BACTERIA IN SALT PANS ALONG THE MISSISSIPPI GULF COAST

By

Lauren Alexis Lawson

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

Oxford
May 2019

Approved by:

Advisor: Colin Jackson

Reader: Peter Zee

Reader: Gregg Roman

ACKNOWLEDGMENTS

I would first like to thank Dr. Colin Jackson for his guidance and patience as my advisor. I am thankful for his invaluable insight and constant encouragement. Thank you for supporting me throughout my undergraduate course load and always believing in me. The experience gained under your advising has helped me achieve my goals thus far and will continue to aid me in the pursuit of my professional career.

I would also like to thank Dr. Peter Zee and Dr. Gregg Roman for acting as my second and third readers. I am incredibly grateful to Eric Weingarten for all of his guidance and advice while simultaneously completing his own doctorate. Professor Marta Chevalier, you have impacted my college career and my future in the best way, merci.

Thank you to my family, specifically my parents and brother for their constant support and love. Though all of my research took place inside of the lab, I could not have completed this project without the support of my family and friends. Thank you for holding me accountable.

Lastly, I would like to express my gratitude to the Sally McDonnell Barksdale Honors College. Thank you for all of the opportunities granted to me over these past three years.

ABSTRACT

LAUREN ALEXIS LAWSON: Bacteria in Salt Pans Along the Mississippi Gulf Coast
(Under the direction of Colin Jackson)

Salt pans form in shallow depressions in the ground where salt water evaporates leaving a hypersaline environment remaining. These pans become flooded during extremely high tides and as time progresses, this additional salt water is evaporated leaving behind more salt deposits. Marine salt pans can provide habitat for halophilic microorganisms and provide an interesting environment for study as conditions can change with both sediment and water depth. Towards the surface of sediment in salt pans, oxygen is still available, however, deeper sediments have limited oxygen availability and are likely anaerobic. Deeper sediments can also have higher salt concentrations so may provide an optimal environment for anaerobic halophiles.

Samples were taken in July and October 2018, when the salt pans were dry and flooded, and from the surface and 30cm deep in the sediment. DNA was extracted and the V4 region of the 16S rRNA gene sequenced to determine the bacterial microbiome. Bacterial communities were compared between the surface and deeper samples and flooded and dry samples. Samples taken from surface sediments had more bacterial sequences than those taken from deeper into the sediment, and surface samples accounted for 73% of the 105,000 sequences in the dataset.

Salt pan bacterial communities were primarily composed of members of the Proteobacteria, Bacteroidetes, Cyanobacteria, and Planctomycetes, although a total of 21 distinct bacterial phyla were detected. These phyla differed in their distributions, with members of the Bacteroidetes, Cyanobacteria, Planctomycetes, and Chloroflexi being mainly associated with surface sediment, and Proteobacteria being more prevalent in deeper sediment. Proteobacteria, Bacteroidetes, Cyanobacteria, and Planctomycetes accounted for a greater proportion of the dry (July) bacterial community, whereas members of the Actinobacteria were more prevalent in the flooded (October) samples.

In conclusion, salt pans along the Mississippi Gulf Coast harbor a diverse bacterial community that differs both spatially (by depth) and temporally (by season and/or flooded versus dry conditions). These environments can become hypersaline when dry, suggesting that this diverse community is adapted to both flooding and high salinity conditions.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

Introduction

Despite bacteria accounting for the majority of Earth's biodiversity, our understanding of the diversity of these organisms is limited (Green et al., 2006). Bacteria were first discovered by Leeuwenhoek in the 17th century, and his discovery of these small life forms established the field of microbiology (Porter, 1976). The study of the diversity of microbes has improved since Leeuwenhoek, with Carl Woese's delineation of a phylogeny that divides Earth's biodiversity into three phyla, Eukarya, Archaea, and Bacteria, based on ribosomal RNA being a particular advance (Woese, 1987). Extending on Woese's work, Norman Pace showed that rRNA genes (particularly 16S rRNA genes) could be amplified directly from environmental samples, overcoming the reliance on cultivated microorganisms that had defined microbiology previously (Pace, 1998). Pace estimated that over 99% of microorganism in nature could not be cultivated using standard techniques, and molecular approaches allow the identification of organisms without cultivation (Pace, 1998). More recently, next-generation gene sequencing techniques have furthered our knowledge of microbes, and allow more detailed studies of microbial diversity in host microbiomes and natural environments (Wain, 2014).

Microorganisms can be found in environments where other organisms cannot survive. These extremophiles extend our view of the range of conditions that life can tolerate, and microbial communities have been found that thrive in a diverse range of conditions such as extreme pH, temperature, pressure, and salinity (van den Burg, 2003). Salt-tolerant microorganisms, or halophiles, are of particular interest and can be classified as slight, moderate, or extreme halophiles depending on their salt requirements

(DasSarma and DasSarma, 2001). Most marine microbes are slight halophiles and are adapted to the 3.5% salinity found in the oceans. Extreme halophiles are more specialized in their locations and require a minimum salt concentration of 10% to survive, with optimal survival and growth at 20% (Lanyi, 1974) These extreme halophiles can be found in underground salt mines, deep-sea brine pools, and in areas where seawater evaporates leaving residual salt deposits (DasSarma and DasSarma, 2001). The latter can be found in coastal areas as naturally occurring hypersaline environments called salt pans.

Salt pans form in shallow depressions in the ground where salt water evaporates leaving a hypersaline environment remaining (Lowenstein, 1985). These pans become flooded during extremely high tides, and as time progresses, this additional salt water is evaporated leaving behind more salt deposits. Marine salt pans can provide habitat for halophilic microorganisms (Lanyi, 1974), and provide an interesting environment for study as conditions can change with both sediment and water depth. Towards the surface of sediment in salt pans, oxygen is still available, however, deeper sediments have limited oxygen availability and are likely anaerobic. Deeper sediments can also have higher salt concentrations so may provide an optimal environment for anaerobic halophiles (Weigelt, 1990).

Salt pans can be found in coastal areas around the world, including along the Mississippi Gulf Coast. This region is part of the coast of the northern Gulf of Mexico and is subject to saltwater intrusion and encroachment during tropical storms and hurricanes (Steyer, 2005). In this study, soil cores were collected from a salt pan along the Mississippi Gulf Coast during a summer dry period, and following tidal inundation after a storm further east along the Gulf Coast. On each date, sediment was collected from both the salt pan surface and deeper into the sediment. DNA was extracted from all

samples and the bacterial community described using culture-independent next generation sequencing approaches. As well as providing a description of the salt pan microbiome, the goal of this study was to assess whether broader environmental conditions (dry, flooded) exert a greater influence on the salt pan bacterial community than depth in the sediment.

Methods

Sample Collection

Samples were collected from a salt pan located in Grand Bay National Estuarine Research Reserve (NERR) along the Mississippi Gulf Coast in Moss Point, Mississippi. This pan, measuring 106 hectares, composes 4.9% of the Reserve's land (Peterson, et al. 2007). Samples were extracted on June 18th (dry conditions) and October 3rd (flooded conditions), 2018. On each date, samples were collected as soil cores using a sterilized (70% ethanol) 38 cm x 2 cm soil corer. Surface samples were taken from <10 cm into the core; deep samples were collected from 30 cm deep. Two cores were taken on each sample date, giving a total of eight samples (2 dates x 2 depths x 2 cores). Soil from each sample was passed through a sterile (70% ethanol) 1 mm pore size sieve to remove larger particles, and stored on ice in sterile 15 mL tubes until returned to the laboratory the same day. Samples were frozen until November 2018, when 0.1 g samples were taken for use in DNA extraction procedures.

DNA Extraction and 16S rRNA Gene Sequencing

DNA was extracted using a PowerSoil DNA Isolation Kit, following the manufacturer's (MO BIO Laboratories, Inc) instructions. Agarose gel electrophoresis was used to confirm the presence of DNA. The V4 region of the 16S rRNA gene was amplified and sequenced by the dual-index barcoded Illumina next-generation sequencing approach described by Kozich et al. (2013) and Jackson et al. (2015). Amplicon concentration was normalized using a SequalPrep Normalization Plate Kit

(Thermofisher) and amplicons pooled. Final sequencing was completed at the University of Mississippi Medical Center Molecular and Genomics Core Facility using the Illumina MiSeq platform

Data Analysis

Sequence data was presented in the form of FASTQ files and was analyzed using mothur (Schloss et al., 2009). A series of system commands were performed as recommended by Schloss et al. (2011) to remove ambiguous sequences and align the sequences against the SILVA v4 16S rRNA database. Chimeras, erroneously combined sequences, were removed via UCHIME software. The remaining aligned sequences were classified following the Ribosomal Database Project database (02.2016) and contaminant sequences (those classified as Eukarya, Archaea, chloroplast, and mitochondrial sequences) removed from the dataset. The remaining bacterial sequences were arranged into operational taxonomic units (OTUs) based on >97% similarity in sequences. Communities were described based on the composition of bacterial taxa and compared based on the presence/absence and relative abundance of OTUs.

Results

The original sequence count across all eight samples was a total of 179,822 sequences. After removing chimeras, potential sequencing errors and sequences that classified as chloroplasts, mitochondria, Archaea, Eukarya, or unknown, the final number of bacterial sequences across all eight samples was 105,825, consisting of 5,829 different sequence types. The number of sequences obtained from each sample was highly variable (Figure 1). The sample taken in June from the surface of the core (Dry Surface 1) yielded the most sequences, 46,008, while the October bottom sample (Hydrated Subsurface 1) had the least amount of sequences, 744.

In terms of bacterial community composition (Figure 2), dry subsurface samples were composed mainly of Proteobacteria (33.9%), unclassified bacteria (19.2%) and Bacteroidetes (10.7%). Dry surface samples were composed similarly, with Proteobacteria (37.6%), unclassified bacteria (22.1%), and Bacteroidetes (10.3%) being the dominant bacteria. Hydrated subsurface samples were largely composed of Proteobacteria (27.7%), unclassified bacteria (19.8%) and Acidobacteria (12.2%), while hydrated surface samples were composed of Proteobacteria (11.1%), Unclassified (8.4%), and Bacteroidetes (5.9%). Subsurface samples contained a greater proportion of Acidobacteria, Chloroflexi, Firmicutes, and Verrucomicrobia, whereas surface samples contained proportionally more Chlamydiae, Cyanobacteria, Parcubacteria, Spirochaetes, and Planctomycetes. Dry samples contained more Bacteroidetes, Chloroflexi, and Firmicutes. Hydrated samples contained more Acidobacteria, Actinobacteria, and Verrucomicrobia (Figure 2). Within the

Proteobacteria, sequences were largely Alphaproteobacteria and Gammaproteobacteria, although the hydrated subsurface samples contained more Epsilonproteobacteria (1.4%), Betaproteobacteria (17.81%) and Deltaproteobacteia (16.82%) than other samples.

Sequences were grouped into 3,380 OTUs based on 97% sequence similarity. 11 OTUs had >1000 reads. Two different OTU's identified as members of the Enterobacteriaceae accounted for almost 15% of all sequences (Table 1). A third member of the Gammaproteobacteria identified as belonging to genus *Pseudomonas* accounted for almost another 7% (Table 1). Other dominant OTUs included a member of class Cyanobacteria (5.2%) and a member of genus *Gaetbulibacter* (2.4%) (Table 1). Of the top 16 OTUs, Gammaproteobacteria accounted for 24.1% (Table 1).

Alpha diversity was determined after subsampling each sample to 744 sequences (the number of sequences in the sample with fewest sequence reads). Coverage (how well the community was sampled) at this level of subsampling was typically around 0.8, which is acceptable but not ideal. Species diversity and richness varied across different depths and hydration (Figure 3). Hydrated Subsurface samples had the highest Inverse Simpson index value (149), while dry surface samples had the lowest (10). Subsurface samples overall had higher Inverse Simpson Index values (Figure 3). Dry surface samples had the highest observed species richness (254), and dry subsurface samples had the lowest (115; Figure 3).

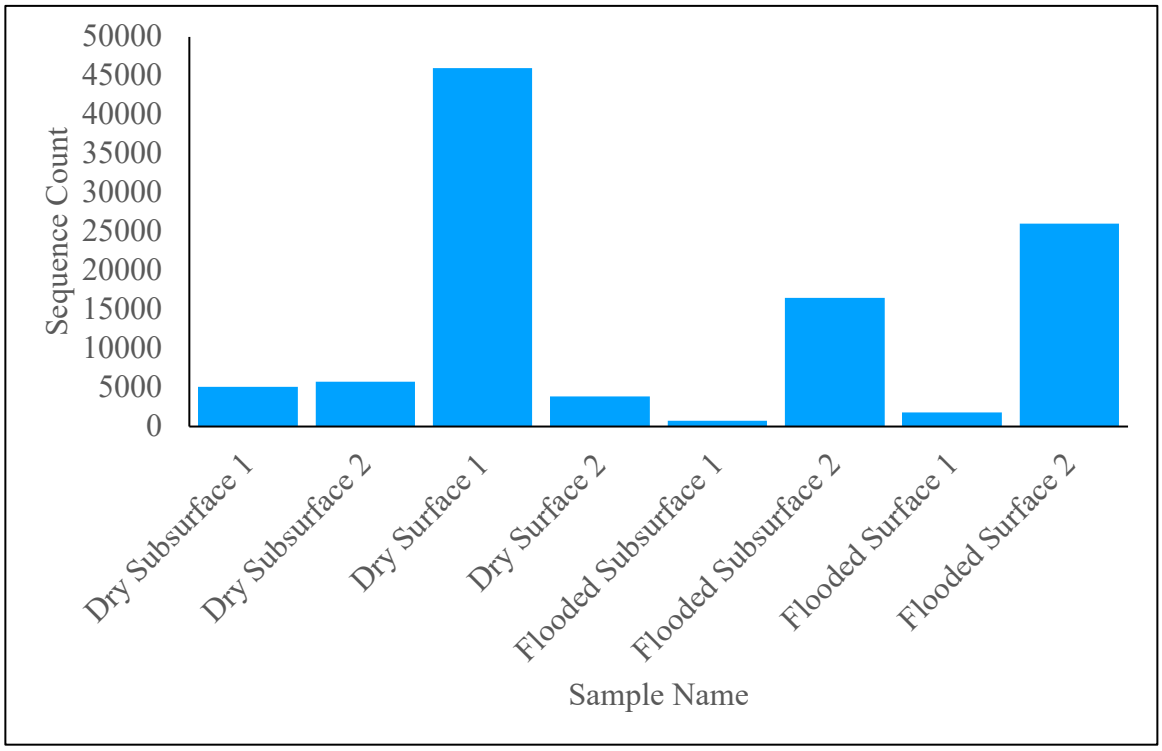

Figure 1) Numbers of 16S rRNA gene sequences obtained from next generation sequencing of bacterial communities of a salt pan located on the Mississippi Gulf Coast. Samples were taken when the salt pan was flooded and dry, from the surface and subsurface. Two replicate samples of each were analyzed.

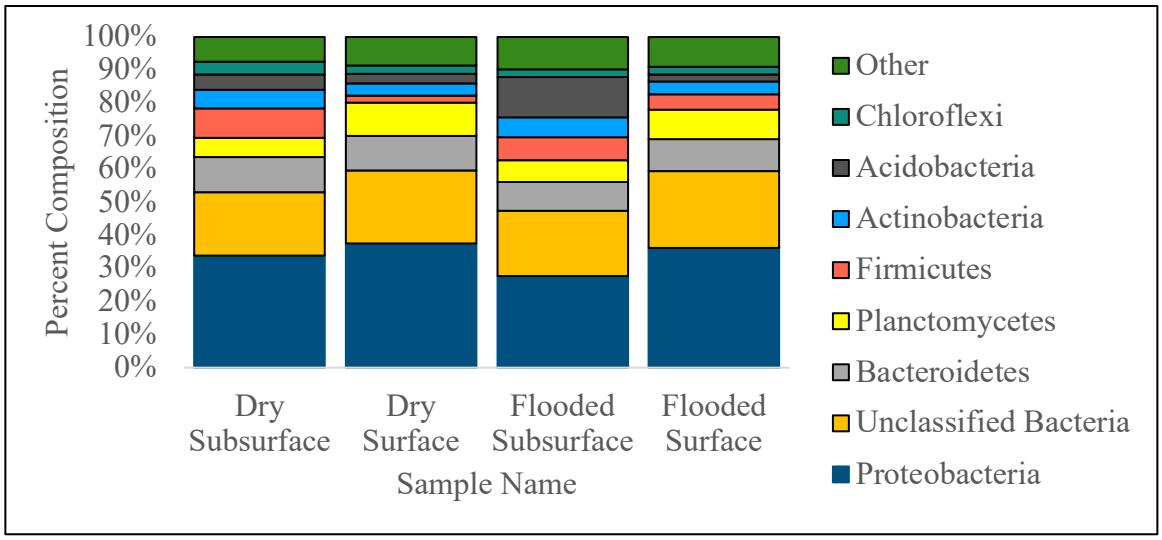

Figure 2) Relative abundance of bacterial phyla in salt pans on the Gulf of Mexico. Chart includes sequences from eight samples taken from the surface and subsurface and in both flooded and dry environments. Bacterial phyla that composed over 90% of the samples are listed, while Other includes 16 different phyla.

OTU	Number of Sequences	Relative Abundance (%)	Phylum	Lowest Taxonomic Classification
Otu0001	8298	7.74	Gammaproteobacteria	(f)Enterobacteriaceae
Otu0002	7408	7.00	Gammaproteobacteria	(f)Enterobacteriaceae
Otu0003	7038	6.65	Gammaproteobacteria	(g) *Pseudomonas*
Otu0004	5413	5.11	Cyanobacteria	(c)Cyanobacteria
Otu0007	2593	2.45	Bacteriodetes	(g) *Gaetbulibacter*
Otu0009	1803	1.70	Gammaproteobacteria	(g) *Providencia*
Otu0011	1416	1.34	Alphaproteobacteria	(f) Rhodobacteraceae
Otu0012	1167	1.10	Verrucomicrobia	(c) Spartobacteria
Otu0013	1124	1.06	Bacteriodetes	(f) Rhodothermaceae
Otu0014	1114	1.05	Gammaproteobacteria	Gammaproteobacteria
Otu0016	1075	1.02	Alphaproteobacteria	(f) Sphingomonadaceae

Table 1) Most abundant bacterial OTUs found in samples taken from a salt pan on the Gulf of Mexico. Only the OTUs with over 1000 sequences are shown. Relative abundance is shown as a percentage of the total number of sequences in the dataset. OTUs are identified by phylum and the lowest taxonomic classification level of each that was possible. (c=class, f=family, g=genus).

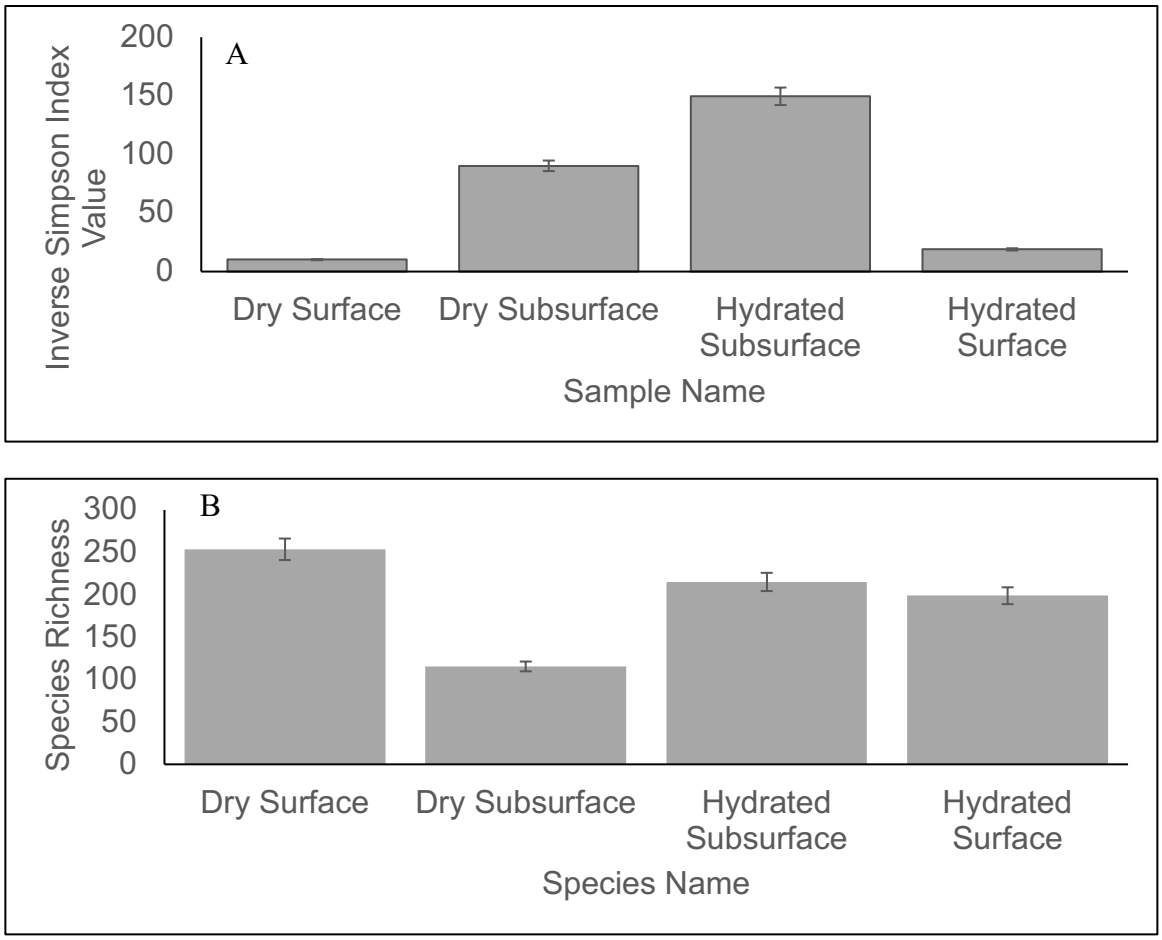

Figure 3) The diversity of bacterial communities in sediments taken from a salt pan on the Gulf of Mexico. Samples were taken in when the pan was flooded and dry, from the surface and subsurface. Diversity was determined by subsampling of 744 sequences and expressed as (A) Inverse Simpson index, and (B) observed species richness.

Differences in overall bacterial community composition between samples were examined using Bray-Curtis measures of dissimilarity (Figure 4). The two hydrated surface samples, were very similar as were the two dry surface samples, based on dendrogram branching patterns (Figure 4A). Subsurface samples varied in their similarity: Dry subsurface 1 and Dry subsurface 2 were closely related, but hydrated subsurface 1 and hydrated subsurface 2 were distant in their branching (Figure 4A). Regardless, subsurface samples branched together and separately from surface samples. NMDS ordination (Figure 4B) showed the same patterns, with surface and subsurface samples separating along the first axis. NMDS did highlight the variability between samples and even replicates were distinct in NMDS space. NMDS revealed the OTUs driving compositional differences between samples. For dry surface samples, *Marivita* and Flammeovirgaceae were the driving OTUs. In flooded surface samples, *Truepera* and Plancomycetaceae were the driving OTUs. For flooded subsurface samples, OTUs identified as *Pseudomonas*, *Aeromonas*, *Alcaligenes*, two species of Enterobacteriaceae, *Strenotrophomonas*, and *Providencia* were the driving forces separating these communities from others.

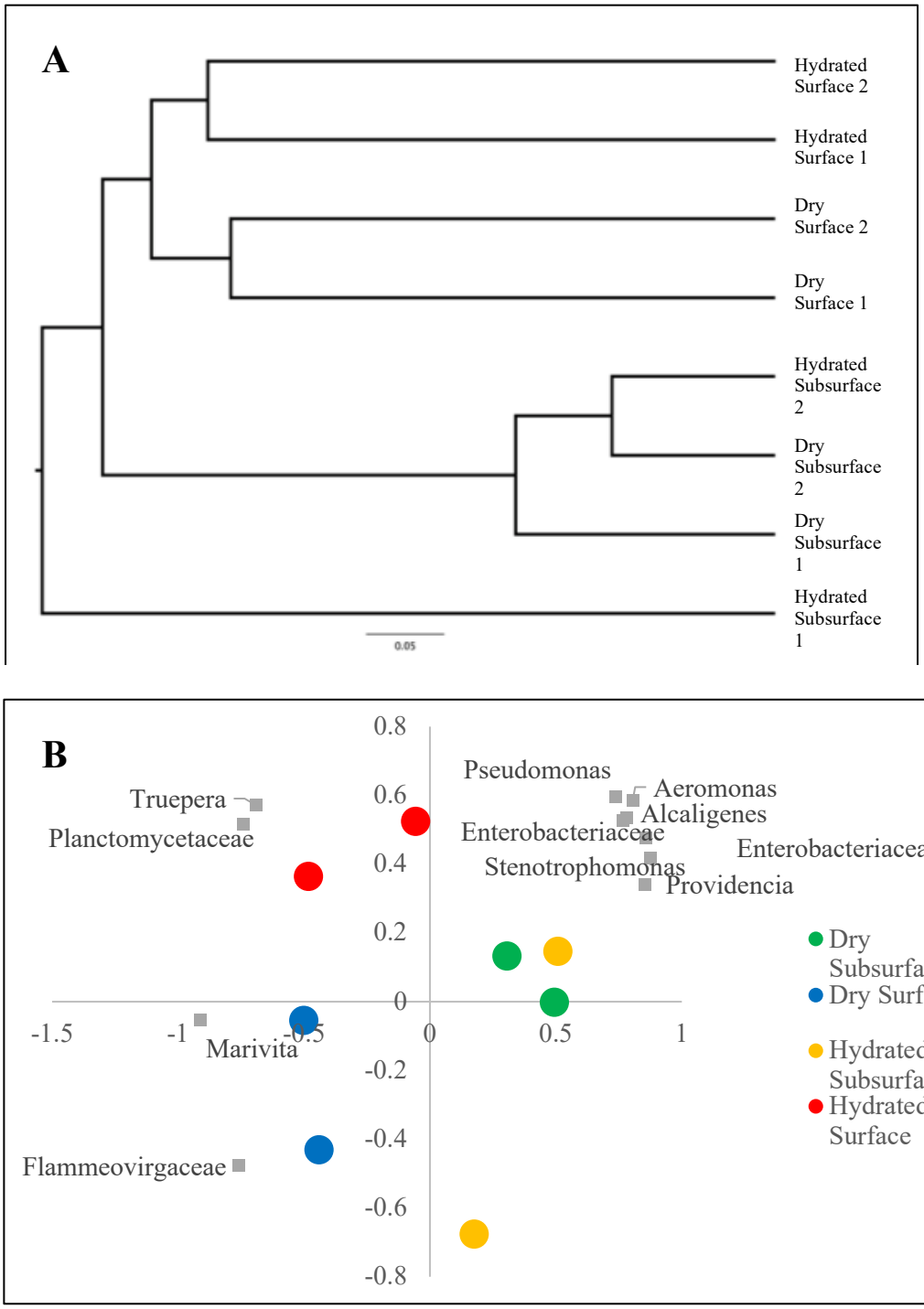

Figure 4) A. Bray-Curtis UPGMA tree to visualize similarity between eight bacterial communities taken from a salt pan on the Gulf of Mexico at different times of the year and different depths. B. NMDS ordination (stress = 0.11) of the same samples with the indicator species driving the dissimilarity shown.

Discussion

This study used 16S rRNA gene sequencing to describe bacterial communities in a salt pan along the Mississippi Gulf Coast. It compared communities from the surface and subsurface when pans were both flooded and dry. Overall, the bacterial community differed more by depth than by hydration, and NMDS ordination showed that samples grouped by depth, and within that grouping, samples clustered by hydration. For the subsurface samples, dry samples clustered closely with one hydrated sample, while the other hydrated subsurface sample was more dissimilar. This particular sample had the lowest number of sequences, making it more susceptible to appearing dissimilar from other samples based on small differences in the community.

Previous studies have shown clear changes in microbial community abundance and composition by depth, as reported here (Fierer, et al., 2003). In previous studies, Acidobacteria have been found predominantly in surface soils, while Proteobacteria are more dominant in the subsurface (de Araujo Pereira et al., 2017). This study, however, found that subsurface samples differed from other samples in their proportion of Acidobacteria, and hydrated subsurface samples, in particular, had a much higher percentage of this phylum than other samples. Acidobacteria are abundant in soil bacterial communities (Jones, et al. 2009) and were found in all four sample types analyzed. The hydrated subsurface samples may have been composed of a greater proportion of Acidobacteria as the subsurface of the salt pans may more closely resemble a soil

environment and is less subject to the frequent flooding and inundation that the surfaces of salt pans are exposed to.

The OTU with the highest sequence count was identified as a member of the Rhodobacteraceae and was in the surface samples. Members of the Rhodobacteraceae are common in marine habitats and some lineages have been found in hypersaline environments. It is mainly composed of aerobic photo- and chemoheterotrophs (Pujalte et al., 2014). Planctomycetaceae were also identified in the surface samples and Plancomycetaceae have been identified as salt, acid, heat, and cold tolerant (Schlesner and Stackebrandt, 1986). They have been found previously in soil and water samples (Schlesner and Stackebrandt, 1986). *Truepera* was identified in the top samples as well, and this genus is composed of aerobic organisms that are extremely ionizing radiation resistant (Albuquerque et al., 2005). Other OTUs identified in the surface samples were *Sphaerobacteraceae, Porphyrobacter, Marivita*, and *Alteromonas*. These species are all common in marine environments. Since surface samples are exposed to both dry and flooded conditions, it is likely that common marine species would be identified in these samples.

OTUs found in the deeper samples included *Aeromonas*, a genus composed of facultative anaerobic bacteria that includes pathogens associated with infections following hurricanes and tsunamis (Janda and Abbott, 2010). Enterobacteriaceae, facultative aerobes, were also identified in subsurface samples. *Providencia*, a genus composed of anaerobes, was found in subsurface samples along with *Stenophomonas* and *Alcaligenes*. Since subsurface samples are likely to have less oxygen available than surface samples, it is not surprising that they contained anaerobic or facultative bacteria.

Through 16S rRNA gene sequencing, bacteria from many different phyla were identified, and the dominant phylum was the Proteobacteria in all samples. However, all samples included a large percentage of unclassified bacteria. Unclassified bacteria are a significant percentage of most environmental microbiome studies, as most bacteria have not yet been discovered or cultured. In previous studies in soils, undiscovered bacteria are commonly one of the most abundant groups in the samples (Uroz, et al., 2010). 16S rRNA has allowed biologist to identify more bacteria, but many species are undiscovered because of our inability to represent environmental conditions in culture (Pablo, et al., 2014). Extremophiles are especially difficult to culture, because the conditions they live in are much more complex than researchers are able to mimic in labs. Salt pans are a complex extreme environment, with a high salinity and irregular water influx. These pans are also exposed to high levels of UV radiation. Bacteria in salt pan environments are adapted to these conditions and most are probably dependent on them, so that culturing these species is likely to be difficult. Thus, that a large number of sequences identified as unclassified bacteria is not surprising for these samples.

Hydrated subsurface samples and dry subsurface samples were more similar to each other than surface samples taken at different times, suggesting that the bacteria on the subsurface of these salt pans do not experience the extreme environmental changes like the surface samples, and are more consistent. These locations provide a stable environment for the bacteria to flourish. Surface samples were less diverse than subsurface samples, which is surprising given that they are exposed to more variable conditions. The surface environment is more likely to change between dry to wet and is also exposed to high levels of UV radiation from both the sun and the reflection from the salt. However, how diversity is assessed is important and while surface samples had low

Inverse Simpson scores, they had typical or even high scores for observed species richness. This suggests a community that contains many bacterial species, but a few species dominate, and may reflect the more variable nature of the surface where many bacterial populations are transient.

Many taxa identified as extremophiles were found at high abundance in the surface community suggesting that this environment exerts a strong selection pressure on the bacteria in these pans. Samples from the surface grouped by their hydration and many extremophiles found in the dry sample were not found in the hydrated samples. This suggests different salt pan surface bacteria are adapted to these different conditions, and the drier environment may be particularly harsh. Regardless, these environments are subject to fluctuations between flooding and drying, and salt pans, as with many coastal wetlands are subject to changes from natural disasters such as hurricanes, as well as sea level rise. That the surface bacterial community differs between these conditions suggest that the impact of such changes on the salt-pan microbiome will largely be seen at the surface level, and the more constant environment in the deeper sediment may buffer some of these impacts.

LIST OF REFERENCES

Albuquerque, L, Simoes, C, Nobre, MF, Pino, NM, Battista, JR, Silva, MT, et al. 2005. Truepera radiovictrix gen. nov., sp. nov., a new radiation resistant species and the proposal of Trueperaceae fam. nov. FEMS Microbiology Letters 247: 161-169.

DasSarma, P, DasSarma, S. 2001. Halophiles. eLS: 1-13. Doi 10.1002/9780470015902.a0000394.pub4

De Araujo Pereira, AP, de Andrade, PAM, Bini, D, Currer, A, Robin, A, Bouillet, JP, et al. 2017. Shifts in the bacterial community composition along deep soil profiles in monospecific and mixed stands of Eucalyptus grandis and Acacia mangium. PloS one: 12: e0180371

Eleuterius, LN, McDaniel, S. 1978. The salt marsh flora of Mississippi. Castanea 43: 86-95.

Fierer, N, Schimel, JP, Holden, PA. 2003. Variation in microbial community composition throughoutt two soil depth profiles. Soil Biology and Biochemistry 35: 167-176.

Green, J, Bohannan, BJ. 2006. Spatial scaling of microbial biodiversity. Trends in Ecology & Evolution 21: 501-507.

Hugenholtz, P, Goebel, BM, Pace, NR. 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. Journal of Bacteriology 180: 4765-4774.

Jackson, CR, Stone, BWG, Tyler, HL. 2015. Emerging perspectives on the natural microbiome of fresh produce vegetables. Agriculture 5: 170-187

Janda, JM, Abbott, SL. 2010. The genus Aeromonas: taxonomy, pathogenicity, and infection. Clinical Microbiology Reviews 23: 35-73.

Jones, RT, Robeson, MS, Lauber, CL, Hamady, M, Knight, R, Fierer, N. 2009. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. The ISME Journal 3: 442-453.

Kamat, T, Savita, K Bacteria from Salt Pans: a potential resource of antibacterial metabolites. 2011. Resent Research in Science and Technology 3: 46-52

Kozich, JJ, Westcott, SL, Baxter, NT, Highlander, SK, Schloss, PD. 2013. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. Applied and Environmental Microbiology 79: 5112-5120.

Lanyi, JK. 1974. Salt-dependent properties of proteins from extremely halophilic bacteria. Bacteriological Reviews 38: 272-290.

Lowenstein, TK, Hardie, LA. 1985. Criteria for the recognition of salt-pan evaporites Sedimentology 32: 627-644.

Peterson, M.S., Waggy, GL, Woodrey MS. 2007. Grand Bay National Estuarine Research Reserve: An Ecological Characterization: 268

Porter, JR. 1976. Antony van Leeuwenhoek: tercentenary of his discovery of bacteria. Bacteriological Reviews 40: 260-269.

Pujalte, MJ, Lucena, T, Ruvira, MA, Arahal, DR, Macian, MC. 2014. The family rhodobacteraceae. The Prokaryotes: Alphaproteobacteria and Betaproteobacteria: 439-512.

Schlesner, H, Stackebrandt, E. 1986. Assignment of the genera Planctomyces and Pirella to a new family Planctomycetaceae fam. nov. and description of the order

Pablo, P, Tilmaz, P, Pruesse, E, Glöckner, FO, Ludwig, W, Schleifer, KH, et al. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. Nautre Reviews Microbiology 12: 635-645.

Planctomycetales ord. nov. Systematic and Applied Microbiology 8: 174-176.

Schloss, PD, Westcott, SL, Ryabin, T, Hall, JR, Hartmann, M, Hollister, EB, et al. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environmental Microbiology 75: 7537-7541.

Schloss, PD, Gevers, D, Westcott, SL. 2011. Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. PloS One 6: e27310 doi: 10.1371/journal/pone.0027310

Sørensen, KB, Canfield, DE, Teske, AP, Oren A. 2005. Community composition of a hypersaline endoevaporitic microbial mat. Applied and Environmental Microbiology 71: 7352-7365.

van den Burg, B .2003. Extremophiles as a source for novel enzymes. Current Opinion in Microbiology 6: 213-218.

Steyer, GD, Perez, BC, Piazza, S, Suir, G. 2005. Potential consequences of saltwater intrusion associated with Hurricanes Katrina and Rita. Science and the storms: The USGS response to the hurricanes of 2005: 138-147.

Uroz, S, Buée, M, Murat, C, Pascale, FK, Martin, F. 2010. Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. Environmental Microbiology Reports 2: 281-288.

Wain, J, Mavrogiorgou, E. 2014. Next-generation sequencing in clinical microbiology. Expert Review of Molecular Diagnostics 3: 225 doi: 10.1586/erm.13.8

Weigelt, M. 1990. Oxygen conditions in the deep water of Kiel Bay and the impact of inflowing salt-rich water from the Kattegat. Meeresforschung-Reports on Marine Research 33:1

Woese, CR. 1987. Bacterial evolution. Microbiological Reviews 51: 221-227