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Origin and Phylogeny of Microbes Living in Permanent Antarctic Lake Ice

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Abstract

The phylogenetic diversity of bacteria and cyanobacteria colonizing sediment particles in the permanent ice cover of an Antarctic lake was characterized by analyses of 16S rRNA genes amplified from environmental DNA. Samples of mineral particles were collected from a depth of 2.5 m in the 4-m-thick ice cover of Lake Bonney, McMurdo Dry Valleys, Antarctica. A rRNA gene clone library of 198 clones was made and characterized by sequencing and oligonucleotide probe hybridization. The library was dominated by representatives of the cyanobacteria, proteobacteria, and Planctomycetales, but also contained diverse clones representing many other microbial groups, including the *Acidobacterium/Holophaga* division, the Green Non-Sulfur division, and the Actinobacteria. Six oligonucleotide probes were made for the most abundant clades recovered in the library. To determine whether the ice microbial community might originate from wind dispersal of the algal mats found elsewhere in Taylor Valley, the probes were hybridized to 16S rDNAs amplified from three samples of terrestrial cyanobacterial mats collected at nearby sites, as well as to bacterial 16S rDNAs from the lake ice community. The results demonstrate the presence of a diverse microbial community dominated by cyanobacteria in the lake ice, and also show that the dominant members of the lake ice microbial community are found in terrestrial mats elsewhere in the area. The lake ice microbial community appears to be dominated by organisms that are not uniquely adapted to the lake ice ecosystem, but instead are species that originate elsewhere in the surrounding region and opportunistically colonize the unusual habitat provided by the sediments suspended in lake ice.

Introduction

The McMurdo Dry Valleys form the largest of the ice-free areas on the Antarctic continent (approximately 4,800 km^2). This polar desert is one of the most extreme environments on the planet, with high winds and bitter cold (average air temperature = −20°C) [18]. Lakes in this region are permanently covered with 3–20 m of ice. This ice cover supports microbial life that is active in liquid occlusions associated with sediment particles of aeolian origin [3, 16]. Liquid water, which arises from solar melting, has been shown to exist

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for about 150 days during each austral summer (November–March) [3]. Microbes in the ice are diverse [14, 13, 4] and can alter the optical properties of the ice [1], thereby influencing primary production in the underlying liquid water column [5]. The unique physical, chemical, and biological features in the permanent ice covers of lakes in the McMurdo Dry Valleys have been proposed as an analogue for other icy worlds [16]. This analogy is particularly relevant to Mars and the Jovian moon Europa, where water ice is known to exist [16].

The objectives of this study were to describe the phylogeny of the microorganisms living within the permanent lake ice, and determine their genomic relationship to microorganisms inhabiting the surrounding terrestrial environment. These objectives address the hypothesis that the aeolian transport of terrestrial material provided the biological seed for the lake-ice microbes. To address this hypothesis we characterized, on a phylogenetic level, the organisms associated with the lake ice and the terrestrial cyanobacterial mats within the Taylor Valley where Lake Bonney is situated. The comparison was made through the analysis of 16S rDNA sequences followed by hybridization experiments using oligonucleotide probes specific to certain genomes found within the ice microbial community [7, 11, 21].

Materials and Methods

Sampling

Approximately 500 grams of sediment was obtained from cores taken from a depth of 2.5 m in the permanent ice cover of Lake Bonney. All sampling was conducted during September–October 1995 when liquid water was absent (the ice temperature was ~−15°C). Samples were lyophilized and stored at −20°C before processing.

Nucleic Acid Extraction

DNA was extracted from 14 g of sediment using a modification of the method described by Pitcher [15]. Briefly, a lyophilized ice sediment sample was resuspended in lysozyme (10 mg sediment ml^{-1}) for 30 min at 37°C. The sample was then lysed with a guanidinium thiocyanate solution. A standard phenol chloroform extraction followed by ethanol precipitation yielded approximately 14 μg of DNA. The resulting pellets were resuspended in water. DNA from lyophilized cyanobacterial mat collected from the terrestrial environment in the vicinity of Lake Bonney was obtained using the same method.

Cloning

Prokaryotic 16S rRNAs were amplified for cloning from the mixed population genomic DNAs by PCR with r*Tth* DNA polymerase XL (PerkinElmer, Foster City, CA) and bacterial 16S primers for DNA coding for rRNA (rDNA primers 27F (AGA GTT TGA TCM TGG CTC AG) and 1522R (AAG GAG GTG ATC CAN CCR CA). The reaction volume was 100 ml, containing 10 ng of template, 200 nm each primer, 1.5 mM $MaCl_2$, 200 mM dNTPs, XL buffer, and 2 units of r*Tth* DNA polymerase XL. Thirty-five cycles were performed on an MJ Research thermocycler under the following conditions: annealing at 55°C for 1.5 min, elongation at 72°C for 3 min, and denaturation at 96°C for 1 min. A Qiaquick column (Qiagen, Chatsworth, CA) was used to purify the PCR product. Ten ml of the PCR product was then incubated at 72°C for 2 h in the presence of 1.5 mM $MgCl_2$, 2 mM dATP, 2 ml 10x *Taq* buffer, 5 units of *Taq* polymerase, and water to 20 ml. The resulting product was passed over another Qiaquick column and resuspended in a volume of 10 ml. This product was ligated into the plasmid vector pCRII (Invitrogen, San Diego, CA) as described in the manufacturer's instructions. The ligation product was used to make a library of 198 clones. Double-stranded plasmid DNA sequencing was performed with an Applied Biosystems 373A automated sequencer (Applied Biosystems, Foster City, CA).

Sequence Analysis

Sequence analysis was performed with the program GDE, supplied by Steve Smith (Millipore, Bedford, MA). Regions of ambiguous alignment were excluded from the phylogenetic analysis. The Phylogeny Inference Package (Phylip) version 3.5 was used to infer phylogenetic relationships [2].

Accession Numbers

Nucleotide sequences were filed in Genbank under the accession numbers AF173817–AF173825.

Oligonucleotide Probe Hybridization

Oligonucleotide probes specific to 16S rDNA sequences originating from the lake ice sediment were designed with the aid of the program ARB [19]. ARB was instructed to design probes at least 18 nucleotides long, with no fewer than two mismatches allowed when compared against a database containing approximately 8000 16S rRNA sequences. Oligonucleotides that met these criteria were then subjected to testing to ensure that hybridization and washing conditions were optimal. Melting points were determined empirically by measuring the hybridization of oligonucleotide to both target and closely related nontarget plasmids bound to membrane at increasing stringencies. Probe sequences, targets, and wash conditions are shown in Table 1. Terrestrial cyanobacterial mat and lake ice sediment DNA was amplified in 30 cycles of a MJ Research thermocycler under the following conditions: annealing at 55°C for 1.5 min, elongation at 72°C for 3 min, and denaturation at 96°C for 1 min. Amplified DNA was cleaned using a Qiagen column (Qiagen, Chatsworth, CA), and, after determining concentration by spectrophotometry, the DNA was blotted on Zetaprobe membrane

Table 1. Probe sequences and hybridization conditions

Name	Target group	Sequence 5′–3′	Length	Temp (°C)[b]	[Z-hyb][b,c]
Chms5[a]	*Chaemisiphon* sp.	AGG CGA TAA ATC TTT CAA CTT	21	37	0.2x
76-156[a]	LB3-76-156 cluster	CTA CAG GCA CTA AAG CTT T	19	40	2x
46-75[a]	LB3-46-75 cluster	AGG CCA AAT AGT TTC ACC TCT	21	37	0.2x
Plancto4	*Planctomycetales*	CCT AAW CCM YGC CGC CAR C	19	37	0.2x
Rhf5	*Rhodoferax* sp.	CAC AGC TTT CGC TGC GTT ATC	21	40	0.2x
1078-3	1078-3 cluster	TAA TCT GCC GCA AGC TCC TC	20	40	0.2x

[a] Affliated with cyanobacteria (see Fig. 1)
[b] Wash temperature determined empirically
[c] Concentration of wash buffer

(Biorad, Hercules, CA) in the following amounts (in triplicate): 50 ng, 25 ng, 10 ng, and 5 ng. Positive control plasmid was blotted at the following amounts (in triplicate): 50 ng, 25 ng, 10 ng, and 5 ng. After hybridization under the appropriate conditions, the degree of hybridization was quantified with a Phosphoimager (Molecular Dynamics, Sunnyvale, Calif).

Results and Conclusions

Approximately 14 μg of high molecular weight DNA was extracted from 14 g of freeze-dried Lake Bonney sediment obtained from the ice at a depth of 2.5 m. 16S rDNA was successfully amplified from the DNA pool, and used to develop a gene clone library. The 16S rDNA gene clone library consisted of 192 full-length clones. The clones were characterized by a combination of sequence analysis and oligonucleotide hybridization. Clones were selected at random for sequencing, and oligonucleotide probes, designed on the basis of the sequence data (Table 1), were subsequently used to identify 20 of the clones. Of the remaining 172 clones, 18 were eventually sequenced nearly in full (approximately 1,400 nucleotides) and the remainder were partially sequenced, usually including approximately 400 nucleotides of both the 5' and 3' regions. These domains of the gene contain both highly conserved and highly variable regions, suitable for preliminary phylogenetic analysis and probe design [8]. The full-length clones were phylogenetically analyzed, and the results of the neighbor joining and parsimony analyses for the cyanobacterial sequences are shown in Fig. 1 [17]. Only one of the gene clusters in Figure 1 is closely affiliated with a well-characterized cyanobacteria species, *Chamaesiphon subglobosus.* This gene cluster is designated *Chms5,* after the oligonucleotide probe that hybridizes specifically to this group. The remaining cyanobacterial gene clusters, 46–75, 76–156, and 53–64, are less than 93% similar to any characterized sequences in public databases. Oligonucleotide probes were designed to specifically hybridize to each of the three gene clusters highlighted in Fig. 1, as well as to the clone LB3-1.

Phylogenetic Analyses

Phylogenetic analyses of the remaining full-length sequences are shown in Fig. 2. The library contained a number of gene clones that were related to previously characterized bacterial groups. The cluster containing *Rhodoferax sp.* included six of the gene clones from the library, one of which was fully sequenced, LB3-27. A third gene cluster is designated as 1078-3 in Fig. 2. These organisms appear to be affiliated with the Acidobacterium/Holophaga group. Their nearest previously described neighbor is clone 11–25, which was isolated from an agricultural soil library [12]. Seven of the gene clones in the library belong to this gene cluster. No full-length sequencing of clones clearly affiliated with the *Planctomycetales* was carried out, but 12 of the partially sequenced clones were found to be affiliated phylogenetically with this group. Oligonucleotide probes specific to the gene clusters in brackets were designed and employed in the screening of the clone library and other experiments.

Two other gene clusters in Fig. 2 are of particular interest. The gene cluster including LB3-13 and LB3-147 appears to be loosely affiliated with the *Planctomycetales.* Eleven signature nucleotides for *Planctomycetales* groups *Pirellula* and *Planctomyces* have been described [20]. Of these 11 signature positions, only five occur in gene clones LB3-13 and LB3-147. The numbers cited below refer to positions in the *E. coli* 16S rRNA, which is commonly used as a frame of reference. All five substitutions (position 115 = G, position 312 = C, position 948 = G, position 1100 = U, and position 1233 = C) are characteristic of the *Pirellula* group [6, 20]. Therefore, the clones LB3-13 and LB3-147 appear to be members of a novel gene cluster, affiliated with the *Planctomycetales.* How-

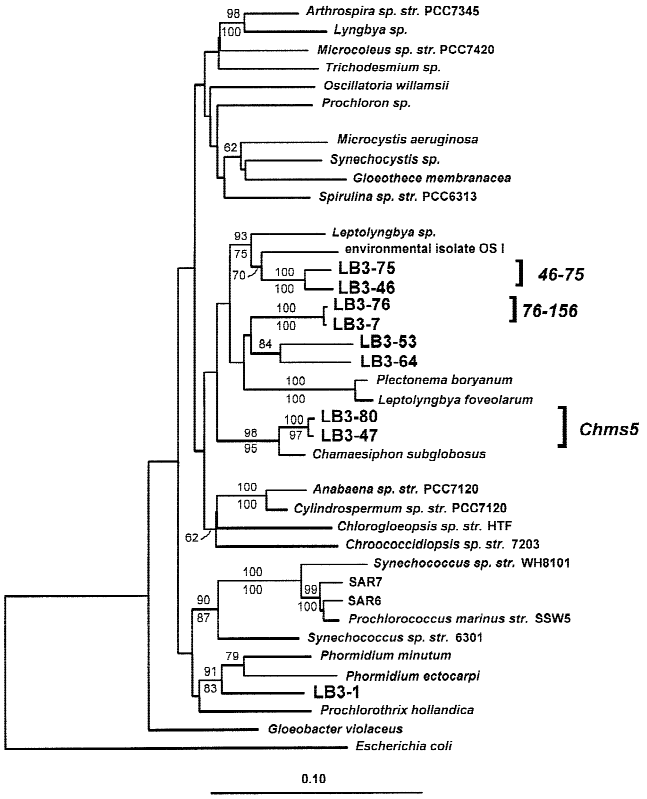

Fig. 1. Phylogenetic tree illustrating relationships among selected 16S ribosomal DNA clones from the ice cover of Lake Bonney (clones from this study are designated LB3) and representative terrestial cyanobacterial sequences. The number of bootstrap replicates that supported each branch, from a total of 100 replicates, is shown above (neighbor joining) and below (parsimony) each node. Bootstrap values below 60% are not shown. The 16S rDNA sequence of *Escherichia coli* was used to root the tree.

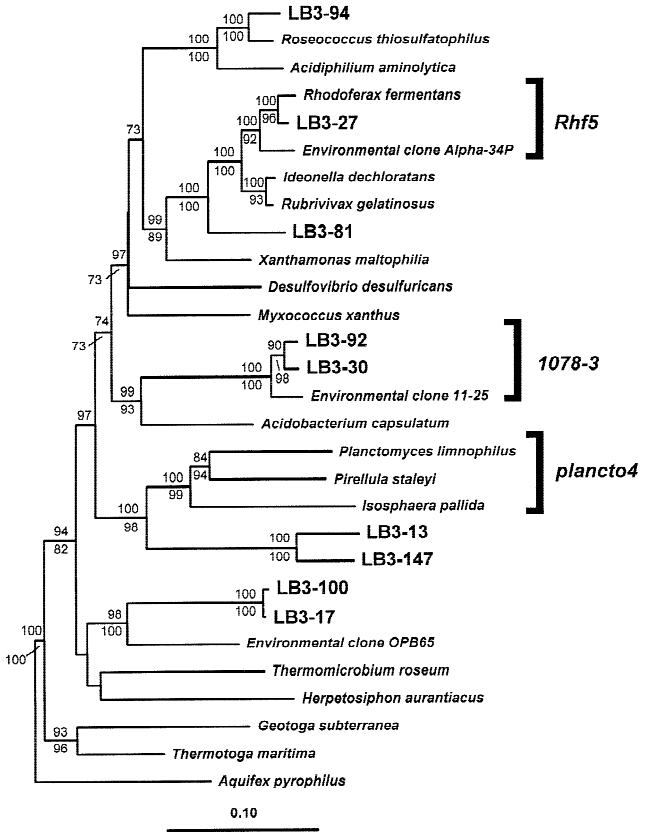

Fig. 2. Phylogenetic tree illustrating relationships among selected 16S ribosomal DNA clones and representative sequences. Gene clusters for which specific oligonucleotide probes have been designed are bracketed. The number of bootstrap replicates that supported each branch, from a total of 100 replicates, is shown above (neighbor joining) and below (parsimony) each node. Bootstrap values below 60% are not shown. The tree was rooted with *Aquifex pyrophilus.*

ever, the oligonucleotide probe designed to hybridize to the *Planctomycetales* does not hybridize to these clones.

The gene cluster composed of LB3-100 and LB3-17 is most closely affiliated with the environmental Clone OPB65 [10]. OPB65 was classified as a green non-sulfur bacterium and was isolated from a Yellowstone hot spring. The phylogenetic position of clones LB3-100 and LB3-17 with the green non-sulfur bacteria is supported by analyses of signature sequences. Two unusual signature features of the rRNA of the green non-sulfur bacteria are a deletion of 15 bases between positions 1123 and 1147, and a loop structure between positions 607 and 630 [9]. This deletion is present in both LB3-100 and LB3-17. Additionally, Woese [22] has described five diagnostic base substitutions that are highly conserved among the green non-sulfur bacteria. Four of these substitutions, G at position 53, A at 906, G at 1202, and G at 1410, are found in both LB3-100 and LB3-17. The last signature substitution of a G at position 1224 occurs in LB3-100, but in LB3-17, this nucleotide is replaced by U.

Oligonucleotide Hybridization

The oligonucleotide probes shown in Table 1 were used to screen 16S rDNA from environmental samples obtained from the terrestrial environment from various locations within the Taylor Valley. Two of the samples were from cyanobacterial mat samples collected in the vicinity of Lake Bonney. A third mat sample, collected about 15 km to the east of Lake Bonney in the Lake Fryxell basin, was also used. The final sample consisted of 16S rDNA from the same lake ice sediment used in the preparation of the gene clone library.

Table 2. Microbial distributions in cyanobacterial mat and lake ice sediment from the Dry Valleys, determined by oligonucleotide probe hybridization

	Chms5	46-75	76-156	Plancto	Rhf5	1078-3
Sediment[a]	2.55 ± 0.86	12.54 ± 0.4	5.92 ± 0.84	3.13 ± 0.33	1.91 ± 0.24	6.16 ± 1.15
Mat 1[b]	4.21 ± 1.8	29.65 ± 3.09	6.46 ± 1.33	2.68 ± 0.51	3.19 ± 0.4	4.53 ± 1.05
Mat 2[c]	7.71 ± 1.05	59.69 ± 1.79	8.19 ± 1.82	3.24 ± 0.51	0.41 ± 0.07	5.55 ± 1.55
Mat 3[d]	23.11 ± 2.87	3.76 ± 0.68	0.65 ± 0.18	2.75 ± 0.69	4.68 ± 0.96	12.75 ± 1.72

[a] Sediment collected from lake ice (2 m), east lobe Lake Bonney
[b] Lake Bonney, east lobe
[c] Lake Fryxell, near Canada glacier
[d] Lake Bonney, west lobe

The hybridization results are given in Table 2. The large data set consisted of triplicate blots of each sample in a dilution series, with a dilution series of positive plasmid controls for each probe. The data are given as the slope of each specific oligonucleotide hybridization divided by the slope of a universal 16S rDNA probe to the identical data set, expressed as a percentage [7]. The r^2 values for the hybridization of the universal probe to the dilution series ranged from 0.93 to 0.99. The results indicate that none of the six gene clusters detected by the specific probes are found exclusively within the lake ice community.

The results of the oligonucleotide probe hybridization experiment directly address the hypothesis this study was designed to test. The data demonstrate that organisms found in the ice community are also found in cyanobacterial mats collected from the terrestrial environment near the lake.

Hybridization revealed no clear pattern of distribution of the organisms targeted by the oligonucleotide probes developed for this study. The probes designed to hybridize to cyanobacterial 16S rRNA genes effectively hybridized to each sample, indicating that the cyanobacterial sequences present in the lake ice sediment are also found in all three of the terrestrial mat samples. The hybridization data for the three other gene clusters, 1078-3, plancto, and Rhf-5, imply that the organisms targeted by these probes also constitute a significant portion of the cyanobacterial mats examined in this study.

Our results demonstrate that the microbial community colonizing sediment particles within the permanent ice cover of Lake Bonney is a diverse assemblage. This assemblage contains organisms that have been well characterized and bacteria not previously encountered, including members of the green non-sulfur bacteria and 16S rRNA genes distantly related to those of the *Planctomycetales*. By employing oligonucleotide probes designed to hybridize to 16S rDNA genes found in a gene clone library, we were able to demonstrate that many of the bacteria from the lake ice sediment layer were found in microbial assemblages from the surrounding terrestrial environment. It appears that the strong katabatic winds that exist in the McMurdo Dry Valleys act as a major dispersal mechanism for microorganisms within this polar desert.

Determining the roles of the organisms described in this study, particularly those that represent novel and as yet uncultured microbial species, is a difficult task. Although it is probably correct to assume that those organisms phylogenetically affiliated with the cyanobacteria have a role as primary producers within the lake ice sediment, it is much more challenging to determine the metabolic or other phenotypic characteristics of the organisms represented by the remaining gene clones. A first attempt at relating genomic composition to phenotypic expression has been made for N_2-fixing organisms [13]. The data we present here provide important information for future studies based on metabolic activity and biogeochemical fluxes within permanent Antarctic lake ice.

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