Community structure and psychrophily in Antarctic microbial ecosystems

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ABSTRACT

Molecular based and traditional microbiological techniques were utilized to determine the structure and degree of cold adaptation of a variety of Antarctic bacterial communities. These studies were performed to obtain a better understanding of Antarctic ecosystems and prokaryotic adaptations to low temperature. A high degree of novel diversity was found associated with sea ice communities, marine-derived lake sediments, and quartz stone subliths. 16S rDNA-based clone library analysis of samples from these sites contained many clones exhibiting novelty at the genus and family-level, and occasionally at the division-level. Most (non-thermophilic) bacterial phylogenetic divisions were represented. Sediment from hyposaline Clear Lake (Vestfold Hills) contained highly unusual archaeal clones possibly representing a novel kingdom. Evidence based on cultivation work indicated quartz stone subliths like sea ice are havens for psychrophilic bacteria. The culturability of bacteria from sea ice communities was also demonstrated to be surprisingly high with molecular data matching cultivation data.

Introduction

Little is known about bacterial communities in polar ecosystems including their species composition, interactions with higher taxa, significance in various biogeochemical processes and mechanisms and degrees of cold adaptation. In this study baseline cultivation-dependent and independent survey information was obtained on the components of three marine and marine-derived Antarctic ecosystems including sea ice microbial communities (SIMCO), quart stone subliths (QSS) and continental lakes. All studies were performed in or in the vicinity of the Vestfold Hills region of Eastern Antarctica (68°S 78°E), an ice-free area formed ~9000 years ago by marine uplifting [6]. The cultivation-dependent analyses were based on universal 16S rRNA clone libraries [5] which were screened using restriction digest analysis (using *Rsa*I, *Nci*I and *Hinf*I or *Msp*I). Clones showing different patterns were then sequenced and analyzed using well established phylogenetic techniques [1, 5].

Sea Ice Microbial Communities (SIMCO)

In our study, an extensive array of bacteria isolates were obtained from bottom ice assemblages as well as from algae-free "clean" ice and under-ice seawater to determine the

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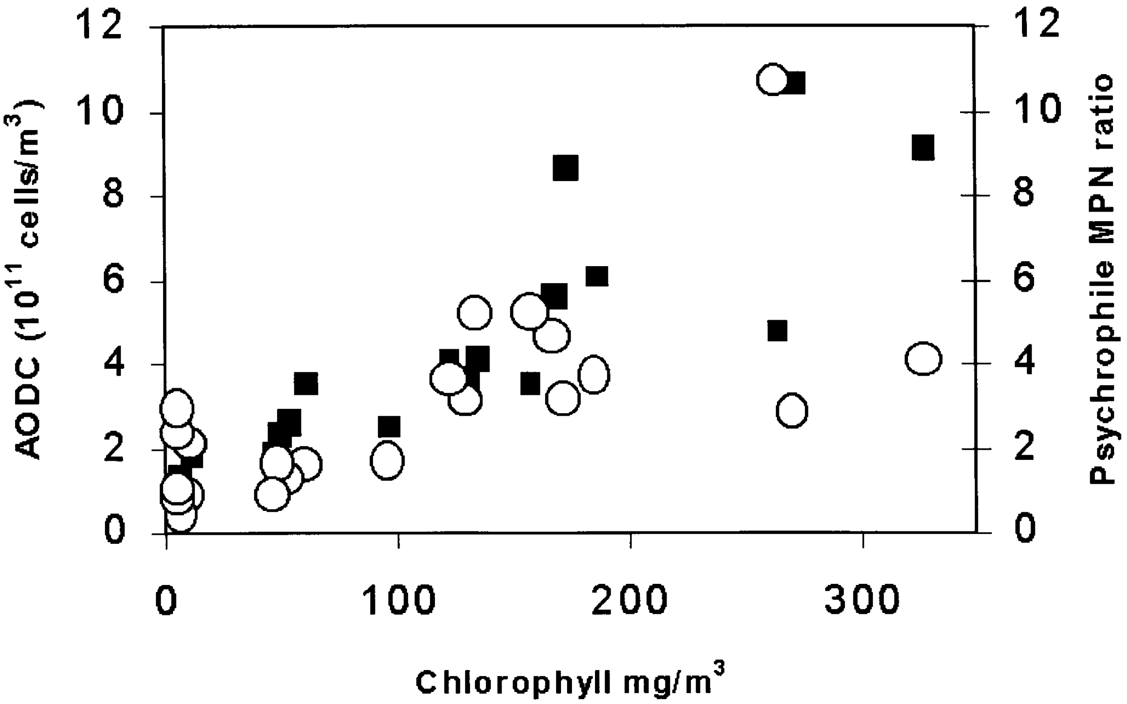

Figure 1. Ratio of acridine orange direct counts (■) and psychrophilic bacterial populations (○) (a ratio of most probable number counts at 2°C and 25°C) in sea ice with different chlorophyll (phytoplanktonic) levels.

influence diatom blooms have on the bacterial inhabitants. Previous results have indicated that biodiversity increases 3 to 7-fold between ice free of algae and under-ice seawater to ice samples rich in algae (>100 mg chlorophyll/m^3) [1]. We can now show a direct correlation between the amount of chlorophyll and bacterial AODC (Acridine Orange Direct Count) with the proportion of psychrophilic bacteria (T_{opt} <15°C, no growth at >20°C) (Figure 1). From this, 35-95% of the bacterial biomass from diatom assemblages is estimated to be psychrophilic. By comparison ice lacking algae and under-ice seawater samples are nearly devoid of psychrophiles which in our study were almost always isolates of *Psychrobacter glacincola* [1]. Identification of sea ice isolates (grouped initially by phenotype) using 16S rRNA indicated the dominant sea ice bacterial groups represented are from the gamma subdivision of the *Proteobacteria* and the *Flavobacterium-Bacteroides-Flexibacter* (flavobacteria) phylum. Psychrophiles included *Polaribacter* spp., *Octadecobacter* spp., *Glaciecola* spp., *Psychrobacter glacincola*, *Psychroflexus torquis*, *Colwellia* spp. and *Shewanella* spp. [1, 2]. Psychrotrophs make up a persistent background population and are found equally commonly in sea ice (with and without algae) and in the under-ice seawater [1]. 16S rRNA clone library data prepared from clean ice samples and from ice diatom assemblages indicate clones from clean sea ice match closely with cultivated isolates. The predominant groups in clean ice include *Shewanella frigidimarina* (52% of clones), *Pseudoalteromonas* spp. (29%) and *Marinobacter* sp. (11%). A significant change in the bacterial community structure and a modest increase in biodiversity occurs between clean ice and algae-rich ice (Table 1) with a marked enhancement in the proportion of flavobacteria

Table 1. Phylogenetic distribution of 16S rRNA clones from a variety of Antarctic samples.

Clonal Lineage	Clonal distribution (%): Clean sea-ice	Algae-rich sea-ice	Quartz stone subliths	Deep Lake sediment	Pendant Lake sediment	Clear Lake sediment	Admin Lake sediment
Archaea: Novel groups	-	-	-	-	8	7	6
Extreme halophiles	-	-	-	96	-	-	-
Bacteria: OP3/OP9	-	-	-	-	10	3	6
Planctomycetales	-	-	10	-	-	10	10
Veruccomicrobiales	-	-	-	-	-	7	3
Flexistipes branch	-	-	-	-	-	-	3
Spirochetes	-	-	-	-	-	2	-
Green Sulfur bacteria	-	-	-	-	3	7	10
Flavobacteria	5	32	20	-	-	4	-
Acidobacterium	-	-	-	-	-	2	-
Holophaga/ *Nitrospira*	-	-	-	-	3	6	10
Cyanobacteria	-	-	20	-	10	2	-
Proteobacteria: Alpha	3	2	5	-	-	-	3
Beta	-	-	-	-	-	-	-
Gamma	92	15	-	-	7	11	19
Delta	-	-	-	-	-	20	6
Epsilon	-	-	-	-	5	-	6
Low G+C gram +ve	-	-	-	-	50	-	-
High G+C gram +ve	-	5	45	-	2	-	3
Unafilliated	-	-	-	-	-	7	-
Eukaryotic:							
18S rRNA	-	32	-	4	2	3	6
Mitochondrial	-	-	-	-	-	6	6
Chloroplasts	-	14	-	-	-	3	3
Salinity (approx. ‰)	10-30	10-30	no data	280	35	20	15

clones. 18S rRNA (mostly of dinoflagellates, stramenopiles and diatoms) and diatom chloroplast 16S rRNA sequences comprised about half the clones of the library. Matches occurred for about half of the clones with cultivated strains. Archaea were not detected in any sea ice samples, either within clone libraries or by using specific archaeal primers.

Quartz Stone Subliths (QSS)

Both cultivation-based and 16S rRNA-based clone library methodologies were utilized to analyze the community structure of Quartz stone subliths (QSS) [3]. This variety of sublithic ecosystem is dominated by filamentous cyanobacteria species with the community concentrating in a narrow band at the base of translucent quartz stone. Cultivation data indicates a relatively high proportion of the community present in Vestfold Hills QSS is psychrophilic (36% of taxa isolated) and halophilic (62% of taxa isolated). Identification by 16S rRNA analysis indicates the heterotrophic bacterial community includes a range of bacterial species with a phylogenetic distribution similar to sea ice but also including several soil bacteria. Some unusual isolates were related to the *Planctomycetales* and to various soil ultramicrobacteria [7]. Cyanobacteria which were cultivated successfully include *Oscillatoria* and *Lyngbya*-like strains which phylogenetically group with a *Lyngbya*-like cyanobacterium inhabiting perennial ice from Dry Valley lakes [9]. Additional cyanobacterial types were also cultivated, including strains with *Pleurocapsa*, *Chroococcidiopsis* and *Synechococcus*-like morphologies. Clone library analysis of QSS material indicated a least a portion of the prokaryotic community are related to species of marine origin and are presumably marine-derived. However in correlation with the cultivation data several clones (related to *Rubrobacter* and *Microthrix* high G+C gram-positive lineages) are related to soil taxa. The QSS ecosystem thus appears to be made up of a mixture of marine organisms surviving in the salty coastal soils and normal soil bacteria. Marine bacteria may have been transported to the soil upon either salt spray or by winds. Studies are continuing to carbon date QSS sample material thus establishing a developmental timeframe of representative QSS from different geographical locations.

Lake Sediments

The Vestfold Hills area contains numerous water bodies recently derived from marine uplifting. Many of the lakes are meromictic and possess a diverse range of limnological and physico-chemical characteristics. In this study the bacterial diversity of several lake sediment grabs were undertaken using clone library analysis. Most of this work is still ongoing, however results to date (Table 1) indicate the following: 1) broad diversity occurs in most lake samples except hypersaline Deep Lake; 2) Pendant lake possesses an apparently high population of *Clostridium estertheticum*, a psychrophilic anaerobe [4]; and 3) detection of unusual A-T rich (35-47% G+C) archaeal clones (Figure 2). The Pendant Lake archaeal clones possess unique large palindromic inserts in two highly conserved sequence regions and have some similarity to clones previously detected in paddy soils [8]. Originally it was thought the clones may have made up a new kingdom of the archaea, however recent data [5] suggests these clones may represent an unusually long-branched division of the Kingdom Euryarchaeoata.

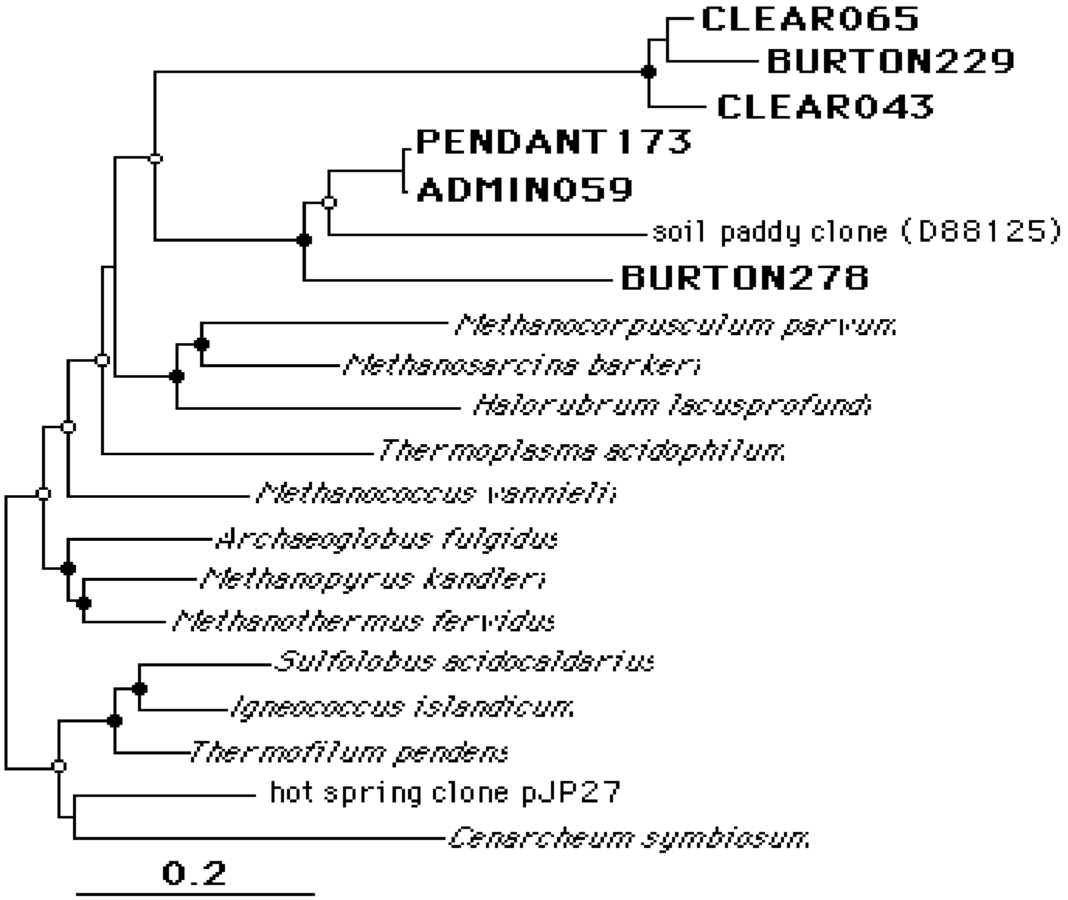

Figure 2. 16S rRNA phylogenetic tree showing position of archaea from Vestfold Hill lakes. The tree is based on Jin and Nei values and clustered by neighbor-joining. Bar equals a distance of 0.20. Branch points supported by both maximum parsimony and distance analysis (>74%) are indicated by filled circles; open circles indicate branch points supported by both or either types of analysis but only marginally supported (50-74%). Branch points lacking circles possess boostrap values of <50%.

Conclusions

Development of ecosystem baseline data coupled with more complete understanding of Antarctic microbial communities and their processes may have some value in predicting or observing climactic changes due to global warming in Antarctica. The presence of rich psychrophilic bacterial diversity in the Antarctic ecosystems studied may make them a significant resource for biotechnology and for future research into the mechanisms of cold adaptation.

Acknowledgements

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