ELSEVIER

FEMS Microbiology Letters 183 (2000) 81–88

FEMS MICROBIOLOGY LETTERS

www.fems-microbiology.org

The microbial composition of three limnologically disparate hypersaline Antarctic lakes

John P. Bowman [a,b,*], Sharee A. McCammon [b], Suzanne M. Rea [a,c], Tom A. McMeekin [a,b]

[a] *Cooperative Research Center for the Antarctic and Southern Ocean, University of Tasmania, Hobart, Tasmania, Australia*
[b] *School of Agricultural Science, University of Tasmania, Hobart, Tasmania, Australia*
[c] *Institute for Antarctic and Southern Ocean Studies, University of Tasmania, Hobart, Tasmania, Australia*

Received 29 September 1999; received in revised form 15 November 1999; accepted 25 November 1999

Abstract

16S rRNA clone library analysis was used to examine the biodiversity and community structure within the sediments of three hypersaline Antarctic lakes. Compared to sediment of low to moderate salinity Antarctic lakes the species richness of the hypersaline lake sediments was 2–20 times lower. The community of Deep Lake (32% salinity, average sediment temperature −15°C) was made up almost entirely of halophilic Archaea. The sediment communities of two meromictic hypersaline lakes, Organic Lake (20% salinity, −7°C) and Ekho Lake (15% salinity, 15°C) were more complex, containing phylotypes clustering within the Proteobacteria and Cytophagales divisions and with algal chloroplasts. Many phylotypes of these lakes were related to taxa more adapted to marine-like salinity and perhaps derive from bacteria exported into the sediment from the lower salinity surface waters. The Ekho Lake clone library contained several major phylotypes related to the Haloanaerobiales, the growth of which appears to be promoted by the comparatively high in situ temperature of this lake.

Keywords: Antarctica; Hypersaline lake; 16S rRNA; Biodiversity; Halophilic Archaeon; Haloanaerobiales

1. Introduction

The Vestfold Hills lake system (68°S 78°E) of Eastern Antarctica was formed during the Holocene by isostatic glacial marine uplift, eustatic sea level rise and depending on location, glacio-eustatic rebound [1]. The lake system includes an immense variety of lacustrine environments; about 20% of the world's meromictic lakes are found here. According to diatom palaeorecords, the lakes have been relatively stable for the last 4000 years [2]. Bacteria which have been cultivated from Vestfold Hill marine salinity lakes are close relatives of cultivated marine bacteria [3]. Limnological studies on various hypersaline lakes in the Vestfold have revealed they are disparate in physical and chemical properties.

This study focuses its attention on three hypersaline lakes, Ekho Lake, Organic Lake and Deep Lake. Ekho Lake is a 40 m deep, heliothermal meromictic lake. Organic Lake is a shallow meromictic lake with unusually high levels of dimethylsulfide in its bottom waters [4]. Deep Lake is a 36 m deep, monomictic lake possessing salinity 10 times that of seawater and extreme frigidity (−14°C to −18°C) [5]. These lakes are nearly or completely perennially ice-free, and were formed by evaporation of seawater. Interest in extreme environments prompted a study of the microbial composition of these lakes using 16S rRNA gene sequences, amplified and cloned from the lake sediments. This strategy has been employed due to the limitations of traditional cultivation methods, which are not sufficient for culturing most Bacteria or Archaea [6].

2. Materials and methods

2.1. Samples

Sediments from the deepest point of Ekho, Organic and

* Corresponding author. Tel.: +61 (3) 6226 2776; Fax: +61 (3) 6226 2642; E-mail: john.bowman@utas.edu.au

0378-1097/00/$20.00
PII: S0378-1097(99)00633-3

Deep Lakes were collected using an Ekman sediment grabber. Sediments were placed immediately into plastic bags and frozen at −20°C within a few hours of collection. Conductivity, depth and temperature (CTD) profiles of the water column were recorded using a submersible data logger (Platypus Engineering, Hobart, Australia).

2.2. DNA extraction and clone library construction

The method of Purdy et al. [7] was utilised for extraction of DNA from sediment material using a combined physical and chemical treatment for cell lysis. All solutions used were prepared with sterilised MilliQ water to reduce the chance of amplifying DNA contaminates. 16S rDNA was amplified using PCR with the following reaction mixes: 200–300 ng of sediment DNA, 50 pmol forward primer 530f (5′-GTG CCA GCM GCC GCG G-3′) and reverse primer 1492r (5′-TAC GGY TAC CTT GTT ACG ACT T-3′), 1.25 mM deoxynucleotides, buffer (Clontech) and 2 μl Advantage polymerase mix (Clontech). PCR was performed in a thermosequencer FTS-960 (Corbett Research) with the following protocol: 95°C for 3 min (×1 cycle); 94°C for 3 min, 50°C for 2 min, 72°C for 6 min (×30 cycles); 72°C for 6 min (×1 cycle). PCR of controls lacking DNA extracts was also performed to determine whether contaminants were being amplified.

The amplified fragment was purified using the Prep-A-Gene kit (Bio-Rad) and cloned using blunt-end ligation by employing the pGEM-T vector system I kit (Promega) according to the manufacturer's instructions using *Epicurian coli* XL ultracompetent cells (Stratagene) for the transformations. Transformants were screened by blue-white selection on Luria agar treated with X-Gal/IPTG and containing 100 μg ml^{-1} ampicillin.

To determine which clones contained 16S rRNA gene inserts a loopful of cells of each clone was suspended and lysed in 2% (w/v) SDS. The lysate was extracted with an equal volume of chloroform-isoamyl alcohol (24:1). A sample from the resultant aqueous layer was electrophoresed in a 1% (w/v) agarose gel and compared with a molecular mass marker. Positive clones were then cultivated overnight on plates and plasmid extracted using the Bres-A-Spin miniplasmid extraction kit (GeneWorks, Adelaide, SA, Australia). Clones were further screened using restriction fragment length polymorphism (RFLP) analysis, with plasmid extracts separately digested with *Nci*I, *Rsa*I and *Hin*fI for 3–4 h at 37°C. Digests were separated on a 3% agarose gel, stained with ethidium bromide and photographed for evaluation. Each enzyme generated 5–10 bands. If more than one clone produced the same RFLP type, triplicate clones were selected at random and sequenced. The distribution of each RFLP type and the corresponding sequence(s) were compiled for each library analysed. Sequence reactions used the Thermosequenase v. 2.0 ready reaction dideoxy cycle sequencing kit (Amersham) and M13 reverse and forward primers. Subsequent electrophoresis and analysis was then performed using an A377 automated sequencer (Applied Biosystems). In most cases, the near complete sequence of each clone (900–1000 nucleotides, spanning the region from *E. coli* equivalent nucleotide positions 519–1507) was obtained.

2.3. Phylogenetic analyses

Clone sequences were compared to the GenBank nucleotide data library (http://www.ncbi.nlm.nih.gov/blast/blast.cgi) to determine their closest phylogenetic neighbours. The clone sequences were aligned manually to selected complete or near complete sequences downloaded from GenBank and from the Ribosomal RNA Database Project (RDP) internet site (http://www.cme.msu.edu/RDP/) [8]. Evolutionary distance was determined using the maximum likelihood algorithm employing the program DNADIST which is part of the PHYLIP v. 3.57c software package [9]. Phylogenetic trees were generated by using the Neighborliness option from the NEIGHBOR program. To determine the statistical stability of branch nodes bootstrap analysis using maximum parsimony (DNAPARS) and maximum likelihood analysis were performed with SEQBOOT and CONSENSE, using 250 replicate analyses. The RDP Chimera-CHECK program was used to detect PCR-amplified hybrid sequences. In addition potential chimerism was also determined by detection of inconsistencies in branching order and secondary structure. Sequences generated in this study are deposited in the GenBank nucleotide library under accession numbers shown in Figs. 1 and 2.

2.4. Diversity indices

For calculations of diversity indices, the libraries were normalised using the rarefaction method [10] by using the program RAREFACT.FOR written by C.J. Krebs (University of British Columbia) and which is available through the Internet at http://gause.biology.ualberta.ca/jbrzusto/rarefact.html. The Shannon-Weaver index [11] for estimating diversity was determined with the following equation: $H' = (n\log n - [\Sigma kN\log N])/n$, where n is the total number of individuals (clones), N is the number of clones per phylotype (equivalent to species) and k is the number clones in a given phylotype. Dominance concentration (Simpson index) [11] was determined from: $SI' = \Sigma(N[N-1]/n[n-1])$. Evenness [11] was based on the Shannon-Weaver index data: $J' = H'/H_{max}$, where H_{max} is the equal to the logarithm of the total number of phylotypes. Species richness was extrapolated from the data by using the non-parametric model of Chao [12]. As sequence data were accumulated collector's curves [11] were created to compare the relative diversity and coverage of each library by plotting the number of phylotypes to the number of clones.

3. Results

3.1. Clone library construction and screening

16S rRNA clone libraries were constructed from DNA extracted from sediment collected from three limnologically disparate Antarctic hypersaline lakes including Ekho Lake, Organic Lake and Deep Lake, of the Vestfold Hills, Eastern Antarctica. The sediments from Deep Lake were aerobic while the others were anoxic though there was a considerable difference in redox value between them. Additional information on the physicochemical characteristics of the samples is shown in Table 1. DNA yields for both Ekho and Organic Lake sediments were relatively high (0.5–1 μg μl^{-1}), but was quite low for Deep Lake sediment (< 0.01 μg μl^{-1}). Clones (74–87 per library) containing 16S rRNA inserts were screened by RFLP analysis using *Nci*I, *Rsa*I and *Hin*fI. When more than one clone was found for a given RFLP type, three clones were chosen at random for sequencing to determine the homogeneity of the RFLP type. In this study we found different RFLP types were internally homogeneous and distinct (with 98–100% sequence similarity) from other RFLP types. We screened 238 clones and identified 43 unique phylotypes. Six chimerical clones were detected during the analyses and were discarded. All 16S rRNA sequences obtained possessed standard secondary structural features.

3.2. 16S rRNA clone distribution

Using universal primers for clone library construction, clones from Bacteria, Archaea and Eukarya were obtained. Fig. 1 shows the distribution of clones between different phylogenetic groups. In Ekho and Organic Lake sediments bacterial clones predominated with only 0–7.8% archaeal clones detected, while no 18S rRNA clones were found. In Deep Lake, Archaea predominated with only a small number of bacterial phylotypes and a single 18S rRNA clone present (Fig. 1). The 18S rRNA clone grouped within the chlorophyte genus *Dunaliella*. The very low yield of DNA from the Deep Lake sediment

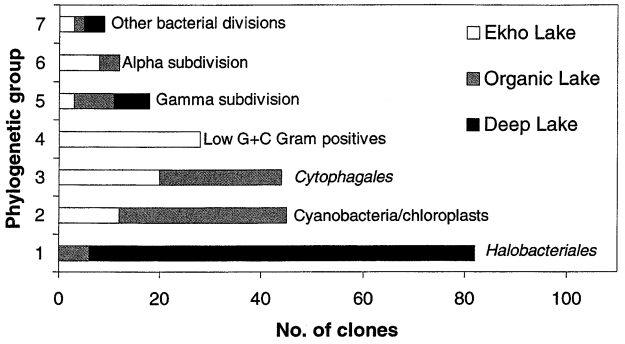

Fig. 1. Distribution of 16S rDNA clones derived from the sediment of three hypersaline Antarctic Lakes across bacterial and archaeal phylogenetic groups.

makes it highly vulnerable to even the slightest contamination during PCR. Thus some Deep Lake and possibly Ekho Lake clones, including those grouping in *Pseudomonas*, *Stenotrophomonas*, *Enterobacter* and *Delftia* (Fig. 2), could represent PCR contaminants [13]. However, as the PCR controls were negative this could not be ascertained conclusively. PCR bias owing to mismatches in the primers, mostly within the popular universal primer 1492r, significantly reduced the chance of detecting 18S rRNA clones (P. Hugenholtz, personal communication).

Clones were grouped within phylogenetic divisions depending on the robustness of bootstrap values ($> 60\%$ was considered good bootstrap support) from the phylogenetic analyses. The biodiversity across the three sediment clone libraries was limited with only eight phylogenetic divisions represented. Most clones clustered within the α and γ subdivisions of the Proteobacteria, order Cytophagales, low G+C Gram-positive bacteria, cyanobacteria/chloroplasts and order Halobacteriales (halophilic Archaea) (Figs. 1 and 2). A few clones also clustered in the class Actinobacteria, order Spirochaetales, order Verrucomicrobiales (Fig. 2) and the order Halobacteriales (Fig. 3). A single clone, designated ORGANIC-1, could not be assigned a phylogenetic group and appears to represent a novel lineage. Analysis of this clone using larger data sets which included all known bacterial divisions and candidate divi-

Table 1
Physicochemical data for three hypersaline lakes of the Vestfold Hills, Antarctica

	Ekho Lake	Organic Lake	Deep Lake
Depth (m)	40	7.5	36
Position of oxycline (m)	12–20	4–5	–
Surface water (0–2 m) salinity (%)	8	15–17	28–32
Sediment salinity (%)	15	21	32
Annual sediment temperature	13–18°C	−6 to −8°C	−14 to −18°C
Redox at deepest point (mV)	−160	+120	+360
pH	n.d.	6.9–7.0	7.3–7.4
H_2S	2 mg l^{-1}	0	0

Data from Franzmann et al. [4], Ferris and Burton [5] and this study; n.d., not determined.

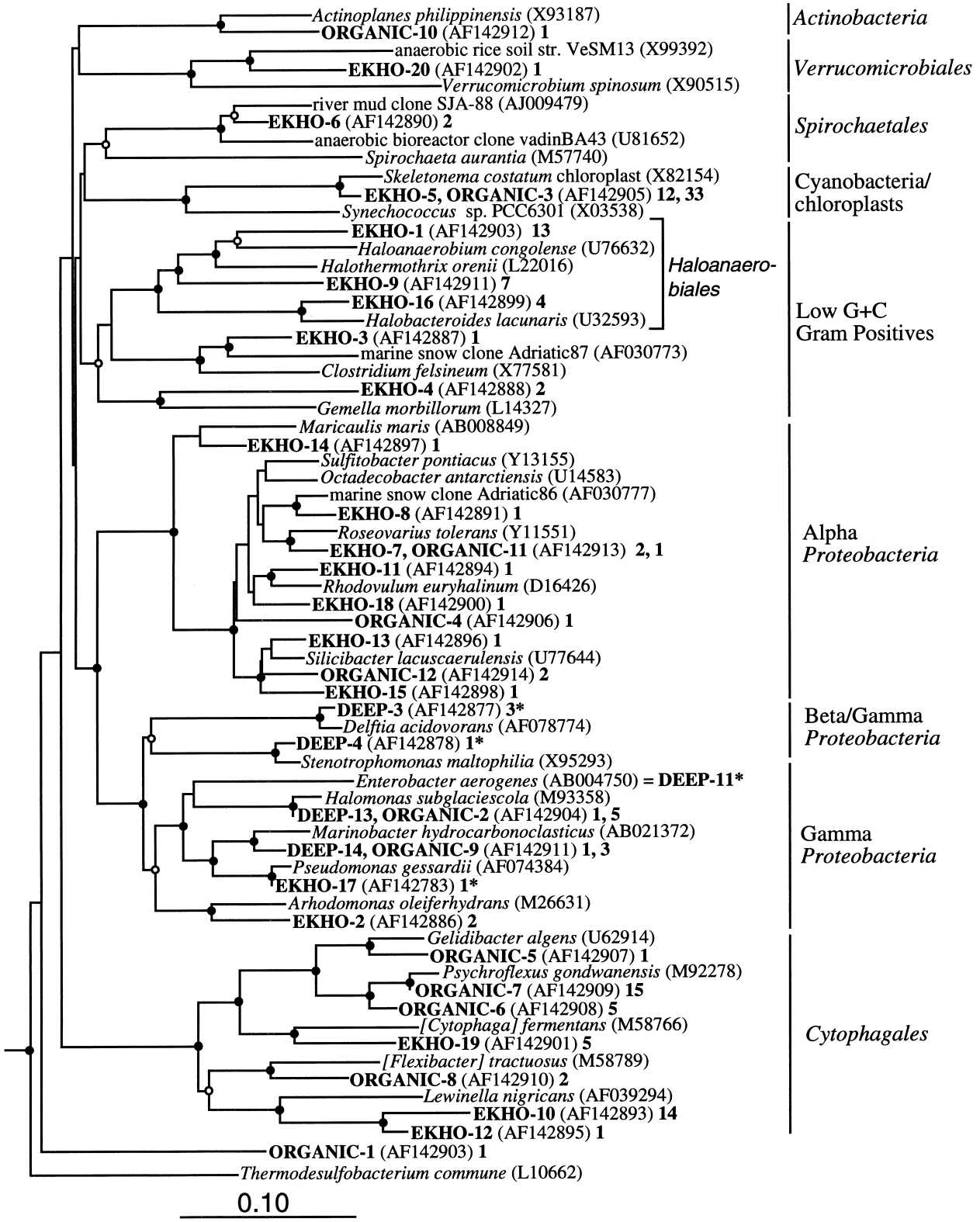

Fig. 2. Phylogenetic tree of bacterial 16S rRNA clones derived from Antarctic hypersaline lake sediment. Names in bold type are representative clones of phylotypes from each clone library with the following numbers indicating the GenBank accession code (in parentheses) and the number of clones detected for each phylotype. Bootstrap values at branch nodes are indicated by closed circles (bootstraps 80%) and open circles (60–79%). The tree was rooted with the sequence of *Aquifex pyrophilus* (M83458). *Phylotypes which may represent PCR contaminant.

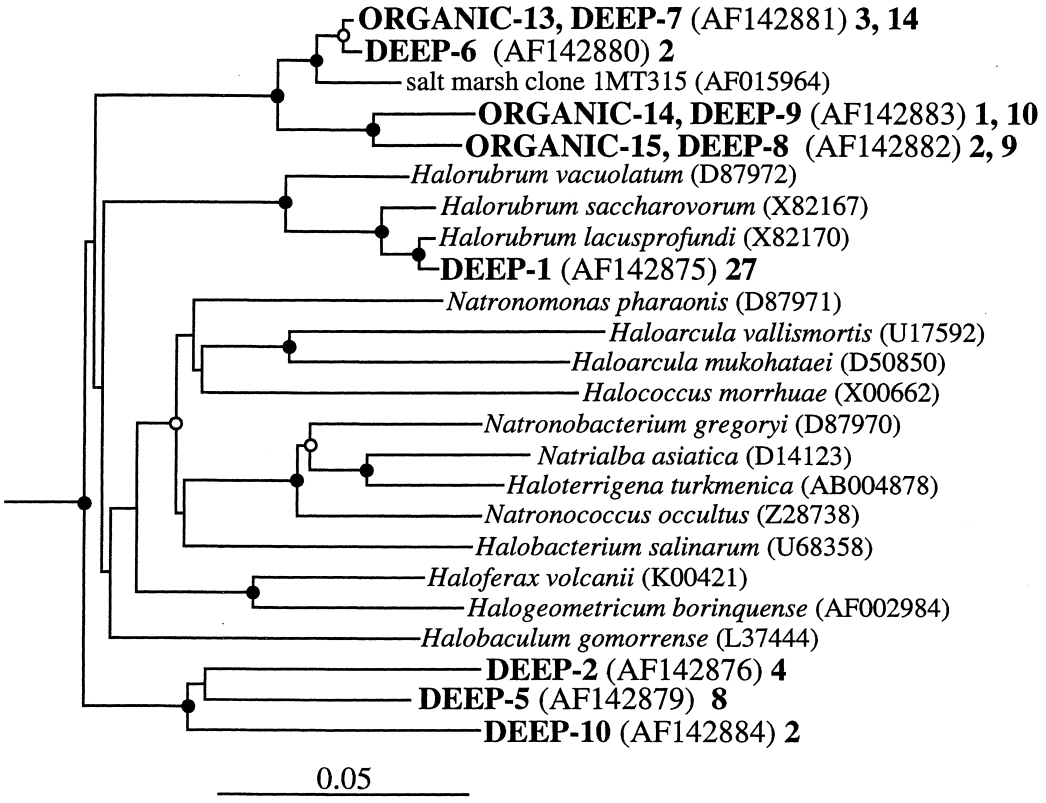

Fig. 3. Phylogenetic tree of archaeal 16S rRNA clones derived from Antarctic hypersaline lake sediment. Names in bold type are representative clones of phylotypes from each clone library with the following numbers indicating the GenBank accession code (in parentheses) and the number of clones detected for each phylotype. Bootstrap values at branch nodes are indicated by closed circles (bootstraps 80%) and open circles (60–79%). The tree was rooted with the sequence of *Thermoplasma acidophilum* (M38637).

sions [6] (data not shown) suggested it was distantly allied with the *Thermodesulfobacterium* division (Fig. 2); however, bootstrap support for this was only weak (40–48%).

A number of individual phylotypes were found abundant in the lake sediment libraries but no individual phylotype was found in all samples. The incidence of clones in some phylogenetic groups showed a similar distribution between some lake sediment samples. The samples from Ekho and Organic Lake both contained phylotypes of the α Proteobacteria, Cytophagales division and diatom chloroplast group. Phylotypes clustering within the genus *Roseovarius* and amongst diatom chloroplasts were common to both Ekho and Organic Lake (Fig. 2). Deep Lake possessed only Archaea of the extremely halophile group which were also present in Organic Lake sediment though at relatively low abundance (three phylotypes present both in Deep and Organic Lakes) and completely absent from Ekho Lake (Fig. 3). Both Organic and Deep Lakes shared phylotypes grouping within the genera *Halomonas* and *Marinobacter* (Fig. 2).

3.3. Biodiversity indices

Diversity indices were determined following normalisation of the clone libraries (by the rarefaction method) so that they could be compared directly. Indices indicating biodiversity coverage [14], diversity (Shannon-Weaver index), dominance (Simpson index), evenness and species richness [12] are shown in Table 2. The coverage of biodiversity was very high, ranging from 87 to 96%. This was

Table 2
Diversity indices for sediments of Antarctic hypersaline lakes compared with Antarctic low to moderate salinity lake and fjord meromictic basins

Diversity index	Ekho Lake	Organic Lake	Deep Lake	Moderate salinity lakes/fjord
Coverage (%)	86.5	90.8	96.6	14.8–35.9
Diversity (H')	1.10	1.01	0.94	1.15–1.72
Dominance (SI')	0.11	0.18	0.14	0.02–0.17
Evenness (J')	0.57	0.52	0.48	0.55–0.83
Species richness (Chao)	36 ± 10	32 ± 12	15 ± 2	68 ± 18 to 361 ± 82

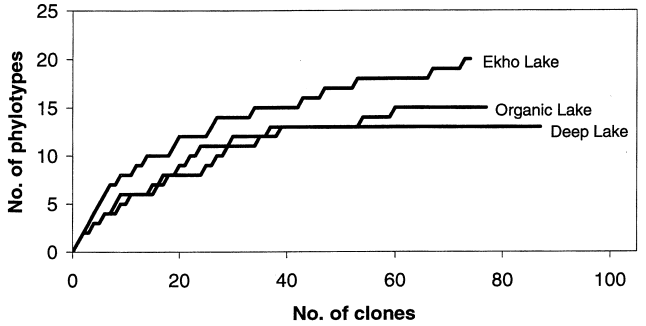

Fig. 4. Collector's curves for Antarctic hypersaline lake sediment clone libraries. Phylotypes are equivalent to groups of clones which share >98% sequence similarity.

also indicated by collector's curves (Fig. 4) in which a plateau-shaped curve, indicating full biodiversity coverage, was evident for each library. The indices showed there was a shift towards lower diversity and species richness as sediment salinity increased (Table 1). The dominance concentration of phylotypes varied considerably and appeared most evident in Organic Lake in which only two phylotypes (ORGANIC-3 and ORGANIC-6) made up 61.5% of the clones screened. Using Chao's non-parametric model for estimating species richness [12], the total number of different phylotypes in the various samples was extrapolated (Table 2). The values would theoretically include phylotypes derived from microbes in or adjacent to the sediment as well as whatever species had been exported into the sediment from the upper water column. The significance of the latter could not be reliably estimated with the data available. The species richness was highest in Ekho Lake sediment (36 ± 10) and lowest in Deep Lake (15 ± 2) (Table 2).

4. Discussion

The aim of this study was to determine the biodiversity and community structure within sediments from Antarctic hypersaline lakes. The end goal was to determine the nature of life forms capable of living, surviving and accumulating in high salinity, low temperature sediments. Clone library analysis was chosen as it provides useful data for further analysis of these and other related environments. The clone sequence data can be utilised for the construction of 16S rRNA oligonucleotide probes which can then be used in experiments for enumerating and studying specific bacterial or archaeal groups.

Only recently have 16S rRNA-based clone libraries utilised mathematical estimations of diversity and richness [15]. In this study, diversity indices were used to compare the three different lake sediments. It is important to remember that the differences in diversity and richness observed in this study have only been measured for one sample in each site and it is possible that there could be horizontal variation in diversity and richness. Differences and similarities are probably also exaggerated by the potential biases inherent in PCR [16]. Thus the phylotype abundances, diversity and richness values probably do not accurately reflect the sediment community structure.

Diversity appears lowest in Deep Lake which has a community dominated by members of the Halobacteriales archaeal division (Fig. 1). The predominant Deep Lake phylotype (DEEP-1) groups closely to a psychrotolerant Archaeon, *Halorubrum lacusprofundi*. This species was originally isolated from Deep Lake [17]. Beyond the presence of the chlorophyte *Dunaliella* [3] no other microorganisms have been successfully isolated from Deep Lake; however, our data indicate a number of Halobacteriales phylotypes are present (Fig. 3). One lineage common to both Deep and Organic Lakes (consisting of phylotypes DEEP-6 to DEEP-9 and ORGANIC-15 to ORGANIC-17) cluster with a clone from a British salt marsh [18], indicating the source Archaeon may have wide geographical distribution in marine-derived ecosystems. A second group of phylotypes (DEEP-2, DEEP-5, DEEP-10) forms another distinct deep branching cluster (Fig. 3).

The community structures in Ekho Lake and Organic Lake were comparatively more complex than Deep Lake. Many clones cluster within the α and γ Proteobacteria, Cytophagales and chloroplast groups (Figs. 1 and 2) with the related taxa all primarily aerobic microorganisms. For example major phylotypes in Organic Lake group within *Halomonas*, *Marinobacter* and *Psychroflexus*, aerobic planktonic bacteria with characteristic extreme halotolerance. Both *Halomonas subglaciescola* and *Psychroflexus gondwanensis* (formerly *Flavobacterium gondwanense*) were dominant populations throughout the year in the surface waters of Organic Lake [19]. Another major phylotype grouped closest to chloroplast 16S rRNA-like sequences and was common to both Ekho and Organic Lakes. The surface waters of both lakes possess low pennate and centric diatom populations [20], while Ekho Lake also contains significant populations of the prasinophyte *Tetraselmis* in its surface waters [21]. Ekho Lake sediment also contained a major phylotype related to the aerobic, marine, sheathed, gliding bacterial genus *Lewinella* (formerly species of the genus *Herpetosiphon*). Many of the α Proteobacterial clones are closely related to halophilic aerobes of the *Rhodobacter* branch including *Sulfitobacter*, '*Silicibacter*' and *Roseovarius*. An Ekho Lake species, *Roseovarius tolerans* [21], was closely related to several clones found in both Ekho and Organic Lakes. It is possible that some of these phylotypes grow only in the upper, less saline water layers and eventually sink into the lake sediment, slowly decomposing en route. Thus much of the clone libraries of Ekho and Organic Lakes includes Bacteria and Archaea not active in the sediment but which instead are being slowly decomposed by anaerobes.

Anaerobic bacterial or archaeal groups were not evident in the sediments of Organic and Deep Lakes. The lack of

H_2S in these sediments indicates a lack of sulfate-reducing bacteria. Although Organic Lake is anoxic, the high redox value precludes much anaerobic respiration, which requires strong reducing conditions. No phylotypes related to taxa known to be strictly anaerobic were detected in the Organic Lake clone library. However, fermentation probably occurs as indicated by the accumulation of organic acids (acetate, lactate and formate at up to 0.3 mM) in the bottom waters of Organic Lake [4]. In Ekho lake several phylotypes group within the low G+C Gram-positive division, with three phylotypes clustering within the Haloanaerobiales, constituting 33.4% of clones (Fig. 2). The combination of relatively warm temperatures (due to the heliothermic nature of Ekho Lake), anoxia and high salinity seems to select for this bacterial group which includes moderate to extreme halophiles with a primarily fermentative metabolism [22]. At least some haloanaerobes can grow by reduction of sulfur compounds [23] and may be responsible for the H_2S detected in Ekho Lake sediments. The sediments of several other low to moderate salinity meromictic Antarctic lakes (salinity 1.4–4.1%, temperatures −1°C to 6°C) have also been analysed using 16S rRNA clone library analysis (Bowman, unpublished data). No members of the Haloanaerobiales have been detected in any of these sediments. Haloanaerobes have been isolated from a variety of saline environments but this is the first report of their existence in polar regions.

Overall our results suggest the diversity is much lower (3–10 times lower) than what is found in sediment from adjacent brackish-marine salinity meromictic basins. Seemingly in contrast to this assessment, the upper water column of Ekho Lake has been shown to contain a surprising range of morphotypes as great as has ever been seen for any other water body examined (P. Hirsch, personal communication; [21,24]). It is possible many microbes in the upper waters of Ekho Lake decompose before reaching the sediments and so remain undetected.

Acknowledgements

This study was supported by grants from the Australian Research Council (Grants A196062517, A09905709, F09905711) and Antarctic Science Advisory Committee (Grant 1012). We would like to thank the Australian Antarctic Division for Antarctic expeditionary logistic support.

References

[1] Peterson, J.A., Finlayson, B.L. and Zhang, Q. (1988) Changing distributions of later quaternary terrestrial lacustrine and littoral environments in the Vestfold Hills, Antarctica. Hydrobiologia 165, 221–226.

[2] Whitehead, J.M. and McMinn, A. (1997) Palaeodepth determination from Antarctic benthic diatom assemblages. Mar. Micropaleontol. 29, 301–318.

[3] Franzmann, P.D. (1996) Examination of Antarctic prokaryotic diversity through molecular comparisons. Biodivers. Conserv. 5, 1295–1305.

[4] Franzmann, P.D., Deprez, P.P., Burton, H.R. and van den Hoff, J. (1987) Limnology of Organic Lake, Antarctica, a meromictic lake that contains high concentrations of dimethyl sulfide. Aust. J. Mar. Freshwater Res. 38, 409–417.

[5] Ferris, J.M. and Burton, H.R. (1988) The annual heat content and mechanical stability of hypersaline Deep Lake, Vestfold Hills, Antarctica. Hydrobiologia 165, 115–128.

[6] Hugenholtz, P., Goebel, B.M. and Pace, N.R. (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. J. Bacteriol. 180, 4765–4774.

[7] Purdy, K.J., Embley, T.M., Takii, S. and Nedwell, D.B. (1996) Rapid extraction of DNA and rRNA from sediments by a novel hydroxyapatite spin-column method. Appl. Environ. Microbiol. 62, 3905–3907.

[8] Maidak, B.L., Cole, J.R., Parker, C.T., Garrity, G.M., Larsen, N., Li, B., Lilburn, T.G., McCaughey, M.J., Olsen, G.J., Overbeek, R., Pramanik, S., Schmidt, T.M., Tiedje, J.M. and Woese, C.R. (1999) A new version of the RDP (Ribosomal Database Project). Nucleic Acids Res. 27, 171–173.

[9] Felsenstein, J. (1993) PHYLIP v. 3.57c (phylogenetic inference program package). University of Washington, Seattle, WA.

[10] Simberloff, D.S. (1972) Properties of the rarefaction diversity measurement. Am. Natur. 106, 414–418.

[11] Krebs, C.J. (1989) Ecological Methodology. Harper and Row, New York.

[12] Chao, A. (1984) Nonparametric estimation of the number of classes in a population. Scand. J. Stat. 11, 265–270.

[13] Tanner, M.A., Goebel, G.M., Dojka, M.A. and Pace, N.R. (1998) Specific ribosomal DNA sequences from diverse environmental settings correlate with experimental contaminants. Appl. Environ. Microbiol. 64, 3110–3113.

[14] Mullins, T.D., Britschgi, T., Krest, B., Lake, R. and Giovannoni, S.J. (1995) Genetic comparisons reveal the same unknown bacterial lineages in Atlantic and Pacific bacterioplankton communities. Limnol. Oceanogr. 40, 148–158.

[15] McCaig, A.E., Glover, A. and Prosser, J.I. (1999) Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. Appl. Environ. Microbiol. 65, 1721–1730.

[16] Hansen, M.C., Tolker-Nielsen, T., Givskov, M. and Molin, S. (1998) Biased 16S rDNA PCR amplification caused by interference from DNA flanking the template region. FEMS Microbiol. Ecol. 26, 141–149.

[17] Franzmann, P.D., Stackebrandt, E., Sanderson, K., Volkman, J.K., Cameron, D.E., Stevenson, P.L., McMeekin, T.A. and Burton, H.R. (1988) *Halobacterium lacusprofundi* sp. nov., a halophilic bacterium isolated from Deep Lake, Antarctica. Syst. Appl. Microbiol. 11, 20–27.

[18] Munson, M.A., Nedwell, D.B. and Embley, T.M. (1997) Phylogenetic diversity of Archaea in sediment samples from a coastal salt marsh. Appl. Environ. Microbiol. 53, 4729–4733.

[19] James, S.R., Burton, H.R., McMeekin, T.A. and Mancuso, C.A. (1994) Seasonal abundance of *Halomonas meridiana, Halomonas subglaciescola, Flavobacterium gondwanense*, and *Flavobacterium salegens*. Antarct. Sci. 6, 325–332.

[20] Roberts, D. and McMinn, A. (1996) Relationship between surface sediment diatom assemblages and water chemistry gradients in saline lakes of the Vestfold Hills, Antarctica. Antarct. Sci. 8, 331–341.

[21] Labrenz, M., Collins, M.D., Lawson, P.A., Tindall, B.J., Schumann, P. and Hirsch, P. (1999) *Roseovarius tolerans* gen. nov., sp. nov., a budding bacterium with variable bacteriochlorophyll *a* production from hypersaline Ekho Lake. Int. J. Syst. Bacteriol. 49, 137–147.

[22] Rainey, F.A., Zhilina, T.N., Boulygina, E.S., Stackebrandt, E., Tourova, T.P. and Zavarzin, G.A. (1995) The taxonomic study of the fermentative halophilic bacteria: description of Haloanaerobiales ord. nov., *Halobacteroidaceae* fam. nov., *Orenia* gen. nov. and further taxonomic rearrangements at the genus and species level. Anaerobe 1, 185–199.

[23] Ravot, G., Magot, M., Ollivier, B., Patel, B.K.C., Ageron, E., Grimont, P.A.D., Thomas, P. and Garcia, J.L. (1997) *Haloanaerobium congolense* sp. nov., an anaerobic, moderately halophilic, thiosulfate- and sulfur-reducing bacterium from an African oil field. FEMS Microbiol. Lett. 147, 81–88.

[24] Ashbolt, N.J. (1990) Antarctic biotechnology – what is the potential? Aust. J. Biotechnol. 13, 103–105.