2007-03-15

Ecology of Culturable Organisms at Rozel Point, Great Salt Lake, Utah

Emily Sarah Haws
Brigham Young University - Provo

ECOLOGY OF CULTURABLE ORGANISMS AT ROZEL POINT, GREAT SALT LAKE, UTAH

by

Emily S. Haws

A thesis submitted to the faculty of

Brigham Young University

in partial fulfillment of the requirements for the degree of

Master of Science

Department of Microbiology and Molecular Biology

Brigham Young University

April 2007

BRIGHAM YOUNG UNIVERSITY

GRADUATE COMMITTEE APPROVAL

of a thesis submitted by

Emily S. Haws

This thesis has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

_____	_____
Date	Alan R. Harker, Chair
_____	_____
Date	Donald P. Breakwell
_____	_____
Date	Byron J. Adams

BRIGHAM YOUNG UNIVERSITY

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_____ _____

Date

Alan R. Harker
Chair, Graduate Committee

Accepted for the Department

_____

Brent L. Nielsen
Chair, Department of Microbiology
and Molecular Biology

Accepted for the College

_____

Rodney J. Brown
Dean, College of Biology
and Agriculture

ABSTRACT

ECOLOGY OF CULTURABLE ORGANISMS

AT ROZEL POINT, GREAT SALT LAKE (UTAH)

Emily S. Haws

Department of Microbiology and Molecular Biology

Master of Science

The study of organisms from extreme environments is an emerging field of research with applications to multiple scientific areas. One of these extreme environments is Great Salt Lake (GSL), whose microbiology has yet to be extensively studied. This dynamic and unique environment offers an excellent opportunity to increase understanding of hypersaline ecology. Cultivation of microorganisms remains an important part of ecology research, as it is essential for understanding microbial physiology. We report here the culturing and characterization of isolates from Rozel Point, located on the northeastern shore of Great Salt Lake. This site was chosen because of the presence of petroleum seeps at Rozel Point and the extreme salinity of the North Arm of GSL. We hypothesize that culturing at GSL will reveal a diverse prokaryotic

population, with both commonly isolated and novel organisms. We would predict that prokaryotes at GSL will share many features in common with other hypersaline microbial communities, but that given the distinctive properties of the site, there will be unique characteristics as well. Samples were taken from Rozel Point and cultured using direct plating, enrichment cultures, and dilution cultures with a variety of minimal and complex halophilic media. Fluorescence *in situ* hybridization (FISH) was used to examine abundance of cultured organisms in the environment. Culturing and characterization has revealed both isolates novel and previously uncultured, with many unique characteristics. FISH demonstrated that, unlike most environments, in GSL the dominant species are culturable. These results show the value of culturing in discovering new organisms and demonstrating diversity at the microbial level. Culturing of these organisms will allow for further research to be done on microbial processes that occur in this system and the unique properties of halophilic microbes.

ACKNOWLEDGEMENTS

I would like to express my appreciation for Dr. Harker for being such a wonderful mentor. He showed a lot of patience and support for a novice researcher, and I'm thankful I was able to work with him, both as an undergraduate and graduate student. I was fortunate to work with someone who seemed more interested in effort and learning experiences than just laboratory results. I would also like to thank Dr. Breakwell for his undying enthusiasm and helpful guidance both in the laboratory and in the classroom as a TA. Dr. Adams for his encouragement and expert advice in helping to shape and move my project along and always being willing to help, regardless of how busy he was. I am indebted to my committee for their guidance and friendship; they helped make my graduate time at BYU a worthwhile and memorable experience.

I would also like to thank Ben, my husband, for all of his support and faith in me. Without it I would not have had the drive or sense of humor to get through. Also, my parents for their excitement and support throughout my college career. Without the personal background of love and support from my family I would never have been able to achieve my ambitions. Thank you!

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INTRODUCTION

Extremophiles

The discovery in the late 1930's that life could exist in environments as extreme as thermal springs, ice, deep-sea vents, and salterns opened the scientific world to a whole new group of fascinating organisms. These organisms, or extremophiles, are able to thrive in hostile environments never imagined to support life. Scientists have been very interested in learning more about the characteristics and processes that allow them to do so. Ecology and taxonomy have been the focus of many studies involving extreme environments. These studies have described which organisms are found in these environments and how they interact with their environment.

The unique properties of microbial extremophiles have also encouraged interest in several other fields beside ecology and taxonomy. Evolutionists have been interested in extreme organisms as the primordial Earth was a very hostile environment, and some believe that extremophiles are the best possible candidates for representing the first organisms to grow and survive (Wiegel, 1998). Another emerging area of extremophile research is astrobiology or looking for alternative life forms on other planets. Comparisons have been drawn between Earth's hostile environments and those found on Mars, Europa, and comets (Rothschild *et al*, 2001). By researching more about the organisms found in Earth's extreme areas, scientists hope to be able to understand more about what other life could be discovered elsewhere. Biotechnological applications of extremophile enzymes and proteins are seen in the common use of *Taq* polymerase, isolated from the thermophile *Thermus aquaticus*, and several other industrial uses (Rodriguez-Valera, 1992).

Halophiles

One class of extreme organisms is the halophiles, which live in saline environments. These organisms can be found in a wide range of environments from low-saline marine environments to hypersaline lakes such as the Dead Sea and GSL. Halophiles fall into three categories depending on the salinity optimal for growth: halotolerant (1-6%), moderate (6-15%), and extreme (15-30%) (Garabito *et al*., 1998). The study of microbial organisms from saline environments is a field of research that has found increased interest in the past few decades. New organisms are being identified and characterized from marine and salt lake environments throughout the world, which has helped shed light on the ecology of these microbes (Ventosa *et al*., 1998, Dyall-Smith, 2004). Within the domain *Archaea*, almost all halophiles belong to the family *Halobacteriaceae*. The *Bacteria* are generally more diverse, with halophilic groups found in several phyla. In extreme salinities halophilic archaea have been shown to be the dominant population and most bacterial species have been shown to prefer lower levels of salinity for optimal growth (Oren, 2002).

Although knowledge about these organisms and their metabolic processes has increased over the years, it is limited by the ability to culture halophiles. Many of these organisms have unique growth requirements that can be hard to duplicate in the laboratory, and this, combined with slow growth rates, can hamper the isolation of certain halophilic strains. Comparisons between non-culturing molecular methods and culturing methods have shown discrepancies in all but the simplest saltern environment (Amann, 1995; Benlloch, *et al.*, 2001; Burns *et al.*, 2004). Fast growers, such as *Haloarcula* species, often overwhelm culture studies, even though they were shown to be very

uncommon or absent in molecular clone libraries or microscope analyses (Oren, 2002). Other species, such as Walsby's square archaeon (*Haloquadra walsbyi*), were dominant in clone libraries and seen frequently in microscope analysis, but were never seen in cultures (Benlloch, *et al*., 2001). However, culturing remains a very important part of understanding any system as many biochemical and metabolic processes cannot be demonstrated or discovered without it.

As culturing is necessary in order to study the unique properties and applications of these organisms, new techniques have since been designed to try and increase the number of cultured organisms. Dilution culturing is one new method that eventually led to the culturing of *H. walsbyi* and other previously uncultured groups, but many dominant populations remain unisolated (Bolhuis *et al*., 2004). Studies have also shown that use of minimal media can be beneficial, as many of the fast growers will be selected out (Dyall-Smith, 2004). Using different carbon sources that simulate the environment has also been shown to help target more fastidious organisms (Dyall-Smith, 2004). By learning more about what they require and finding better ways to imitate their environment, culturing techniques have improved over the past few years and more "uncultivables" are being isolated (Kaeberlein *et al*., 2002).

Great Salt Lake

One halophilic system that has not been extensively studied is GSL. The lake is a remnant of the ancient Lake Bonneville and depending on water levels covers approximately 4,400 km^2. This makes it the largest salt lake in the western hemisphere and the fourth largest terminal lake in the world (Baxter *et al*., 2005). It is one of the most influential water systems in the Western United States. The salinity in the lake can

range from 5-30% (30% is considered saturation) and varies depending on location in the lake and water levels (Stephens, 1997). Sodium chloride is the most common salt in GSL, as opposed to the Dead Sea, which has high amounts of sodium and magnesium (Post, 1977). There is a railroad causeway dividing the lake into two parts, which was constructed in 1959 (Waddell and Bolke, 1973). The South Arm is significantly less saline than the north because all main sources of freshwater are introduced into the southern areas. The South Arm salinity is usually around 5-15%, varying with water levels, while the North Arm stays consistently around saturation with salinity approaching 30% (Gwynn, 2002). Within the lake are many different microenvironments, including thermal and freshwater springs, and petroleum seeps (Gwynn, 2002). Humans have influenced this environment heavily through construction of the causeway, drilling for oil, and processing plants for salt removal and purification (Waddell and Bolke, 1973). The ecosystem surrounding GSL is home to millions of shorebirds and waterfowl and provides important habitat for migratory birds. Other organisms found in GSL include brine shrimp, *Artemia franciscana*, brine flies (*Ephedra* spp.), several types of algae, protozoa, yeast, and cyanobacteria (Baxter *et al*., 2005). With the exception of the *Dunaliella* algae group, all known eukaryotes are found only in the less saline southern arm (Post, 1977).

Surprisingly, there has not been a great deal of microbiological research performed on Great Salt Lake system, and a complete picture of microbial diversity and ecology is lacking (Baxter *et al*., 2005). The last study done on culturable prokaryotic ecology was by Post almost three decades ago, who was only able to isolate a few culturable organisms using a single nutrient rich medium (Post, 1977). Unfortunately,

these strains were not deposited and preserved at the time and all have subsequently been lost, except one. Post's GSL-11 strain was recultured and identified as *Haloterrigena turkmenica* (Ventosa *et al*., 1999). Earlier studies identified eleven culturable bacteria, *Serratia salinaria, Cellulomonas subcreta, Bacillus freudenreichii, Achromobacter solitarium, Bacillus cohaerens, Flavobacterium arborescens, Micrococcus sulflavus, Achromobacter hartlebii, Bacteriodes rigidus, Bacillus mycoides,* and *Achromobacter album* (Frederick, 1924). However, these studies were done before the causeway and alteration of the lake's salinity, and the names in many cases are no longer recognizable (Gwynn, 1980). More recent studies on GSL have targeted specific groups including *Proteobacteria* (Fendrich, 1988), anaerobes (Tsai *et al*, 1998), and sulfur-reducing bacteria (Brandt *et al*., 2001). In total twelve species have been isolated from GSL, with all but three isolated from the South Arm (see Table 1). Though these studies added to the number of novel organisms isolated from GSL, no molecular or biodiversity study was ever performed. This leads us to believe that there is great potential for new discoveries and an increased understanding of GSL ecology.

Name	Location	Reference
	Aerobic	
Gracilibacillus halotolerans	South Arm	Waino, *et al*., 1999
Halobacillus litoralis	South Arm	Spring *et al*., 1996
Halobacillus trueperi	South Arm	Spring *et al*, 1996
Halorhabdus utahensis	South Arm	Waino, *et al*., 1990
Halomonas variabilis	North Arm	Fendrich, 1988
Pseudomonas halophila	North Arm	Fendrich, 1988
Haloterrigena turkmenica	North Arm	Ventosa, *et al*., 1999
	Anaerobic	
Desulfocella halophila	South Arm	Brandt *et al*., 1997
Desulfobacter halotolerans	South Arm	Brandt *et al*., 1997
Haloanaerobium praevalens	South Arm	Zeikus *et al*., 1983
Haloanaerobium alcaliphilum	South Arm	Tsai *et al*., 1995
Methanohalophilus mahii	South Arm	Ingvorsen and Brandt, 2002

Table 1. **List of GSL isolates**. Referenced in Baxter *et al*., 2005.

Rozel Point was chosen as the site of sampling due to its location in the extremely saline North Arm (Fig. 1), ease of access to the site for repeated sampling, and the presence of several naturally occurring oil seeps. The petroleum seeps at Rozel Point are irregularly distributed for about a half a mile. The oil slowly migrates up through cracks in the lake bedrock, and is either deposited on neighboring rocks or eventually on the lake bottom since it is denser than water. Many commercial attempts at oil drilling occurred at Rozel Point in the past, but the low pressure and oil composition made it difficult and costly to produce (Chidsey, 1995). The petroleum is very high in sulfur and nitrogen (Chidsey, 1995), making it a potentially rich source of energy for microbes.

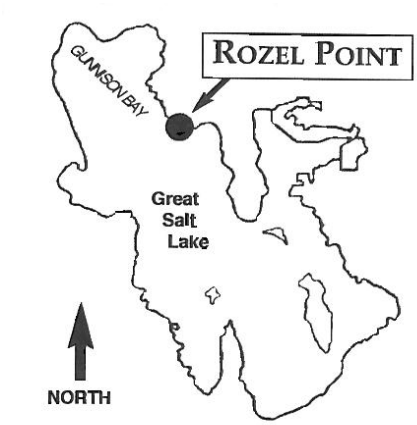

Figure 1. Rozel Point site. (Chidsey, 1995)

Present Study

Three things are needed to be accomplished in order to provide an ecological understanding of any system: identification of organisms that live in the environment, discovery of their population abundance, and identification of which ones can be cultured (Burns, 2004). The goal of this project was to explore and address all three aspects at Rozel Point. It is hypothesized that organisms seen in other hypersaline environments will also be cultured from Rozel Point. It is also hypothesized that due to the unique properties of this site (petroleum seeps, dynamic water levels, salinity, etc) that novel characteristics or species may also be found.

Different media and culturing techniques were used to encourage the growth of a variety of halophiles, and plates were incubated at long enough intervals to encourage growth of slow-growers. Culturing revealed several members of the *Halobacteriaceae* family and also members of the gamma and alpha subclasses of *Proteobacteria* and *Firmicutes* phyla. Phenotypic tests demonstrated unique characteristics of cultured isolates and showed potential for high metabolic activity at GSL. Fluorescence *in situ* hybridization was used to examine abundance of specific groups of organisms and revealed that the majority of the prokaryotic population represented culturable organisms.

These results give us a greater understanding of prokaryotic ecology in the GSL and how it compares to other hypersaline systems. Culturing of isolates will allow for further characterization and assessment of the potential for unique applications to biochemistry and biotechnology. This study will provide a framework for further culturing and ecological work that will be done in the GSL and potentially other hypersaline systems.

MATERIAL AND METHODS

Sampling

Rozel Point oil field, located on the northeastern shore of Great Salt Lake, was the sampling site. Samples were collected in September 2004, November 2004, June 2004, September 2005, May 2005, March 2006, and June 2006.

Water samples were collected in sterile 7 oz whirl-pak bags (Nasco) and stored in a cooler with ice until brought to the laboratory. High salt is inhibitory for common bacterial contaminants so sterility was not of high concern while sampling (Burdy and Post, 1979). All fresh samples were either used immediately for cultivation and microscopy or stored at -20^{o} C in the laboratory. Samples were also taken from a laboratory microcosm, which was a 340 liter aquarium containing frequently replenished mixtures of sediment and water from GSL.

Cultivation

Traditional direct plating was done with 100µl taken from a water sample. The three complex media used in direct plating were HM (DSMZ media 97), VN (ATCC media 217), and MGM (Dyall-Smith, 2004) (See Appendix A for recipes). HM and MGM are both common nutrient rich halophile media with high Mg^{2+} concentrations, to encourage growth of a variety of fast growing halophiles. VN media had a lower Mg^{2+} concentration and higher pH to encourage growth of alkaliphilic halophiles. For all three media types a variety of enrichment and salt concentrations were used to encourage the growth of unique microbes (see Table 2). One of the most important aspects of culturing

halophiles is time, as many of these organisms grow very slowly. Maximum recovery of growth usually occurs at 8-12 week incubation times so plates were left for up to eight weeks in a 30 ^{o} C incubator and checked for growth periodically (Burns, 2004). To achieve pure cultures individual colonies were isolated and regrown five times on solid media plates.

Complex Media	Chemical Concentration Compared to Standard Media Recipe	NaCl Concentration
MGM media	Peptone at 25%, 50%, 75%, and 100%	5%, 12%, 18%, 23%, 25%
VN media	Yeast at 25%, 50%, 75%, and 100%	5%, 12%, 18%, 23%, 25%
HM media	Casamino acids, citrate and yeast at 25%, 50%, 75%, and 100%	5%, 12%, 18%, 23%, 25%

Table 2. Nutrient and salt modifications to complex media.

Dilution methods of culturing were also used to encourage isolation of organisms that are typically overwhelmed by fast growing species in regular plate cultures. 96-well plates with 2ml depth (ISC Bioexpress) were used with the dilution cultures. Seven minimal media using a variety of carbon sources were used for dilution cultures; MA, MB, MC, MD, SWA, SWG, and MAM (Burns *et al*., 2004; Dyall-Smith, 2004, see Appendix A for recipes). Cultures were inoculated with 100µl of a water sample in 900µl of media and diluted out to extinction. The 96-well plates were incubated and shaken at 37 ^{o} C in a closed humid chamber. The chamber was opened and growth checked every few days to create an aerobic environment. Dilution cultures were allowed to grow for up to twelve weeks. The last well in a dilution series that demonstrated turbidity and the well diluted a tenth more were chosen for DNA extraction. The non-turbid well was chosen to check purity of the extinction culture and also try to culture halophiles that grow slowly and don't result in turbidity. Turbid wells

were additionally plated on solid media using 100µl and checked for the growth of colonies.

Enrichment cultures were accomplished using M63 media (Garcia, 2004; see appendix A) with 10% and 20% NaCl and different aromatic substances to try and isolate degraders for bioremediation purposes. As the site is on a petroleum seep, it was thought the potential for aromatic degradation should be high. Aromatic substances tested were phenol, benzoic acid, xylene, toluene, and phenylpropionic acid (Garcia, 2004). Liquid cultures were inoculated with fresh water samples and observed for turbidity.

Cultures were also grown in sealed mason jars, with a mix of soil and water taken from GSL. Lids were modified with plastic septum to allow for sampling of head space and cultures.

Frozen stock of all pure cultures were made following protocols outlined by Dyall-Smith (2004), and stored at -80 ^{o} C.

DNA Extraction

Plated axenic cultures were harvested and placed into a 1.5 ml microcentrifuge tube. For dilution cultures, 200µl was taken from a well and centrifuged for 2 minutes at high speed. The cells were resuspended in 400µl of cell lysis solution (10 mM Tris, 100 mM EDTA, and 2% SDS). In cases where the cells do not lyse directly, a make-shift mortar and pestle was used to grind up chunks and promote cell lysis. After all the cell debris was dissolved, 9µl of proteinase K (20mg/mL) was added, followed by 400µl more cell lysis solution. The microcentrifuge tubes were vortexed, wrapped in parafilm, and incubated/shaken in a water bath overnight at 55 ^{o} C. The samples were centrifuged

to pellet the unwanted cell debris and 180µl of 5M NaCl was added to the supernatant and vortexed. The samples were centrifuged again and the supernatant transferred to a clean tube. The DNA was precipitated with 420µl of ice-cold isopropanol and placed in the freezer for 2-4 hours to help with precipitation. The DNA was washed several times with 70% ethanol. The pellets were dried for 10-15 minutes and 20-100 µl of TE was added. The amount of DNA successfully extracted was checked with either gel electrophoresis or a NanoDrop® ND-1000 UV-Vis Spectrophotometer (BYU Research Instrumentation Core Facility). If the extraction was unsuccessful, possibly due to low cell counts or tough cell walls, it was again tried using an Alkaline/SDS procedure (Birnboim and Doly, 1979; Ish-Horowitz and Burke, 1981).

PCR

The sample tubes were set up with a master mix (DMSO, 5X buffer, MgCl2, primers, dNTPs), *Taq* polymerase (Promega), and DNA. Three different types of primers were used for 16S and 18S rDNA analysis (Table 3).

	Sequence	**Annealing temp.**	**Reference**
Bacterial			
63F	CAGGCCTAACACATGCAAGTC	65ºC	Marchesi *et*
882R	GTTTTAACCTTGCGGCCGTACTCC		*al.*, 1998
Archaeal			
6F	CGGTTGATCCYGCCGGM	60ºC	Tazi,
741R	GACTACCSGGGTATCTAATCC		unpublished
Eukaryotic			
EukF	AACCTGGTTGATCCTGCCAGT	43ºC	Medlin *et al.*,
EukR	TGATCCTTCTGCAGGTTCACCTAC		1988

Table 3. PCR probes.

The basic PCR set up was as follows: initial denaturation of 95^{o} C for 3 min, 35 cycles of 94^{o} C for 30 sec., 43^{o} C for 1 min. (for eukaryotic primers), and 72^{o} C for 2.5 min., and a final extension of 72^{o} C for 20 min. A different annealing temperature was used for the other two primers; 60^{o} C for the 6F primers and 65^{o} C for the 63F. The PCR product size was ~800 bp and was purified by Gene Clean methods. The DNA could then be submitted to the BYU DNA Sequencing Center for analysis, which was done using the ABI Big-Dye Terminator v3.1 Cycle Sequencing Kit on an ABI Prism 3730XL automated sequencer. A few samples were also cloned and transformed using Topo TA Cloning Kit (Invitrogen) and then screened with vector-specific primers. This was done to verify that all clones were the same in a pure culture.

Phylogenetics

Sequences were viewed using Sequence Navigator software and were compared to those deposited in GenBank and RDPII databases. Isolate sequences representing identical sequences were pruned out and not included in phylogenetic analysis. Overall thirty-nine archaeal (with thirteen reference species) and twenty-six bacterial sequences (with ten reference species) were used. *Methanosarcina siciliae* was used as an outgroup in archaeal trees and *Chlamydia abortus* for bacterial trees. *Methanosarcina* belongs to the class *Methanococci*, which is sister to the halophilic class *Halobacteria*. The phylum *Chlamydiae* was used, as it is separate from halophilic phyla. Alignments were done with the program MUSCLE (Edgar, 2004) and visually checked in Mesquite v1.12 (Maddison and Maddison, 2006). PHYLIP v3.65 was used to create maximum likelihood and distance phylogenies (Felsenstein, 2005). Maximum likelihood (ML) was performed using default parameters that allow for different transition/transversion rates

and nucleotide frequencies, as calculated under a Hidden Markov Model. Distance tree construction was done using the Fitch-Margoliash method with random sequence addition and global rearrangements, assuming a F84 model. Bootstrap analyses were performed on ML and distance trees using 500 bootstrap replicates with 10 random additions. Mr. Bayes v3.04b was used to perform Bayesian phylogenetic analyses under a GTR model with gamma distribution (Ronquist and Huelsenbeck, 2003), as determined through Modeltest (Posada and Crandall, 1998). Four chains were run for 3.0 X 10^6 cycles, sampling every 1000^{th} generation. Trees were viewed in TreeView v1.6.6 (Page, 1996) and exported to Mesquite for aesthetic editing.

Characterization

API 20NE strips (Biomerieux) and BIOLOG EcoPlates™ were used to characterize isolates. API 20NE were done following the recommended protocols except the inoculation medium used was 20% salt water. A *Pseudomonas stutzeri* strain capable of growing at 0-20% salt was used as a control. One strip was inoculated with the 20% ASW and another inoculated following the recommended protocols and compared to the known identification code for *Pseudomonas stutzeri*. BIOLOG EcoPlates™ have been shown to be inconsistent above 15% salinity (Litchfield *et al*., 2001), so characterizations were only performed on those isolates that could be grown at a 15% salinity or lower.

FISH

Fluorescence *in situ* hybridization was conducted on samples taken in September 2006 and February 2007. Fixation of cells and hybridization followed protocols outlined in the HaloHandbook (Dyall-Smith, 2006). Cell counts were done using a GTTP

Millipore filter and were repeated five times with at least 200 cells counted each time using a Zeiss AxioSkop 2 with AttoArc2 light source. Total cell counts were done using DAPI staining. Several probes were used to look at and quantify different populations (Table 4). *Salinibacter ruber* and *Halobacterium salinarum* probes were chosen to target that group of organisms that is presently considered to be the dominant population in GSL for *Bacteria* and *Archaea* respectively. Probes targeting the *Pseudomonas halophila* and *Haloarcula* groups were chosen to represent the dominant species found in cultures. Each probe was labeled with either Alexa488 fluor or Texas Red fluor (Invitrogen). The HBS and HBN probes were designed using the ARB database. Possible probe sequences were tested in GenBank and compared to the *Escherichia coli* 16S rDNA gene sites to try and account for inaccessibility (Fuchs *et al*., 1998). Both probes were optimized using a pure culture of *H. salinarum* at different formamide concentrations. The other probes were hybridized at recommended formamide concentrations. Pictures of cell fluorescence were done using direct slides, as opposed to filters. Protocols followed those in the HaloHandbook (Dyall-Smith, 2006) and pictures were taken using an AxioCamHRC and Zeiss AxioVision Imaging System.

Name	Probe Sequence	Fluor	Target group	Reference
ARC915	GTGCTCCCCCGCCAATTCCT	Texas Red	*Archaea*	Stahl and Amann, 1991
EUB388	GCTGCCTCCCGTAGGAGT	Alexa 488	*Bacteria*	Amann, 1990
NON338	CGACGGAGGGCATCCTCA	Alexa 488	Nonspecific hybridization	
EHB586	ACATCCGACTTGCTGCCC	Texas Red	*Salinibacter ruber*	Anton, 2000
EHB1451	GCCGCTCTCCCTCCGGCT	Texas Red	*Salinibacter ruber*	
HBN3738	CCTGTATCGCGCTCCGTTT	Alexa 488	*Halobacterium* NRC-1	Present study
HBS531	GTTCAGCCAGCTACGGACG	Alexa 488	*Halobacterium salinarum*	
HRCU1502	ATTCCTCTACGGCTACCT	Alexa 488	*Haloarcula* group	Anton, 1999
PH659	ATTCCACTGCCCTCTGCT	Texas Red	*Pseudomonas halophila*-like group	Maturrano 2005

Table 4. FISH probes.

Species (Genbank accession number)	*Sequence*
HBS531 probe	**GTTCAGCCAGCTACGGACG**
Halobacterium salinarum str. VKMM029 (DQ915836)	**GTTCAGCCAGCTACGGACG**
Halobacterium salinarum (AB219965)	GTTCAGCCAGCTACGGACG
Halobacterium salinarum DSM3754T (AJ496185)	GTTCAGCCAGCTACGGACG
Halobacterium salinarum str. cerca (AY994198)	GTTCAGCCAGCTACGGACG
Halobacterium NRC-1 (AE005128)	GTTCAGCCAGCTACGGACG
Halobacterium salinarum (AJ420167)	GTTCAGCCAGCTACGGACG
Halobacterium saccharovorum (U17364)	GT***G***C***G***GCCAGCTACGGACG
HBN3738 probe	**CCTGTATCGCGCTCCGTTT**
Halobacterium NRC-1 (AE005128)	CCTGTATCGCGCTCCGTTT
Natronomonas pharaonis DSM 2160 (CR936257)	CCTGTATCGCGCTCCG***GGG***

Table 5. *Halobacterium salinarum* probe alignments. List of species that aligned with probe sequences (shown in bold) in GenBank, and species that had the fewest misalignments (shown in italics), in order to demonstrate probe specificity.

RESULTS

Cultivation

The results of direct plating of *Archaea* are shown in Fig. 2. There were 38 archaeal isolates identified from direct plates of HM, MGM, and VN. Sequencing and comparison with GenBank and RDPII showed that seven different genera were represented and that species similarity ranged from 95-100% (Appendix B). Decreased salt and altered nutrient concentrations did not seem to result in the isolation of different types. All isolates cultured at lower salt or nutrient concentrations were also cultured at the higher ranges.

Dilution culturing of *Archaea* with minimal medias resulted in 18 isolates (Fig. 2). When identified through 16S sequencing and comparisons with Genbank and RDPII, species similarities ranged from 90-99% (Appendix B). There were six genera represented, three of which (*Haloarcula*, *Halobacterium*, and *Halorubrum*) were also cultured from direct plates. The most commonly isolated archaeal genus was *Haloarcula* (see Appendix B).

The results of isolation of *Bacteria* by direct plating is shown in Fig. 3. There were 37 bacterial isolates identified, with a species similarity of 88-100% when compared with GenBank sequences. The isolates represented nine genera of *Bacteria*, with the HM plates being the most productive. Lower salt concentrations resulted in higher number of cultures for the bacteria, whereas altered nutrient concentrations decreased the number of cultures isolates.

Bacterial isolation results from dilution cultures with minimal media are shown in Fig. 3. There were 4 isolates identified, which showed a species similarity of 96-99% when compared with GenBank sequences. Only two genera were isolated from dilution cultures: *Salicola* and *Pseudomonas*. The most commonly isolated bacterial genus was *Pseudomonas* (see Appendix B).

Colonies grown in jar cultures were identified through 18S rDNA sequencing as algae belonging to the species *Dunaliella viridans* and *Dunaliella salina*. Both species have previously been the only eukaryotic organisms isolated from the North Arm of GSL (Baxter, 2005).

Several media did not result in any isolation of organisms. Minimal media with acetate, glycerol, and methylamine as the sole carbon source (SWA, SWG, MAM) did not promote any growth. Enrichment cultures using aromatic compounds and M63 media also did not result in any isolation of microorganisms.

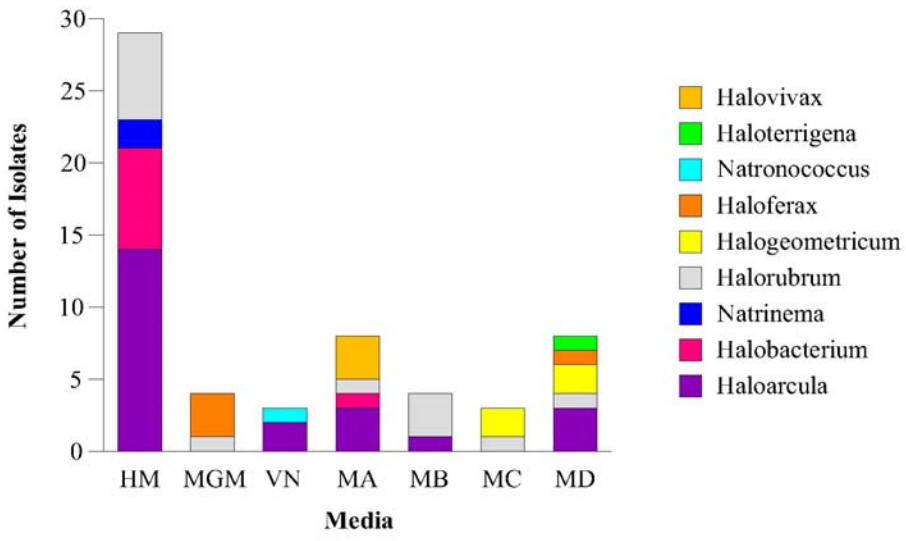

Figure 2. **Results of archaeal culturing**. The first three (HM, MGM, and VN) are direct plating results from complex media, the last four (MA, MB, MC, and MD) are dilution culture results from minimal media.

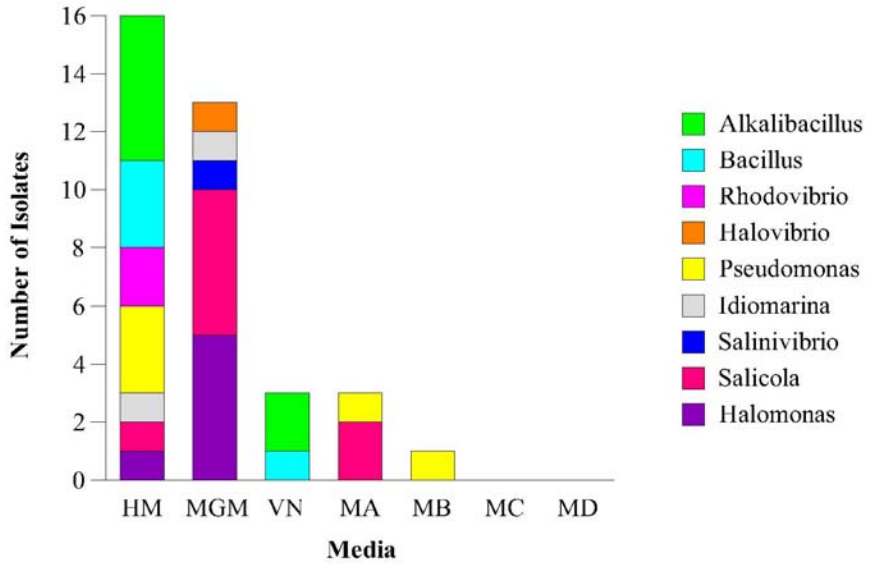

Figure 3. **Results of bacterial culturing**. The first three (HM, MGM, and VN) are direct plating results from complex media, the last four (MA, MB, MC, and MD) are dilution culture results from minimal media.

Phylogenetics

A combined strict consensus tree of the maximum likelihood and Bayesian phylogenies are shown in Fig. 4 and Fig. 6. There was high level of agreement among the two methods and differences were mainly a question of resolution and not placement. Distance trees are shown in Fig. 5 and Fig. 7, with branch lengths showing substitution rates. Results indicated that the majority of bacterial isolates fell into the gamma subclass of the *Proteobacteria*, with the exception of a few from alpha-*Proteobacteria* (*Rhodovibrio* sp.) and *Firmicutes* (*Alkalibacillus* sp.) groups. All of the archaeal isolates belonged to *Halobacteriaceae*. Most culture sequences clustered in clades with closely related reference sequences, however a few isolates did not. These isolates include MedCp1B12, MedDp1H10, MedDp1I11, and MedBp2B2 for the archaeal tree. For the

bacterial tree isolates HM18p1C6, HM18p1C8, and HM23p1H11 appeared to cluster outside the main group.

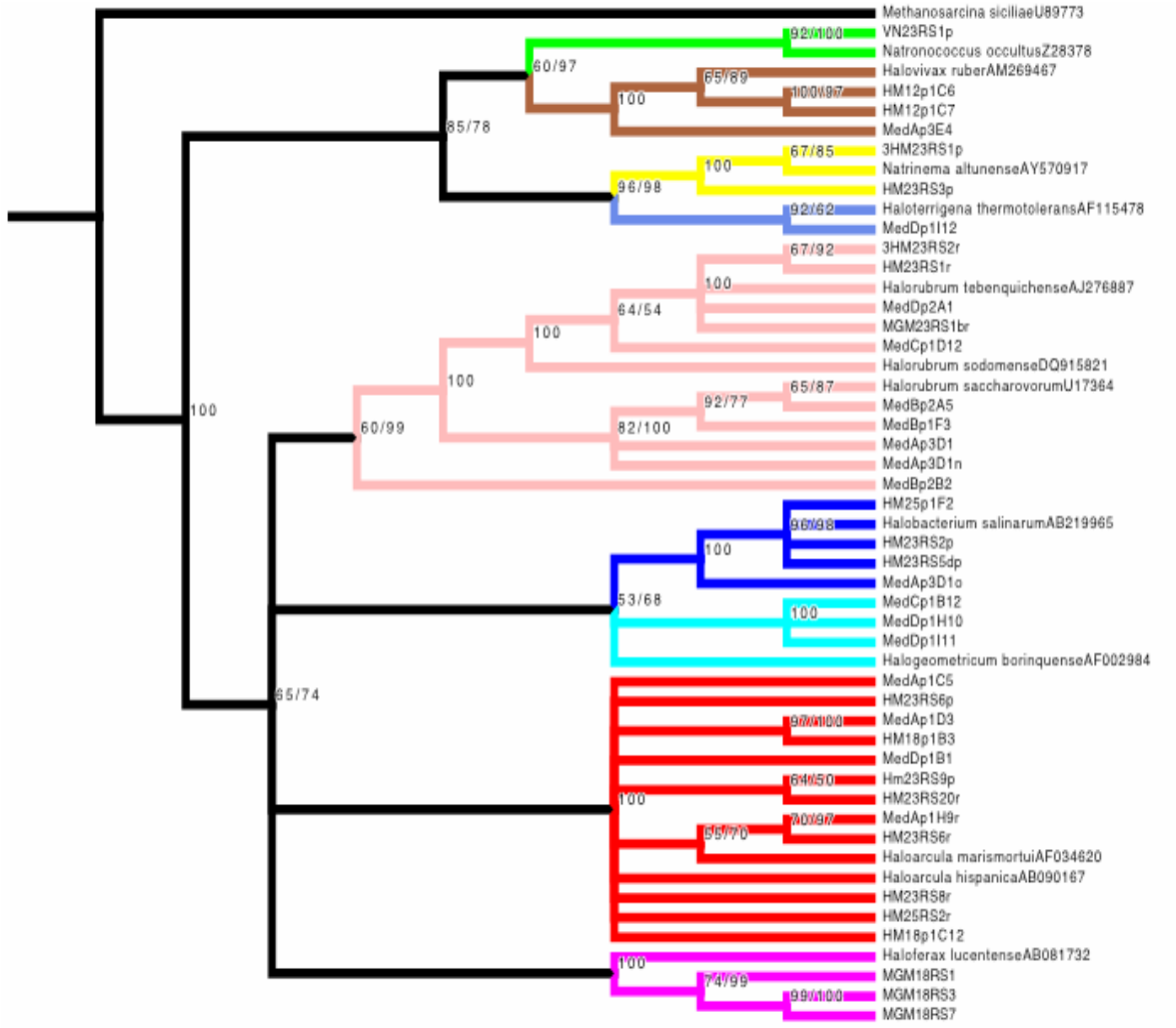

Figure 4. **Archaeal phylogenetic tree**. Archaeal consensus tree of nodes in agreement between ML trees and Bayesian trees. The first number at the node is the bootstrap probability for ML and the second is a posterior probability for Bayesian. Colors coordinate to different genera, with isolates grouped according to 16S rDNA similarity in GenBank.

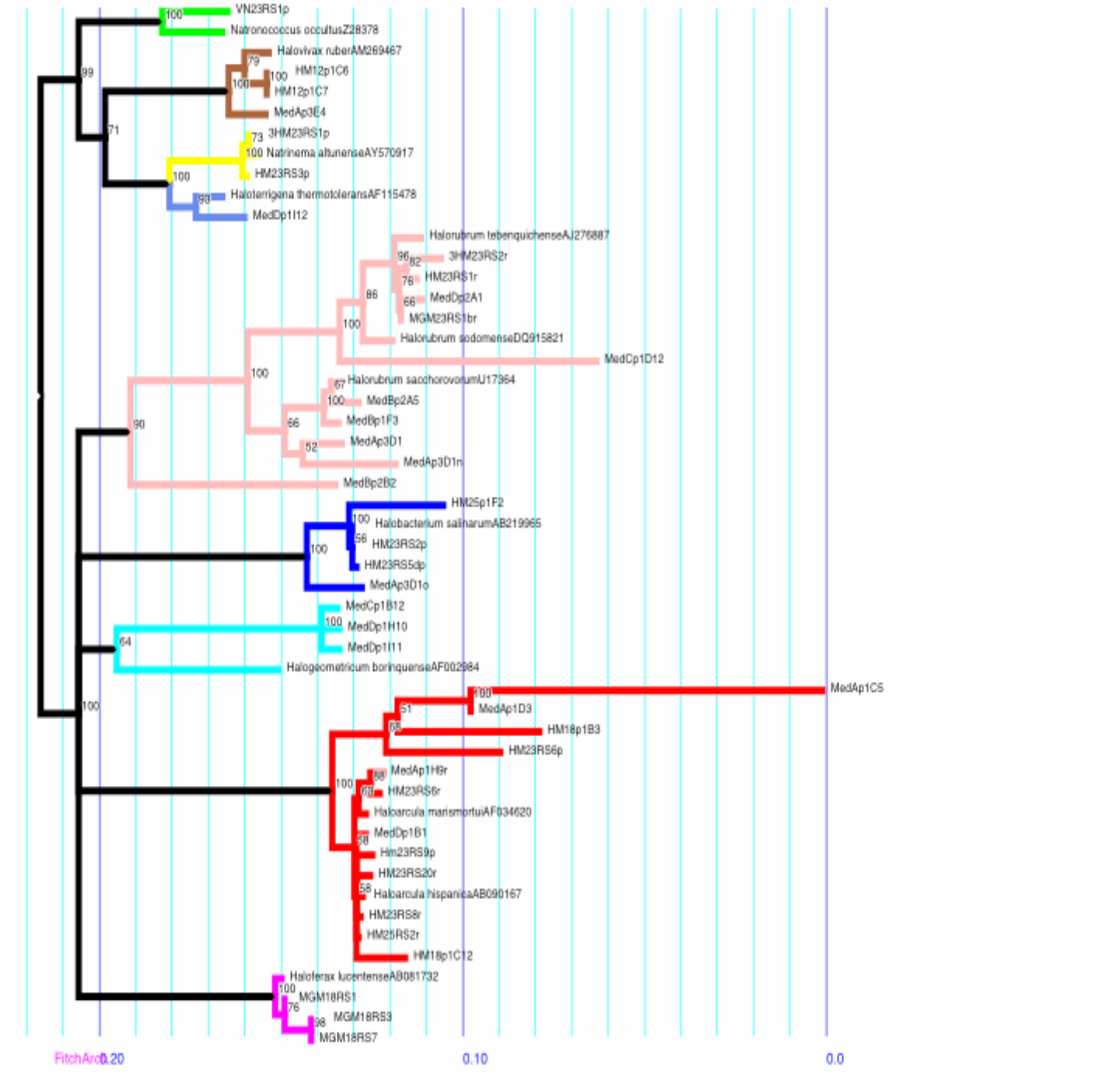

Figure 5. Archaeal distance tree. Bootstrap numbers are shown at the nodes. Branch lengths are scaled to the number of substitutions per site. Colors coordinate to different genera, with isolates grouped according to 16S rDNA similarity in GenBank.

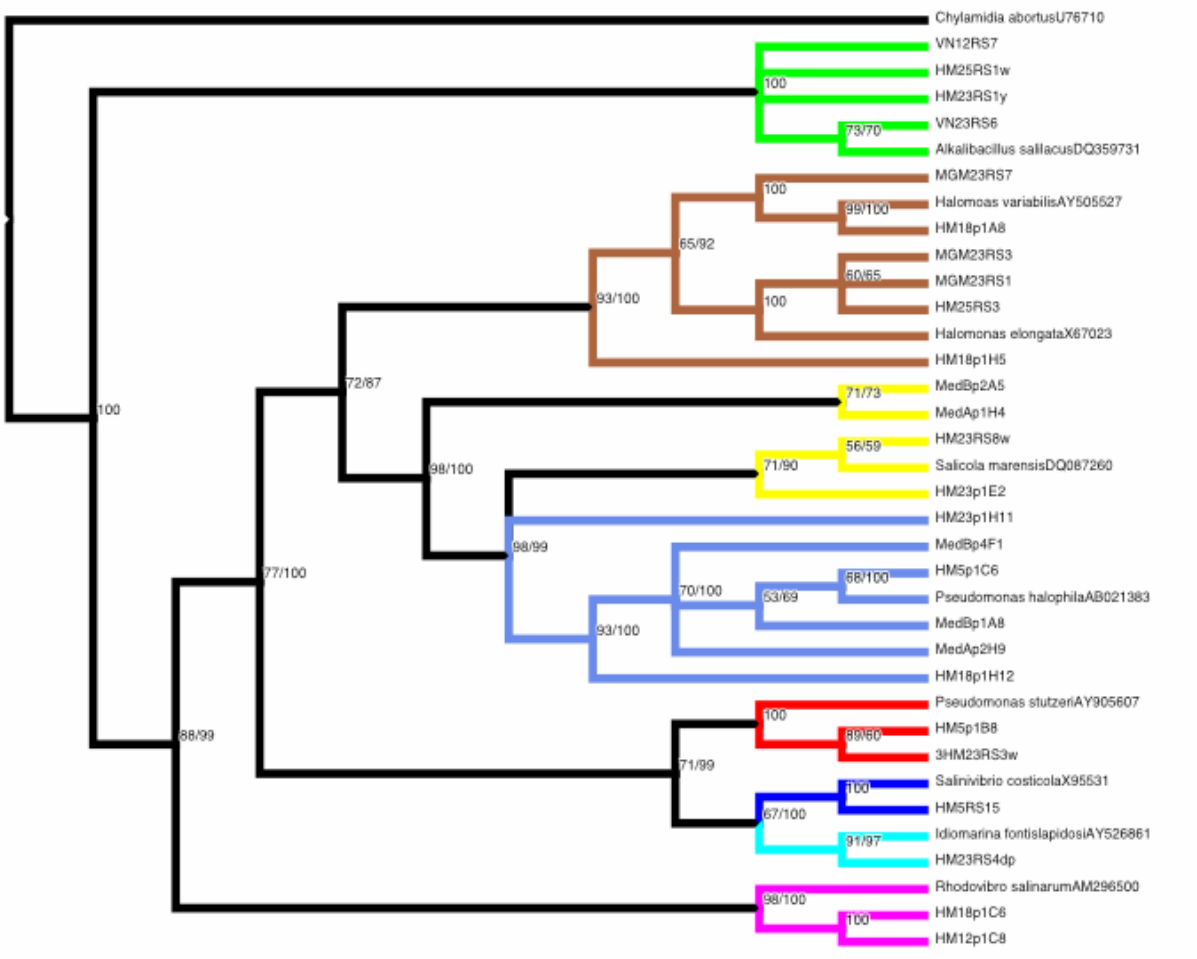

Figure 6. **Bacterial phylogenetic tree**. Bacterial consensus tree of nodes in agreement between ML trees and Bayesian trees. The first number at the node is the bootstrap probability for ML and the second is a posterior probability for Bayesian. Colors coordinate to different genera groups, with the exception of *Pseudomonas*, which is split. Isolates are grouped according to 16S rDNA similarity in GenBank.

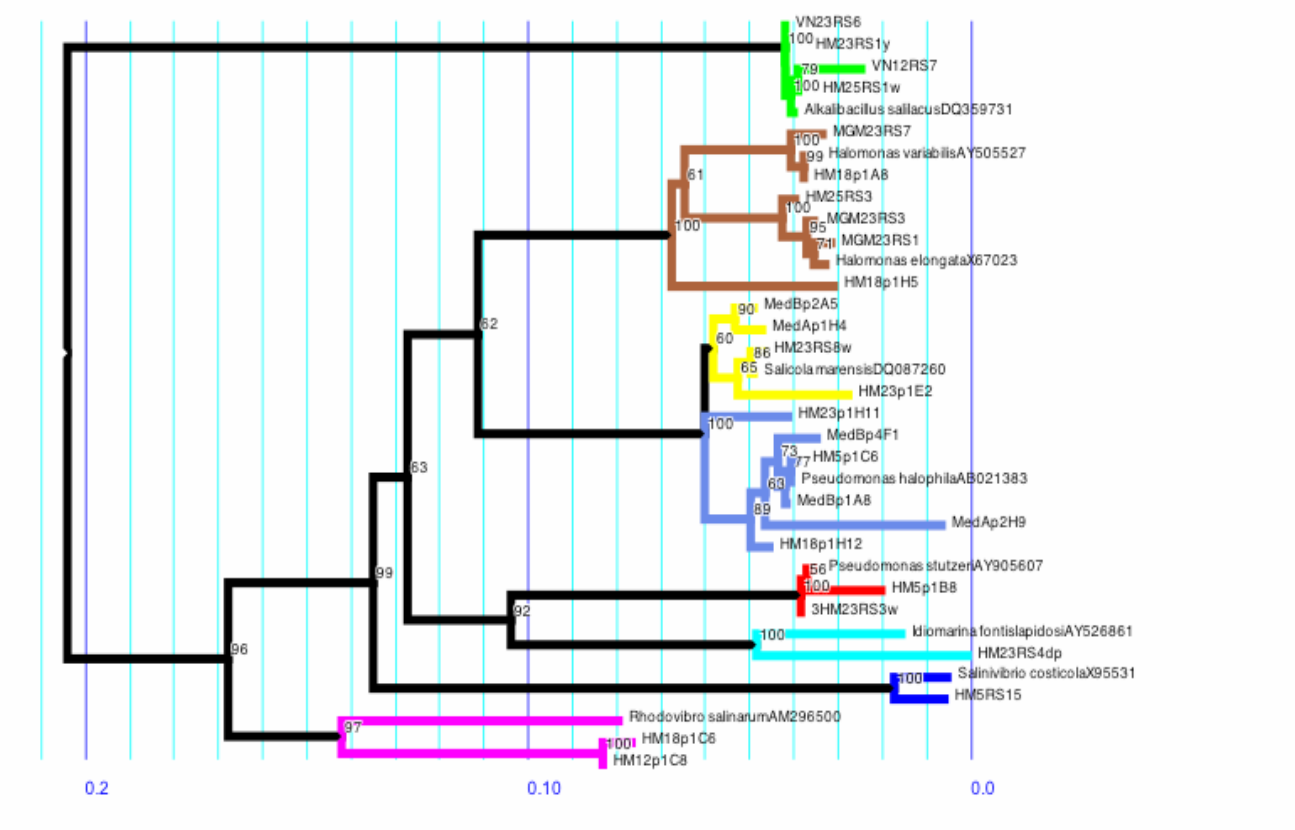

Figure 7. Bacterial distance tree. Bootstrap numbers are shown at the nodes. Branch lengths are scaled to the number of substitutions per site. Colors coordinate to different genera, with isolates grouped according to 16S rDNA similarity in GenBank.

Characterization

Results of the controls showed similar profiles between isolate 3HM23RS3w at no salt and at 20% salt with the exception of the nitrate reduction test. While the strip run at normal parameters gave a positive for the NO_3 test, the strip run at high salt gave a negative reaction. The API 20 NE profiles are shown in Appendix C with differences between isolates and the known profiles of the closest related species highlighted.

The BIOLOG EcoPlate™ tests showed that thirteen of the possible thirty-one carbon sources were utilized by isolates. These included Tween-40, Tween-80, D-galacturonic

acid, α-ketobutyric acid, glycogen, i-erythritol, N-acetyl-glucosamine, glucose-1-phosphate, α-glycerol phosphate, hydroxybutyric acid, L-serine, L-asparagine, and D-malic acid. The most commonly utilized substrate was Tween-40, which was used by 77% of the isolates tested.

FISH

Probe optimization of HBS531 found that 25% formamide concentration was optimal for hybridization. Probe HBN3738 was optimally hybridized at a concentration of 45% formamide.

Total cell counts, as shown by DAPI, were found to be approximately 3-4 X $10^{\wedge 7}$ cells/ml (Table 6 and Fig. 8A). *Archaea* were shown to be the majority, representing 72% of the population, with *Bacteria* accounting for 22% (Table 6 and Figs. 8C-F). The remaining cells were thought to be eukaryotic algae based on appearance (Fig. 8B). Of the archaeal cells, 24% were shown to be *Halobacterium salinarum*, with 10% of that population *Halobacterium* NRC-1, and 61% were shown to be *Haloarcula* spp (Table 7 and Figs. 8G-H). Of the bacterial cells, 80% were shown to be *Salinibacter ruber* and 15% were shown to be *Pseudomonas halophila*-like organisms (Table 7 and Figs. 8I-J).

Probe	DAPI	ARCH915	EUB388
Cells/mL	$3.60x10^{\wedge 7} \pm 0.84x\ 10^{\wedge 7}$	$2.54x10^{\wedge 7} \pm 0.56x10^{\wedge 7}$	$8.25x10^{\wedge 6} \pm 2.3x10^{\wedge 6}$

Table 6. FISH total cell count results. Cell count results for total (DAPI), archaeal (Arch915), and bacterial cell numbers (Eub388).

Probe	HBS531	HBN3738	HRCU1502	EHB586 + EHB 1451	PH659
Percentage of Total cells	17%	7%	43%	18%	3%
Percentage of Archaea	24% ± 9%	10% ± 8%	61% ± 15%	-	-
Percentage of Bacteria	-	-	-	80% ± 10%	15% ± 3%

Table 7. **FISH specific probe results**. Percentage of specific groups in the archaeal or bacterial population.

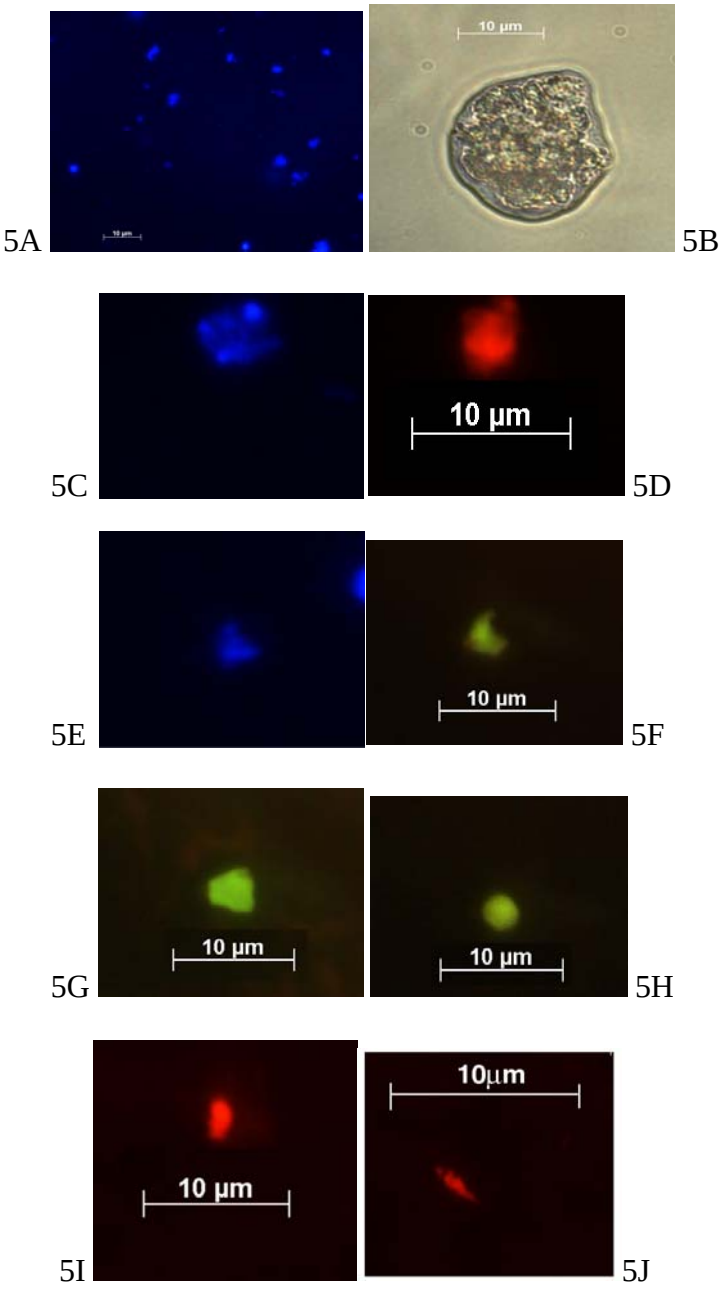

Figure 8. **FISH pictures**. 8A is a microscope field stained with DAPI. 8B is a phase-contrast picture of a *Dunaliella* species. 8C and 8D are DAPI and ARCH915 of the same cell. 5E and 5F are DAPI and EUB388 of the same cells. 8G-H are cells probed with HRCU1502 and HBS531, targeting *Haloarcula* and *Halobacterium salinarum* cells respectively. 8I-J are cells probes with PH659 and EHB 586/1481, targeting *Pseudomonas halophila*-like and *Salinibacter ruber* cells respectively.

DISCUSSION

The question of whether or not culturing studies fairly represent what is actually present in the environment has been debated and looked at in almost every habitat on Earth. Hypersaline systems are no exception and several studies have tried to address concerns about culturing bias in these environments (Benlloch *et al*., 2001; Burns *et al*., 2004; Orchenresiter, 2002). These studies have found conflicting results that suggest it is dependant on the habitat studied. In general, hypersaline scientists have found that dominant and ecologically important isolates are yet to be cultured (Oren, 2002). However, in GSL, results show that those organisms most commonly found in the environment were the ones most commonly cultured, or at least found to be culturable. This disagrees with previous thinking and supports culturing in the GSL as fairly representing the environment. However, while culturing was able to capture a lot of diversity in GSL, discrepancies were seen in comparing molecular and culture libraries (Tazi, unpublished). These discrepancies suggest that use of both culturing and nonculturing methods will give a more complete picture of biodiversity at GSL.

Culturing at Rozel Point revealed that the biodiversity appears to be similar to, if not more diverse, than that found in culture libraries from other hypersaline environments. While common archaeal genera were isolated (*Halobacterium*, *Haloarcula*, *Halorubrum*, and *Haloferax*) many genera were isolated that are not commonly seen in culture studies (*Natronococcus*, *Halovivax*, *Haloterrigena*, and *Halogeometricum*). Members of the gamma subclass of *Proteobacteria* are commonly isolated bacteria from saline environments, in particular the genus *Halomonas*, however

Rhodovibrio and *Alkalibacillus* are uncommon to most culturing studies from a salt-saturated environment.

Archaeal isolates were mainly cultured from media containing 18% or higher NaCl, though a few isolates were cultured from media with 12% NaCl. Bacterial isolates were cultured from a wider range of NaCl salinity (5-25%), with the lower ranges being more favorable. This supports previous studies that indicate extreme halophilic archaea have a higher salinity requirement and smaller range than most bacteria (Ventosa *et al*., 1998; Oren, 2002). The type of medium and method used was influential in archaeal culturing, with complex media high in nutrients (HM and MGM media) shown to be the most productive, but more unique groups of organisms isolated from other media types, particularly those with altered pH levels and carbon sources (VN and MA-MD). This contrasts with culturing studies done in crystallizer ponds, which indicated that medium composition was not important for isolate diversity (Burns *et al.*, 2004). Unlike the archaeal isolation, minimal media were not effective in isolating unique bacterial groups. This indicates that traditional plating methods are still the best methods to date for culturing halophilic bacteria. This agrees with previous studies that indicate most moderately halophilic aerobic bacteria have more demanding nutritional requirements than aerobic archaea (Ventosa *et al*., 1998).

It was surprising that minimal media with pyruvate (MA and MC) were successful, while acetate and glycerol as the sole carbon source were not (SWA and SWG). Studies done by Fendrich in the GSL found that addition of acetate increased bacterial numbers in liquid cultures (Fendrich, 1988). Tests performed in jar cultures with different carbon sources seemed to indicate glycerol promoted high rates of metabolic

activity (Alan Harker, personal communication). Also, as glycerol is present in high amounts at GSL due to production by *Dunaliella* at high salts, it is surprising that it was not better utilized in cultures (Post, 1977). However in both the jar cultures and the Fendrich study the acetate and glycerol were not sole carbon sources, but were added to either lake water or complex media. This could indicate that while acetate and glycerol are beneficial for growth, other nutrients are needed. It is also believed that trimethylamine, as opposed to methylamine, would have been a better choice for a carbon source, as it is more easily utilized (Oren, 2002). However jar culture results showed that trimethlyamine did not promote increased metabolic activity (Alan Harker, personal communication), so these compounds do not appear to be a good carbon source for GSL isolates.

It was also disappointing that enrichment cultures were unsuccessful. Ward and Brock also failed to cultivate microbes from Rozel Point using enrichment cultures with mineral oil (1978). Petroleum degradation has not conclusively been shown to occur in GSL. Their study found that increased salinity led to decreased turnover of hydrocarbons, suggesting that extreme salinities inhibit or reduce hydrocarbon biodegradation (1978). However, several halophilic organisms have been shown to have petroleum degradative capabilities (Bertrand *et al*., 1990; Emerson *et al*., 1994; Garcia, 2004; Margesin and Schinner, 2001). The aromatic compounds chosen were those shown to be effective in culturing from other halophilic environments (Garcia, 2004), and perhaps using only compounds common to GSL petroleum would be more successful for future enrichments.

Fluorescence *in situ* hybridization (FISH) revealed that the most abundant population at Rozel Point is archaeal, particularly of the *Haloarcula* group. This differed from previous thinking that *Halobacterium* was the most abundant species (Baxter, 2005). As far as we know this is the first time that *Haloarcula* have been demonstrated to be the most abundant organism in the community. Most studies have shown that it is found frequently in culturing, but not in the environment (Anton, 1999). The bacterial population was almost entirely dominated by the species *Salinibacter ruber*, as previously thought (Carol Litchfield, personal communication). After FISH revealed the high percentage of *Salinibacter ruber*, efforts were made to target this group for culturing through use of specific media and antibiotics, but have not been successful to date. Though we were unsuccessful in culturing *Salinibacter*, several other studies have done so (Anton *et al*., 2002; Bolhuis *et al*., 2004; Peña *et al*., 2005). FISH results support the use of culturing as an effective tool for identifying the most abundant players in both archaeal and bacterial communities in hypersaline environments. However, culturing may introduce bias and miss certain groups, as seen with *Salinibacter*, and again demonstrates that it should be combined with non-culturing methods in order to study biodiversity.

Phenotypic characterization demonstrated that many of these isolates show unique properties when compared to similar species. Even those isolates with almost identical ribosomal genes did not give the same profile. This would indicate that though they appear genetically similar they might represent a unique species or strain. Both halophilic archaea and bacteria appeared to assimilate different carbon sources well, confirming studies analyzing metabolic capabilities in salterns (Litchfield, *et al*., 2001).

The nitrate reduction test needs to be looked at further due to the control giving different responses based on salt levels. Also, the indole test should be examined further as it was consistently negative for all isolates. All previous isolates from the North Arm of GSL have been found to be indole negative (Fendrich, 1988; Ventosa *et al*., 1998; Waino *et al*., 1990), however several extreme archaea are known to be indole positive (see reference profiles in Appendix C), so it is hard to determine if this is a pattern of GSL isolates or a problem with testing methods.

Culturing in GSL revealed a diverse group of organisms, including nine archaeal genera and eight bacterial genera, two of which represent novel genera. Standards for prokaryotes have set a 16S rDNA similarity above 97% as belonging to the same species, and above 95% belonging to the same genus (Stackebrandt and Goebel, 1994). However, most scientists suggest a polyphasic approach of using genetic, phenotypic, and metabolic characteristics to identify new organisms (Vandamme *et al*., 1996). Bacterial isolates HM18p1C6 and HM12p1C8 were much lower than the 95% similarity generally seen at the genus level (Appendix B) and possessed many unique phenotypic properties (Appendix C), supporting the placement of these two isolates in a separate genus. Archaeal isolates MedCp1B12, MEdDp1H10, and MedDp1I11 were also lower than the 95% standard, phylogenetically separate (Fig. 3), and phenotypically different (Appendix C), supporting the placing of these three isolates in a new genus. A more extensive characterization of the morphology, growth requirements, physiological traits, and genetics of these two groups needs to be completed before they can be formally recognized (Imhoff and Caumette, 2004; Oren *et al*., 1997). This would increase the number of GSL isolates to fourteen (Table 8). Several other isolates with low sequence

similarity and unique phenotypic profiles were also cultured and should be further examined as possibly representing novel species. This study demonstrates Rozel Point's potential as a site for novel taxonomic discoveries.

Name	Location	Reference
	Aerobic	
Gracilibacillus halotolerans	South Arm	Waino, *et al*., 1999
Halobacillus litoralis	South Arm	Spring *et al*., 1996
Halobacillus trueperi	South Arm	Spring *et al*, 1996
Halorhabdus utahensis	South Arm	Waino, *et al*., 1990
Halomonas variabilis	North Arm	Fendrich, 1988
Pseudomonas halophila	North Arm	Fendrich, 1988
Haloterrigena turkmenica	North Arm	Ventosa, *et al*., 1999
Unnamed Archaea (MedCp1B12, MEdDp1H10, and MedDp1I11)	North Arm	Present study
Unnamed Bacteria (HM18p1C6, HM18p1C8)	North Arm	Present study
	Anaerobic	
Desulfocella halophila	South Arm	Brandt *et al*., 1997
Desulfobacter halotolerans	South Arm	Brandt *et al*., 1997
Haloanaerobium praevalens	South Arm	Zeikus *et al*., 1983
Haloanaerobium alcaliphilum	South Arm	Tsai *et al*., 1995
Methanohalophilus mahii	South Arm	Ingvorsen and Brandt, 2002

Table 8. **Modified List of GSL isolates**.

Overall this study has demonstrated many similarities with other hypersaline environments, including a dominant archaeal population, culturing of the commonly seen fast-growers, and difficulty culturing certain microbial populations. In contrast, there was more diversity seen in cultures when compared to many simpler hypersaline environments, a large culturable population demonstrated through FISH, and the presence of many potentially novel species. These results will allow for a more extensive

understanding of the prokaryotic biodiversity in GSL and similar hypersaline environments. Characterization of these isolates will help us to understand more about what these microbes are doing and how that compares to other hypersaline and non-hypersaline environments. More extensive studies of those isolates that appear to be novel or have novel properties can be made. Culturing of GSL isolates will also allow further studies to be done on the biochemical and metabolic processes occurring in this system.

REFERENCES CITED

Amann R.I., Binder B.J., Olson R.J., Chisholm R.D., and Stahl D.A. (1990) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed populations. *Appl. Environ. Microbiol*. **56**: 1919-1925.

Amann R.I., Ludwig W., and Schleifer K. (1995) Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol. Rev*. **59**: 143-169.

Anton J., Llobet-Brossa E., Rodriguez-Valera F., and Amann R. (1999) Fluorescence *in situ* hybridization analysis of the prokaryotic community inhabiting crystallizer ponds. *Environ. Microbiol*. **1**: 517-523.

Anton J., Rossello-Mora R., Rodriguez-Valera F., and Amann R. (2000) Extremely halophilic bacteria in crystallizer ponds from solar salterns. *Appl. Environ. Microbiol*. **66**: 3052-3057.

Anton J., Oren A., Benlloch S., Rodriguez-Valera F., Amann R., and Rossello-Mora R. (2002) *Salinibacter ruber* gen. nov., sp. nov., a novel, extremely halophilic member of the *Bacteria* from saltern crystallizer ponds. *Int. J. Syst. Evol. Microbiol*. **52**: 485-491.

Baxter B., Litchfield C.D., and Sowers K., Griffith J.D., Dassarma P.A., and Dassarma S. (2005) Great Salt Lake Microbial Diversity. In: *Adaptation to life in High Salt Concentrations in Archaea, Bacteria, and Eukarya* (ed. Gunde-Cimerron, N., Oren, A., Plemenita, A. (eds.)). Springer, The Netherlands.

Benlloch S., Acinas S. G., Anton J., Lopez-Lopez, Luz S.P., and Rodriguez-Valera F. (2001) Archaeal biodiversity in crystallizer ponds from a solar saltern: culture versus PCR. *Microb. Ecol*. **41**: 12-19.

Birnboim H.C. and Doly J. (1979) *Nucleic Acids Res*. **7**: 1513-23.

Bolhuis H., te Poele E.M., and Rodriguez-Valera F. (2004) Isolation and cultivation of Walsby's square archaeon. *Environ. Microbiol*. **6**: 1287-1291.

Brandt K.K. and Ingvorsen K. (1997) *Desulfobacter halotolerans* sp. nov., a halotolerant acetate-oxidizing sulfate-reducing bacterium isolated from sediments of the Great Salt Lake, Utah. *Syst. Appl. Microbiol*. **20**: 366-373.

Brandt K K., Patel B.K.C., and Ingvorsen K. (1999) Desulfocella halophila gen. nov., sp. nov., a halophilic fatty-acid oxidizing, sulfate-reducing bacterium isolated from sediments of the Great Salt Lake. *Int. J. Syst. Bacteriol*. **49**: 193-200.

Burdy P. and Post F.J. (1979) Survival of *Escherichia coli* in Great Salt Lake water. *Water, Air, and Soil Pollution* **12**: 237-246.

Burns D.G., Camakaris H.M., Janssen P.H., and Dyall-Smith M.L. (2004) Combined use of cultivation-dependent and cultivation-independent methods indicates that members of most haloarchaeal groups in an Australian crystallizer pond are cultivable. *Appl. Environ. Microbiol*. **70**: 5258-5265.

Chidsey T.C. (1995) Rozel Point: harbinger of petroleum potential. *Utah Geological Survey* **27**: 10-11.

Dyall-Smith M. (2004) The Halohandbook: Protocols for halobacterial genetics. Downloaded from www.microbiol.unimelb.edu.au/micro/staff/mds/HaloHandbook

Dyall-Smith M. (2006) The Halohandbook: Protocols for halobacterial genetics. Downloaded from www.microbiol.unimelb.edu.au/people/dyallsmith/resources/halohandbook

Edgar R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*. **32**: 1792-1797.

Felsenstein J. (2005) PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Fendrich C. (1988) *Halovibrio variabilis* gen. nov. sp. nov., *Pseudomonas halophila* sp. nov. and a new halophilic aerobic coccoid *Eubacterium* from Great Salt Lake, Utah, USA. *Syst. Appl. Microbiol*. **11**: 36-43.

Frederick E. (1924) On the bacterial flora of Great Salt Lake and the viability of other microorganisms in Great Salt Lake water: M.S. Thesis (unpublished), University of Utah, Salt Lake City, Utah.

Fuchs B.M., Wallner G., Bisker W., Schwippl I., Ludwig W. and Amann R. (1998) Flow cytometric analysis of the *in situ* accessibility of *Escherichia coli* 16S rRNA for fluorescently labeled oligonucleotide probes. *Appl. Environ. Microbiol*. **64**: 4973-4982.

Garabito M.J., Marquez M.C., and Ventosa A. (1998) Halotolerant *Bacillus* diversity in hypersaline environments. *Can. J. Microbiol*. **44**: 95-102.

Garcia M.T., Mellado E., Ostos J.C., and Ventosa A. (2004) *Halomonas organivorans* sp. nov., a moderate halophile able to degrade aromatic compounds. *Int. J. Syst. Evol. Microbiol*. **54**: 1723-1728.

Gwynn J.W. (1980) Great Salt Lake. A scientific historical and economic overview. In: *Utah Department of Natural Resources, bull. 116.* Salt Lake City, Utah.

Gwynn J.W. (2002) Great Salt Lake, an overview of change. In: *Special publication of the Utah Department of Natural Resources*. Salt Lake City, Utah.

Horikoshi K. (1995) Discovering novel bacteria with an eye to biotechnological application. *Current Opinion in Biotechnology*, **6**: 292-297.

Imhoff J.F. and Caumette P. (2004) Recommended standards for the description of new species of anoxygenic phototrophic bacteria. *Int. J. Syst. Evol. Microbiol*. **54**: 1415-1421.

Ish-Horowitz D. and Burke J.F. (1981) *Nucleic Acids Res*. **9**: 2989-98.

Kaeberlein T., Lewis K., and Epstein S.S. (2002) Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. *Science* **296**: 1127-1129.

Litchfield C.D., Irby A., Kis-Papo T., and Oren A. (2001) Comparative metabolic diversity in two solar salterns. *Hydrobiologia* **466**: 73-80.

Maddison W.P. and Maddison D.R. (2006) Mesquite: a modular system for evolutionary analysis. Version 1.12 http://mesquiteproject.org

Marchesi J.R., Sato T., Weightman A.J., Martin T.A., Fry J.C., Hiom S.J., Dymock D., and Wade W.G. (1998) Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Appl. Environ. Microbiol*. **64**: 795-799.

Maturrano L., Santos F., Rossello-Mora R., and Anton J. (2005) Microbial diversity in Maras Salterns, a hypersaline environment in the Peruvian Andes. *Appl. Environ. Microbiol*. **72**: 3887-3895.

Medlin L., Elwood H.J., Stickel S., and Sogin M.L. (1998) The characteristics of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene* **71**: 491-499.

Ochsenreiter T., Pfeifer F., and Schleper C. (2002) Diversity of Archaea in hypersaline environments characterized by molecular-phylogenetic and cultivation studies. *Extremophiles* **6**: 267-274.

Oren A., Ventosa A., and Grant W.D. (1997) Proposed minimal standards for description of new taxa in the order *Halobacteriales*. *Int. J. Syst. Bacteriol*. **47**: 233-238.

Oren A. (2002) Molecular ecology of extremely halophilic Archaea and Bacteria. *FEMS Microbial Ecol*. **39**: 1-7.

Page R.D.M. (1996) TREEVIEW: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12**: 357-358.

Peña A., Valens M., Santos F., Buczolits S., Anton J., Kampfer P., Busse H., Amann R., and Rossello-Mora R. (2005) Instraspecific comparative analysis of the species *Salinibacter ruber*. *Extremophiles* **9**: 151-161.

Posada D. and Crandall K. A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817-818.

Post F.J. (1977) The microbial ecology of the Great Salt Lake. *Microbial. Ecol*. **3**: 143-65.

Post F.J. (1981) Microbiology of the Great Salt Lake north arm. *Hydrobiologia* **81**: 59-69.

Rodriguez-Valera F. (1992) Biotechnological potential of halobacteria. *Biochemical Society Symposium* **58**: 135-147.

Ronquist F. and Huelsenbeck J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572-1574.

Rothschild L.J. and Mancinelli R.L. (2001) Life in extreme environments. *Nature*, **409**: 1092-1101.

Stephens D.W. (1997) Changes in lake levels, salinity, and the biological community of Great Salt Lake (Utah, USA), 1847-1987. *Hydrobiologia* **197**: 139-146.

Spring S., Ludwig W., Marquez M.C., Ventosa A., and Schleifer K.H. (1996) *Halobacillus* gen. nov., with descriptions of *Halobacillus litoralis* sp. nov. and *Halobacillus trueperi* sp. nov., and transfer of *Sporosarcina halophila* to *Halobacillus halophilus* comb. nov. *Int. J. Syst. Bacteriol*. **46**: 492-496.

Tsai C.R., Garcia J.L., Patel B.K., Cayol J.L., Baresi L., and Mah F.A. (1995) *Haloanaerobium alcaliphilum* sp. nov., and anaerobic moderate halophile from the sediments of Great Salt Lake, Utah. *Int. J. Syst. Bacteriol*. **45**: 301-7.

Vandamme P., Bot B., Gillis M., De Vos P., Kersters K., and Swings J. (1996) Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiol. Rev*. **60**: 407-438.

Ventosa A., Gutierrez M.C., Kamekura M., and Dyall-Smith M.L. (1999) Proposal to transfer *Halococcus turkmenicus, Halobacterium trapanicum* JCM 9743 and strain GSL-11 to *Haloterrigena turkmenica* gen. nov., comb. nov. *Int. J. Syst. Bacteriol*. **49**: 131-136.

Ventosa A., Nieto J.J., and Oren A. (1998) Biology of moderately halophilic aerobic bacteria. *Microbiol. Mol. Biol. Rev*. **62**: 504-544.

Waddell K.M. and Bolke E.L. (1973) The effect of restricted circulation on the salt balance of Great Salt Lake, Utah. In: *Utah Geological and Mineral Survey, Water Resources Bull,* p. 54.

Waino M., Tindall B.J., Schumann P., and Ingvorsen K. (1999) *Gracilibacillus* gen. nov., with description of *Gracilibaciilus halotolerans* gen. nov., sp. nov.; transfer of *Bacillus dipsosauri* to *Gracilibacillus dipsosauri* comb. nov., and *Bacillus salexigens* to the genus *Salibacillus* gen. nov., as *Salibacillus salexigens* comb. nov. *Int. J. Syst. Bacteriol*. **49**: 821-831.

Waino M., Tindall B.J., and Ingvorsen K. (2000) *Halorhabdus utahensis* gen. nov., sp. nov., an aerobic, extremely halophilic member of the Archaea from Great Salt Lake, Utah. *Int. J. Syst. Evol. Microbiol*. **50**: 183-90.

Ward D.M. and Brock T.D. (1978) Hydrocarbon biodegradation in hypersaline environments. *Appl. Environ. Microbiol*. **35**: 353-359.

Wiegel J. and Adams M. (eds). (1998) Thermophiles: The Keys to Molecular Evolution and the Origion of Life? Taylor and Francis: London.

APPENDIX A

Halophilic Media (HM)

(DSMZ Media 97, www.dsmz.d/media)

Casamino acids	7.5g
Yeast extract	10g
Trisodium citrate	3g
KCl	2g
$MgSO_4.7H_2O$	20g
$FeSO_4.7H_2O$	0.05g
$MnSO_4.4H_2O$	0.25mg
NaCl	X g (depending on % salt desired)
Agar	20g
Distilled water	up to 1L

Dissolve all ingredients except the agar and adjust pH to 7.4. Add the agar and autoclave

Modified Growth Medium (MGM)

(Halohandbook, p. 11)

<u>30% Concentrated Salt Water:</u>
Dissolve the following in 1 liter of water and adjust pH to 7.5; autoclave

NaCl	240g
$MgCl_2.6H_2O$	30g
$MgSO_4.7H_2O$	35g
KCl	7g
$CaCl_2.2H_2O$	5 mL from a 1M sterile stock

	12%MGM	**18%MGM**	**23%MGM**	**25%MGM**
Salt Water (30% stock)	400 mL	600 mL	767 mL	833 mL
Distilled water	567 mL	367 mL	200 mL	134 mL
Peptone	5g	5g	5g	5g
Yeast Extract	1g	1g	1g	1g

Stir to dissolve and adjust the pH to 7.5. Add 15g agar/L and autoclave 30 minutes

Van Niel's Yeast Agar with Salt

(ATCC media 217, www.atcc.org)

K_2HPO_4	1g
$MgSO_4.7H_2O$	0.5g
Yeast extract	10g

Agar powder	15g
NaCl	250g (for 25% salt plates)
Distilled water	up to 1L

Dissolve all ingredients and adjust pH to 8.0, autoclave

Media A-Media D (MA, MB, MC, MD)

(Burns *et al.,* 2004*)*

MA = 5 μM amino acids and 5 μM pyruvate in autoclaved lake water
MB = 5 μM amino acids, 5 μM acetate, and 5 μM pyruvate in autoclaved lake water
MC = 50 μM amino acids and 0.5% (wt/vol) pyruvate in autoclaved lake water
MD = 50 μM amino acids, 50 μM acetate, and 50 μM pyruvate in autoclaved lake water

Salt Water with Acetate (SWA)

(Halohandbook, p. 14)

23% SW stock	up to 1 liter (or autoclaved lake water)
1M NH_4Cl	5 mL
$NaC_2H_3O_2$	0.1% (w/v)
0.25 M K_2HPO_4 buffer	2 mL

The K_2HPO_4 buffer is made up by mixing 36 mL of 0.5 M K_2HPO_4 and 14 mL of 0.5 M KH_2PO_4 and diluting to 100 mL final volume. Adjust pH to about 7 and autoclave.

Salt Water with Glycerol or Methylamine (SWG, MAM)

Same as SWA recipe just replace acetate with glycerol or methylamine at 0.5% (w/v)

M63 mineral Media with 10% or 20% salt

(Garcia, *et al.*, 2004)

KH_2PO_4	13.6 g
NH_4SO_4	2.0 g
$FeSO_4$ X $7H_20$	0.5 mg
NaCl	100 g (for 10%) or 200g for (20%)
Distilled water	up to 1 Liter

After autoclaving add 1 mL of a 1M solution of $MgSO_4$ X $7H_20$ and 0.05% (w/v) of carbon source

APPENDIX B

Archaeal culturing results

Name	**Species**	**Similarity to GenBank**
HM23RS2Ar	*Haloarcula hispanica*	99%
HM23RS6br	*Haloarcula hispanica*	99%
HM18p1C6	*Haloarcula hispanica*	99%
HMRS5br	*Haloarcula hispanica*	99%
HM23RS2Ep	*Haloarcula hispanica*	99%
MedDp1B1	*Haloarcula hispanica*	99%
HM23RZ1r	*Haloarcula hispanica*	99%
MGM23pink	*Haloarcula hispanica*	99%
HM23RS6p	*Haloarcula hispanica*	98%
HM25RS2r	*Haloarcula hispanica*	99%
HM18p1B3	*Haloarcula argentinensis*	98%
MedAp1D3	*Haloarcula argentinensis*	98%
MedAp1C5	*Haloarcula hispanica*	97%
HM23RS8r	*Haloarcula hispanica*	99%
HM18C6r	*Haloarcula marismortui*	98%
HM23RS20r	*Haloarcula marismortui*	99%
MedDp1B1	*Haloarcula marismortui*	99%
MedAp1H9r	*Haloarcula marismortui*	99%
HM23RS9p	*Haloarcula marismortui*	99%
HM18p1C12	*Haloarcula marismortui*	98%
HM23RS4r	*Haloarcula marismortui*	99%
VN23RS9r	*Haloarcula marismortui*	99%
MedAp3E4	*Halovivax ruber*	98%
HM12p1C6	*Halovivax ruber*	99%
HM12p1C7	*Halovivax ruber*	98%
MedDp1i12	*Haloterrigena thermotolerans*	98%
MedDp1i11	*Halogeometricum borinquense*	89%
MedBp2B2	*Halogeometricum borinquense*	93%
MedCp1B12	*Halogeometricum borinquense*	90%
MedDp1H10	*Halogeometricum borinquense*	89%
VN23RS1p	*Natronoccus occultus*	95%
MGM18RS7	*Haloferax lucentense*	98%

MGM18RS1	*Haloferax lucentense*	99%
MGM18RS3	*Haloferax lucentense*	98%
HM23RS3p	*Natrinema altunense*	99%
3HM23RS1p	*Natrinema altunense*	99%
3HM23RS2r	*Halorubrum tebenquichense*	97%
MedDp2A1	*Halorubrum tebenquichense*	98%
HM23RS1r	*Halorubrum tebenquichense*	98%
MGM23RS1br	*Halorubrum tebenquichense*	99%
MedCp1D12	*Halorubrum sodomense*	95%
MedAp3D1n	*Halorubrum saccharovorum*	94%
MedBp2A5	*Halorubrum saccharovorum*	98%
MedAp3D1	*Halorubrum saccharovorum*	97%
MedBp1F3	*Halorubrum saccharovorum*	99%
HM25p1F2	*Halobacterium salinarum*	99%
MedAp3D1o	*Halobacterium salinarum*	97%
HM23RS5dp	*Halobacterium salinarum*	99%
HM23RW8p	*Halobacterium salinarum*	99%
HM23RS6r	*Halobacterium sp.* NRC-1	99%

Bacterial culturing results

Isolate Name	**Species**	**Similarity to GenBank**
HM12p1C8	*Rhodovibrio salinarum*	90%
HM18p1C6	*Rhodovibro salinarum*	89%
HM12C6p	*Rhodovibrio salinarum*	90%
HM12C8p	*Rhodovibrio salinarum*	93%
HM5p1C6	*Halovibrio denitrificans*	99%
MedBp4F1	*Halovibrio denitrificans*	98%
MedAp2H9	*Pseudomonas halophila*	96%
MedBp1A8	*Pseudomonas halophila*	99%
HM18p1H12	*Pseudomonas halophila*	98%
HM23p1A8	*Pseudomonas halophila*	99%
3HM23RS3w	*Pseudomonas stutzeri*	100%
HM5p1B8	*Pseudomonas stutzeri*	98%
HM18p1B7	*Pseudomonas stutzeri*	100%
HM23p1E2	*Salicola marensis*	97%
MedBp2A5	*Salicola marensis*	98%
HM23p1H11	*Salicola marensis*	97%
MedAp1H4	*Salicola marensis*	97%
HM23RS8w	*Salicola marensis*	99%
MGM23F3	*Salicola marensis*	100%

MGM23E5	*Salicola marensis*	99%
MGM23D2	*Salicola marensis*	99%
MGM23F4	*Salicola marensis*	100%
HM23p1I5	*Salicola marensis*	99%
HM23RS4dp	*Idiomarina fontislapidosi*	93%
HM5RS15	*Salinivibrio costicola*	98%
MGM23RS3	*Halomonas sp. 40*	99%
HM23RS5w	*Halomonas sp. 40*	100%
HM23RS4w	*Halomonas sp. 40*	99%
HM25RS3	*Halomonas sp. 40*	98%
MGM23RS1	*Halomonas elongata*	99%
HM23opaq	*Halomonas elongata*	100%
HM23RSW4	*Halomonas elongata*	100%
MGM23RS7	*Halomonas variabilis*	99%
HM18p1A8	*Halomonas variabilis*	100%
HM18p1H5	*Halomonas ventosae*	98%
VN12RS7	*Alkalibacillus salilacus*	95%
HM23RS1y	*Alkalibacillus salilacus*	99%
HM25RS1w	*Alkalibacillus salilacus*	99%
VN23RS6	*Alkalibacillus salilacus*	100%
HM12A1y	*Alkalibacillus salilacus*	99%
HM12B6w	*Alkalibacillus salilacus*	99%
HM10C6w	*Alkalibacillus salilacus*	99%
VN23RS9	*Alkalibacillus salilacus*	100%

APPENDIX C

API 20 NE results. Yellow and blue highlighted cells indicate disagreement with reference profile. Reference species profiles were obtained through Bergey's manual.

Species	Sample	NO3	Trp	Glu	Adh	Ure	Esc	Gel	PPG	Glu
Haloarcula hispanica	Reference	+	+/-	?	+	-	?	+	?	+
	HM23RS20r	+	-	+	+	-	+	+	-	+
	HM23RS8r	+	-	+	+	-	+	+	-	+
	HM18p1B3	+	-	+	-	+	-	+	-	+
	MedDp1B1	+	-	+	-	+	-	+	-	+
	HM25RS2r	-	-	+	+	-	+	+	-	+
Haloarcula marismortui	Reference	+	-	?	+	+	?	-	?	+
	MedAp1H9r	+	-	+	+	+	+	+	-	+
	HM23RS6r	+	-	+	+	+	+	+	-	+
Halobacterium salinarum	Reference	-	+	-	+	-	?	+	?	-
	HM23RS5dp	-	-	-	-	+	-	+	-	-
	MedAp3D1o	-	-	-	-	-	-	+	-	+
	HM23RS2p	+	-	-	-	+	-	+	-	-
Halorubrum tebenquichense	Reference	+	+	-	?	?	+	-	?	-
	3HM23RS2r	-	-	-	-	-	-	-	-	-
	MGM23R1br	-	-	-	-	-	+	-	-	-
Halorubrum saccharovorum	Reference	-	-	+	?	?	-	-	?	+
	MedBp2A5	-	-	+	-	-	-	-	-	-
	MedAp3D1n	-	-	+	-	-	-	+	-	+
	MedBp2B2	-	-	+	-	-	+	-	+	+
Halorubrum sodomense	Reference	+	-	+	?	?	+	-	?	+
	MedCp1D12	-	-	-	-	-	-	-	+	+
Natrinema altunense	Reference	+	-	+	+	?	?	?	?	+
	3HM23RS1p	-	-	+	-	-	-	+	-	+
	HM23RS3p	-	-	+	-	-	-	+	-	+
Haloferax lucentense	Reference	+	+	+	-	?	?	+	?	+
	MGM18RS7	-	-	+	-	-	-	+	-	+
	MGM18RS1	-	-	+	-	-	-	+	-	+
Halogeometricum borinquense	Reference	+	+	-	-	?	?	+	?	+
	MedDp1H10	-	-	-	+	-	-	+	-	-
	MedCp1B12	-	-	-	-	-	-	+	-	+
	MedDp1I11	-	-	-	-	-	-	+	-	+
Natronococcus occultus	Reference	+	+	-	?	?	?	+	?	+
	VN23RS1p	-	-	-	+	+	+	+	+	+
Halovivax ruber	Reference	-	-	-	-	+	-	+	?	+
	MedAp3E4	-	-	-	-	-	-	+	-	+

Archaeal results for tests 1-9

SAMPLE	Ara	Mne	Man	Ng	Mal	Gnt	Cp	Adi	Mlt	Cit	Pac
H. hispanica	?	?	+	?	+	?	?	?	+	+	?
HM23RS20r	-	+	+	+	+	+	+	+	-	+	-
HM23RS8r	-	+	+	+	+	+	+	+	+	+	+
HM18p1B3	-	+	+	+	+	+	-	+	+	+	+
MedDp1B1	-	+	+	+	+	+	+	+	+	+	-
HM25RS2r	-	+	+	+	+	+	+	+	-	+	-
H. marismortui	?	+	+	?	+	?	?	?	+	+	?
MedAp1H9r	-	+	+	+	+	+	-	-	+	+	-
HM23RS6r	-	+	+	+	+	+	-	+	+	+	-
H. salinarum	-	-	-	-	-	?	?	?	+	?	?
HM23RS5dp	-	-	-	-	+	+	-	+	+	+	+
MedAp3D1o	-	+	-	+	+	+	-	+	+	+	+
HM23RS2p	-	-	+	-	+	+	-	-	+	+	-
H. tebenquichense	-	+	-	?	-	?	?	?	-	-	?
3HM23RS2r	-	-	-	-	-	-	-	-	-	-	-
MGM23R1br	-	-	-	-	-	-	-	-	-	-	-
H. saccharovorum	-	+	?	?	+	-	-	?	?	?	?
MedBp2A5	-	+	-	-	+	-	-	-	+	+	-
MedAp3D1n	-	-	-	+	+	-	-	-	-	-	-
MedBp2B2	+	-	-	-	+	-	-	-	+	+	-
H. sodomense	-	-	?	?	+	-	?	?	?	-	?
MedCp1D12	-	-	+	+	+	-	-	-	-	-	-
N. altunense	?	-	?	?	+	?	?	?	+	+	?
3HM23RS1p	+	-	+	-	+	+	+	+	+	+	+
HM23RS3p	+	-	+	-	-	+	+	+	+	+	+
H. lucentense	+	?	+/-	?	+	?	?	?	+	+	?
MGM18RS7	+	+	+	+	+	-	-	+	+	+	+
MGM18RS1	+	+	+	+	+	-	-	+	-	+	+
H. borinquense	+	+	?	?	+	?	?	?	+	+	?
MedDp1H10	-	+	+	+	+	-	-	+	+	+	+
MedCp1B12	+	+	+	+	+	+	-	+	+	+	-
MedDp1I11	+	+	+	+	+	+	-	+	+	+	+
N. occultus	+	+	+	+	+	?	?	?	?	-	?
VN23RS1p	+	+	+	+	+	-	-	-	-	-	-
H. ruber	-	-	-	?	-	?	?	?	+	-	?
MedAp3E4	-	-	+	-	-	-	+	-	+	+	-

Archaeal results for tests 10-21

Species	Sample	NO3	Trp	Glu	Adh	Ure	Esc	Gel	PnPG	Glu
Pseudomonas stutzeri	Reference	+	-	-	-	-	-	-	-	+
	3HM23RS3w **(20%)**	-	-	-	-	-	-	-	-	+
	3HM23RS3w **(0%)**	+	-	-	-	-	-	-	-	+
	HM5p1B8	-	-	-	-	-	-	-	-	+
Pseudomonas halophila	Reference	?	-	-	-	+	+	+	+	+
	HM18p1H12	+	-	-	-	-	-	+	-	+
	MedBp1A8	+	-	+	-	-	-	+	-	-
	MedBp2A5	-	-	+	-	-	-	-	-	-
	MedBp4F1	-	-	+	-	-	-	-	-	+
Halomonas variabilis	Reference	+	-	?	?	-	-	-	?	+
	HM18p1A8	+	-	+	-	-	-	-	-	+
	MGM23RS7	+	-	+	+	-	+	-	+	+
Halomonas elongata	Reference	+	-	+	?	+	+	+	+	+
	HM25RS3	+	-	+	+	+	+	+	+	+
	MGM23RS1	+	-	+	+	+	+	+	-	+
Salicola marensis	Reference	-	-	+	?	+	+	+	+	+
	HM23RS8w	-	-	+	+	+	+	+	+	+
	HM23p1E2	-	-	-	+	+	+	+	+	+
Salinivibrio costicola	Reference	-	-	+	+	?	?	+	?	+
	HM5RS15	-	-	+	+	-	-	+	-	+
Rhodovibrio salinarum	Reference	-	-	-	?	-	?	-	-	-
	HM18p1C6	-	-	+	-	-	-	-	-	+
	HM18p1C8	-	-	+	-	-	-	-	-	+
Alkalibacillus salilacus	Reference	-	-	-	-	?	?	?	?	-
	HM23RS1y	-	-	-	-	-	-	-	-	-
	VN12RS7	-	-	-	-	+	-	-	-	-

Bacterial results for tests 1-9

Sample	Ara	Mne	Man	Nag	Mal	Gnt	Cap	Adi	Mlt	Cit	Pac
P. stutzeri	-	-	+	+	+	+	+	+	+	-	+
3HM23RS3w **(20%)**	-	-	+	+	+	+	+	-	+	+	-
3HM23RS3w **(0%)**	-	-	+	-	+	+	+	-	+	+	-
HM5p1B8	-	-	+	-	+	+	+	-	+	+	-
P. halophila	+	-	+	?	+	?	+	?	?	+	?
HM18p1H12	+	-	-	-	+	-	-	-	+	+	+
MedBp1A8	+	-	-	-	+	-	-	-	+	+	+
MedBp2A5	-	+	-	-	+	-	-	-	+	+	-
MedBp4F1	+	-	-	-	+	-	-	-	+	+	+
H. variabilis	-	-	+	?	+	+	?	?	?	+	?
HM18p1A8	+	-	+	+	+	+	+	+	+	+	+
MGM23RS7	+	+	+	+	+	+	-	-	+	+	+
H. elongata	?	+	+	?	?	+	?	?	?	?	?
HM25RS3	+	+	+	+	+	+	+	+	+	+	+
MGM23RS1	+	+	+	+	+	+	+	+	+	+	+
S. marensis	+	-	+	?	+	?	+	?	-	+	?
HM23RS8w	+	+	+	-	+	-	+	-	-	+	+
HM23p1E2	+	+	+	-	+	-	+	-	+	+	+
S. costicola	-	+	-	+	-	?	?	?	-	-	?
HM5RS15	-	+	-	+	-	+	+	+	+	-	-
R. salinarum	+	-	+	-	-	?	?	?	?	+	?
HM18p1C6	-	-	-	-	-	-	-	-	-	-	-
HM18p1C7	-	-	-	-	-	-	-	-	-	-	-
A. salilacus	+	-	-	?	-	-	?	?	-	-	?
HM23RS1y	-	-	-	-	-	-	-	-	-	-	-
VN12RS7	-	-	-	-	-	-	-	-	-	-	-

Bacterial results for test 10-21