSM 10	98	NR_027552.1	

Table 6 continued

	Shala3	1) *Virgibacillus salarius* strain SA-Vb1	99	NR_044880.1	1426
		2) *Virgibacillus marismortui* strain 123	99	NR_042069.1	
		3) *Virgibacillus olivae* strain E308	99	NR_042256.1	
	Shala4	1) *Halomonas venusta* strain DSM 4743	97	NR_042069.1	1378
		2) *Halomonas hydrothermalis* strain Slthf2	97	NR_027220.1	
		3) *Halomonas alkaliphila* strain : 18bAG	97	NR_042256.1	
IMBM culture collection (from Lake Shala)	ERV9	1) *Bacillus pseudofirmus* OF4 strain OF4	99	NR_102774.1	1414
		2) *Bacillus pseudofirmus* strain DSM 8715	99	NR_026139.1	
		3) *Bacillus akibai* strain 1139	96	NR_028620.1	
	HS3	1) *Paracoccus aestuarii* strain B7	99	NR_044342.1	1312
		2) *Paracoccus marinus* strain KKL-A5	98	NR_041234.1	
		3) *Paracoccus homiensis* strain DD-R11	97	NR_043733.1	

B. licheniformis is a Gram-positive, spore-forming bacterium, widely distributed as a saprophytic organism in the environment. *B. licheniformis* is a facultative anaerobe, a feature that allows it to grow in diverse ecological niches (Alexander, 1977). The bacterium grows in the presence of NaCl of up to 10% and pH of 5-6. The colonies are cream coloured on tyrosine agar and reddish-brown on glycerol/glutamate agar (Palmisano *et al*., 2001). *V. salarius* is a moderately halophilic Gram-positive bacterium having rod-shaped motile cells, isolated originally from Gharsa salt lake (Chott el Gharsa), Tunisia. The cells occur singly, in pairs or in chains. Colonies in solid medium containing 10% NaCl appear white in colour with a diameter of 2-2.5 mm after 48 hours at 30 to 35^{o}C. This halophilic bacterium can grow at temperatures between 10 to 50^{o}C and salt concentrations of 0.5 to 25% (w/v) NaCl and pH is of 5.5 to 10. Optimally growth occurs at 30 to 35^{o}C, 7 to 10% (w/v) NaCl and pH 7.5 (Hua *et al*., 2008). *H. venusta* was originally isolated from marine waters in Hawaii, USA. They are rod-shaped Gram-negative bacteria. These halophilic bacteria can grow at temperatures between 4 and 40^{o}C (Arahal *et al*., 2002).

Two bacterial hosts from Lake Shala obtained from the IMBM culture collection ERV9 and HS3 were described as *Bacillus pseudofirmus* and *Paracoccus aestuarii*, respectively. *B. pseudofirmus* is a rod-shaped Gram-positive bacterium. This bacterium produces yellow colonies on solid medium. The strain showed growth at tempertures ranging from 10 to 45^{o}C in the presence of 16 and 17% (w/v) NaCl concentrations. No growth was observed at pH 7 and the optimum pH was 9 indicating it is strictly alkaliphilic (Nielsen *et al*., 1995). *P. aestuarii* is a Gram-negative bacterium having a rod-shaped non-motile cell

which was initially isolated from tidal flat sediments from Yeosu, South Korea. This strain produces orange circular colonies with the diameter of 1 to 2.5 mm after 1 day growth on TSB (tryptic soy broth) agar at 30^{o}C. The growth temperature range is from 15 to 37^{o}C, with no growth at higher than 5% (w/v) NaCl concentrations. Growth occurs at pH's of 7.5 to 9.5 with an optimum pH of 9 (Roh *et al*., 2009).

A phylogenetic tree showing the relationship between the strains used in this study and Genbank sequences of closely related isolates from other soda lakes is shown in Figure 10. The phylogenetic tree shows that isolates MGK1, Shala1 and Shala4 fall within the same cluster as other strains of *Bacillus* species. Shala2 and Shala4 are most closely related to a cluster comprising of *Halomonas* species. In another cluster, strain HS3 appears to be more closely related to *Paracoccus carotinifaciens.*

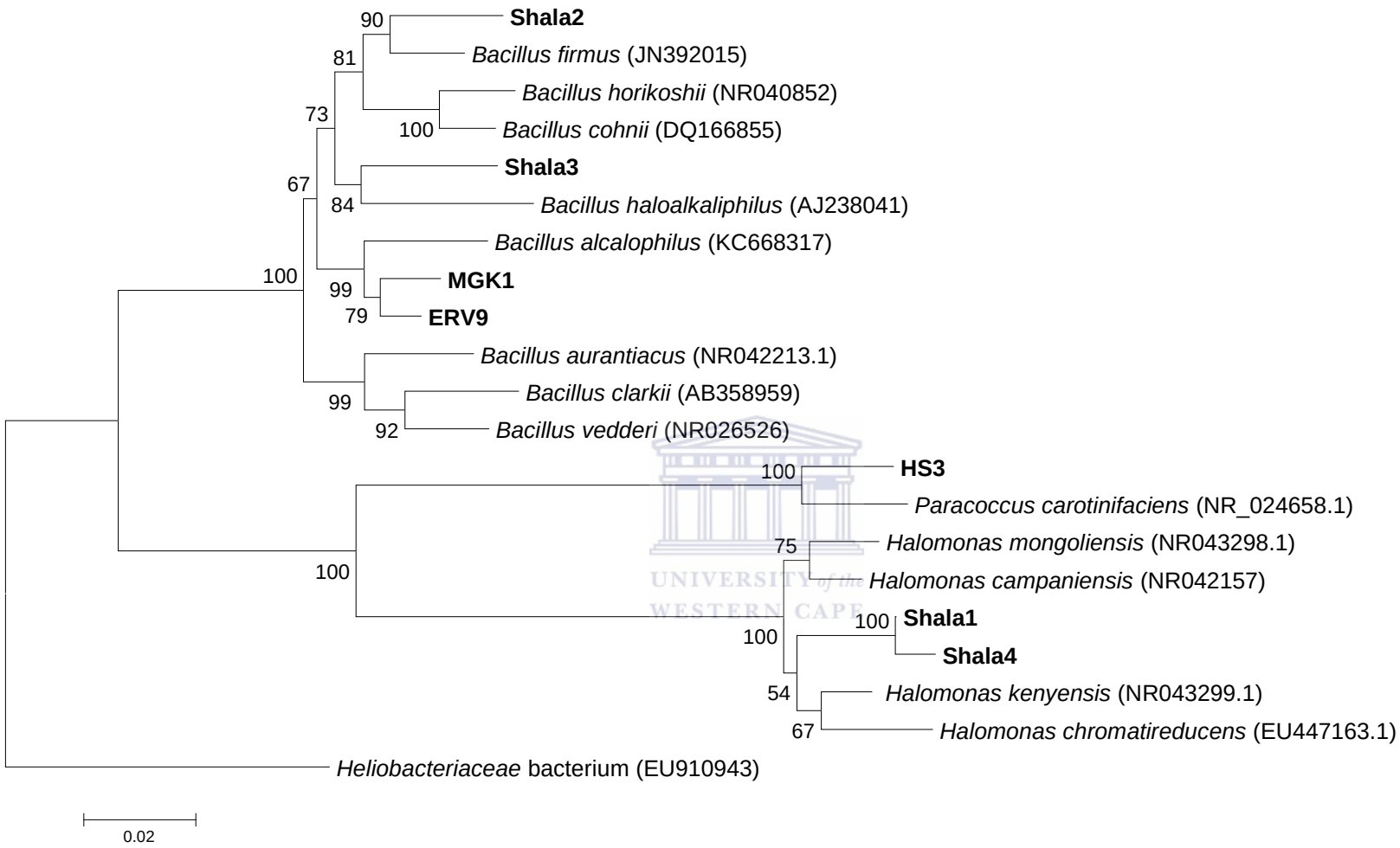

Figure 10: Phylogenetic analysis based on the alignment of 16S rRNA sequences of bacterial isolates from this study and other 13 isolates from different soda lakes. Isolates from this study are indicated in bold font. The tree was constructed by the neighbor-joining method with 1000 bootstrap replicates using the MEGA 5 software. The 16S rRNA gene sequence of *Helibacteriaceae bacterium* SLH was used as an out group. The scale bar of the tree represents a 0.2% difference in nucleotide sequences.

3.3. Bacteriophage isolation

Bacterial strains were screened for susceptibility to phage infection using a standard plaque assay. Soil sediments from both Lake Magadi and Lake Shala were tested against the seven bacterial isolates from the lakes to determine if bacteriophage plaques would form.

Formation of plaques was observed using both Lake Magadi and Lake Shala soil sediments (Table 8). When screening for phage against MGK1 host strain plaques were observed with sediments from both Lake Magadi and Lake Shala. No plaques were formed when the Lake Shala bacterial strains Shala1-4 were used as host strains against soil sediments from both Lake Magadi and Lake Shala. Plaque formation was observed in strains from the IMBM culture collection (ERV 9 and HS3) which originated from Lake Shala against Lake Shala sediments.

Phage MGBH1 isolated from Lake Magadi sediments using strain MGK1 as a host, produced clear plaques (Figure 11, Table 9) as did phages SHBH1 and SHPA, isolated from Lake Shala sediments using strains MGK1 and HS3 respectively as hosts. Phage SHBH2 isolated from Lake Shala sediments using strain ERV9 as a host, produced turbid plaques (Figure 11).

Table 8: Bacteriophage isolated from Lake Shala and Lake Magadi soil sediments.

Bacterial strains		Source of sediment	
Strain	**Origin**	**Lake Magadi**	**Lake Shala**
MGK1	Lake Magadi (This study)	+	+
ERV 9	Lake Shala (IMBM culture	-	+
HS3	collection)	-	+
Shala 1-4	Lake Shala (This study)	-	-

+: plaques; - : no plaques.

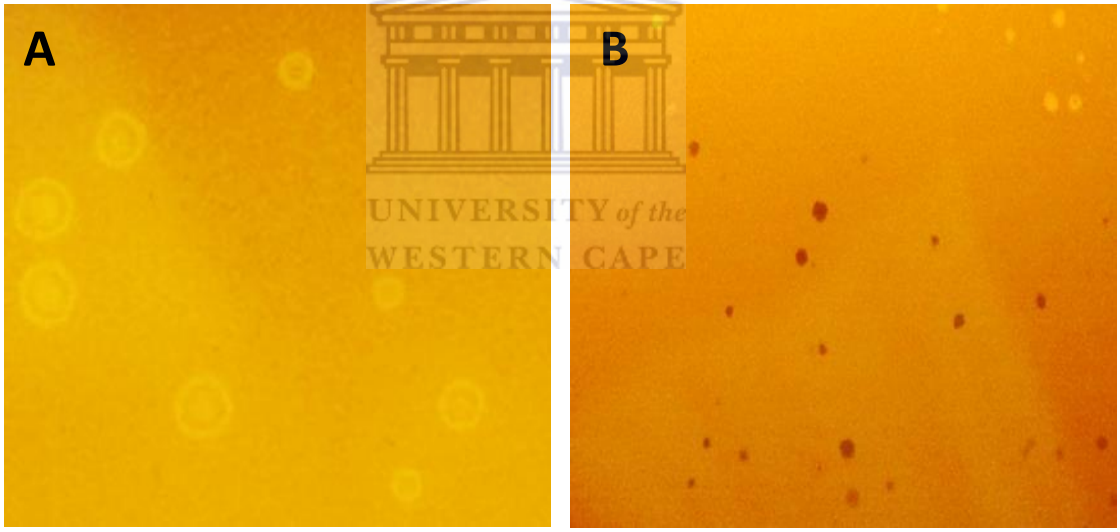

Figure 11: (A) A bacteriophage plaque plate showing the turbid plaques formed by phage SHBH1 infecting strain ERV9 (B) Clear plaques formed by phage SHBH1 infecting MGK1 bacterium.

Phage preparations produced phage titers ranging from $6X10^5$ to $2X10^{10}$ following isolation using different bacterial hosts (Table 9).

Table 9: Bacteriophage plaque characterization

Phage sample source	Bacteria	Phage	Plaque morphology	Phage titer PFU/ml
Lake Magadi	MGK1	MGBH1	Clear	$1X10^9$
Lake Shala	MGK1	SHBH1	Clear	$4X10^9$
	ERV9	SHBH2	Turbid	$6X10^5$
	HS3	SHPA	Clear	$2X10^{10}$

Phage SHBH1 and SHBH2 were shown to be the same phage after TEM (section 3.4) and genome restriction digests were performed (section 3.8). Very few phages have been isolated from haloalkaline environments using culture dependant approaches because little attention has been paid to the study of phage diversity in this environment (Jiang *et al*., 2004; Sabet *et al*., 2006; Moulton *et al*., 2011). In this study only four potentially different phages were isolated from the sediments of the two lakes, from seven bacteria used in the screening process.

3.4. TEM morphological characterization

The morphologies of the four phage virions isolated during this study were determined by TEM microscopy. All were tailed. Lake Magadi phage MGBH1 was identified as a member of the family *Siphoviridae,* with a long non-contractile tail (Figure 12) (Ackermann, 2007). At this point of the study it was unknown that it was the same phage (SHBH1) that infected the 2 different hosts, MGK1 and ERV9. TEM images of the phage(s) using the two different hosts are shown (A and B). Both phage preparations were identified as members of the family *Myoviridae* (Ackermann, 2007) with contractile tails (Figure 13). Phage SHPA was identified as a member of the family *Podoviridae* (Figure 13) (Ackermann, 2007).

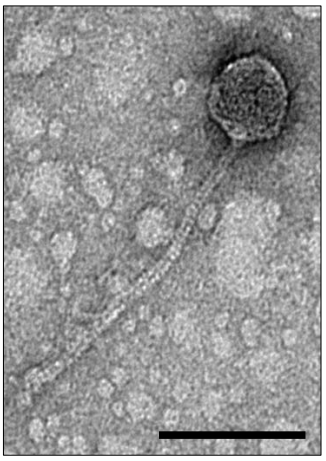

Figure 12: TEM image of phage MGBH1 isolated from Lake Magadi. Scale bar represents 100 nm.

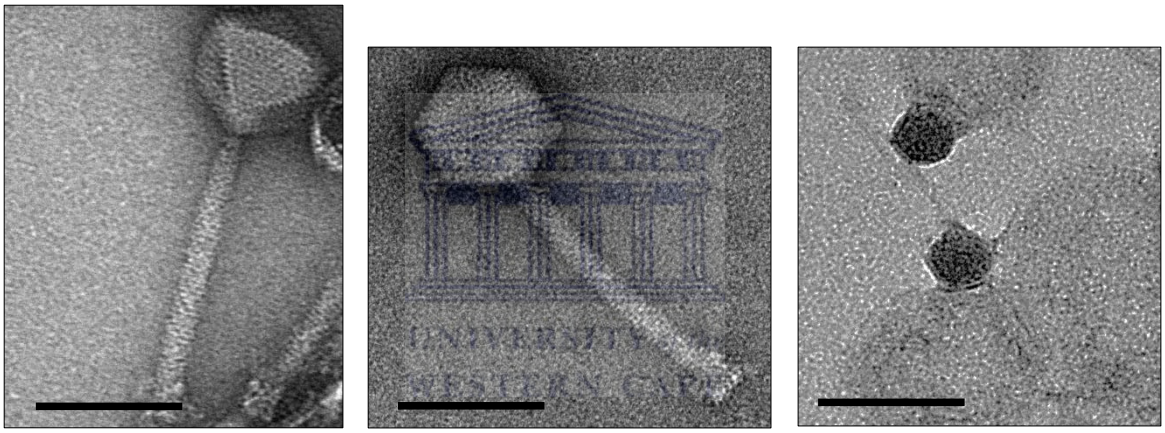

Figure 13: Electron micrographs of phages isolated from Lake Shala. A: phage SHBH1 purified from MGK1, B: phage SHBH1 purified fron ERV9, C: phage SHPA. Scale bar represents 100 nm.

The isolated phages had morphological characteristics similar to phages previously isolated from soda lakes. Brum & Steward (2010) reported a high percentage of tailed phages from Mono Lake. It is not surprising that the dsDNA phages *Siphoviridae*, *Myoviridae* and *Podoviridae* were observed in these environments as they comprise the vast majority of phage that have been reported in any environment (Ackermann, 2007).

In this study, phage MGBH1 had a capsid diameter of 49 nm and a tail length of approximately 227 nm (Table 10), whereas the capsid diameters of phages SHBH1 (prepared from both bacterial hosts) and SHPA were 92 and 37 nm, respectively. The tail lengths of phage SHBH1 was 200 nm (Table 10). At this point of the study, based on the TEM analysis, we suspected that the 2 phage preparations using the MGK1 and ERV9 hosts might represent the same phage. This was confirmed in subsequent genome analysis, and from here on the discussions will pertain to the phage SHBH1.

In aquatic environments approximately 65% of viruses have capsid diameters between 30 to 60 nm (Wommack & Colwell, 2000). Two of the phages fall within this range while phage SHBH1 displayed a larger capsid diameter of 92 nm.

Table 10: Bacteriophage TEM morphology and genome size characterization

Sample source	Bacteria	Bacteriophage	Genome size (kb)	Family	Dimensions (nm)	
					Head diameter	Tail length
Lake Magadi	MGK1	MGBH1	57	*Siphoviridae*	49	227
Lake Shala	MGK1	SHBH1	111	*Myoviridae*	92	200
	HS3	SHPA	25	*Podoviridae*	37	N/D

N/D: not determined, there was no noticeable tail.

3.5. Phage host specificity

Bacteriophages were tested for host specificity using the agar spot assay (Armon and Kott, 1993) described in Section 2.4.6. The results were verified by the plaque assay method (Adams, 1959) as described in Section 2.4.4. The phages isolated in this study were tested for the ability to lyse all seven bacterial strains. Clear zones were observed using the agar spot test while clear and turbid plaques were observed on plaque assay plates (Table 11).

Table 11: Bacteriophage host specificity

Sample source	Bacteria	Phage		
		MGBH1	SHBH1	SHPA
Lake Magadi	MGK1	+	+	-
Lake Shala	Shala1- 4	-	-	-
	ERV9	+	+	-
	HS3	-	-	+

+: lytic activity observed; -: no plaque formation

None of the phages used in this study were able to lyse any of the strains isolated from Lake Shala (Shala1-4). Strain MGK1, identified as *Bacillus halodurans*, and strain ERV9, identified as *Bacillus pseudofirmus* were sensitive to lytic viral infection by two phages (MGBH1 and SHBH1). Phage SHPA was only able to lyse the original host bacterial strain *Paracoccus aestuarii* (HS3). Studies have shown that infection by bacteriophage is initiated

when the phage virion interacts with the bacterial host cell surface receptor. Most phages require highly specific receptor molecules on the bacterial cell surfaces and show little or no interaction with bacterial cells bearing slightly different receptor molecules (Sundar *et al*., 2009).

The most studied broad host range phages are phage P1, a phage that infects *E. coli* and other enteric bacteria (Łobocka *et al.,* 2004), and phage Mu, a temperate phage that infects several enteric bacteria (Morgan *et al.,* 2002). Most of the phages which display broad host range capabilities have been isolated from aquatic environments (Sundar *et al*., 2009). Many phages are able to infect closely related species within a genus (Brodetsky & Romig, 1965; Hemphill & Whiteley, 1975). The results from this study showed that phage MGBH1 and SHBH1 were able to infect both the *Bacillus* host strains used in this study, which were isolated from 2 different lakes.

3.6. Determining bacterial host growth curves

Bacterial growth curves were performed as described in Section 2.4.2 to determine the optical density equivalent to 10^8 CFU/ml for use in the phage one step growth curve. This cell density corresponded to an OD_{600} of 0.5. All bacterial hosts were grown at 37^{o}C with shaking at 120 rpm to stationary phase in medium A at pH9 and 5% (w/v) NaCl (Figure 14). The duration of the lag phase of cultures of both strains MGK1 and ERV 9 were similar with the exponential phase of growth starting after 5 hours incubation. Strain HS3 had a longer lag phase with the exponential phase of growth starting after 6 hours.

After 12 and half hours cultures of strain MGK1 reached the stationary growth phase, whereas strains HS3 and ERV 9 reached the stationary phase of growth after 13 and 15 hours, respectively.

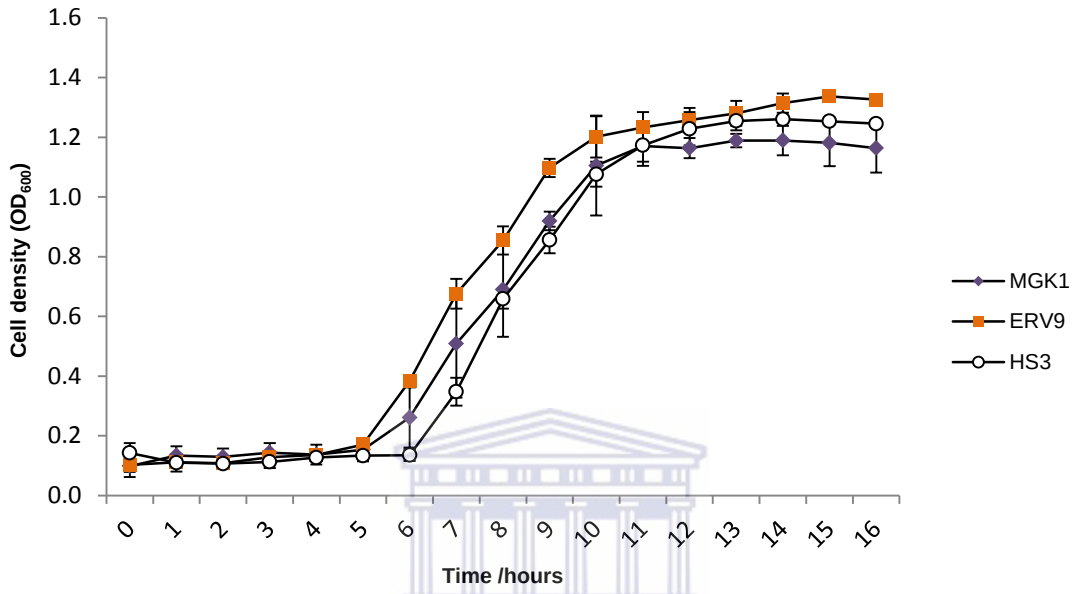

Figure 14: Growth curves of bacterial host strains MGK1, ERV9 and HS3 grown at pH9 and 5% (w/v) NaCl concentration in Medium A at 37°C. Error bars represent the standard error of results from triplicate cell cultures.

3.7. Phage one step growth curve

A one step growth curve of all four phage isolates used in this study was individually determined as described in Section 2.4.7. to identify the different phases of phage infection (Figures 15 & 16). The one step growth curve experiment was first performed by Ellis and Delbrück in the late 1930's (Ellis & Delbrück 1939). In the 1940's, Luria and Delbrück reported that the burst size number varied depending on the bacterium and phage used in the study (Luria & Delbrück, 1943). This test reveals the average number of phage progeny being released from infected cells following a single infection (Delbrück, 1945), and the phage life cycle parameters (Eclipse, latent period, generation time and burst size) were determined (Table 12).

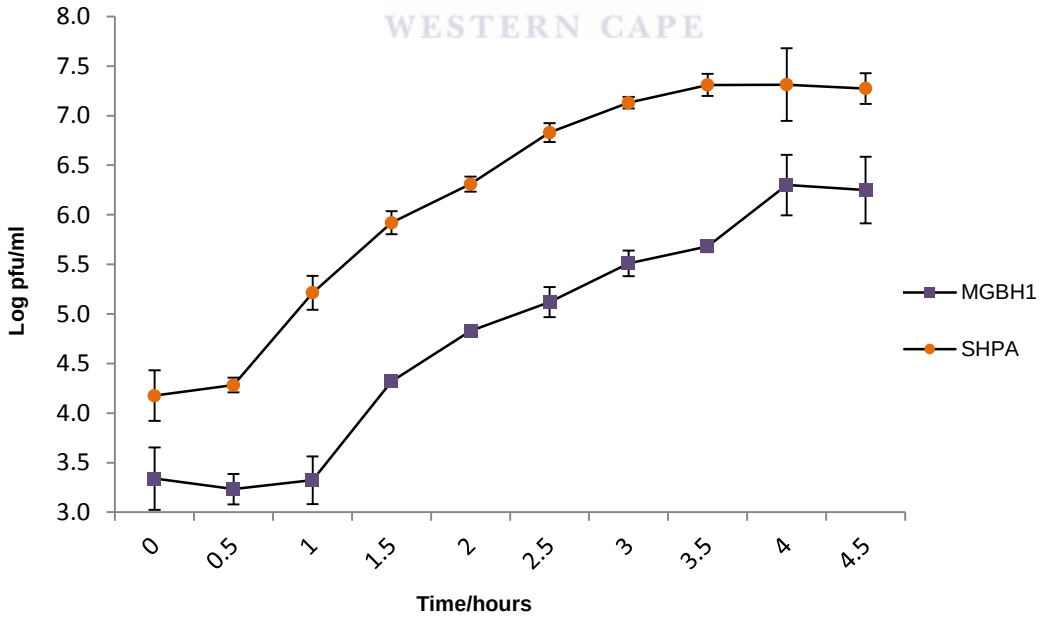

Figure 15: One-step growth curve of phages MGBH1 and SHPA. The total pfu/ml represents infected cells as well as free phage. Error bars represent the standard error of results from triplicate plaque assays.

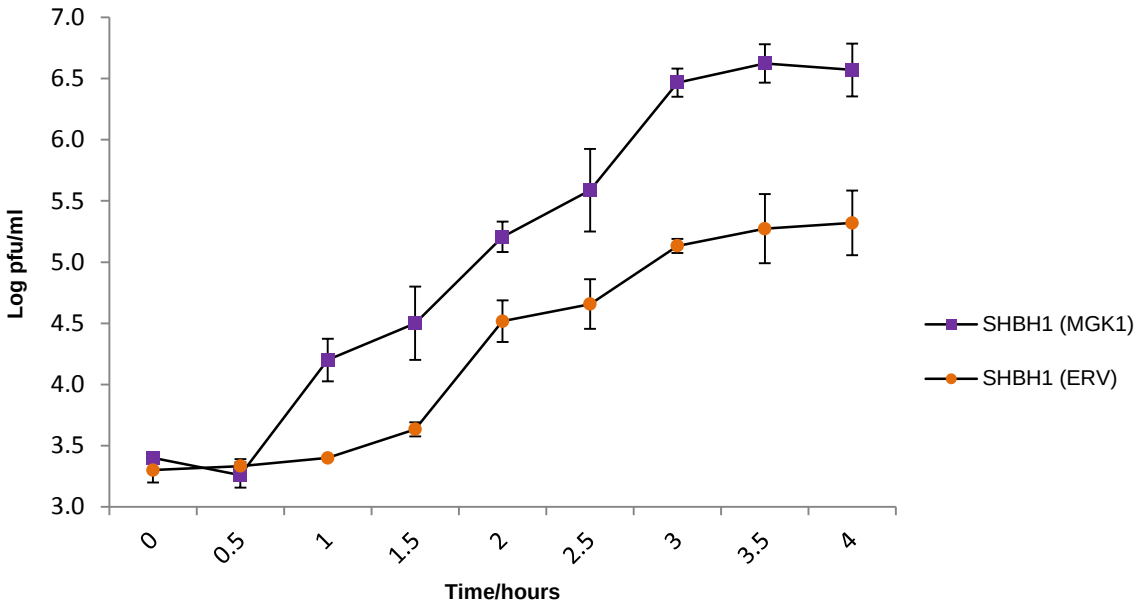

Figure 16: One-step growth curve of phage SHBH1 (with both MGK1 and ERV9 as hosts). The total pfu/ml represents the sum of infected cells and free phage. Error bars represent the standard deviation of results from triplicate plaque assays.

Bacterial hosts, medium composition, incubation temperature, physiological conditions and the specific phage growth rate can influence the latent period and burst size of phage infections (Abedon *et al.,* 2003; Carey-Smith *et al.,* 2006). Ellis & Delbrück (1939) found that phage latent and generation time have no effect on burst size. Bacterial lysis time is also affected by host cell quantity or quality (Abedon *et al*., 2001).

The burst size of phage is calculated as the final count of released phage particles divided by the initial count of infected bacterial cells during the latent period (Adams, 1959). Delbrück (1945) reported that the infection of a single strain of *E. coli* with a single phage can release in the order of 20 to over 1000 phage progeny per host cell. Phage MGBH1 yielded a burst size of 882 phage per infected cell. The burst sizes of the two phages from Lake Shala were

approximately 1722 (SHPA), 1550 (SHBH1 with MGK1 as a host) and 106 (SHBH1 with ERV9 as a host) phage particles per infected cell (Table 12). The burst size of a particular phage may increase due to high nutrient concentration in the environment which facilitates an increased high host growth rate, allowing the bacterial cells to produce more phages (Zachary, 1978). Therefore under these conditions these burst sizes could be considered the maxima that could be reached, and do not necessarily reflect their activity in their natural environments.

Table 12: Phage life cycle parameters

Sample source	Bacteria	Phage	Burst size per infected cell	Eclipse (hours)	Latent period (hours)	Generation time (hours)
Lake Magadi	MGK1	MGBH1	882	1	4	3
Lake Shala	MGK1	SHBH1	1550	0.5	3.5	3
	ERV9	SHBH1	106	1	3.5	2.5
	HS3	SHPA	1722	0.5	4	3.5

The burst size of phage SHBH1 (ERV9 as a host) of 106 phage particles per single cell was similar to that reported for the haloalkaliphilic virus ϕCh1 (*Myoviridae*), a phage with turbid plaques and a burst size of approximately 150 phage particles per infected cell (Witte *et al.,* 1997). Phage SHBH1 (MGK1 as a host) and phage SHPA from this study showed high burst sizes of more than

1000 particles/cell. While SHBH1 is capable of infecting both MGK1 (*B. Halodurans*) and ERV9 (*B*. *pseudofirmus*), more phages were produced after infection using MGK1. Phages can infect only one or a few bacterial hosts whose cell surface contains an appropriate binding site (Smith & Trevino, 2009). Phage Bc431v3 with *Bacillus cereus* as a host was found to infect closely related species such as *B. anthracis, B. licheniformis* and *B. weihenstephanensis* strains also with different degrees of lysis (El-Arabi *et al*., 2013). Phages with broad-host-range may promote genetic exchange and genetic diversity in microbial communities (Jensen *et al*., 1998).

3.8. Phage genome analysis

A 500-1000 ml phage lysate of a well-studied lambda phage in high titer of 10^{10} pfu/ml is sufficient to obtain sufficient DNA for downstream analysis (Scheif & Wensink, 1981; Brown, 2006). Enough DNA to use for further investigation was obtained from each phage except for the phage SHPA (*Podoviridae*). Even though the phage titer of phage SHPA suspensions was the highest obtained of all phage used in this study (10^{10} pfu/ml), a very low concentration of DNA was generated using the same extraction conditions as was used for the larger phages. However, a higher concentration of DNA (sufficient for downstream analysis) was obtained by increasing the centrifuge speed from 13000 x g for 30 min to 18000 x g for 1 hour, and increasing the culture volume from 100 ml to 200 ml. Phage genomic DNA was digested for 6 hours at 37^{o}C using *Hind*III restriction enzyme (Figure 17) and phage SHPA was also digested using *Alu*I restriction enzyme (Figure 18).

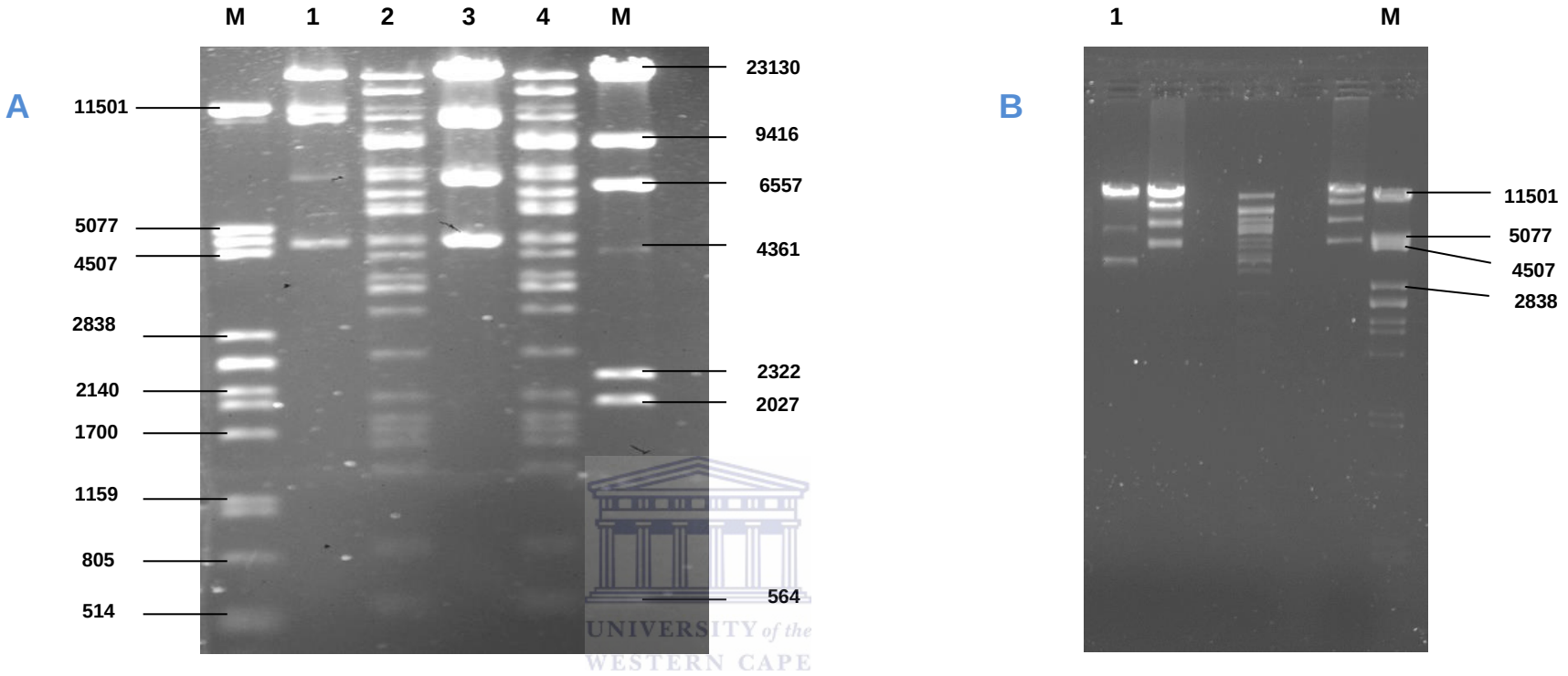

Figure 17: Agarose gel (0.8%) electrophoresis showing restriction patterns of phages generated from digesting phage DNA with *Hind*lll restriction enzyme. (A) Lane M, Lambda phage DNA marker cut with *Pst*I; Lane 1 & 3, phage MGBH1; Lane 2, phage SHBH1 purified from MGK1; Lane 4, SHBH1 purified from ERV9; Lane M, Lambda phage digested with *Hind*lll. (B) Lane 1, phage SHPA; Lane M, Lambda phage DNA marker cut with *Pst*I.

The genome size of viruses isolated from Mono Lake range between 14 and more than 400 kb, with the majority of viruses having genome sizes between 30 and 60 kb (Jiang *et al.,* 2004). The genome sizes of all the phages isolated in this study fit into this range. Phage MGBH1 isolated from Lake Magadi, was identified as a member of the family *Siphoviridae* and had a genome size of 57 kb (Table 10). The average genome size of *Siphoviridae* is approximately 50 kb (Petrovski *et al.,* 2012). *Bacillus* phage SPBc2 is the largest isolated siphovirus and has a genome size of approximately 134 kb (Hatfull, 2008).

TEM analysis showed that phage SHBH1 isolated from Lake Shala belongs to the family *Myoviridae* and this phage has a genome size of 111 kb (Table 10). This is not surprising as the viruses with the largest genomes are generally *Myoviridae* with genome sizes of greater than 125 kb (Hatful, 2008). Phage SHPA was identified as a member of the *Podoviridae* and had a small genome size of 25 kb (Table 10). The average *Podoviridae* genome size is approximately 45 kb (Wichels *et al*., 1998).

3.9. Phage genomic DNA library construction

Due to the small genome size, the 25 kb genome of phage SHPA was selected for sequencing. DNA fragments generated using restriction enzyme *Alu*I between 0.6 bp and 2.5 kb in length were cloned and sequenced (Figure 18).

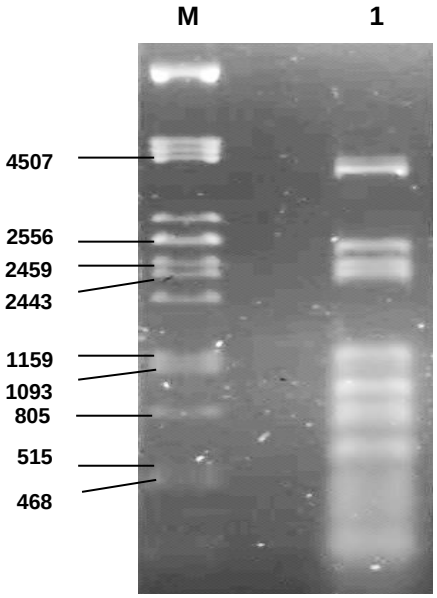

Figure 18: Restriction patterns of phage SHPA DNA generated by the restriction enzyme *Alu*I and separated by 0.8% agarose gel electrophoresis. Lane M, lambda DNA cut with *Pst*I; Lane 1, phage SHPA cut with *Alu*I.

Transformants were analysed by plasmid digestion with *Bgl*II in order to determine whether the correct inserts were cloned. Clones containing inserts of different sizes were sequenced. Inserts of 1.1, 0.8, 0.5, 0.7, 0.9, 1.0 and 1.7 kb were identified from lanes 1, 2, 3, 8, 12, 13 and 20 (Figure 19), and designated as clones 1, 2, 3, 4, 5, 6 and 7, respectively. The inserts in lanes 1, 2, 3 and 5 (Figure 20) were cloned and designated as clones 8, 9, 10 and 11 respectively. They contained inserts of sizes 0.8, 1.2, 1.0 and 2.4, respectively.

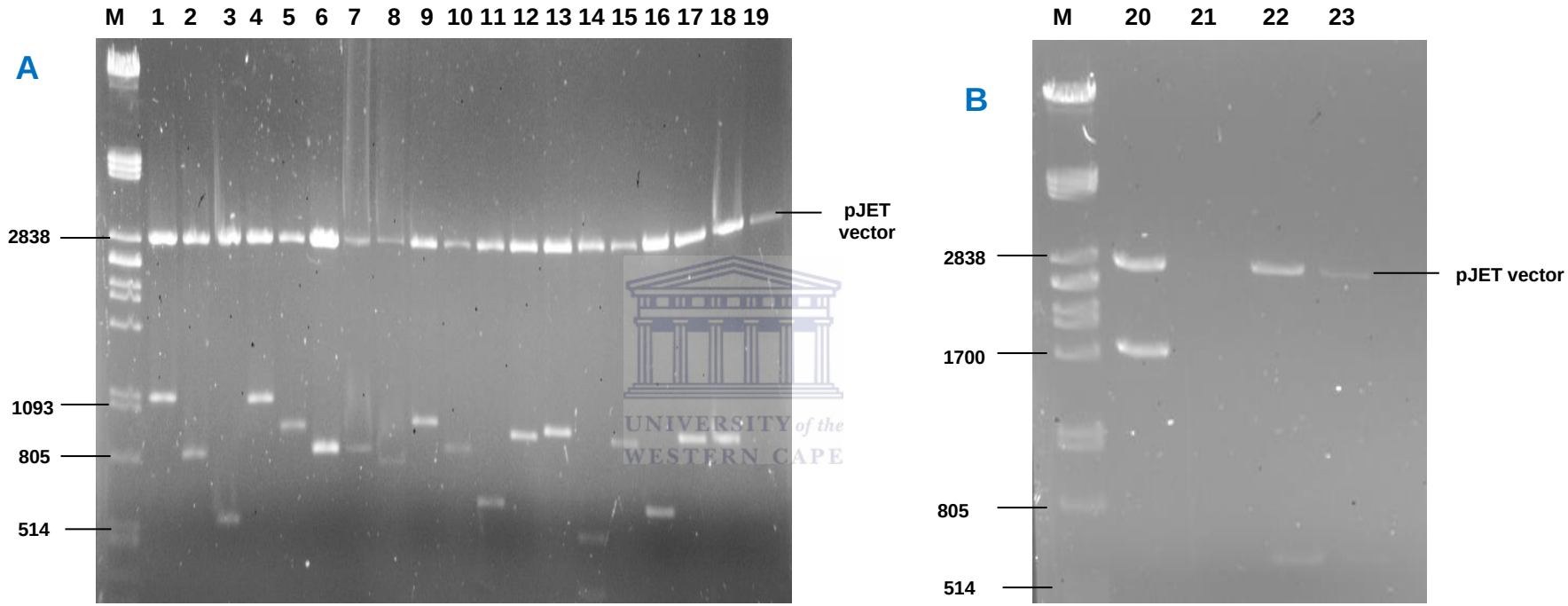

Figure 19: Agarose gel (0.8%) showing the restriction digest analysis of recombinant pJet vectors containing phage SHPA DNA fragment inserts between 0.5 and 1.7 kb in length. (A) Lane M, Lambda DNA cut with *Pst*I marker ; Lane 1-19, plasmids cut with *Bgl*II. (B) Lane M, Lambda DNA cut with *Pst*I marker; Lane 20-23, plasmid digested with *Bgl*II restriction enzyme.

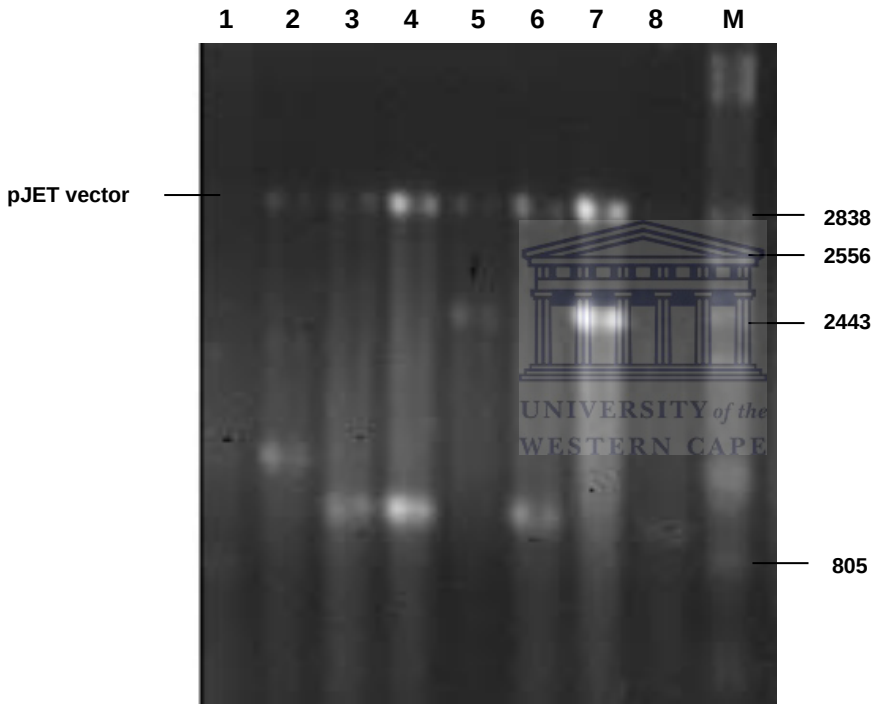

Figure 20: 0.8% agarose gel showing the restriction digest analysis of phage SHPA DNA cloned into the pJet vector. Insert sizes of between 0.8-2.4 kb in length. Lane 1-8, plasmid digested with *Bgl*II restriction enzyme; Lane M, Lambda DNA cut with *Pst*I marker.

3.10. Phage DNA sequence analysis

Sequence analysis indicated that only phage-related proteins were identified. The sequence homologies of the cloned inserts showing the highest sequence hits and phage-related proteins are shown in Table 13. The graphic representations showing the positions of ORFs of phage SHPA sequences are shown in Figure 21 & 22. Sequence homologies of the phage sequences showed that phage SHPA DNA contains both bacterial and phage genes. Lysozyme (on the reverse strand) from *Brevundimonas diminuta* and *Pseudomonas* phage phi15 (*Podoviridae*) was found in clone 1. Lysozyme helps to release mature phage from bacterial cells by breaking down the peptidoglycan layer of the bacterial cell (Santamaria, R.I., Bustos, P., unpublished data). Clone 2 contained three open reading frames (ORFs) (Figure 21), running on the reverse sequence. Two code for a hypothetical protein PaTRP_19434 and a helicase domain-containing protein, both from *Paracoccus* sp. TRP, another codes for a hypothetical protein RAZWK3B_15493 from *Roseobacter* sp. AzwK-3b. Helicases are proteins that play a role in many cellular processes involved in nucleic acid strand separation. These include DNA replication, DNA repair, transcription, translation and recombination. Phages that carried this gene might use the helicase gene product during their own or their host's replication (Jarvis *et al.,* 1996).

Clone 4 contained three possible ORFs (on the reverse strand). The first gene showed high identity to a putative phage head-tail adaptor from *Rhodobacter*

sphaeroides ATCC 17029, the second showed identity to a hypothetical protein NB311A_00470 from *Nitrobacter* sp. Nb-311A, and the third for phage protein DNA packaging protein from *Roseibium* sp. TrichSKD4. Phage head-tail adaptor enzymes help in the attachment of the phage head-tail during phage replication (Copeland, A., Lucas, S., unpublished data). Sequence 5 contained an ORF coding for the phage terminase from both *Rhodobacter sphaeroides* ATCC 17029 and *Achromobacter piechaudii* (running on the negative strand). Terminase is an enzyme that is involved in phage DNA packaging (Copeland, A., Lucas, S., unpublished data). Clone 7 contained two ORFs, phage protein gp12 from *Burkholderia* phage Bcep1 (*Myoviridae*) and hypothetical protein from *Methylobacterium* sp. GXF4, both running on the reverse strand. Phage protein Gp 12 is a tail fiber protein (summer *et al*., 2006).

Clone 8 contained one ORF coding for either phage-type endonuclease from *Hyphomicrobium denitrificans* ATCC 51888 or phage-related exonuclease from *Liberibacter crescens* BT-1 (on the negative strand). Endonucleases catalyse the hydrolysis of ester linkages within nucleic acids by creating internal breaks. Exonucleases catalyse the hydrolysis of ester linkages within nucleic acids by removing nucleotide residues from the 3' or 5' ends of the DNA molecule (Leonard *et al*., 2012; Lucas, S., Copeland, A., unpublished data). Clone 10 contained an ORF running on the positive strand, coding for the phage tail fiber protein from *Nitratireductor pacificus* pht-3B. Phage tail fiber proteins help in attachment of the phage to the bacterial cell during phage replication (Lai *et al*., 2012). Clone 11 contained an ORF coding for the phage major capsid protein, HK97, from *Ruegeria* sp. TM1040 or putative head protein from *Xanthomonas*

phage C1 (running on the positive strand). This protein assists with DNA packaging (Moran *et al*., 2007).

Phage proteins can be found in bacterial genomes due to lysogenic replication, the process that leads to prophage formation. Bacteriophage genomes are comprised of a high proportion of novel genetic sequences of unknown function (Hatful, 2008). Therefore it is not surprising that the functions of some of the phage genes identified by homology searches in this study are unknown.

Table 13: Bacteriophage SHPA sequence identity results of cloned inserts (June 2013).

Clone no.	Sequence length	ORF Strand	ORF Position	ORF length/ predicted protein length (aa)	Match	Score	E-value	% identity/ similarity	Accession no.
1	726	-	416-721	102/152	Lyzozyme [*Brevundimonas diminuta*]	116	9e-29	42/51	WP_003165060.1
		-	419-718	100/151	Putative N-acetylmuramoyl-L-alanine amidase [*Pseudomonas* phage phi15]	99.9	3e-22	41/47	WP_016033323.1
2	747	-	187-426	80/83	Hypothetical protein PaTRP_19434 [*Paracoccus* sp. TRP]	91.3	3e-20	32/65	WP_010400601.1
		-	11-187	59/232	Helicase domain-containing protein, partial [*Paracoccus* sp. TRP]	72.0	3e-17	23/59	WP_010400602.1
		-	435-719	95/315	Hypothetical protein RAZWK3B_15493 [*Roseobacter* sp. AzwK-3b]	72.8	4e-12	38/50	WP_007816087.1
3	500	-	268-423	52/774	Xaa-Pro aminopeptidase [*Treponema pallidum* subsp. pertenue str. Gauthier]	36.2	4.5	31/40	YP_005222753.1
4	773	-	24-302	93/111	Phage head-tail adaptor, putative [*Rhodobacter sphaeroides* ATCC 17029]	100	2e-23	36/54	WP_011842266.1
		-	509-766	86/196	Phage protein DNA packaging protein [*Roseibium* sp. TrichSKD4]	60.8	2e-08	33/40	WP_009759185.1
5	781	-	98-778	227/583	Phage terminase [*Rhodobacter sphaeroides* ATCC 17029]	243	1e-72	52/87	WP_011840500.1
		-	155-778	208/523	Terminase, partial [*Achromobacter piechaudii*]	182	3e-50	45/79	WP_006228214.1
6	905	-	126-413	96/432	Histidinol dehydrogenase [*Crocosphaera watsonii*]	38.5	2.8	31/33	WP_007303853.1

Table continued

7	745	-	10-276	89/231	Hypothetical protein [*Nitratireductor indicus*]	155	1e-42	35/78	WP_009756129.1
		-	52-276	75/181	Gp12 [*Burkholderia* phage Bcep1]	80.9	2e-15	33/53	WP_015974549.1
8	1088	-	27-482	152/214	Phage-type endonuclease [*Hyphomicrobium denitrificans* ATCC 51888]	209	2e-62	41/64	WP_013214170.1
		-	27-479	151/210	Phage-related exonuclease [*Liberibacter crescens* BT-1]	196	4e-57	41/61	WP_015272888.1
9	1051	-	205-954	250/541	Hypothetical protein ALIPUT_01592 [*Alistipes putredinis* DSM 17216]	137	3e-33	46/78	WP_004327624.1
		-	100-684	195/269	Oxidoreductase subunit [*Citrobacter rodentium* ICC168]	88.2	4e-17	33/84	WP_012905650.1
10	1054	+	61-1032	324/1221	Hypothetical protein Rsph17025_1315 [*Rhodobacter sphaeroides* ATCC 17025]	263	6e-76	48/92	YP_001168281.1
		+	67-996	310/886	Phage tail fiber protein [*Nitratireductor pacificus* pht-3B]	229	2e-64	42/88	WP_008599054.1
11	602	+	10-519	170/403	Phage major capsid protein, HK97 [*Ruegeria* sp. TM1040]	158	5e-41	61/84	WP_011538996.1
		+	61-498	146/394	Putative head protein [*Xanthomonas* phage CP1]	112	3e-26	51/72	WP_016066045.1

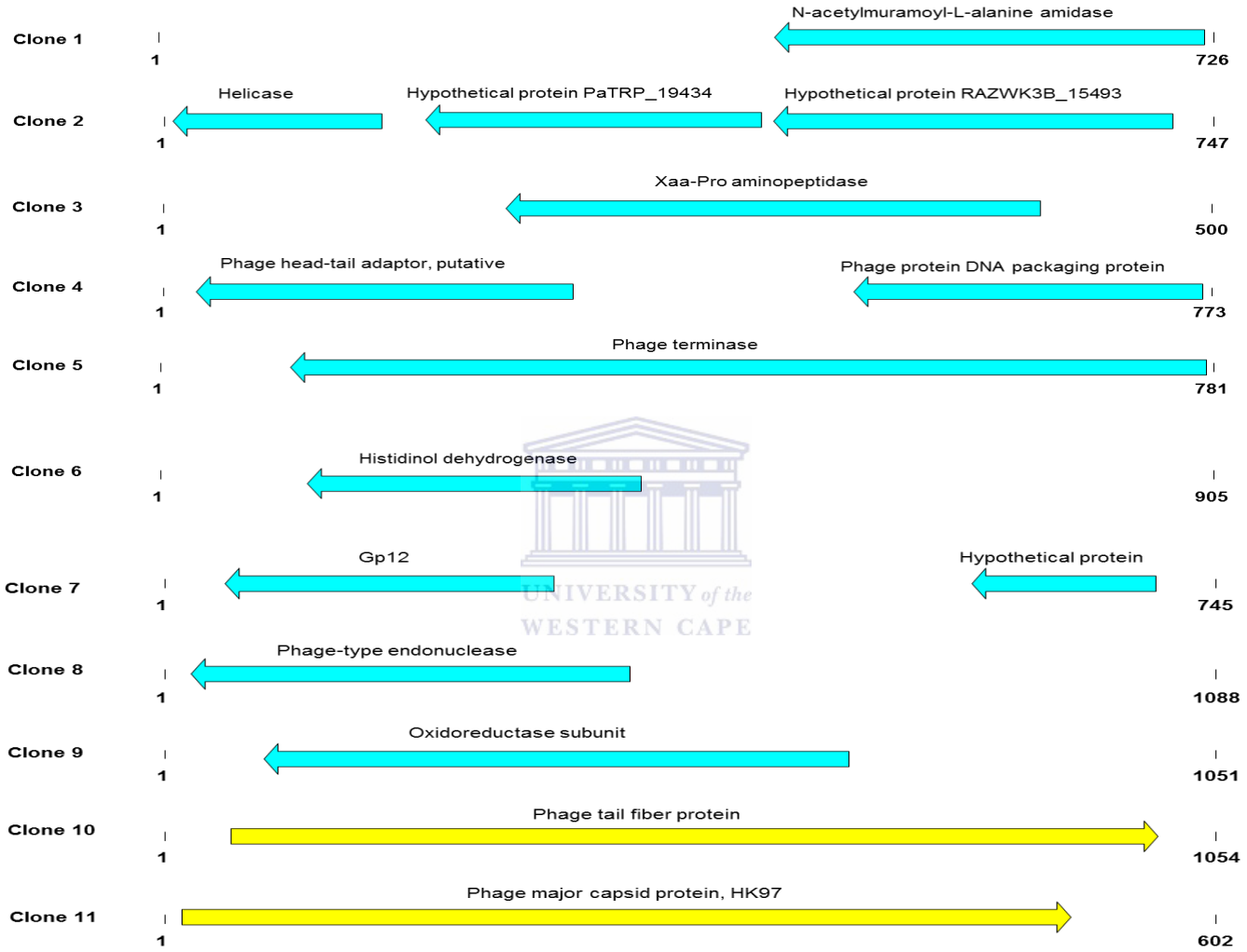

Figure 21: A graphic representation showing the positions of ORFs on phage SHPA DNA sequences. Blue arrow, ORF running on the left strand; yellow arrow, ORF running on the positive strand. The length of the sequences in base pairs (bp).

Chapter 4: General discussion and conclusion

Soda lakes are characterized by a high concentration of sodium carbonate (Na_2CO_3) with varying degrees of salinity and high pH values. Lake Magadi and Lake Shala have NaCl concentrations of approximately 30% and 18% (Jones *et al.,* 1998; Legesse *et al.,* 2004), with pH's of 11 and 9, respectively (Zinabu, 2002; Denson *et al*., 2010). Despite the extreme conditions soda lakes are well populated almost entirely by prokaryotes (Sorokin & Kuenen, 2005) and are the most productive aquatic environments on earth (Grant, 2003). Phages have been isolated from extreme environments such as thermophilic and psychrophilic sources. Thermophilic and hyperthermophilic lakes have proved to be rich in novel phages (Sharp *et al*., 1986; Arnold *et al*., 2000; Rice *et al*., 2004; Prangishvili *et al*., 2006). However, in comparison, there are few studies reporting the microbial diversity in soda lakes, and none exist for estimating phage diversity, therefore nothing is known of the phage community in these environments. This largely unstudied phage population may contain a large reservoir of important and unknown genes which probably play a critical role in the microbial population dynamics and geochemical processes.

Haloalkaliphilic microbes are important as they play the role in remineralization of organic matter within the ecosystem. They also play an important role in transforming carbon, sulfur, nitrogenous compounds and metals in nutrient cycling and food webs under aerobic and anaerobic conditions (Joshi *et al*., 2008). Experimental data and food web models have proved that converting cellular biomass to dissolved organic carbon leads to stimulation of microbial

growth and respiration by viral lysis with a decrease in the transfer of carbon to higher trophic level via grazing, proving that viruses have an effect on carbon flow in soda lakes (Brum *et al*., 2005). Viruses are capable of species succession, transduction, lysogenic processes and help in the maintenance of microbial diversity (Fuhrman, 1999) and they play an important role in the mortality of their bacterial hosts (Wommack & Colwell, 2000). Because phages can affect their hosts by lysogeny and transduction they effect gene exchange within microbial communities in the environment (Wommack & Colwell, 2000).

Phage classification is based on the morphology and genome type (Nelson, 2004). Unlike bacterial classification, phage classification cannot be based on the analysis of molecular marker sequence like the 16S rRNA gene because no single gene that can be used as the basis for a classification system is shared by all phages (Rohwer & Edwards, 2002). There is a lack of sequence data in the databases because only a few phage sequences mostly related to known phage such as T4 and T7, have been sequenced. At least 50–60% of metagenomic virus sequences have been reported to have no similarities to the current database sequences, indicating that most viruses remain uncharacterized (Kristensen *et al*., 2010).

The aim of this study was to isolate and characterize phages from soda lake soil sediment samples. An alkaline medium, Medium A, adapted from Sato *et al*. (1983) was used to isolate these organisms, and the isolates were incubated at 37^{o}C. This medium is similar to the basal medium Horikoshi-I (Horikoshi, 1991). Because the samples were from haloalkaline environments,

the organisms require both high pH and NaCl concentrations for growth. In this study, very few bacteria were isolated using this medium. Although this medium is rich in organic carbon, it is hypothesised that more isolates might have been detected using a medium prepared with lake water. Mwirichia *et al.* (2010) found more novel isolates using different media with varying compositions prepared using soda lake water. As the number of phage isolates depends on the isolated bacteria used to screen them, it is not surprising that very few phages were isolated from both lakes. Metagenomic studies showed that less than 1% of microorganisms in a given environment are culturable (Streit and Schmitz, 2004), therefore culturable isolates represent the minority of the species in microbial communities (Sabet *et al*., 2006). Unculturability of microorganisms might be part of why so few microorganisms were isolated from both lakes. However, this cannot count for isolating only 5 bacteria in both environments. The samples used in this study had been stored at -80^{o}C for some time, therefore we speculate that significant cell viability was lost, especially since the samples were not stored in glycerol (Gundersen *et al*., 1996).

Five different bacterial hosts were identified, one bacterial strain (MGK1) from Lake Magadi and four (Shala1-4) from Lake Shala. Two bacterial hosts ERV9 and HS3 from the IMBM culture collection both collected from Lake Shala were also used to isolate phages. All these bacteria were moderately halophilic and alkalitolerant as they were able to grow at up to 10% (w/v) NaCl concentrations with an optimum pH of 10 (Nowlan *et al*., 2006; Ventosa, 2006).

Morphological characterization and 16S rRNA gene sequencing were used to identify the bacteria. Different bacterial morphologies were identified and the results of 16S rRNA gene sequence showed 96 to 99% identity to existing database sequences. All seven bacterial hosts showed identities to species from four different genera in the database *Bacillius, Halomonas, Virgibacillus* and *Paracoccus.*

Only one phage (MGBH1) was isolated from Lake Magadi sediment sample, and two different phages (SHBH1 and SHPA) were identified from Lake Shala sediment samples. One phage from Lake Shala (SHBH1) was isolated using the Lake Magadi bacterial host (MGK1). No phage was isolated using the isolated Lake Shala bacterial strains (Shala1-4) as host strains against both soil sediment samples. Bacterial strains ERV9 and HS3 (IMBM culture collection) showed plaque formation when screening using Lake Shala sediments.

Phage images were taken by transmission electron microscopy. Despite the advantages of relatively new approaches such as flow cytometry (Brussaard *et al.*, 2000) and epifluorescence microscopy (Noble & Fuhrman, 1998), TEM remains the principal instrument used to reveal information about virus morphology and size (Pearce & Wilson, 2003). In this study three different viral morphologies were observed: *Siphoviridae*, *Myoviridae* and *Podoviridae* with variously sized capsids and tails. To date over 6000 phages have been examined by electron microscopy (Ackermann, 2011). Most of these phages are tailed, representing 96% of phage and only 3.7% are polyhedral, filamentous or pleomorphic (Ackermann, 2007; 2011). *Siphoviridae* are the

most abundant phages known in the biosphere and have also been extensively characterized (Guttman *et al*., 2005). About 61% of the isolated *Siphoviridae* and 24.5% of *Myoviridae* phages have been examined by electron microscopy (Ackermann, 2007). As tailed phages are the most commonly found phages in the environment, their sequence information is growing and has strongly influenced the current view of viral genomics (Brüssow & Hendrix, 2002; Pedulla *et al*., 2003). These dsDNA phages are suspected to infect most if not all bacteria in the biosphere.

Phage genomic analysis and morphological analysis are complementary approaches which provide a more complete picture of phage characteristics. Using one without the other will cause environmental phage diversity to be severely underestimated (Auguet *et al.,* 2006). TEM is also very useful in differentiating phages with a high degree of sequence identity (Jenkins & Hayes, 2006) as it is not possible to describe the viral phenotype from its genomic sequence (Büchen-Osmond, 2003). Although morphological information from electron microscopy alone is unreliable for classifying phages, it is still an important technique used in characterizing viral diversity in the environment (Brum & Steward, 2010).

The smallest phage isolated in this study, phage SHPA (*Podoviridae*), was selected for sequencing. SHPA phage DNA fragments were cloned, and the clones showed homology to some phage related proteins from both phage and bacteria. Phage SHPA is the first phage known to infect *Paracoccus aestuarii*. Although only one phage was sequenced, all the isolated phages may share

similar genetic homology because they are from the same environment. Sabet *et al*. (2006) found that phages from the same environment could share genetic homology as it could relate to increased survivability or better infection in the habitat even though they might infect very diverse hosts.

Podoviruses have linear double stranded DNA genomes of approximately 40-45 kb in length and encodes approximately 55 genes (Zhang *et al*., 2011; Flores *et al*., 2012; Karumidze *et al.,* 2012; Kim *et al.,* 2012; Sajben-Nagy *et al.,* 2012). The genome size of phage SHPA was found to be 25 kb, most similar to the genome of *Helicobacter* phage KHP30 isolated from Japanese patients with the size of 26 kb. *Helicobacter* phage KHP30 is a *Podoviridae* that infects *Helicobacter pylori,* a Gram-negative spiral bacterium that is found in the human stomach (Uchiyama *et al*., 2012). *Podoviridae* are found to infect mostly Gram-negative bacteria (Plunkett *et al*., 1999; Das *et al*., 2012; Sillankorva *et al.*, 2012; Abbasifar *et al*., 2013; Kęsik-Szeloch *et al*., 2013) with very few infecting Gram-positive bacteria (Kotsonis *et al.,* 2008; Kleppen *et al*., 2012; Morales *et al*., 2012).

Most *Podoviridae* that have been classified are from sewage samples (Zhu *et al*., 2010; Drulis-Kawa *et al*., 2011; Fan *et al*., 2012; Karumidze *et al*., 2012; Kęsik-Szeloch *et al*., 2013; Thien *et al.,* 2013), while several have been isolated from marine environments (Breitbart *et al*., 2004; Holmfeldt *et al*., 2007; Labonté *et al*., 2009). Although some phage particles have been detected and isolated from Mono Lake, California, USA (a shallow saline soda lake) (Jiang *et al*., 2004; Sabet *et al*., 2006), these phage particles have not been classified as

they are not fully characterized. Other than these, no *Podoviridae* from haloalkaline environments have been classified so far. Moreover, only 20% of *Podoviridae* phage genomes have been completely sequenced (Deschavanne *et al*., 2010). This highlights the sparcity of available information on this family of bacteriophages, and draws attention to the need to conduct further research.

From the sequence analysis of phage SHPA it is evident that this phage, isolated from the extreme environment sediments of Lake Shala, is a novel phage and, like many other environmental viruses (Sabet *et al.,* 2006), it is not fully characterized.

This study indicates that haloalkaline environments such as Lake Magadi and Lake Shala harbour a diversity of phages. A further investigation of haloalkaliphilic phages using culture independent methods such as metagenomic strategies is important to fully understand the diversity of phages in these environments. Another, less costly approach, would be to conduct sequence analysis of representative phage genes. The genetic diversity of specific groups of viruses in different environments has been examined using the sequence analysis of the structural genes *g20* and *g23* (Fuller *et al*., 1998; Hambly *et al*., 2001; Short & Suttle, 2002; Dorigo *et al*., 2004; Filee *et al*., 2005; Short & Suttle, 2005). Although it is not universally present, family A DNA polymerase has been identified as a good target for examining the diversity of podoviruses (Breitbart *et al*., 2004). Considering the gap in the number of podovirus sequences with which to conduct genome comparisons, perhaps a diversity survey of structural genes would be useful to identify environments

rich in podoviruses to inform on further analyses of this poorly studied group of phages.

References

Abbasifar, R., Kropinski, A.M., Sabour, P.M., Ackermann, H.-W., Villa, A.A *et al*. (2013). The genome of *Cronobacter sakazakii* bacteriophage vB_CsaP_GAP227 suggests a new genus within the *Autographivirinae*. *Genome Announcements* **1**, 1-2.

Abd-el-Malek, Y. & Rizk, S.G. (1963). Bacterial sulfate reduction and the development of alkalinity. III. Experiments under natural conditions in the Wadi Natrun. *Journal of Applied Bacteriology* **26**, 20-26.

Abedon, S.T., Herschler, T.D. & Stopar, D. (2001). Bacteriophage latent-period evolution as a response to resource availability. *Applied & Environmental Microbiology* **69**, 4233-4241.

Abedon, S.T., Hyman, P. & Thomas, C. (2003). Experimental examination of latent-period evolution as a response to bacterial availability. *Applied and Environmental Microbiology* **69**, 7499-7506.

Abedon, S.T. (2011). Size does matter – distinguishing bacteriophages by genome length (and 'breadth'). *Microbiology Australia*, 95-96.

Ackermann, H.-W. (2003). Bacteriophage observations and evolution. *Research in Microbiology* **154**, 245-251.

Ackermann, H.-W. (2007). 5500 Phages examined in the electron microscope. *Archives of Virology* **152**, 227–243.

Ackermann, H.-W. (2011). Bacteriophage taxonomy. *Microbiology Australia* 90-94.

Adams, M.H. (1959). Bacteriophages. Interscience. New York.

Adriaenssens, E.M., Ceyssens, P.J., Dunon, V., Ackermann, H.W., Vaerenbergh, J.V. *et al.* (2011). Bacteriophages LIMElight and LIMEzero of *Pantoea agglomerans*, belonging to the "phiKMV-like viruses". *Applied & Environmental Microbiology* **77**, 3443–3450.

Alexander, M. (1977). Introduction to soil microbiology New York: John Wiley.

Arahal, D.R., Ludwig, W., Schleifer, K.H. & Ventosa, A. (2002). Phylogeny of the family *Halomonadaceae* based on 23S and 16S rDNA sequence analyses. *International Journal of Systematic & Evolutionary Microbiology* **52**, 241–249.

Armon, R. & Kott, Y. (1993). A simple, rapid and sensitive presence/absence detection test for bacteriophage in drinking water. *Journal of Applied Bacteriology* **74**, 490- 496.

Arnold, H.P., Ziese, U. & Zillig, W. (2000). SNDV, a novel virus of the extremely thermophilic and acidophilic archaeon *Sulfolobus*. *Virology* **272**, 409-416.

Asao, M., Takaichi, S. & Madigan, M.T. (2012). Amino acid-assimilating phototrophic heliobacteria from soda lake environments: *Heliorestis acidaminivorans* sp. Nov. and '*Candidatus* Heliomonas lunata'. *Extremophiles* **16**, 585-595.

Auguet, J.C., Montanié, H. & Lebaron, P. (2006). Structure of virioplankton in the Charfente Estuary (France). Transmission Electron Microscopy versus Pulsed Field Gel Electrophoresis. *Microbial Ecology* **50**, 337-349.

Baker, B.H. (1958). Geology of the Magadi area. *Geological Survey Kenya Report* **42**, 1-81.

Baker, G.C., Smith, J.J. & Cowan, D.A. (2003). Review and re-analysis of domain-specific 16S primers. *Journal of Microbiological Methods* **55**, 541-555.

Balding, C., Bromley, S.A., Pickup, R.W. & Saunders, J.R. (2005). Diversity of phage integrases in *Enterobacteriaceae*: development of markers for environmental analysis of temperate phages. *Environmental Microbiology* **7**, 1558–1567.

Bastías, R., Higuera, G., Sierralta, W. & Espejo' R.T. (2010). A new group of cosmopolitan bacteriophages induce a carrier state in the pandemic strain of *Vibrio parahaemolyticus*. *Environmental Microbiology* **12**, 990–1000.

Baumann, A., Förstner, U. & Rohde, R. (1975). Lake Shala: Water chemistry, mineralogy and geochemistry of sediments in an Ethiopian Rift Lake. *International Journal of Earth Science* **64**, 593-609.

Beadle, L.C. (1932). Scientific results of the Cambridge expedition to the East African lakes 1930. IV. The water of some East African lakes in relation to their flora and fauna. *Zoological Journal of the Linnean Society* **38**, 157–211.

Beck, E., Sommer, R., Auerswald, E.A., Kurz, C., Zink, B., Osterburgt , G. *et al.* (1978). Nucleotide sequence of bacteriophage fd DNA. *Nucleic Acids Research* **5**, 4495-4503.

Benhar, I. (2001). Biotechnological applications of phage and cell display. *Biotechnology Advances* **19**, 1-33.

Benvenutia, M., Carnicellib, S., Belluominic, G., Dainellia, N., Di Graziaa, S *et al.* (2002). The Ziway–Shala lake basin (main Ethiopian rift, Ethiopia): a revision of basin evolution with special reference to the Late Quaternary. *Journal of African Earth Sciences* **35**, 247–269.

Bettarel, Y., Kan, J., Wang, K., Williamson, K.E., Cooney, S *et al.* (2005). Isolation and preliminary characterisation of a small nuclear inclusion virus infecting the diatom *Chaetoceros* cf. *gracilis*. *Aquatic Microbial Ecology* **40**, 103–114.

Blattner, F.R. & Dahlberg, J.E. (1972). RNA synthesis start-points in bacteriophage lambda: are the promoter and operator transcribed? *Nature* **237**, 227-232.

Boldareva, E.N., Akimov, V.N., Boĭchenko, V.A., Stadnichuk, I.N., Moskalenko, A.A. *et al*. (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**, 241-54.

Boltianskaia, I.V., Kevbrin, V.V., Lysenko, A.M., Kolganova, T.V., Turova, T.P. *et al*. (2007). *Halomonas mongoliensis* sp. nov. and *Halomonas kenyensis* sp. nov., new haloalkaliphilic denitrifiers capable of reducing N_2O, isolated from soda lakes. *Microbiology* **76**, 834-43.

Boone, D.R., Worakit, S., Mathrani, I.M & Mah, R.A. (1986). Alkaliphilic methanogens from high pH lake sediments. *Systematic & Applied Microbiology* **7**, 230-234.

Born, Y., Fieseler, L., Marazzi, J., Lurz, R., Duffy, B. & Loessner, M.J. (2011). Novel virulent and broad-host-range *Erwinia amylovora* bacteriophages reveal a high degree of mosaicism and a relationship to *Enterobacteriaceae* phages. *Applied and Environmental Microbiology* **77**, 5945–5954.

Bradley, D.E. (1967). Ultrastructure of bacteriophages and bacteriocins. *Bacteriology Reviews* **31**, 230–314.

Breitbart, M. & Rohwer, F. (2005). Here a virus, there a virus, everywhere the same virus? *Trends in Microbiology* **13**, 278–284.

Breitbart, M., Salamon, P., Andresen, B., Mahaffy, J.M., Segall, A. M *et al*. (2002). Genomic analysis of uncultured marine viral communities. *Proceedings of the National Academy of Sciences* **99**, 14250–14255.

Breitbart, M., Miyake, J.H. & Rohwer, F. (2004). Global distribution of nearly identical phage-encoded DNA sequences. *FEMS Microbiology Letters* **236**, 249–256.

Brodetsky, A. M. & Romig, W. R. (1965). Characterization of *Bacillus subtilis* bacteriophages. *Journal of Bacteriology* **90**, 1655–1663**.**

Brown, T.A. (2006). Gene cloning and DNA analysis: an introduction. Edition: 5. Wiley-Blackwell, USA, pp 15-48.

Brum, J.R., Steward, G.F., Jiang, S.C & Jellison, R. (2005). Spatial and temporal variability of prokaryotes, viruses, and viral infections of prokaryotes in an alkaline, hypersaline lake. *Aquatic Microbial Ecology* **41**, 247–260.

Brum, J.R. & Steward, G.F. (2010). Morphological characterization of viruses in the moderately hypersaline stratified Mono Lake. *Microbial Ecology* **60**, 636-643.

Brussaard, C.O.D., Marie, D. & Bratbak, G. (2000). Flow cytometric detection of viruses. *Journal of Virological Methods* **85**, 175-182

Brüssow, H. & Hendrix, R.W. (2002). Phage genomics: small is beautiful. *Cell Press* **108**, 13–16.

Bryantseva, I.A., Gorlenko, V.M., Kompantseva, E.I. & Imhoff, J.F. (2000). *Thioalkalicoccus limnaeus* gen. nov., sp. nov., a new alkaliphilic purple sulfur bacterium with bacteriochlorophyll b. *International Journal of Systematic Bacteriology* **50**, 2157-2163.

Büchen-Osmond, C. (2003). The universal virus database ICTVdB. *Computing in Science & Engineering* **5**, 16-25.

Burnet, F.M. (1933). The classification of dysentery-coli bacteriophages. A correlation of the serological classification with certain biochemical tests. *Journal of Pathology & Bacteriology* **37**, 179–184.

Byrne, M. & Kropinski, A.M. (2005). The genome of the *Pseudomonas aeruginosa generalized* transducing bacteriophage F116. *Gene* **346**, 187–194.

Campbell, A. & Bostein, D. (1983). Evolution of the lambdoid phage. In *Lambda II* (Hendrix, R., Ed.), Cold Spring Harbor Laboratory Press. 365-380.

Canchaya, C., Fournous, G., Chibani-Chennoufi, S., Dillmann, M. & Brüssow, H. (2003). Phage as agents of lateral gene transfer. *Current Opinion in Microbiology* **6**, 417-424.

Carey-Smith, G.V., Billington, C., Cornelius, A.J., Hudson, J.A. & Heinemann, J.A. (2006). Isolation and characterization of bacteriophages infecting *Salmonella* spp. *FEMS Microbiology Letters* **258**, 182–186.

Casjens, S. (2003). Prophages and bacterial genomics: what have we learned so far? *Molecular Microbiology* **49**, 277–300.

Casjens, S.R. (2005). Comparative genomics and evolution of the tailed-bacteriophages. *Current Opinion in Microbiology* **8**, 451-458.

Casjens, S., Hatfull, G.F. & Hendrix, R.W. (1992). Evolution of dsDNA tailed-bacteriophage genomes. *Virology* **3**, 383-397.

Casjens, S., Winn-Stapley, D.A., Gilcrease, E.B., Morona, R., Kühlewein, C *et al.* (2004). The chromosome of *Shigella flexneri* bacteriophage sf6: complete nucleotide sequence, genetic mosaicism, and DNA packaging. *Journal of Molecular Biology* **339**, 379–394.

Ceyssens, P.-J., Lavigne, R., Mattheus, W., Chibeu, A., Hertvelt, K *et al.* (2006). Genomic analysis of *Pseudomonas aeruginosa* phages LKD16 and LKA1: establishment of the ϕKMV Subgroup within the T7 Supergroup. *Journal of Bacteriology* **188**, 6924–6931.

Ceyssens, P.-J., Brabban, A., Rogge, L., Lewis, M.S., Pickard, D *et al.* (2010). Molecular and physiological analysis of three *Pseudomonas aeruginosa* phages belonging to the “N4-like viruses”. *Virology* 405, 26–30.

Chauthaiwale, V.M., Therwath, A. & Deshpande, V.V. (1992). Bacteriophage lambda as a cloning vector. *Microbiological Reviews* ***56***, 577-591.

Chen, F. & Lu, J. (2002). Genomic sequence and evolution of marine cyanophage P60: a new insight on lytic and lysogenic phage. *Applied and Environmental Microbiology* **68**, 2589–2594.

Chibani-Chennoufi, S., Bruttin, A., Dillmann, M. & Brüssow, H. (2004). Phage-host interaction: an ecological perspective. *Journal of Bacteriology* **186**, 3677–3686.

Chopin, A., Deveau, H., Ehrlich, S.D., Moineau, S. & Chopin, M.-C. (2007). KSY1, a lactococcal phage with a T7-like transcription. *Virology* **365**, 1–9.

Clark, A.J., Inwood, W., Cloutier, T. & Dhillon. T.S. (2001). Nucleotide sequence of coliphage HK620 and the evolution of lambdoid phages. *Journal of Molecular Biology* **311**, 657-679.

Clark, J.R. & March, J.B. (2006). Bacteriophages and biotechnology: vaccines, gene therapy and antibacterials. *TRENDS in Biotechnology* **24**, 212-218.

Clokie, M.R.J., Millard, A.D., Letarov, A.V. & Heaphy, S. (2011). Phages in nature. *Bacteriophage* **1**, 31-45.

Collins, J. & Hohn, B. (1978). Cosmids: a type of plasmid gene-cloning vector that is packageable *in vitro* in bacteriophage lambda heads. *Proceedings of the National Academy of Sciences* **75**, 4242-4246.

Comeau, A.M., Bertrand, C., Letarov, A., Tetart, F. & Krisch, H.M. (2007). Modular architecture of the T4 phage superfamily: a conserved core genome and a plastic periphery. *Virology* **362**, 384-396.

Cornelissen, A., Ceyssens, P., Krylov, V.N., Noben, J., Volckaert, G. & Lavigne, R. (2012). Identification of EPS-degrading activity within the tail spikes of the novel *Pseudomonas putida* phage AF. *Virology* 434, 251–256.

Das, M., Nandy, R.K., Bhowmick, T.S., Yamasaki, S., Ghosh, A *et al*. (2012). *Vibrio cholerae* typing phage N4: Genome sequence and its relatedness to T7 viral supergroup. *Intervirology* **55**, 185–193.

Degnan, P.H. & Moran, N.A. (2008). Diverse phage-encoded toxins in a protective insect endosymbiont. *Applied Environmental Microbiology* **74**, 6782-6791.

Delbrück, M. (1945). The burst size distribution in the growth of bacterial viruses (bacteriophages). *Journal of Bacteriology* **50**, 131–135.

Delisle, A.L., Barcak, G.J. & Guo, M. (2006). Isolation and expression of the lysis genes of *Actinomyces naeslundii* phage Av-1. *Applied & Environmental Microbiology* **72**, 1110–1117.

Demuth, J., Neve, H. & Witzel, K. P. (1993). Direct electron evidence study on the morphological diversity of bacteriophage populations in Lake Plußsee. *Applied Environmental Microbiology* **59**, 3378–3384.

Denson, J.D., Ivey, D.M., Leuko, S., Rothschild, L.J. & Hand, K.L. (2010). A survey of the microbial diversity of an alkaline biofilm associated with a unique geological feature within a hot spring of the hypersaline alkaline basin of Lake Magadi, Kenya, *Astrobiology Science Conference.*

Deresinski, S. (2009). Bacteriophage therapy: exploiting smaller fleas. *Clinical Infectious Diseases* **48**, 1096–1101.

Deschavanne, P., DuBow, M.S. & Regeard, C. (2010). The use of genomic signature distance between bacteriophages and their hosts displays evolutionary relationships and phage growth cycle determination. *Virology Journal* **7**, 163.

d'Hérelle, F. (1917). Sur un microbe invisible antagoniste des bacillus dysentériques. *Comptes Rendus de l'Académie des Sciences* **165**, 373–375.

d'Herelle, F. (1918). Technique de la recherche du microbe filtrant bacteriophage (*Bacteriophagum intestinale*). *Comptes Rendus des Séances de la Société de Biologie et de ses Filiales* **81**, 1160–1162.

Dobbins, A.T., George, M. Jr., Basham, D.A., Ford, M.E., Houtz, J.M *et al*. (2004). Complete genomic sequence of the virulent *Salmonella* bacteriophage SP6. *Journal of Bacteriology* **186**, 1933-1944.

Dorigo, U., S. Jacquet, and J. F. Humbert. 2004. Cyanophage diversity, inferred from *g20* gene analyses, in the largest natural lake in France, Lake Bourget. *Applied Environmental Microbiology* **70,** 1017–1022.

Dotto, G.P. & Zinder, N.D. (1983). The morphogenetic signal of bacteriophage f1. *Virology* **130**, 252-256.

Dotto, G.P. & Zinder, N.D. (1984). Increased intracellular concentration of an initiator protein markedly reduces the minimal sequence required for initiation of DNA synthesis. *Proceedings of the National Academy of Sciences* **81**, 1336-1340.

Dotto, G.P., Horinuchi, K. & Zinder, N.D. (1984). The functional origin of bacteriophage fl DNA replication: its signal and domains. *Journal of Molecular Biology* **172**, 507-521.

Drulis-Kawa, Z., Mackiewicz, P., Kęsik-Szeloch, A., Maciaszczyk-Dziubinska, E *et al*. (2011). Isolation and characterisation of KP34—a novel 8KMV-like bacteriophage for *Klebsiella pneumonia*. *Applied Microbiology & Biotechnology* **90**, 1333–1345.

Dubinin, A.V., Gerasimenko, L.M. & Zavarzin, G.A. (1995). Ecophysiology and species diversity of cyanobacteria from Lake Magadi. *Microbiology* **64**, 717-721.

Dublanchet, A. & Bourne, S. (2007). The epic of phage therapy. *The Canadian Journal of Infectious Diseases & Medical Microbiology* **18**, 15-18.

Duckworth, A.W., Grant, W.D., Jones, B.E. & Van Steenbergen, R. (1996). Phylogenetic diversity of soda lake alkaliphiles. *FEMS Microbiology Ecology* **19**, 181-191.

Duckworth, A.W., Grant, S., Grant, W.D., Jones, B.E. & Meijer, D. (1998). *Dietzia natronolimnaios* sp. nov., a new member of the genus *Dietzia* isolated from an East African Soda Lake. *Extremophiles* **2**, 359-66.

Dunn, J.J. & Studier, F.W. (1983). Complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. *Journal of Molecular Biology* **166**, 477–535.

Earnshaw, W.C. & Harrison, S.C. (1977). DNA arrangement in isometric phage heads. *Nature* **268**, 598-602.

Edwards, R.A. & Rohwer, F. (2005). Viral metagenomics. *Nature Reviews Microbiology* **3**, 504-10.

Efrony, R., Loya, Y., Bacharach, E. & Rosenberg, E. (2007). Phage therapy of coral disease. *Coral Reefs* **26**, 7-13.

Eisenstark, A. (1967). Bacteriophage techniques. In: Maramorosch, K., Koprowski, H (eds). Methods in virology, Vol 1. Academic Press, New York, 449-525.

El-Arabi, T.F., Griffiths, M.W., She., Y.-M., Villegas, A., Lingohr, E.J. & Kropinski, A.M. (2013). Genome sequence and analysis of a broad-host range lytic bacteriophage that infects the *Bacillus cereus* group. *Virology Journal* **10**, 48.

Eller, M.R., Salgado, R.L., Vidigal, P.M., Alves, M.P., Dias, R.S *et al*. (2013). Complete genome sequence of the *Pseudomonas fluorescens* bacteriophage UFV-P2. *Genome Announcements* **1**, 1-2.

Ellis, E.L. & Delbrück, M. (1939). The growth of bacteriophage. *Journal of General Physiology* **22**, 365-384.

Elvin, C.M., Thompson, P.R., Argall, M.E., Hendry, P., Stamford, N.P.J *et al.* (1990). Modified bacteriophage lambda promoter vectors for overproduction of proteins in *Escherichia coli*. *Gene* **87**, 123-126.

Eugster, H.P. (1986). Lake Magadi, Kenya: A model for Rift Valley hydrochemistry and sedimentation? In Geol. Soc Spec. Publ. Edited by L.E Frostick R.W Renaut, I. & Tiercelin, J.J. Oxford: Blackwell Scientific.

Eugster, H.P. & Hardie, L.A. (1978). Saline lakes. In: Lerman A. (ed.), Lakes: Chemistry, Geology, Physics. Springer, New York, pp. 237–289.

Fan, H., Fan, H., An, X., Huang, Y., Zhang, Z *et al.* (2012). Complete genome sequence of IME11, a new N4-like bacteriophage. *Journal of Virology* **86**, 13861.

Feng, J., Zhou, P., Zhou, Y., Liu, S.J. & Warren-Rhodes, K. (2005). *Halorubrum alkaliphilum* sp. nov., a novel haloalkaliphile isolated from a soda lake in Xinjiang, China**.** *International Journal of Systematic & Evolutionary Microbiology* **55**, 149–152

Fiers, W., Conterra, R. & Duerinck, F. (1976). Complete nucleotide sequence of bacteriophage MS2 RNA: primary and secondary structure of the replicase gene. *Nature* **260**, 500–507.

Filee, J., Tetart, F., Suttle, C.A. & Krisch, H.M. (2005). Marine T4-type bacteriophages, a ubiquitous component of the dark matter of the biosphere. *Proceedings of the National Academy of Sciences* **102**, 12471–12476**.**

Flores, V., López-Merino, A., Mendoza-Hernandez, G. & Guarneros, G. (2012). Comparative genomic analysis of two brucellaphages of distant origins. *Genomics* **99**, 233-240.

Fortier, L.C. & Moineau, S. (2007). Morphological and genetic diversity of temperate phages in *Clostridium difficile*. *Applied Enviromental Microbiology* **73**, 7358-7366.

Foti, M., Ma, S., Sorokin, D.Y., Rademaker, J.L.W., Kuenen G. & Muyzer, G. (2006). Genetic diversity and biogeography of haloalkaliphilic sulphur oxidizing bacteria belonging to the genus *Thioalkalivibrio. FEMS Microbiology Ecology* **56**, 95–101.

Frank, H. & Moebus, K. (1987). An electron microscopic study of bacteriophages from marine waters. *Helgoländer Meeresuntersuchungen* **41**, 385–414.

Freire R., Serrano M., Salas M. & Hermoso J. (1996). Activation of replication origins in phi29-related phages requires the recognition of initiation proteins to specific nucleoprotein complexes. *Journal of Biological Chemistry* **271**, 31000-31007.

Frilander, M., Poranen, M. & Bamford, D.H. (1995). The large genome segment of dsRNA bacteriophage phi6 is the key regulator in the in vitro minus and plus strand synthesis. *RNA* **1**, 510–518.

Fuhrman, J.A. (1999). Marine viruses and their biogeochemical and ecological effects. *Nature* **399**, 541-548.

Fuller, N. J., Wilson, W.H., Joint, I.R. & Mann, N.H. (1998). Occurrence of a sequence in marine cyanophages similar to that of T4 *g20* and its application to PCR-based detection and quantification techniques. *Applied Environmental Microbiology* **64,** 2051–2060.

Gani, J. & Yeo, G.F. (1965). Some birth-death and mutation models for phage reproduction. *Journal of Applied Probability* **2**, 150-161.

Gao, J., Wang, Y., Liu, Z. & Wang, Z. (2010). Phage display and its application in vaccine design. *Annals of Microbiology* **60**, 13-19.

Gao, E.-B., Gui, J.-F. & Zhang, Q.Y. (2011). A novel cyanophage with a cyanobacterial nonbleaching protein a gene in the genome. *Journal of Virology* **86**, 236–245.

Garcia, E., Elliott, J.M., Ramanculov, E., Chain, P.S.G., Chu, M.C. & Molineux, I.J. (2003). The genome sequence of *Yersinia pestis* bacteriophage phiA1122 reveals an intimate history with the coliphage T3 and T7 genomes. *Journal of Bacteriology* **185**, 5248–5262.

Gasse, E. &. Street, F.A. (1978). Late quaternary lake-level fluctuations and environments of the northern Rift valley and far region (Ethiopia and Djibouti). *Palaeogeography, Palaeoclimatology, Palaeoecology* **24**, 279-325.

Gill, J.J., Summer, E.J., Russell, W.K., Cologna, S.M., Carlile, T.M *et al.* (2011). Genomes and characterization of phages Bcep22 and Bcepil02, founders of a novel phage type in *Burkholderia cenocepacia*. *Journal of Bacteriology* **193**. 5300–5313.

Glukhov, A.S., Krutilina, A.I., Shlyapnikov, M.G., Severinov, K., Lavysh, D *et al*. (2012). Genomic analysis of *Pseudomonas putida* phage tf with localized single-strand DNA interruptions. Pros ONE **7**, 1-10.

González, J.M. & Suttle, C.A. (1993). Grazing by marine nanoflagellates on viruses and viral-sized particles: ingestion and digestion. *Marine Ecology Progress Series* **94**, 1–10.

Gorlenko, V.M., Bryantseva, I.A., Rabold, S., Tourova, T.P., Rubtsova, D. *et al*. (2009). *Ectothiorhodospira variabilis* sp. nov., an alkaliphilic and halophilic purple sulfur bacterium from soda lakes. *International Journal of Systematic & Evolutionary Microbiology* **59**, 658-664.

Grant, W.D. (1992). Alkaline Environments. In Encyclopaedia of Microbiology, vol 1. (Ed. J. Lederberg), Academic Press. London, 73-80.

Grant, W.D. (2003). Alkaline environments and biodiversity. In Extremophiles: Basic Concepts; Gerdsy, C., Glansdorff, N., Eds.; Encyclopedia of Life Support Systems: Paris, France.

Grant, W.D. & Tindall, B.J. (1986). The alkaline, saline environment. In microbes in extreme environments, (Eds. R. A. Herbert and G. A. Codd), 22-54. Academic Press. London.

Grant, W.D., Jones B.E. & Mwatha W.E. (1990). Alkaliphiles: ecology, diversity and applications. *FEMS Microbiology Letters* **75**, 255–270.

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.

Griffiths, A.D. & Duncan, A.R. (1998). Strategies for selection of antibodies by phage display. *Current Opinion in Biotechnology* **9**, 102-108.

Gundersen, K., Bratbak, G. & Heldal, M. (1996). Factors influencing the loss of bacteria in preserved seawater samples. *Marine Ecology Progress* **137**, 305-310.

Guttman, B., Raya, R. & Kutter, E. (2005). Basic phage biology. In: bacteriophages: Biology and Applications. *CRC Press,* 29-66.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95-98.

Hambly, E., Tetart, F., Desplats, C., Wilson, W.H., Krisch, H.M. & Mann, N.H. (2001). A conserved genetic module that encodes the major virion components in both the coliphage T4 and the marine cyanophage S-PM2. *Proceedings of the National Academy of Sciences* **98**, 11411–11416.

Hambly, E. & Suttle, C.A. (2005). The viriosphere, diversity, and genetic exchange within phage communities. *Current Opinion in Microbiology* **8**, 444-450.

Hankin, E. (1896). The bactericidal action of the waters of the Jumna and the Ganges on *Vibrio cholerae. Annales de l'Institut Pasteur* **10**, 511.

Hansen, M.C., Tolker-Nelson, T., Givskov, M. & Molin, S. (1998). Biased 16S rDNA PCR amplification caused by interference from DNA flanking template region. *FEMS Microbiology Ecol*ogy **15**, 25-36.

Haq, I.U., Chaudhry, W.N., Akhtar, M.N., Andleeb, S. & Qadri, I. (2012). Bacteriophages and their implications on future biotechnology: a review. *Virology Journal* **9**, 9.

Hardies, S.C., Comeau, A.M., Serwer, P. & Suttleb, C.A. (2003). The complete sequence of marine bacteriophage VpV262 infecting *Vibrio parahaemolyticus* indicates that an ancestral component of a T7 viral supergroup is widespread in the marine environment. *Virology* **310**, 359–371.

Hardies, S.C., Comeau, A.M., Serwer, P. & Suttleb, C.A. (2007). Whole genome sequencing of a novel temperate bacteriophage of *P. aeruginosa*: evidence of tRNA gene mediating integration of the phage genome into the host bacterial chromosome. *Cellular Microbiology* **9**, 479–491.

Hatfull, G.F. (2006). In: Calendar, R., editor. The Bacteriophages. Oxford University Press, 602-620.

Hatfull, G.F. (2008). Bacteriophage genomics. *Current Opinion in Microbiology* **11**, 447–453.

Hatfull, G. F., Pedulla, M.L., Jacobs-Sera, D., Cichon, P.M., Foley, A. *et al.* (2006). Exploring the mycobacteriophage metaproteome: phage genomics as an educational platform. *PLoS Genetics* **2**, 835-847.

Häusler, T. (2006). Viruses vs. superbugs: a solution to the antibiotics crisis, Macmillan Publishers, 248-251.

Heilmann, S., Sneppen, K. & Krishna, S. (2010). Sustainability of virulence in a phage-bacterial ecosystem. *Journal of Virology* **84**, 3016-3022.

Hemphill, H. E. & Whiteley, H. R. (1975). Bacteriophages of *Bacillus subtilis*. *Bacteriology Review* **39**, 257–315.

Hendrix, R.W. (2002). Bacteriophage: evolution of the majority. *Theoretical Population Biology* **61**. 471-480.

Hendrix, R.W. (2003). Bacteriophage genomics. *Current Opinion in Microbiology* **6**, 506–511.

Hendrix, R.W., Smith, M.C.M., Burns, R.N., Ford, M.E. & Hatful, G.F. (1999). Evolutionary relationships among diverse bacteriophages and prophages: all the world's a phage. *Proceedings of the National Academy of Sciences* **96**, 2192–2197.

Hendrix, R.W., Lawrence, J.G., Hatfull, G.F. & Casjens, S. (2000). The origins and ongoing evolution of viruses. *Trends in Microbiology* **8**, 504-507.

Hendrix, R.W., Hatfull, G.F. & Smith, M.C.M. (2003). Bacteriophages with tails: chasing their origins and evolution. *Research in Microbiology* **154**, 253-257.

Hershey, A. & Chase, M. (1952). Independent functions of viral protein and nucleic acid in growth of bacteriophage. *Journal of General Physiology* **36**, 39–56.

Ho, N., Lingohr, E.J., Villegas, A., Cole, L., Kropinski, A.M *et al.* (2012). Genomic characterization of two new *Salmonella* bacteriophages: vB_SosS_Oslo and vB_SemP_Emek. *Annals of Agrarian Science* **10**, 18-23.

Hoeft, S.E., Blum, J.S., Stolz, J.F., Tabita, F.F., Witte, B. *et al.* (2007). *Alkalilimnicola ehrlichii* sp. nov., a novel, arseniteoxidizing haloalkaliphilic gammaproteobacterium capable of chemoautotrophic or heterotrophic growth with nitrate or oxygen as the electron acceptor. *International Journal of Systematic and Evolutionary Microbiology* **57**, 504–512.

Holmfeldt, K., Middelboe, M., Nybroe, O. & Riemann, L. (2007). Large variabilities in host strain susceptibility and phage host range govern interactions between lytic marine phages and their *Flavobacterium* hosts. *Applied and Environmental Microbiology* **73**, 6730–6739.

Horikoshi, K. (1971). Production of alkaline enzymes by alkalophilic microorganisms. Part I. Alkaline protease produced by *Bacillus* no. 221. *Agricultural and Biological* Chemistry **36**, 1407–1414.

Horikoshi, K. (1991). General view of alkaliphiles and thermophiles. In: Horikoshi K, Grant WD (eds) Superbugs: micro-organisms in extreme environments. Springer, Berlin, 3–13.

Horikoshi, K. (1999). Alkaliphiles: some applications of their products for biotechnology. *Microbiology & molecular biology reviews* **63**, 735–750.

Horikoshi, K. (2006). Alkaliphiles: genetic properties and applications of enzymes; Springer: Berlin, Germany.

Hua, N., Hamza-Chaffai, A., Vreeland, R.H., Isoda, H. & Naganuma, T. (2008). *Virgibacillus salarius* sp. nov., a halophilic bacterium isolated from a Saharan salt lake. *International Journal of Systematic & Evolutionary Microbiology* **58**, 2409–2414.

Huang, Y., Fan, H., Pei, G., Fan, H., Zhang, Z *et al.* (2012). Complete genome sequence of IME15, the first T7-like bacteriophage lytic to pan-antibiotic-resistant *Stenotrophomonas maltophilia*. *Journal of Virology* **86**, 13839 –13840.

Imhoff J.F., Sahl H.G., Soliman, G.S.H. & Trüper H.G. (1979). The Wadi Natrun: Chemical composition and microbial mass developments in alkaline brines of Eutrophic Desert Lakes. *Geomicrobiology Journal* **1**, 219-234.

Jarvis, C.D., Geiman, T., Vila-Storm, M.P., Osipovich, O., Akella, U *et al*. (1996). A novel putative helicase produced in early murine lymphocytes. *Gene* **169**, 203-207.

Jenkin P.M. (1932). Reports of the Percy Sladen expedition to some Rift Valley lakes in Kenya in 1929, Summary of the ecological results with special reference to the alkaline lakes. *Annals & Magazine of Natural History* **8**, 133–181.

Jenkins, C.A. & Hayes, P.K. (2006). Diversity of cyanophages infecting the heterocystous filamentous cyanobacterium *Nodularia* isolated from the brackish Baltic Sea. *Journal of the Marine Biological Association of the United Kingdom* **86**, 529-536.

Jensen, E.C. Schrader, H.S., Rieland, B., Thompson, T.L., Lee, K.W *et al*. (1998). Prevalence of broad-host-range lytic bacteriophages of *Sphaerotilus natans*, *Escherichia coli*, and *Pseudomonas aeruginosa. Applied & Environmental Microbiology* **64**,575–580.

Jepson, C.D. & March, J.B. (2004). Bacteriophage lambda is a highly stable DNA vaccine delivery vehicle, *Vaccine* **22**, 2413–2419.

Jiang, S., Steward, G., Jellison, R., Chu, W. & Choi, S. (2004). Abundance, distribution, and diversity of viruses in alkaline, hypersaline Mono Lake, California. *Micriobial Ecology* **47**, 917.

Jones, B.E. & Grant, W.D. (2000). Microbial diversity and ecology of the Soda Lakes of East Africa. Ecology and Diversity of Extremophiles, Bell, C.R., Brylinsky., M, Johnson-Green, P. (eds) Atlantic Canada Society for Microbial Ecology, Canada.

Jones, B.E., Grant, W.D., Collins, N.C. & Mwatha, W.E. (1994). Alkaliphiles: diversity and identification, In: Priest F.G., Ramos-Cormenzana A. & Tindall, B.J. (eds) Bacterial diversity and systematic, Plenum, New York, 195–229.

Jones, B.E., Grant, W.D., Duckworth, A.W. & Owenson, G.G. (1998). Microbial diversity of soda lakes. *Extremophiles* **2**, 191-200.

Joshi, A.A., Kanekar, P.P., Kelkar, A.S., Shouche, Y.S., Vani, A.A *et al.* (2008). Cultivable bacterial diversity of alkaline Lonar Lake, India. *Microbial Ecology* **55**, 163–172.

Juhala, R.J., Ford, M.E., Duda, R.L., Youlton, A., Hatful, G.F. & Hendrix, R.W. (2000). Genomic sequences of bacteriophages HK97 and HK022: pervasive genetic mosaicism in the lambdoid bacteriophages. *Journal of Molecular Biology* **299**, 27-51.

Kaluzhnaya, M., Khmelenina, V., Eshinimaev, B., Suzina, N., Nikitin, D. *et al.* (2001). Taxonomic characterization of new alkaliphilic and alkalitolerant methanotrophs from soda lakes of the Southeastern Transbaikal region and description of *Methylomicrobium buryatense* sp.nov. *Systematic & Applied Microbiology* **24**, 166-176.

Kanal, H., Kobayashi, T., Aono, R. & Kudo, T. (1995). *Natronococcus amylolyticus* sp. nov., a haloalkaliphilic archaeon. *International Journal of Systematic Bacteriology* **45**, 762-766.

Karumidze, N., Thomas, J.A., Kvatadze, N., Goderdzishvili, M., Hakala, K.W *et al.* (2012). Characterization of lytic *Pseudomonas aeruginosa* bacteriophages via biological properties and genomic sequences. *Applied Microbiology & Biotechnology* **94**, 1609-1617.

Kawasaki, T., Shimizu, M., Satsuma, H., Fujiwara, A., Fujie, M *et al.* (2009). Genomic characterization of *Ralstonia solanacearum* phage ϕRSB1, a T7-like wide-host-range phage. *Journal of Bacteriology* **191**, 422–427.

Kaye, J.Z., Ma´ rquez, M.C., Ventosa, A. & Baross, J.A. (2004). *Halomonas neptunia* sp. nov., *Halomonas sulfidaeris* sp. nov., *Halomonas axialensis* sp. nov. and *Halomonas hydrothermalis* sp. nov.: halophilic bacteria isolated from deep-sea hydrothermal-vent environments. *International Journal of Systematic & Evolutionary Microbiology* **54**, 499–511.

Kęsik-Szeloch, A., Drulis-Kawa, Z., Weber-Dąbrowska, D., Kassner, J., Majkowska-Skrobek, G *et al.* (2013). Characterising the biology of novel lytic bacteriophages infecting multidrug resistant *Klebsiella pneumonia*. *Virology Journal* **10**, 100.

Kevbrin, V.V., Zhilina, T.N., Rainey, F.A. & Zavarzin, G.A. (1998). *Tindallia magadii* gen. nov., sp. nov.: an alkaliphilic anaerobic ammonifier from soda lake deposits. *Current Microbiology* **37**, 94-100.

Khmelenina, V.N., Kalyuzhnaya, M.G., Starostina, N.G., Suzina, N.E & Trotsenko, Y.A. (1997). Isolation and characterization of halotolerant alkaliphilic methanotrophic bacteria from Tuva soda lakes. *Current Microbiology* **35**, 257-261.

Kim, J.H., Son, J.S., Choresca, C.H., Shin, S.P., Han, J.E *et al*. (2012). Complete genome sequence of bacteriophage phiAS7, a T7-like virus that infects *Aeromonas salmonicida* subsp. *Salmonicida*. *Journal of Virology* **86**, 2894-2895.

Kleppen, H.P., Holo, H., Jeon, S.R, Nes, I.F., Yoon, S.S *et al*. (2012). Novel *Podoviridae* family bacteriophage infecting *Weissella cibaria* isolated from Kimchi. Applied and Environmental Microbiology **78**, 7299 –7308.

Klovins, J., Overbeek, G.P., Van den Worm, S.H., Ackermann, H.-W. & Van Duin, J. (2002). Nucleotide sequence of a ssRNA phage from *Acinetobacter* kinship to coliphages. *Journal of General Virology* **83**, 1523–1533.

Kotsonis, S.E., Powell, I.B., Pillidge, C.J., Limsowtin, G.K.Y., Hillier, A.J. & Davidson, B.E. (2008). Characterization and genomic analysis of phage asccϕ28, a phage of the family *Podoviridae* infecting *Lactococcus lactis*. *Applied & Environmental Microbiology* **74**, 3453–3460.

Kovalyova, I.V. & Kropinski, A.M. (2003). The complete genomic sequence of lytic bacteriophage gh-1 infecting *Pseudomonas putida*—evidence for close relationship to the T7 group. *Virology* **311**, 305–315.

Kristensen, D.M., Mushegian, A.R. & Koonin, E.V. (2010). New dimensions of the virus world discovered through metagenomics. Trends in Microbiology 18, 11-19.

Kropinski, A.M., Kovalyova, I.V., Billington, S.J., Patrick, A.N., Butts, B.D *et al.* (2007). The genome of epsilon15, a serotype-converting, Group E1 *Salmonella enterica*-specific bacteriophage. *Virology* **369**, 234-244.

Krulwich, T.A., Ito, M., Hicks, D.B., Gilmour, R. & Guffanti, A.A. (1988). pH homeostasis and ATP synthesis: studies of two processes that necessitate inward proton translocation in extremely alkaliphilic *Bacillus* species. *Extremophiles* **2**, 217-222.

Kulakov, L.A., Ksenzenko, V.N., Kochetkov, V.V., Mazepa, V.N., Boronin, A.M *et al.* (1985). DNA homology and adsorption specificity of *Pseudomonas aeruginosa* virulent bacteriophages. *Molecular & General Genetics* **200**, 123-127.

Kulikov, E., Kropinski, A.M., Golomidova, A., Lingohr, E., Govorun, V. *et al*., (2012). Isolation and characterization of a novel indigenous intestinal N4-related coliphage vB_EcoP_G7C. *Virology* **426**, 93-99.

Kwan, T., Liu, J., Dubow, M., Gros, P., Pelletier, J *et al.* (2005). The complete genomes and proteomes of 27 *Staphylococcus aureus* bacteriophages. *Proceedings of the National Academy of Sciences*. **102**, 5174-5179.

Kwan, T., Liu, J., DuBow, M., Gros, P & Pelletier, J *et al*. (2006). Comparative genomic analysis of 18 *Pseudomonas aeruginosa* bacteriophages. *Journal of Bacteriology* 188, 1184–1187.

Kwon, H.-J., Cho, S.-H., Kim, T.-E., Won, Y.-J., Jeong, J *et al.* (2008). Characterization of a T7-like lytic bacteriophage (ϕSG-JL2) of *Salmonella enterica* serovar Gallinarum Biovar Gallinarum. *Applied & Environmental Microbiology* **74**, 6970–6979.

L'Abée-Lund, T.M., Jørgensen, H.J., O'Sullivan, K,. Bohlin, J., Ligård, G *et al*. (2012). The highly virulent 2006 Norwegian EHEC O103:H25 outbreak strain is related to the 2011 German O104:H4 outbreak strain. *PLoS ONE* **7**, 1-11.

Labonté, J.M., Reid, K.E. & Suttle, C.A. (2009). Phylogenetic analysis indicates evolutionary diversity and environmental segregation of marine Podovirus DNA polymerase gene sequences. *Applied & Environmental Microbiology* **75**, 3634–3640.

Lai, Q., Li, G. & Shao, Z. (2012). Genome sequence of *Nitratireductor pacificus* type strain pht-3B. *Journal of Bacteriology* **194**, 6958.

Lammens, E., Ceyssens, P.J., Voet, M., Hertveldt, K., Lavigne, R. & Volckaert, G. (2009). Representational Difference Analysis (RDA) of bacteriophage genomes. *Journal of Microbiological Methods* **77**, 207-213.

Lavigne, R., Bourkaltseva, M.V., Robben, J., Sykilinda, N.N., Kurochkina, L.P *et al*. (2003). The genome of bacteriophage phiKMV, a T7-like virus infecting *Pseudomonas aeruginosa*. *Virology* **312**, 49-59.

Lawrence, J.G., Hatfull, G.F. & Hendrix, R.W. (2002). Imboglios of viral taxonomy: genetic exchange and failing of phenetic approaches. *Journal of Bacteriology* **184**, 4891-4905.

Leonard, M.T., Fagen, J.R., Davis-Richardson, A.G., Davis, M.J. & Triplett, E.W. (2012). Complete genome sequence of *Liberibacter crescens* BT-1. *Standards in Genomic Sciences* **7**, 271-283.

Lee, J.H., Shin, H., Ji, S., Malhotra, S., Kumar, M *et al*. (2012). Complete genome sequence of phytopathogenic *Pectobacterium carotovorum* subsp. *carotovorum* bacteriophage PP1. *Journal of Virology* **86**, 8899–8900.

Legesse, D., Vallet-Coulomb, C. & Gasse F. (2004). Analysis of the hydrological response of a tropical terminal lake, Lake Abiyata (Main Ethiopian Rift Valley) to changes in climate and human activities. *Hydrological Processes* **18**, 487–504.

Lingohr, E.J. Villegas, A., She, Y-M., Ceyssens, P.-J. & Kropinski, A.M. (2008). The genome and proteome of the *Kluyvera* bacteriophage Kvp1 – another member of the T7-like *Autographivirinae*. *Virology Journal **5**, 122.*

Liu, Y., Boone, D.R. & Choy, C. (1990). *Methanohalophilus oregonense* sp. nov. a methylotrophic methanogen from an alkaline, saline aquifer. *International Journal of Systematic Bacteriology* **40**, 111-116.

Liu, M., Gingery, M., Doulatov, S.R., Liu, Y., Hodes, A *et al*. (2004). Genomic and genetic analysis of bordetella bacteriophages encoding reverse transcriptase-mediated tropism-switching cassettes. *Journal of Bacteriology* **186**, 1503–1517.

Liu, X., Shi, M., Kong, S., Gao, Y. & An, C. (2007). Cyanophage Pf-WMP4, a T7-like phage infecting the freshwater cyanobacterium *Phormidium foveolarum*: Complete genome sequence and DNA translocation. *Virology* **366**, 28–39.

Liu, X., Kong, S., Shi, M., Fu, L., Gao, Y & An, C. (2008). Genomic analysis of freshwater cyanophage Pf-WMP3 infecting *Cyanobacterium phormidium* foveolarum: the conserved elements for a phage. *Microbial Ecology* **56**, 671-680.

Łobocka, M.B., Rose, D.J., Plunkett, G., Rusin, M. & Samojedny, A. (2004). A genome of bacteriophage P1. *Journal of Bacteriology* **186**, 7032–7068.

Lorch, A. (1999). Bacteriophages: an alternative to antibiotics. *Biotechnology and Development Monitor* **39**, 14-17.

Luo, C.H., Chiou, P.Y., Yang, C.Y & Lin, N.T. (2012). Genome, integration, and transduction of a novel temperate phage of *Helicobacter pylori*. *Journal of Virology* **86**, 8781-8792.

Luria, S.E. & Delbrück, M. (1943). Mutations of bacteria from virus sensitivity to virus resistance. *Genetics* **28**, 491–511.

Lwoff, A., Horne, R.W. & Tournier, P. (1962). A system of viruses. *Cold Spring Harbor Symposia on Quantitative Biology* **27**, 51–62.

Lynch, K.H., Stothard, P. & Dennis, J.J. (2012). Characterization of DC1, a broad-host-range Bcep22-like podovirus. *Applied & Environmental Microbiology* **78**, 889 – 891.

Madigan, T.M., Martinko, J.M., Dunlap, P.V. & Clark, D.P. (2009). Brock biology of microorganisms. 12th ed., Pearson Benjamin Cunnings.

Martín, A.C., López, R. & García, P. (1996). Analysis of the complete nucleotide sequence and functional organization of the genome of *Streptococcus pneumonia* bacteriophage Cp-1. *Journal of Virology* **70**, 3678–3687.

Martins, I., Pardal, M.A., Lillebø, A.I., Flindt, M.R. & Marques, J.C., (2001). Hydrodynamics as a major factor controlling the occurrence of green macroalgae blooms in an eutrophic estuary: a case study. *Estuarine, Coastal & Shelf Science* **52**, 165–177.

McAuliffe, O., Ross, R.P. & Fitzgerald, G.F. (2007). The new phage biology: from genomics to applications. In: McGrath, S. & van Sinderen, D. (eds) Bacteriophage: genetics and molecular biology, Caister Academic Press.

McNair, K., Bailey, B.A. & Edwards, R.A. (2012). Phacts, a computational approach to classifying the lifestyle of phages. *Bioinformatics* **28,** 614–618.

Mead, D.A., Szczesna-Skorupa, E. & Kemper, B. (1986). Single-stranded DNA "blue" T7 promoter plasmids: A versatile tandem promoter system for cloning and protein engineering. *Protein Engineering* **1**, 67-74.

Melack, J.M. & Kilham, P. (1974). Photosynthetic rates of phytoplankton in East African alkaline, saline lakes. *Limnology and Oceanography* **19**, 743-755.

Mertens, H. & Hausmann, R. (1982). Coliphage BAI4: a new relative of phage T7. *Journal of general Virology* **62**, 331-341.

Middelboe, M., Chan, A.M. & Bertelsen, S.K. (2010). Isolation and life cycle characterization of lytic viruses infecting heterotrophic bacteria and cyanobacteria. *Manual of Aquatic Viral Ecology* **13**, 118-133.

Mmolawa, P.T., Schmieger, H., Tucker, C.P. & Heuzenroeder, M.W. (2003). Genomic structure of the *Salmonella enterica* serovar Typhimurium DT 64 bacteriophage ST64T: evidence for modular genetic architecture. *Journal of Bacteriology* **185**, 3473–3475.

Moineau, S., Pandian, S. & Klaenhammer, T. (1994) Evolution of a lytic bacteriophage via DNA acquisition from the *Lactococcus lactis* chromosome. *Applied and Environmental Microbiology* **60**, 1832– 1841.

Moineau, S., Pandian, S. & Klaenhammer, T.R. (1995) Specific chromosomal sequences can contribute to the appearance of a new lytic bacteriophage in *Lactococcus*. *Development in Biological Standardization* **85**, 577–580.

Morales, C.A., Oakley, B.B., Garrish, J.K., Siragusa, G.R., Ard, M.B. & Seal, B.S. (2012). Complete genome sequence of the podoviral bacteriophage ΦCP24R, which is virulent for *Clostridium perfringens*. *Archives of Virology* **157**, 769-772.

Moran, M.A., Belas, R., Schell, M.A., Gonzalez, J.M., Sun, F *et al*. (2007). Ecological genomics of marine *Roseobacters*. *Applied & Environmental Microbiology* **73**, 4559-4569.

Morgan, G.J. (2008). Evolution without Species: The case of mosaic bacteriophages. *British Journal for Philosophy of Science* **59**, 745-765.

Morgan, G.J., Hatfull, G.F., Casjens, S. & Hendrix, R.W. (2002). Bacteriophage Mu genome sequence: analysis and comparison with Mu-like prophages in *Haemophilus*, *Neisseria* and *Deinococcus*. *Journal of Molecular Biology* **317**, 337-359.

Morris, P., Marinelli, L.J., Jacobs-Sera, D., Hendrix, R.W. & Hatfull, G.F. (2008). Genomic characterization of mycobacteriophage Giles: evidence for phage acquisition of host DNA by illegimate recombination. *Journal of Bacteriology* **190**, 2172-2182.

Moulton, K.D., Movassaghi, M., Jamison, J.L., Lobo, N., Lobo, C. *et al*. (2011). Isolation and initial study of Ø1M3-16, a bacteriophage infecting an alkaliphilic *Vibrio metschnikovii* isolate from Lake Magadi, a soda lake in Kenya's Great Rift Valley. *Microscopy and Microanalysis* **17**, 350-351.

Müller, I., Kube, M., Reinhardt, R., Jelkmann, W. & Geider, K. (2011). Complete genome sequences of three *Erwinia amylovora* phages isolated in North America and a bacteriophage induced from an *Erwinia tasmaniensis* strain. *Journal of Bacteriology* **193**, 795.

Murray, N.E. (1983a). Lambda vectors. In R. W.Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 677-684.

Murray, N.E. (1983b). Phage lambda and molecular cloning, In R. W. Hendrix, J. W. Roberts, F. W. Stahl, & R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 395-432.

Mwatha, W.E & Grant, W.D. (1993). *Natronobacterium vacuolata* sp. nov., a haloalkaliphilic archaeon isolated from Lake Magadi, Kenya. *International Journal of Systematic Bacteriology* **43**, 401-404

Mwirichia, R., muigai, A.W., Tindall, B., Boga, H.I. & Stackebrandt, E. (2010). Isolation and characterization of bacteria from the haloalkaline Lake Elmenteita, Kenya. *Extremophiles* **14**, 339-348.

Nelson, D. (2004). Phage taxonomy: we agree to disagree. *Journal of Bacteriology* **186**, 7029-7031.

Nelson, D., Schuch, R., Chahales, P., Zhu, S. & Fischetti, V.A. (2006). PlyC: a multimeric bacteriophage lysine. *Proceedings of the National Academy of Sciences* **103**, 10765-10770.

Nho, S.W, Ha, M.A, Kim, K.S, Kim, T.H, Jang, H.B *et al.* (2012). Complete genome sequence of the bacteriophages ECBP1 and ECBP2 isolated from two different Escherichia coli strains. *Journal of Virology* **86**, 12439 –12440.

Nielsen, P., Fritze, D. & Priest, F.G. (1995). Phenetic diversity of alkaliphilic *Bacillus* strains: proposal for nine new species. *Microbiology* **141**, 1745-1761.

Nigro, O.D., Culley, A.I. & Steward, G.F. (2012). Complete genome sequence of bacteriophage VvAW1, which infects *Vibrio vulnificus*. *Standards in Genomic Sciences* **6**, 415-426.

Nissenbaum, A. (1980). Hypersaline brines and evaporatic environments. *Elsevier Scientific Publishing Company* **28**, 23-38.

Noble, R.T & Fuhrman, J.A. (1998). Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. *Aquatic Microbial Ecology* **14**, 113-118.

Nowlan, B., Dodia, M.S., Singh, S.P. & Patel, B.K.C. (2006). *Bacillus okhensis* sp. nov., a halotolerant and alkalitolerant bacterium from an Indian saltpan. *International Journal of Systematic and Evolutionary Microbiology* **56**, 1073–1077.

Pajunen, M.I., Kiljunen, S.J., Söderholm, M.E.L. & Skurnik, M. (2001). Complete Genomic Sequence of the Lytic Bacteriophage ϕYeO3-12 of Yersinia enterocolitica Serotype O:3 *Journal of Bacteriology* **183**, 1928–1937.

Pajunen, M.I., Elizondo, M.R., Skurnik, M., Kielczawa, J., Molineux, I.J *et al.* (2002). Complete nucleotide sequence and likely recombinatorial origin of bacteriophage T3. *Journal of Molecular Biology* **319**, 1115-1132.

Palmisano, M. M., Nakamura, L. K., Duncan, K.E., Istock., C. A & Cohan, F. M. (2001). *Bacillus sonorensis* sp. nov., a close relative of *Bacillus licheniformis*, isolated from soil in the Sonoran Desert, Arizona. *International Journal of Systematic and Evolutionary Microbiology* **51**, 1671–1679.

Paterson, S., Vogwill, T., Buckling, A., Benmayor, R., Spiers, A.J *et al*. (2010). Antagonistic coevolution accelerates molecular evolution. *Nature* **464**, 275-279.

Pearce, D.A. & Wilson, W.H. (2003). Viruses in Antarctic ecosystems. *Antarctic Science* **15**, 319-331.

Pečenková, T., Beneš, V., Pačes, J., Vlček, C. & Pačes, V. (1997). Bacteriophage B103: complete DNA sequence of its genome and relationship to other *Bacillus* phages. *Gene* **199**, 157–163

Pedulla, M.L., Ford, M.E, Houtz, J.M., Karthikeya, T., Wadsworth, C., *et al*. (2003). Origins of highly mosaic mycobacteriophage genomes. *Cell* **113**, 171–182.

Pedulla, M.L., Ford, M.E., Karthikeyan, T., Houtz, J.M., Hendrix, R.W. *et al*. (2003). Corrected sequence of the bacteriophage p22 genome. *Journal of Bacteriology* **185**, 1475–1477.

Petrovski, S., Dyson, Z.A., Seviour, R.J. & Tillett, D. (2012). Small but Sufficient: the *Rhodococcus* phage RRH1 has the smallest known *Siphoviridae* genome at 14.2 kilobases. *Journal of Virology* **86**, 358 –363.

Petty, N.K., Evansa, T.J., Finerana, P.C. & Salmond, G.P.C. (2006). Biotechnological exploitation of bacteriophage research. *Trends in Biotechnology* **25**, 7-15.

Pickard, D., Toribio, A.L., Petty, N.K., van Tonder, A., Yu, L *et al.* (2010). A conserved acetyl esterase domain targets diverse bacteriophages to the Vi capsular receptor of *Salmonella enterica* Serovar Typhi. *Journal of Bacteriology* **192**, 5746–5754.

Plunkett, G., Rose, D.J., Durfee, T.J. & Blattner, F.R. (1999). Sequence of shiga toxin 2 phage 933W from *Escherichia coli* O157:H7: shiga toxin as a phage late-gene product. *Journal of Bacteriology* **181**, 1767–1778.

Pope, W.H., Weigele, P.R., Chang, J., Pedulla, M.L., Ford, M.E *et al*. (2005). Genome sequence, structural proteins, and capsid organization of the cyanophage Syn5: a "horned" bacteriophage of marine *synechococcus*. *Journal of Molecular Biology* **368**, 966-981.

Prangishvili, D., Forterre, P. & Garrett, R.A. (2006). Viruses of the archaea: a unifying view. *Nature Reviews Microbiology* **4**, 837-848.

Price-Carter1, M., Roy-Chowdhury, P., Pope, C.E., Paine, S., De Lisle, G.W *et al.* (2011). The evolution and distribution of phage ST160 within *Salmonella enterica* serotype Typhimurium. *Epidemiology & Infection* **139**, 1262–1271.

Pringsulaka, O., Chavanich, S., Doi, K. & Ogata, S. (2003). Antibody against actinophages isolated from soil in Thailand. *The Natural History Journal of Chulalongkorn University* **3**, 1-6.

Proctor, L. (1997). Advances in the study of marine viruses. *Microscopy Research & Technique* **37**, 136–161.

Rainey, F.A. & Oren, A. (2006). Extremophiles. *Methods in Microbiology* **35**, 439-450.

Ravin, V., Ravin, N., Casjens, S, Ford, M.E., Hatfull, G.F. & Hendrix, R.W. (2000). Genomic sequence and analysis of the atypical temperate bacteriophage N15. *Journal of Molecular Biology* **299**, 53–73.

Remaut, E., Stanssens, P. & Fiers, W. (1981). Plasmid vectors for high-efficiency expression controlled by the p_L promoter of coliphage lambda. *Gene* **15**, 81-93.

Rice, G., Tang, L., Stedman, K., Roberto, F., Spuhler, J. *et al.* (2004). The structure of a thermophilic archaeal virus shows a double-stranded DNA viral capsid type that spans all domains of life. *Proceedings of the National Academy of Sciences* **101**, 7716–7720.

Rich, F. (1933). Scientific results of the Cambridge expedition to the East African lakes 1930. VII. The algae. *Zoological Journal of the Linnean Society* **38**, 249–275.

Roh, S.W., Nam, Y., Chang, H., Kim, K., Kim, M *et al.* (2009). *Paracoccus aestuarii* sp. nov., isolated from tidal flat sediment. *International Journal of Systematic and Evolutionary Microbiology* **59**, 790–794.

Rohwer, F.L., Segall, A.M., Steward, G., Seguritan, V., Breitbart, M. *et al.* (2000). The complete genomic sequence of the marine phage Roseophage SIO1 shares homology with nonmarine phages. *Limnology & Oceanography* **45**, 408-418**.**

Rohwer, F. & Edwards, R. (2002). Phage proteomic tree: a genome-based taxonomy for phage. *Journal of Bacteriology* **184**, 4529–4535.

Romancer, M., Gaillard, M., Geslin, C. & Prieur, D. (2007). Viruses in extreme environments. *Environmental Science Biotechnology* **6**, 17–31.

Rössler, N., Klein, R., Scholz, H. & Witte, A. (2004). Inversion within the haloalkaliphilic virus ϕCh1 DNA results in differential expression of structural proteins. *Molecular Microbiology* **52**, 413–426.`

Ruska, H. (1943). Versuch zu einer Ordnung der Virusarten. *Archiv für die Gesamte Virusforschung* **2**, 480–498.

Russel, M. (1991). Filamentous phage assembly. *Molecular Microbiology* **5**, 1607-1613.

Sabehi, G. & Lindell, D. (2012). The P-SSP7 cyanophage has a linear genome with direct terminal repeats. *PLoS ONE* **7**, 36710.

Sabet, S., Chu, W. & Jiang, S.C. (2006). Isolation and genetic analysis of haloalkaliphic bacteriophages in a North American soda lake. *Microbial Ecology* **51**, 543-554.

Sajben-Nagy, E., Maróti, G., Kredics, L., Horváth,, B., Párducz, A *et al.* (2012). Isolation of new *Pseudomonas tolaasii* bacteriophages and genomic investigation of the lytic phage BF7. *FEMS Microbiology Letters* **332**, 162-169.

Sambrook, J. & Russel. D.W. (2001). Molecular cloning: A Laboratory Manual. 3rd ed., NY: Cold Spring Habour Laboratory Press.

Sambrook, J., Fritch E.F. & Maniatis T. (1982). Molecular Cloning: A Laboratory Manual, NY: Cold Spring Harbour Laboratory Press.

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sanger, F., Air, G.M., Barrell, B.G., Brown, N.L., Coulson, A.R *et al.* (1977). Nucleotide sequence of bacteriophage ϕ174 DNA. *Nature*, **265**, 687–695.

Sanger, F., Coulson A.R., Hong, G.F., Hill, D.F. & Petersen, G.B. (1982). Nucleotide sequence of bacteriophage lambda DNA. *Journal of Molecular Biology* **162**, 729–773.

Sato, M., Beppu, T. & Arima, K. (1983). Studies on antibiotics produced at high alkaline ph. *Agricultural & Biological Chemistry* **47**, 2019-2027.

Savalia, D., Westblade, L.F., Goel, M., Florens, L., Kemp, P *et al.* (2008). Genomic and proteomic analysis of phiEco32, a novel *Escherichia coli* phage. *Journal of Molecular Biol*ogy **377**, 774–789.

Scheif, R.F. & Wensink, P. (1981). Lambda DNA purification. In: Practical methods in molecular Biology (Manor, E.P., ed.). Springer-Verlag, New York.

Scholl, D. & Merril, C. (2005). The genome of bacteriophage K1f, a T7-like phage that has acquired the ability to replicate on K1 Strains of *Escherichia coli*. *Journal of Bacteriology* **187**, 8499–8503.

Scholl, D., Kieleczawa, J., Kemp, P., Rush, J., Richardson, C.C *et al.* (2004). Genomic analysis of bacteriophages SP6 and K1-5, an estranged subgroup of the T7 supergroup. *Journal of Molecular Biology* **335**, 1151–1171.

Scott, J.J., Renaut, R.W. & Owen, R.B. (2012). Impacts of flamingos on saline lake margin and shallow lacustrine sediments in the Kenya Rift Valley. *Sedimentary Geology* **277**, 32–51.

Seed, K. D., Bodi, K. L., Kropinski, A.M., Ackermann, H.-W. Calderwood, S. B *et al.* (2011). Evidence of a dominant lineage of *Vibrio cholerae*-specific lytic bacteriophages shed by cholera patients over a 10-year period in Dhaka, Bangladesh. *mBio* **2**, 334.

Shapiro, O.H. & Kushmaro, A. (2011). Bacteriophage ecology in environmental biotechnology processes. *Current Opinion in Biotechnology* **22**, 449-455.

Sharp, R.J., Ahmad, S.I., Munster, A., Dowsett, B. & Atkinson, T. (1986). The isolation and characterization of bacteriophages infecting obligately thermophilic strains of *Bacillus*. *Journal of General Microbiology* **132**, 1709-1722.

Shen, P.S., Domek, M.J., Sanz-García, E., Makaju, A., Taylor, R.M *et al.* (2012). Sequence and structural characterization of Great Salt Lake bacteriophage CW02, a member of the T7-Like supergroup. *Journal of Virology* **86**, 7907–7917.

Shin, H., Lee, J.-H., Lim, J.A., Kim, H. & Ryua, S. (2012). Complete genome sequence of *Salmonella enterica* serovar Typhimurium bacteriophage SPN1S. Journal of Virology **86**, 1284–1285.

Short, J.M., Fernandez, J.M., Orge, J.A. & Huse, W.D. (1988). Lambda ZAP, a bacteriophage lambda expression vector with *in vivo* excision properties. *Nucleic Acids Research* **16**, 7583-7599.

Short, S. M. & Suttle, C.A. (2002). Sequence analysis of marine virus communities reveals that groups of related algal viruses are widely distributed in nature. *Applied Environmental Microbiology* **68**, 1290–1296.

Short, C. M. & Suttle, C.A. (2005). Nearly identical bacteriophage structural gene sequences are widely distributed in both marine and freshwater environments. *Applied Environmental Microbiology* **71**, 480–486.

Sidhu, S.S. (2000). Phage display in pharmaceutical biotechnology. *Current Opinion in Biotechnology* **11**, 610-616.

Sillankorva, S., Kluskens, L.D., Lingohr, E.J., Kropinski, A.M., Neubauer, P. & Azeredo, J. (2011). Complete genome sequence of the lytic *Pseudomonas fluorescens* phage jIBB-PF7A. *Virology* **8**, 142.

Sillankorva, S., Kropinski, A.M. & Azeredoa, J. (2012). Genome sequence of the broad-host-range *Pseudomonas* phage ϕ-S1. *Journal of Virology* **86**, 10239.

Smith, G.P. (1985). Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science* **228**, 1315–1317.

Smith, H.L. & Trevino, R.T. (2009). Bacteriophage infection dynamics: Multiple host binding sites. *Mathematical Modelling of Natural Phenomena* **4**, 111-136.

Son, J-S., Lee, S-J., Jun, S.Y., Yoon, S.J., Kang, S.H *et al*. (2010). Antibacterial and biofilm removal activity of a *Podoviridae Staphylococcus aureus* bacteriophage SAP-2 and a derived recombinant cell-wall-degrading enzyme. *Applied Microbiology & Biotechnology* **86**, 1439-1449.

Sorokin, D.Y. & Kuenen, J.G. (2005). Chemolithotrophic haloalkaliphiles from soda lakes. *FEMS Microbiology Ecology* **52**, 287–295.

Sorokin, Y.S., Muyzer, G., Brinkhoff, T., Kuenen, J.G. & Jetten, M.S.M. (1998). Isolation and characterization of a novel facultatively alkaliphilic *Nitrobacter* species, N. alkalicus sp. nov. *Archives of Microbiology* **170**, 345-352.

Sorokin, D.Y., Lysenko, A.M., Mityushina, L.L., Tourova, T.P., Jones, B.E. *et al.* (2001). *Thioalkalimicrobium aerophilum* gen. nov., sp. nov. and *Thioalkalimicrobium sibericum* sp. nov., and *Thioalkalivibrio versutus* gen. nov., sp. nov., *Thioalkalivibrio nitratis* sp.nov., novel and *Thioalkalivibrio denitrificancs* sp. nov., novel obligately alkaliphilic and obligately chemolithoautotrophic sulfur-oxidizing bacteria from soda lakes. *International Journal of Systematic and Evolutionary Microbiology* **51**, 565-80.

Sorokin, D.Y., Tourova, T.P., Lysenko, A.M., Mityushina, L.L. & Kuenen, G.J. (2002). *Thioalkalivibrio thiocyanoxidans* sp. nov. and *Thioalkalivibrio paradoxus* sp. nov., novel alkaliphilic, obligately autotrophic, sulfuroxidizing bacteria capable of growth on thiocyanate, from soda lakes. *International Journal of Systematic & Evolutionary Microbiology* **52**, 657–664.

Sorokin, D.Y., Tourova, T.P., Kolganova, T.V., Detkova, E.N., Galinski, E.A. & Muyzer, G. (2011). Culturable diversity of lithotrophic haloalkaliphilic sulfate-reducing bacteria in soda lakes and the description of *Desulfonatronum thioautotrophicum* sp. nov., *Desulfonatronum thiosulfatophilum* sp. nov., *Desulfonatronovibrio thiodismutans* sp. nov., and *Desulfonatronovibrio magnus* sp. nov. *Extremophiles* **15**,391-401.

Spanka, R. & Fritze, D. (1993). *Bacillus cohnii* sp. Nov., a new, obligately alkaliphilic, oval spore-forming *Bacillus* species with ornithine and aspartic acid instead of diaminopimelic acid in the wall. *International Journal of Systematic Bacteriology* **43**, 150-156.

Stummeyer, K., Schwarzer, D., Claus, H., Vogel, U., Gerardy-Schahn, R. & Mühlenhoff, M. (2006). Evolution of bacteriophages infecting encapsulated bacteria: lessons from *Escherichia coli* K1-specific phages. *Molecular Microbiology* **60**, 1123–1135.

Streit, W.R. & Schmitz, R.A. (2004). Metagenomics – the key to the uncultured microbes. *Current Opinion in Microbiology* **7**, 492–498.

Studier, F.W., Rosenberg, A.H., Dunn, J.J. & Dubendorff, J.W. (1990). Use of T7 RNA polymerase to direct expression of cloned genes. *Methods in Enzymol*ogy **185**, 60–89.

Su, L.K., Lu, C.P., Wang, Y., Cao, D.M., Sun, J.H. & Yan, Y.X. (2010). Lysogenic infection of a shiga toxin 2-converting bacteriophage changes host gene expression, enhances host acid resistance and motility. *Molecular Biology* **44**, 54-66.

Sulakvelidze, A., Alavidze, Z. & Morris, J.G. (2001). Bacteriophage therapy. *Antimicrobial Agents & Chemotherapy* **45**, 649-659.

Summer, E.J., Gonzalez, C.F., Bomer, M., Carlile, T., Embry, A *et al.* (2006). Divergence and mosaicism among virulent soil phages of the *Burkholderia cepacia* complex. *Journal of Bacteriology* **188**, 255-268.

Summer, E.J., Enderle, C.J., Ahern, S.J., Gill, J.J., Torres, C.P *et al*. (2010). Genomic and biological analysis of phage Xfas53 and related prophages of *Xylella fastidiosa. Journal of Bacteriology* **192**, 179-190.

Summers, W.C. (1999). Felix d'Herelle and the origins of molecular biology. Yale University Press, New Haven, Conn.

Sundar, M.M., Nagananda G.S., Das, A., Bhattacharya, S. & Suryan, S. (2009). Isolation of host-specific bacteriophages from sewage against human pathogens. *Asian Journal of Biotechnology* **1**, 163-170.

Suttle, C.A. (2005). Viruses in the sea. *Nature* **437**, 356–361.

Tamura K., Peterson K., Peterson N., Stecher G., Nei M. & Kumar S. (2011). MEGA 5: Molecular evolutionary genetics analysis using likelihood, distance, and parsimony methods. *Molecular Biology and Evolution*. (In Press).

Tanaka, K., Nishimori, K., Makino, S-I., Nishimori, T., Kanno, T *et al.* (2004). Molecular characterization of a prophage of *Salmonella enterica* serotype *Typhimurium* DT104. *Journal of Clinical Microbiology* **42**, 1807-1812.

Thien, H.V., Vergnaud, G. & Pourcel, C. (2013). The susceptibility of *Pseudomonas aeruginosa* strains from cystic fibrosis patients to bacteriophages. *PLoS One* **8**, 60575-60575.

Tindall, B.J. (1988). Prokaryotic life in the alkaline, saline athalassic environment. In Halophilic bacteria. (Ed) Rodriguez-Valera, F. Alicante, Spain: CRC Press, Inc., Boca Raton, Forida, 31-67.

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 & Applied Microbiology* **5**, 41–57.

Trun, N. & Trempy, J. (2004). Fundamental bacterial genetics, Blackwell Publishing, 105-125.

Tu, A-H.T., Voelker, L.L., Shen, X. & Dybvig, K. (2001). Complete nucleotide sequence of the Mycoplasma Virus P1 Genome. *Plasmid* **45**, 122–126.

Tudorancea, C. & Harrison, A.D. (1988). The benthic communities of the saline lakes Abijata and Shala (Ethiopia). *Hydrobiologia* **158**, 117-123.

Twort, F.W. (1915). An investigation on the nature of ultramicroscopic viruses. *Lancet* **2**, 1241–1243.

Uchiyama, J., Takeuchi, H., Kato, S., Takemura-Uchiyama, I., Ujihara, T *et al*. (2012). Complete genome sequences of two *Helicobacter pylori* bacteriophages isolated from Japanese patients. *Journal of Virology* **86**, 11400–11401.

Ulukanli, Z. & Diúrak, M. (2002). Alkaliphilic micro-organisms and habitats. *Turkish Journal of Biology* **26**, 181-191.

Vandenbergh, P.A. & Cole, R.L. (1986). Cloning and expression in *Escherichia coli* of the polysaccharide depolymerase associated with bacteriophage-infected *Erwinia amylovora*. *Applied & Environmental Microbiology* **51**, 862-864.

Ventosa, A. (2006). Unusual microorganisms from unusual habitats: hypersaline environments, prokaryotic diversity-mechanisms and significance, Cambridge University Press, 223-253.

Vieira, J. & Messing, J. (1987). Production of single-stranded plasmid DNA. *Methods in Enzymology* **153**, 3-11.

Villafane, R., Zayas, M., Gilcrease, E.B., Kropinski, A.M. & Casjens, S.R. (2008). Genomic analysis of bacteriophage ε^{34} of *Salmonella enterica* serovar Anatum (15+). *BMC Microbiology* **8**, 227.

Volozhantsev, N.V., Oakley, B.B., Morales, C.A., Verevkin, V.V., Bannov, V.A *et al.* (2012). Molecular characterization of Podoviral bacteriophages virulent for *Clostridium perfringens* and their comparison with members of the *Picovirinae*. *PLoS ONE* **7**, 1-12.

Von Damm, K.L. & Edmond, J.M. (1984). Reverse weathering in the closed basin lakes of the Ethiopian Rift. *American Journal of Science* **284**, 835-862.

Vybiral, D., Takáč, M., Loessner, M., Witte, A., von Ahsen, U. & Bläsi, U. (2003). Complete nucleotide sequence and molecular characterization of two lytic *Staphylococcus aureus* phages: 44AHJD and P68. *FEMS Microbiology Letters* **219**, 275-283.

Wahl, G.M., Lewis, K.A., Ruiz, J.C., Rothenberg, B., Zhao, J. & Evan, G.A. (1987). Cosmid vectors for rapid genomic walking, restriction mapping and gene transfer. *Proceedings of the National Academy of Sciences* **84**, 2160-2164.

Weinbauer, M.G. & Höfle, M.G. (1998). Significance of viral lysis and flagellate grazing as controlling factors of bacterioplankton production in an eutrophic lake. *Applied and Environmental Microbiology* **64**, 431–438.

Weinbauer, M.G. (2004). Ecology of prokaryotic viruses. *FEMS Microbiology Reviews* **28**, 127–181.

Wichels, A., Biel, S.S., Gelderblom, H.R., Brinkhoff, T., Muyzer, G. & Schütt., C. (1998). Bacteriophage diversity in the North Sea. *Applied & Environmental Microbiology* **64**, 4128–4133.

Wildy, P. (1971). Classification and Nomenclature of Viruses: First Report of the International Committee on Nomenclature of Viruses, In: Monographs in Virology **5**, Karger, Basel.

Willats, W.G.T. (2002). Phage display: practicalities and prospects. *Plant Molecular Biology* **50**, 837–854.

Willis, S.H., Kazmierczak, K.M., Carter, R.H. & Rothman-Denes, L.B. (2002). N4 RNA polymerase II, a heterodimeric RNA polymerase with homology to the single-subunit family of RNA polymerases. *Journal of Bacteriology* **184**, 4952–4961.

Winter, C., Bouvier, T., Weinbauer, M.G. & Thingstad, T.F. (2010). Trade-offs between competition and defense specialists among unicellular planktonic organisms: the "killing the winner" hypothesis revisited. *Microbiology & Molecular Biology Reviews* **74**, 42–57.

Witte, A., Baranyi, U., Klein, R., Sulzner, M., Luo, C *et al.* (1997). Characterization of *Natronobacterium magadii* phage ϕCh1, a unique archaeal phage containing DNA and RNA. *Molecular Microbiology* **23**, 603–616.

Wolf, A., Zheng, T., Witzel, K. & Jost, G. (2004). Impact of initial phage/host ratio and nutrient addition on coexistence in a phage-host system. *Aquatic Microbial Ecology* **35**, 131–139.

Wommack, K.E. & Colwell, R.R. (2000). Virioplankton: viruses in aquatic ecosystems. *Microbiology & Molecular Biology* **64**, 69–114.

Xu, Y., Zhou, P. & Tian, X. (1999). Characterization of two novel haloalkaliphilic archaea *Natronorubrum bangense* gen. nov., sp. nov. and *Natronorubrum tibetense* gen. nov., sp. nov. *International Journal of Systematic Bacteriology* **49**, 261–266.

Zachary, A. (1978). An ecological study of bacteriophages of *Vibrio natriegens*. *Applied & Environmental Microbiology* **24**, 321-324.

Zavarzin, G.A., Zhilina, T.N. & Kevbrin, V.V. (1999). The alkaliphilic microbial community and its functional diversity. *Microbiology* **68**, 503-521.

Zhang, S., Flores-Cruz, Z., Zhou, L., Kang, B.H., Fleites, L.A *et al*. (2011). *Ca. Liberibacter asiaticus'* carries an excision plasmid prophage and a chromosomally integrated prophage that becomes lytic in plant infections. *The American Phytopathological Society* **24**, 458–468.

Zhao, Y., Wang, K., Jiao, N. & Chen, F. (2009). Genome sequences of two novel phages infecting marine roseobacters. *Environmental Microbiology* **11**, 2055–2064.

Zhao, Y., Temperton, B. , Thrash, J.C., Schwalbach, M.S., Vergin, K.L *et al*. (2013). Abundant SAR11 viruses in the ocean. *Nature* **494**, 357-360.

Zhilina, T.N., Zavarzin, G.A., Detkova, E.N. & Rainey, F.A. (1996a). *Natroniella acetigena* gen. nov. sp. nov., an extremely haloalkaliphilic, homoacetic bacterium: a new member of haloanaerobiales. *Current Microbiology* **32**, 320-326.

Zhilina, T.N., Zavarzin, G.A., Rainey, F.A., Kevbrin, V.V., Kostrikina, N.A. & Lysenko, A.M. (1996b). *Spirochaeta alkalica* sp. nov., *Spirochaeta aficana* sp. nov., and *Spirochaeta asiatica* sp. nov., Alkaliphilic anaerobes from the continental soda lakes in central Asia and the East African Rift. *International Journal of Systematic Bacteriology*, 305-312.

Zhilina, T.N., Zavarzin, G.A., Rainey, F.A., Pikuta, E.N., Osipov, G.A. & Kostrikina, N.A. (1997). *Desulfonatronovibrio hydrogenovorans* gen. nov., sp. nov., an alkaliphilic, sulfate-reducing bacterium. *International Journal of Systematic Bacteriology* **47**, 144-149.

Zhilina, T.N., Garnova, E.S., Tourova, T.P., Kostrikina, N.A. & Zavarzin, G.A. (2001a). *Amphibacillus fermentum* sp. nov. and *Amphibacillus tropicus* sp. nov., new alkaliphilic, facultatively anaerobic, saccharolytic bacilli from Lake Magadi. *Microbiology* **70**, 711–722.

Zhilina, T.N., Garnova, E.S., Tourova, T.P., Kostrikina, N.A. & Zavarzin, G.A. (2001b). *Halonatronum saccharophilum* gen. nov. sp. nov.: a new haloalkaliphilic bacterium of the order haloanaerobiales from Lake Magadi. *Microbiology* **70**, 64-72.

Zhilina, T.N., Zavarzina, D.G., Osipov, G.A., Kostrikina, N.A. & Tourova, T.P. (2009). *Natronincola ferrireducens* sp. Nov., and *Natronincola peptidovorans* sp. Nov. New anaerobic alkaliphilic peptolytic Iron-reducing bacteria isolated from soda lakes. *Microbiology* **78**, 455-467.

Zhu, J., Rao, X., Tan, Y., Xiong, K., Hu, Z *et al*. (2010). Identification of lytic bacteriophage MmP1, assigned to a new member of T7-like phages infecting *Morganella morganii*. *Genomics* **96**, 167–172.

Zinabu, G.M. (2002). The effects of wet and dry seasons on concentrations of solutes and phytoplankton biomass in seven Ethiopian riftvalley lakes. *Limnologica* **32**, 169–179.