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ORIGINAL PAPER

Life in extreme environments: microbial diversity in Great Salt Lake, Utah

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Abstract Great Salt Lake (GSL) represents one of the world's most hypersaline environments. In this study, the archaeal and bacterial communities at the North and South arms of the lake were surveyed by cloning and sequencing the 16S rRNA gene. The sampling locations were chosen for high salt concentration and the presence of unique environmental gradients, such as petroleum seeps and high sulfur content. Molecular techniques have not been systematically applied to this extreme environment, and thus the composition and the genetic diversity of microbial communities at GSL remain mostly unknown. This study led to the identification of 58 archaeal and 42 bacterial operational taxonomic units. Our phylogenetic and statistical analyses displayed a high biodiversity of the microbial communities in this environment. In this survey, we also showed that the majority of the 16S rRNA gene sequences within the clone library were distantly related to previously described environmental halophilic archaeal and bacterial taxa and represent novel phylotypes.

Keywords *Archaea* · *Bacteria* · GSL · Biodiversity · Halophiles · 16S rRNA

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Introduction

Molecular evidence has greatly increased the appreciation of microbial biodiversity and has contributed to a general consensus that the microbial world is much more diverse and complex than anyone had anticipated (Pace 1997; Harris et al. 2013). Such studies are particularly important in extreme environments where the microbial biodiversity is little explored and particularly unique.

Great Salt Lake (GSL) represents one of the world's most extreme environments, it is the fourth largest terminal lake and the second most saline lake in the world (Hassibe and Keck 1978). In the late 1950s, a railroad causeway was built, which subsequently separated the lake into a North Arm and a South Arm and induced an environmental evolution of the habitat (Cannon and Cannon 2002). In historical time, the lake's salinity has ranged from a little less than 5 % to nearly 27 % (Utah geological survey, online publications). This lake has indeed unique characteristics when compared to other hypersaline environments. Whereas the South Arm maintains a salinity level of about 9 %, the North Arm is characterized by high levels of salinity (as high as 30 % w/v). The North Arm, near Rozel Point, is also influenced by numerous petroleum seeps that introduce into the lake nitrogen (0.5 %) and high sulfur (14 %) containing asphaltic oil (Gwynn 1980; Sinninghe Damsté et al. 1987). Moreover, the North Arm of the lake has no significant freshwater input, which prevents important fluctuations in salinity. The petroleum seeps at Rozel Point were discovered in the late 1800s, and oil production attempts began in 1904 (Eardley 1963).

Extensive studies of hypersaline environments in various geographical locations have led to the isolation and characterization of the microbial communities found in these environments (e.g., Litchfield and Gillevet 2002;

Burns et al. 2004; Maturrano et al. 2006; Mesbah et al. 2007; Clementino et al. 2008; Pagaling et al. 2009; Boutaiba et al. 2011; Ghai et al. 2011; Oren 2012; Podell et al. 2013). Classical biological and microbiological studies have found in GSL, only a limited variety of identifiable halophiles that have adapted to the various levels of salinity (Post 1977, 1981; Tsai et al. 1995; Wainø et al. 2000; Weimer et al. 2009). However, molecular characterization of the microbial biodiversity in GSL still remains very limited (Baxter et al. 2005; Parnell et al. 2011; Meuser et al. 2013). This type of study is warranted to shed the light on the microbial structure in GSL and will provide greater insights into the microbial diversity by sampling genomes from organisms not amenable to classical approaches.

In this study, we performed a survey of the archaeal and bacterial communities present in the North Arm and the South Arm of GSL by analyzing 16S rRNA gene sequences using phylogenetic approaches. We assessed the biodiversity detected in the lake and compared it with the microbial communities previously characterized in other hypersaline environments. We also examined the biogeographical patterns displayed by the microbial communities in this ecosystem. GSL represents a unique environment and it is clearly understudied in terms of its microbial ecology. Such examination of the microbial life in GSL provides a new perspective on the structure and composition of the microbial communities found in this extreme environment.

Materials and methods

Sample collection and extraction of genomic DNA

Surface water samples (a total volume of 100 ml for each sample) were collected in September and November 2004 from 20 different sampling sites located at GSL North Arm (Rozel Point) and GSL South Arm (Antelope Island State Park). The samples were collected from 17 various locations at Rozel Point, near the Spiral Jetty (41°25′N 112°39′W 1,281 m), in the immediate vicinity of oil seeps and from three different locations at Antelope Island State Park, near the shore of the lake (40°57′N 112°36′W 1,280 m). We received permission to collect in GSL from the Utah Division of Natural Resources (DNR) and this permit is on file at Brigham Young University. Our activities were not regulated by any other agency. In this study, our field research had no impact on vertebrate or protected species. During this sampling period, the water level in the lake was very low and the salinity had reached its maximum value in GSL North Arm (30 % w/v). By comparison to Antelope Island, Rozel Point is an oil seep environment; indeed the production rates of 5–10 barrels of oil per day were reported by Eardley (1963). This site is also known for its unusually high sulfur content up to 14 % per weight (Sinninghe Damsté et al. 1987). All water samples were stored in sterile Whirl–Pak plastic bags (Fisher Scientific) and kept at −20 °C until further analysis.

In order to obtain microbial DNA for sequencing, water samples (30 ml each) were centrifuged at 20,000 rpm for 1 h (Sorvall RC 5 Plus Superspeed Centrifuge, GMI Inc., Ramsey, MN, USA). After centrifugation, the supernatant was discarded and the rest of the water sample was added to the corresponding pellet for another round of centrifugation. Total genomic DNA was extracted from the pellets by the methods described in Crandall et al. (1999). The quantity of DNA from each sample was evaluated by measuring the optical density at 260 nm, and its quality was checked by electrophoresis in an agarose gel (1 %). The DNA (100 ng/µl) was stored at −20 °C in TE buffer (pH 8.0) prior to analysis.

16S rRNA clone library construction

In order to characterize the microbial communities present in this ecosystem, we generated 16S rRNA sequences from all 20 water samples collected in this study. PCR amplification of the 16S rRNA gene was performed using the primers 6F (5′-CGGTTGATCCYGCCGGM-3′) and 741R (5′-GACTACCSGGGTATCTAATCC-3′) for *Archaea*, and the primers 38F (5′-CAGGCCTAACACATGCAAGTC-3′) and 882R (5′-GTTTTAACCTTGCGGCCGTACTCC-3′) for *Bacteria*. These primers amplify a segment of the 16S rRNA gene (5′-end) with an average size of 850 bp. The size of the fragment analyzed is sufficiently discriminating to assess the microbial diversity in GSL, and is comparable to that used in other studies and therefore allows for a broad comparison (Cytryn et al. 2000; de Souza et al. 2001; Souza et al. 2006). PCR conditions were 95 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 60 °C (for *Archaea*) or 65 °C (for *Bacteria*) for 1 min, and 72 °C for 2 min 30 s, with a final 20-min extension at 72 °C.

The cloning and transformation procedure for all the samples under study were performed using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer recommendations. The successfully cloned fragments were sequenced with included M13 vector primers (forward and reverse primers) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on an ABI 3730XL DNA Analyzer.

16S rRNA gene library analysis

The 16S sequences were analyzed using Sequence Navigator Software (PE Applied Biosystems) and were checked

for chimera formation using DECIPHER (Wright et al. 2012). Sequences were compared with each other and those with >97 % identity were clustered as operational taxonomic units (OTUs) using MOTHUR software v.1.31.2 (Schloss et al. 2009). This cut-off is a commonly used level for comparative analysis in microbial communities (Nam et al. 2011). For the grouping into OTUs, we used 175 archaeal clone sequences (149 sequences from GSL North Arm and 26 sequences from GSL South Arm) and 212 bacterial clone sequences (172 sequences from GSL North Arm and 40 sequences from GSL South Arm). We then compared our 16S rRNA sequences to the NCBI GenBank database (December 2013) and to the ribosomal database project (RDP, Release 11, Update 1, December 2013) (Cole et al. 2009). Since RDP is known to be more informative than GenBank for taxonomic assignment (Cole et al. 2007), we chose to use the comparison results from RDP to identify environmental sequences with the highest sequence identity for inclusion in our analyses. In addition, we included sequences from microbial species found in other hypersaline environments and/or those already characterized from GSL. Taxa found in other archaeal and bacterial phyla and in petroleum environments were also added to this collection for comparison purposes.

Each data set was aligned using MUSCLE, a multiple sequence alignment software (Edgar 2004), and the resulting alignments were checked using MacClade (Maddison and Maddison 2005). Because many environmental sequences used for the analyses are extremely divergent from the archaeal and bacterial ingroup taxa, and therefore difficult to align reliably, Gblocks v0.91b was used to select the conserved blocks in the alignments prior to phylogenetic analyses (Castresana 2000).

Phylogenetic analyses

The phylogenetic relationships from the combined data set were estimated using maximum likelihood analysis (Felsenstein 1981) with nodal support assessed via bootstrapping (100 pseudo-replicates) (Felsenstein 1985) as implemented in PhyML v3.0 (Guindon et al. 2010). Different sister groups to the archaeal and bacterial communities were chosen as outgroups for rooting our phylogenetic trees. For the archaeal sequence analysis, we selected the phyla *Crenarchaeota*, *Korarchaeota*, and *Nanoarchaeota* to serve as outgroups. For the bacterial sequence analysis, we selected *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Cyanobacteria*, *Spirochaetes*, *Chlamydiae*, *Deinococcus-Thermus*, and *Aquificae* to serve as outgroups. These outgroups confirmed the stability of the topology of the trees. Model selection for these analyses followed the procedure outlined by Posada and Buckley (2004) as implemented in ModelTest v3.7 (Posada and Crandall 1998). The model chosen for both communities was GTR+I+G selected by the Akaike information criterion (AIC). Theoretically, AIC reduces the number of unnecessary parameters that contribute little to describing the data by penalizing more complex models.

Statistical analyses

The MOTHUR software was used to calculate archaeal and bacterial richness and diversity (Schloss et al. 2009). The diversity indices included the non-parametric richness estimators ACE, Chao1, the Simpson diversity index and the Shannon–Weaver diversity index. The library coverage was also calculated using MOTHUR software (Schloss et al. 2009). For all these estimates, we used the default setting with a 3 % cut-off in MOTHUR. Species evenness (species' relative abundance) was calculated using Pielou's evenness index (Pielou 1975). A value of evenness close to 1 means that the community is diverse, whereas a value close to 0 means that the community is not diverse.

Nucleotide sequence accession numbers

All the archaeal and bacterial sequences of the 16S rRNA gene clone library generated in this study were deposited in NCBI GenBank database under accession numbers KF160960 to KF161059 and KF585215 to KF585501.

Results

Biodiversity of the archaeal and bacterial clone libraries

In this study, we used a total of 175 archaeal and 212 bacterial clones. 58 OTUs were identified from the archaeal library and 42 from the bacterial library (Table 1). A comparison with environmental 16S sequences deposited in RDP website showed a different distribution in the archaeal and bacterial OTUs in GSL (Fig. 1). Of the total archaeal OTUs, 5 % showed less than 60 % identity with formally described lineages referenced in RDP and the majority of archaeal OTUs (57 %) displayed identity levels ranging from 60 to 90 % (Table 1; Fig. 1). Only 38 % of the total archaeal OTUs showed higher sequence identities (≥90 % identity). A different scenario was observed in the bacterial communities in GSL. The bacterial OTUs included a higher proportion of sequences (62 % of the total OTUs analyzed) displaying a high sequence identity in RDP (≥90 % identity). However, only 38 % of the total bacterial OTUs sequences showed in RDP a sequence identity ranging from 60 to 90 %, and none of the bacterial clone sequences showed a sequence identity lower than 60 % (Table 1; Fig. 1). In comparison with the archaeal

Table 1 16S rRNA clone sequences identified in Great Salt Lake, UT

OTUs	Accession No.	No. of clones	Closest sequence match with RDP (Accession No.)	% identity
Archaea				
ArchA1	KF160960	1	Uncultured archaeon (HQ197772)	96
ArchA2	KF160961	1	Uncultured haloarchaeon (FN391240)	84
ArchA3	KF160962	1	Uncultured haloarchaeon (FN391240)	82
ArchA4	KF160963	1	Uncultured haloarchaeon (FN391240)	76
ArchA5	KF160964	1	Uncultured haloarchaeon (GQ374968)	92
ArchA6	KF160965	1	*Halobacteriaceae* archaeon R35 (HM159597)	85
ArchA7	KF160966	2	Uncultured haloarchaeon (FN391198)	97
ArchA8	KF160967	1	Uncultured *Haloquadratum* sp. (AM947447)	92
ArchA9	KF160968	1	Uncultured haloarchaeon (GQ375030)	80
ArchA10	KF160969	1	Uncultured haloarchaeon (GQ375030)	85
ArchA11	KF160970	1	Uncultured haloarchaeon (GQ375030)	94
ArchA12	KF160971	17	*Haloquadratum walsbyi* C23 (FR746099)	98
ArchA13	KF160972	2	*Halobacteriaceae* archaeon EB27 (HQ197980)	68
ArchA14	KF160973	1	Uncultured haloarchaeon (JN714408)	61
ArchA15	KF160974	1	*Halobacteriaceae* archaeon EB27 (HQ197980)	69
ArchA16	KF160975	1	*Halobacteriaceae* archaeon EB27 (HQ197980)	75
ArchA17	KF160976	1	*Natronomonas moolapensis* (AB576127)	96
ArchA18	KF160977	1	*Halobacteriaceae* archaeon TNN50 (GQ282622)	83
ArchA19	KF160978	1	Uncultured archaeon (HQ425185)	90
ArchA20	KF160979	1	Uncultured haloarchaeon (FN669144)	74
ArchA21	KF160980	1	*Halorhabdus utahensis* (FN994968)	89
ArchA22	KF160981	1	Uncultured euryarchaeote (FN391283)	88
ArchA23	KF160982	1	Uncultured euryarchaeote (FN391283)	86
ArchA24	KF160983	1	Uncultured haloarchaeon (JN714408)	78
ArchA25	KF160984	1	Uncultured archaeon (EU722673)	90
ArchA26	KF160985	1	Uncultured *Halobacterium* sp. (FN391290)	94
ArchA27	KF160986	1	*Haloarcula marismortui* ATCC 43049 (AY596298)	78
ArchA28	KF160987	1	*Haloarcula hispanica* (DQ089683)	93
ArchA29	KF160988	3	*Haloarcula japonica* (EF645686)	91
ArchA30	KF160989	1	*Halorubrum* sp. SS5-7 (JN196484)	87
ArchA31	KF160990	1	Uncultured haloarchaeon (AY498640)	84
ArchA32	KF160991	4	*Halorubrum chaoviator* (D32081)	98
ArchA33	KF160992	1	Haloarchaeon CSW5.28.5 (AY498647)	81
ArchA34	KF160993	1	Uncultured *Halobacterium* sp. (FN994969)	97
ArchA35	KF160994	8	Uncultured haloarchaeon (FN391245)	92
ArchA36	KF160995	1	Uncultured haloarchaeon MSP23 (FN994985)	78
ArchA37	KF160996	1	Uncultured haloarchaeon (AM947489)	76
ArchA38	KF160997	1	Uncultured haloarchaeon MSP23 (FN994985)	94
ArchA39	KF160998	4	Uncultured euryarchaeote (FN391272)	99
ArchA40	KF160999	1	Uncultured haloarchaeon (AM947482)	79
ArchA41	KF161000	1	Uncultured haloarchaeon (GQ375029)	83
ArchA42	KF161001	1	Uncultured haloarchaeon (FN391228)	75
ArchA43	KF161002	1	Uncultured haloarchaeon (AM947458)	83
ArchA44	KF161003	1	Uncultured haloarchaeon (FN391247)	86
ArchA45	KF161004	1	Uncultured haloarchaeon (AM947462)	90
ArchA46	KF161005	3	Uncultured haloarchaeon (AM947461)	98
ArchA47	KF161006	8	Uncultured haloarchaeon (FN391232)	89

Table 1 continued

OTUs	Accession No.	No. of clones	Closest sequence match with RDP (Accession No.)	% identity
ArchA48	KF161007	8	*Halonotius pteroides* (HM159612)	91
ArchA49	KF161008	11	*Halonotius pteroides* (AY498646)	97
ArchA50	KF161009	3	Uncultured haloarchaeon (GQ374975)	87
ArchA51	KF161010	1	Uncultured haloarchaeon (FN391228)	88
ArchA52	KF161011	1	Uncultured haloarchaeon (AM947471)	83
ArchA53	KF161012	1	Uncultured archaeon (HM187576)	83
ArchA54	KF161013	57	Uncultured archaeon (HM187576)	94
ArchA55	KF161014	1	Uncultured euryarchaeote (HQ692057)	30
ArchA56	KF161015	1	Uncultured euryarchaeote (FN391261)	83
ArchA57	KF161016	1	Uncultured euryarchaeote (HQ658682)	46
ArchA58	KF161017	1	Uncultured SA1 group euryarchaeote (AJ347785)	39
Bacteria				
BactB1	KF161018	5	Uncultured *Alteromonadales* bacterium (JN038319)	89
BactB2	KF161019	3	*Idiomarina loihiensis* L2TR (AE017340)	96
BactB3	KF161020	1	Uncultured *Idiomarina* sp. (DQ234155)	93
BactB4	KF161021	9	*Idiomarina seosinensis* (AY635468)	90
BactB5	KF161022	1	Uncultured bacterium (JQ800711)	89
BactB6	KF161023	2	*Halomonas* sp. HE0.1.1 (GU228486)	92
BactB7	KF161024	1	Uncultured bacterium (HM128406)	97
BactB8	KF161025	22	*Halomonas* sp. YCSA31-1 (HQ536998)	93
BactB9	KF161026	3	*Halomonas sediminis* (EU135707)	94
BactB10	KF161027	1	Uncultured bacterium (HM128202)	75
BactB11	KF161028	1	*Salicola marasensis* (DQ087262)	89
BactB12	KF161029	15	*Salicola marasensis* (DQ087262)	93
BactB13	KF161030	1	*Salicola* sp. PV3 (FJ042665)	75
BactB14	KF161031	8	Uncultured *Halorhodospira* sp. (FN393473)	91
BactB15	KF161032	4	Uncultured *proteobacterium* (AY862776)	95
BactB16	KF161033	9	*Pseudomonas fluorescens* (GU198110)	98
BactB17	KF161034	20	*Pseudomonas* sp. R-37908 (HE586385)	98
BactB18	KF161035	1	*Pseudomonas* sp. BU (AF482684)	90
BactB19	KF161036	1	*Pseudomonas putida* (JQ782896)	96
BactB20	KF161037	1	*Pseudomonas* sp. 38B (AB638853)	96
BactB21	KF161038	1	Uncultured *Microbulbifer* sp. (JF421174)	91
BactB22	KF161039	1	*Alteromonadales* bacterium G-He6 (EF554905)	77
BactB23	KF161040	25	Uncultured bacterium (HQ190498)	73
BactB24	KF161041	2	*Providencia* sp. IICDBZ10 (JN836927)	96
BactB25	KF161042	4	*Enterobacter cloacae* (JN969314)	96
BactB26	KF161043	3	*Salinivibrio costicola* (AJ640132)	99
BactB27	KF161044	4	*Alteromonas* sp. JAM-GA13 (AB526336)	94
BactB28	KF161045	2	*Pseudoalteromonas* sp. BSw10020 (DQ789376)	90
BactB29	KF161046	1	*Pseudoalteromonas* sp. TA010_2 (EU308472)	97
BactB30	KF161047	3	*Stenotrophomonas maltophilia* (AB294557)	99
BactB31	KF161048	8	Uncultured bacterium (HM128070)	98
BactB32	KF161049	1	Uncultured bacterium (HM630158)	81
BactB33	KF161050	1	Uncultured alphaproteobacterium (EF105797)	98
BactB34	KF161051	1	*Marispirillum indicum* (EU642410)	69
BactB35	KF161052	2	*Rhodospirillaceae* bacterium Ia16 (JN605361)	71
BactB36	KF161053	1	Uncultured bacterium (HQ864210)	61

Table 1 continued

OTUs	Accession No.	No. of clones	Closest sequence match with RDP (Accession No.)	% identity
BactB37	KF161054	1	Uncultured bacterium (JX884996)	78
BactB38	KF161055	23	Uncultured bacterium (JX884557)	93
BactB39	KF161056	15	Uncultured bacterium (JX883961)	85
BactB40	KF161057	1	Uncultured bacterium (JX883165)	82
BactB41	KF161058	2	Uncultured bacterium (JX883165)	86
BactB42	KF161059	1	Uncultured bacterium (JQ989609)	63

In this table, the closest sequence match with RDP indicates the name designations provided in this database of the environmental sequences with the highest sequence identity with our archaeal and bacterial OTUs

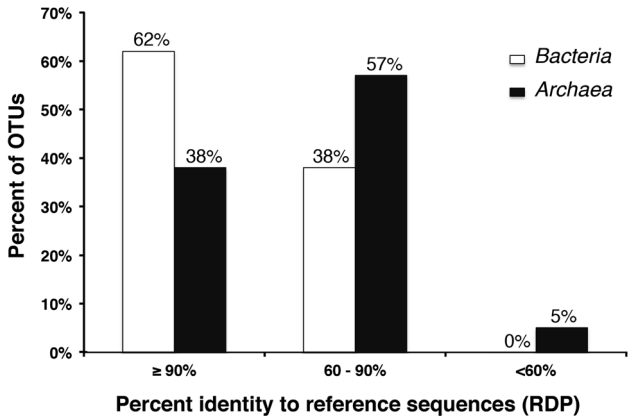

Fig. 1 Archaeal and bacterial community composition in Great Salt Lake, UT, based on sequence identity found in RDP

OTUs in GSL, the majority of the bacterial clone sequences (62 % in comparison with only 38 % in archaeal OTUs) were more closely related to previously described environmental lineages. Furthermore, the comparison of our clone library with the sequences deposited in RDP showed that 78 % of the total archaeal OTUs (45 archaeal OTUs) and 43 % of the total bacterial OTUs (18 bacterial OTUs) did not associate with any of the previously known taxa and might represent novel environmental species yet to be determined (Table 1).

Phylogenetic analyses showed also a very high level of biodiversity among the archaeal and bacterial communities in GSL (Figs. 2, 3). All archaeal OTUs belonged to the phylum *Euryarchaeota* (Fig. 2). Even though most of the OTUs did not cluster with environmental sequences, representatives of the following genera: *Natronomonas*, *Halorhabdus*, *Halorubrum*, *Haloquadratum*, *Haloferax*, *Halogeometricum*, *Haloarcula* and *Halobacterium* were represented in our archaeal clone library (Fig. 2). Bacterial OTUs showed, in contrast to archaeal OTUs, a higher proportion of clones clustered with environmental sequences (Fig. 3). All bacterial OTUs grouped within the phylum *Proteobacteria*, except a single OTU that clustered within the phylum *Firmicutes*. Moreover, the majority of the bacterial OTUs clustered with environmental taxa known to degrade hydrocarbons: *Shewanella*, *Halomonas*, *Idiomarina*, *Alcanivorax*, *Pseudomonas* and *Marinobacter* (Fig. 3).

Diversity and richness analyses

Richness estimators (Chao1 and ACE) were significantly higher than the observed number of OTUs in both archaeal and bacterial communities (Table 2). These results indicate that the diversity observed in this study is, by far, an underestimate of the total archaeal and bacterial diversity present in GSL. A further sampling would yield a more accurate assessment of the diversity in these communities. Also, Shannon–Weaver and Simpson analyses indicated a high biodiversity within the archaeal (3.99 and 0.0018, respectively) and bacterial (3.67 and 0.0023) clone libraries (Table 2). The low level found in library coverage in both communities represents another indication for an incomplete representation of the biodiversity in these microbial communities (Table 2). A more extensive sampling in GSL is warranted. Moreover, values obtained of evenness for both archaeal and bacterial libraries (0.98) reinforce the statement of high biodiversity in GSL (Table 2).

Discussion

In this study, a culture-independent survey of the archaeal and bacterial communities was performed in the North (Rozel Point) and South (Antelope Island) Arms of GSL. Since the studies by Post in the 1970s (Post 1977), little information is available on the microbial community structure in GSL (Weimer et al. 2009; Parnell et al. 2009, 2011; Baxter et al. 2005). A very recent study (Meuser et al. 2013) examined the community assembly in the stratified water column in the South Arm of GSL, however knowledge on species composition of the archaeal and bacterial communities in this extreme environment still

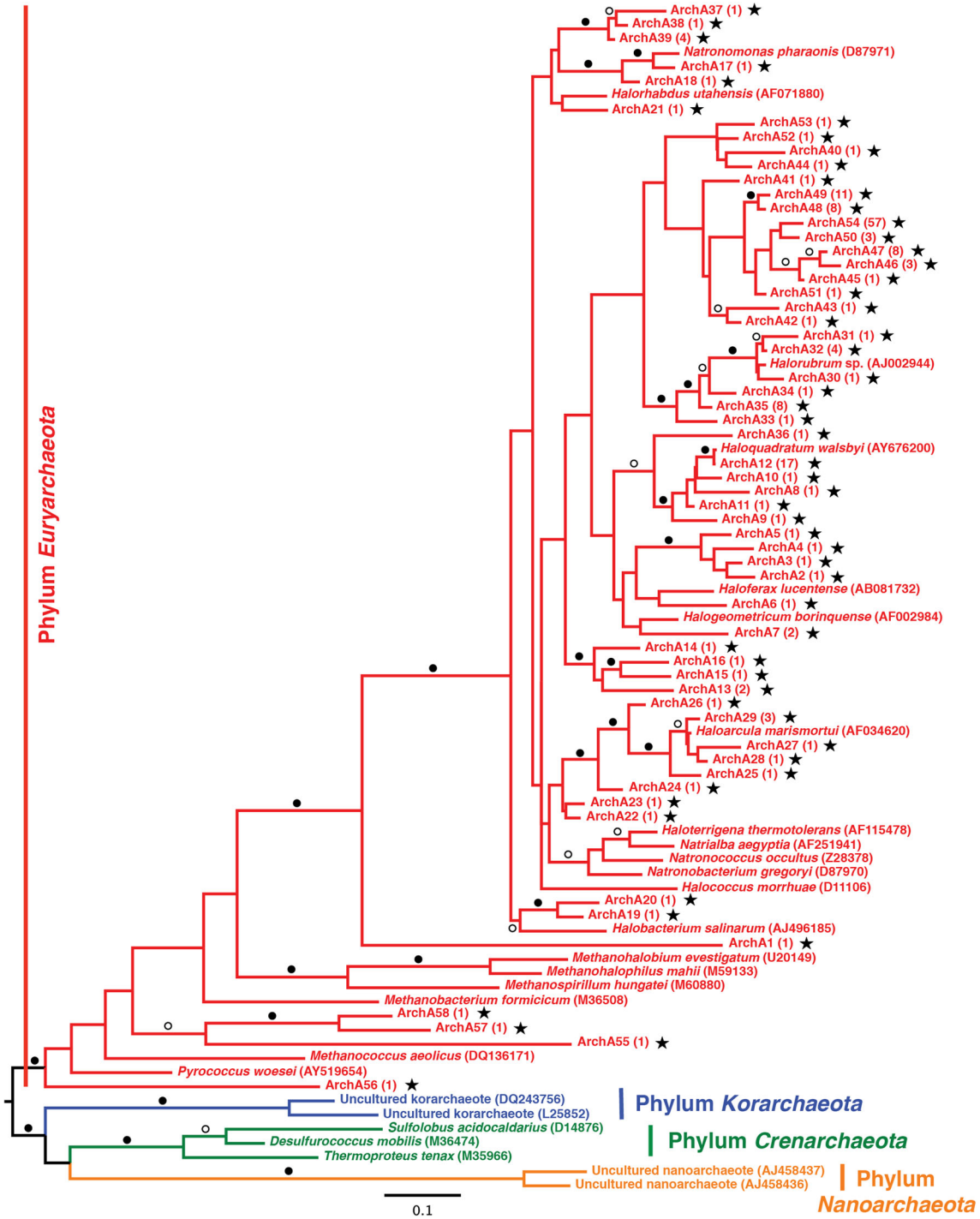

Fig. 2 Phylogenetic analysis showing the biodiversity of the archaeal clone library in Great Salt Lake, UT, along with reference sequences belonging to different phyla. The archaeal OTUs described in this study are marked with a *star symbol*. Branch points with bootstrap values of >90 and 70–90 % are shown in *dark* and *clear circles*, respectively. Branch points *without circles* are not resolved (bootstrap values <70 %). An uncultured nanoarchaeote (AJ458436) was used as outgroup in this tree

remains very limited. This ecosystem remains indeed understudied, especially when its large size and high number of microenvironments are taken into account. In this study, our analysis revealed a high biodiversity in this ecosystem and an abundance of yet-to-be described archaeal and bacterial taxa.

Many of our OTU clone libraries from GSL are unequivocally different and unique from other hypersaline

Fig. 3 Phylogenetic analysis showing the biodiversity of the bacterial clone library in Great Salt Lake, UT, along with reference sequences belonging to different phyla. The bacterial OTUs described in this study are marked with a *star symbol*. Branch points with bootstrap values of >90 and 70–90 % are shown in *dark* and *clear circles*, respectively. Branch points *without circles* are not resolved (bootstrap values <70 %). *Chlamydophila abortus* (AB001816) was used as outgroup in this tree

environments, with 45 archaeal and 18 bacterial OTUs not affiliated with any of the previously described taxa. Similar findings have been observed in other extreme environments where diverse and novel microbial communities were also detected (Harris et al. 2013; Mesbah et al. 2007; Podell et al. 2013; Walker et al. 2005; Boujelben et al. 2012; Huber et al. 2002; Daffonchio et al. 2006).

The diversity indices in addition to the other statistical parameters estimated in this study also indicated that both the archaeal and bacterial communities in GSL were highly diverse and that a more extensive sampling in this environment was warranted to allow a better estimate of species richness. While this survey was modest compared to some environmental sampling efforts (Venter et al. 2004), it still

Table 2 Statistical estimates for archaeal and bacterial 16S rRNA clone sequences from Great Salt Lake, UT

Parameters	*Archaea*	*Bacteria*
Total of sequenced clones	175	212
OTUs	58	42
Chao1	386.5	274.3
ACE	531.7	420
Shannon–Weaver's diversity index	3.99	3.97
Simpson's diversity index	0.0018	0.0023
Library coverage (%)	10	9
Evenness	0.98	0.98

A cut-off value of 3 % is used in these analyses

represents a prerequisite step to assess the biodiversity in the microbial communities recovered from GSL.

Since a large proportion of the bacterial clones were clustered with petroleum-degrading bacteria, one might hypothesize that the abundance of yet-to-be described archaeal and bacterial OTUs in GSL could be explained by their ability to benefit from the presence and metabolism of petroleum hydrocarbons, particularly at Rozel Point. These new microbial communities might have evolved in this ecosystem to use petroleum as a carbon source. Furthermore, though oil degradation by halophilic bacteria has been well documented (Riis et al. 2003; Brakstad and Lødeng 2005), such a biological process has yet to be conclusively demonstrated in archaeal communities (Head et al. 2006; Tapilatu et al. 2010) despite some studies showing that these microorganisms are able to grow in presence of hydrocarbons (Raghavan and Furtado 2000; Tischer et al. 2012).

The current survey performed on microbial communities in GSL demonstrates the intriguing abundance of novel phylotypes in archaeal and bacterial clone libraries in addition to the high diversity in the overall microbial composition in this ecosystem. Similar findings have been also documented in recent studies (Weimer et al. 2009; Parnell et al. 2009, 2011). Based on the comparison with environmental sequences deposited in RDP and the low sequence identity found with previously described taxa, these new lineages seem to be exclusively present in GSL and might represent distinct microbial communities than the ones found in other hypersaline environments (Litchfield and Gillevet 2002; Sørensen et al. 2005; Lay et al. 2012).

Microbial biodiversity studies have led to two major conflicting hypotheses (Martiny et al. 2006). The first is the "Global Dispersal" hypothesis, which suggests that microorganisms are ubiquitous and have few barriers to gene flow resulting in similar microbial communities across different spatial scales and habitats (Baas-Becking 1934; de Wit and Bouvier 2006; O'Malley 2007; Finlay 2002). The alternative is the "Barriers to Dispersal" hypothesis, which suggests microbial community differentiation is driven by ecological and/or geographic barriers, showing mostly similar patterns as those seen in plants and animals (Whitaker et al. 2003; Horner-Devine et al. 2004; Bell et al. 2005; Fierer and Jackson 2006). Since this study showed an abundance of novel phylotypes that have not yet been described in other hypersaline environments, this finding supports the "Barriers to Dispersal" hypothesis, in which local factors in an environment dictate the selection of adapted organisms from a global pool (Daffonchio et al. 2006; Fierer and Jackson 2006; Rengefors et al. 2012; Logares et al. 2013). However, the mechanism driving "Barriers to Dispersal" requires further investigation. Adaptation driven by ecological parameters and/or mere physical isolation could be responsible for driving the microbial biodiversity in GSL (Whitaker et al. 2003; Papke and Ward 2004). Therefore, both ecological and geographic barriers may be the driving forces creating and maintaining biodiversity in GSL, and thus responsible for the development of global microbial community structure in this unique ecosystem. A recent study also showed that adaptation to high salt content was associated with a specific genome signature (Paul et al. 2008), and this finding may support the "Barriers to Dispersal" hypothesis in the GSL microbial community. An extensive sampling in this ecosystem combined with a thorough comparison with similar environments in other locations throughout the world is therefore needed to elucidate the questions related to the microbial biogeography in GSL. Furthermore, the composition of microbial assemblages in GSL points out spatial and evolutionary relationships of the microbiota in extreme environments, and suggests low levels of gene flow that maintains the distinct microbial community structure in this ecosystem (Souza et al. 2006; Rengefors et al. 2012). A better understanding of the microbial diversity in GSL will also shed the light into the biogeochemical processes in this ecosystem. The findings of this study provide a glimpse into the ecological relationships among microbial communities in GSL, as well as an additional perspective on the nature of life in such extreme environments.

Given our results, we are also attempting to culture these novel species belonging to the different new phylotypes to characterize better their biological properties and to elucidate the differences between these new microbial communities found in GSL and all the previously described archaeal and bacterial communities. The characterization of these new archaeal and bacterial lineages found in GSL will allow the preparation of probes for these specific communities (FISH analysis) to estimate their relative abundance and to monitor the quantitative changes in the

community composition in this unique ecosystem over seasons and multiple years (Konno et al. 2013).

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