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SHORT COMMUNICATION

Phylotype diversity in a benthic cyanobacterial mat community on King George Island, maritime Antarctica

Cecilia Callejas · Paul R. Gill · Ana I. Catalán · Gastón Azziz · Susana Castro-Sowinski · Silvia Batista

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Abstract Cyanobacterial 16S ribosomal RNA gene diversity was examined in a benthic mat on Fildes Peninsula of King George Island (62°09′54.4″S, 58°57′20.9″W), maritime Antarctica. Environmental DNA was isolated from the mat, a clone library of PCR-amplified 16S rRNA gene fragments was prepared, and amplified ribosomal DNA restriction analysis (ARDRA) was done to assign clones to seven groups. Low cyanobacterial diversity in the mat was suggested in that 83% of the clones were represented by one ARDRA group. DNA sequences from this group had high similarity with 16S rRNA genes of *Tychonema bourrellyi* and *T. bornetii* isolates, whose geographic origins were southern Norway and Northern Ireland. Cyanobacterial morphotypes corresponding to *Tychonema* have not been reported in Antarctica, however, this morphotype was previously found at Ward Hunt Lake (83°N), and in western Europe (52°N). DNA sequences of three of the ARDRA groups had highest similarity with 16S rDNA sequences of the *Tychonema* group accounting for 9.4% of the clones. Sequences of the remaining three groups (7.6%) had highest similarity with 16S rRNA genes of uncultured cyanobacteria clones from benthic mats of Lake Fryxell and fresh meltwater on the McMurdo Ice Shelf.

Keywords Antarctic environmental gradient · 16S rRNA gene · ARDRAs · Fildes Peninsula · *Tychonema* · Oscillatoriales

Introduction

Life in Antarctica is greatly restricted in that only 0.3% of the surface becomes ice-free during summer. Some central arid regions of continental Antarctica may support very simple ecosystems that are highly reduced in complexity (Wall and Virginia 1999). Most studies on microbial diversity in Antarctica have been done in the McMurdo Dry Valleys and Ice Shelf. Cyanobacteria are the predominant terrestrial and freshwater phototrophs in the McMurdo region and were found in terrestrial and in benthic habitats of lakes and meltwater ponds on sea ice (Nadeau et al. 2001; Taton et al. 2003; Jungblut et al. 2005). In sharp contrast with the McMurdo region, the Fildes Peninsula becomes essentially ice-free during the austral summer and has a much milder and a moist climate. The landscape of this Peninsula, however, remains almost completely barren during the entire summer, with widely dispersed occurrence of lichen and less frequent bryophyte growth arising during summer. The development and succession of prominent cyanobacterial–bacterial mat consortia that may ultimately give rise to growth of bryophytes routinely arise at isolated glacial seepage and other benthic sites during summer.

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C. Callejas · P. R. Gill (✉) · A. I. Catalán · G. Azziz · S. Castro-Sowinski · S. Batista
Unidad de Microbiología Molecular, Instituto de Investigaciones Biológicas Clemente Estable (MEC), Unidad Asociada a Facultad de Ciencias, Ministerio de Educación y Cultura, Av. Italia 3318, 11600 Montevideo, Uruguay
e-mail: prgill@iibce.edu.uy

C. Callejas · P. R. Gill
Laboratorio de Tecnología Molecular, Facultad de Ciencias, UdelaR, Iguá 4225, 11400 Montevideo, Uruguay

S. Castro-Sowinski
Sección Bioquímica, Facultad de Ciencias, UdelaR, Iguá 4225, 11400 Montevideo, Uruguay

Microbial communities of cyanobacterial–bacterial mats on King George Island were previously characterized in detail morphologically and were discussed in several reports (Komárek 1999, 2007; Comte et al. 2007). Eight principal biotopes were clearly delimited in location and exhibited specific compositions of cyanobacterial species. Glacial seepages and adjoining creeks had the highest cyanobacterial diversity, including some morphotypes possibly unique to maritime Antarctica. Like the McMurdo region, cyanobacteria, including nitrogen-fixing forms, were the predominant genera found in these mats using well-established morphological taxonomic criteria. In many cases cyanobacterial mats of the Fildes Peninsula can be dominated by *Leptolyngbya* or *Phormidium*. In addition, smaller dark mats can be found, which were dominated by *Nostoc* sp. (or possibly other nostocalean genera) and characteristic heterocysts could be observed in the filaments by microscopy. *Tolypothrix* was also a dominant morphotype in some mats (Komárek and Komárek 2003). Sample IRJ6, used in this study, was collected from a mat that appeared to be dominated by *Phormidium*-like morphotypes. Compared with others, this mat had an unusually high level of orange pigmentation. As it was well-established that cyanobacteria with related morphotypes dominate these mats as well as those in the McMurdo region, we wanted to determine what phylotypes (ribotypes) might be common in the two regions. The perspective of this report is not taxonomy *per se*, but to establish a reference point for similar exploration of other cyanobacterial mat communities at different sites along the Antarctic Peninsula, which itself forms a natural gradient of climate change (Convey 2003; Yergeau et al. 2007).

Although there have been extensive morphological descriptions of cyanobacterial communities on King George Island, the use of molecular approaches for analysis of microbial communities on the island have only recently been reported (Xiao et al. 2007; Flocco et al. 2009; Foong et al. 2010; Strunecký et al. 2010). In this report we focus on DNA sequence and phylogenetic analysis of cyanobacterial 16S RNA gene fragments derived from a sample from an apparently healthy (near pristine) benthic microbial mat. The benthic mat was in a creek in an area with several similar creeks on the Fildes Peninsula, was distant from a research station, human activity in general and was *ca.* 500 m from the Drake Passage coastline (see Fig. 1).

Materials and methods

Sampling and environmental DNA extraction

Sample IRJ6 was collected from a complex creek system close to a slope that rose to form a ridgeline perpendicular to the coastline of Drake Passage, and was on a very gentle slope to the coastline shown in Fig. 1. The creek system meandered through a patchwork of bryophytes, and there was no obvious evidence of eutrophication. The mat system was 3–5 mm thick, appeared structured, and was loosely attached to large pebbles at the bottom of the creek. Among other *Phormidium*-like mats on the Fildes Peninsula, the mat from which sample IRJ6 was taken was unusual in having a high level of orange pigmentation (noted above).

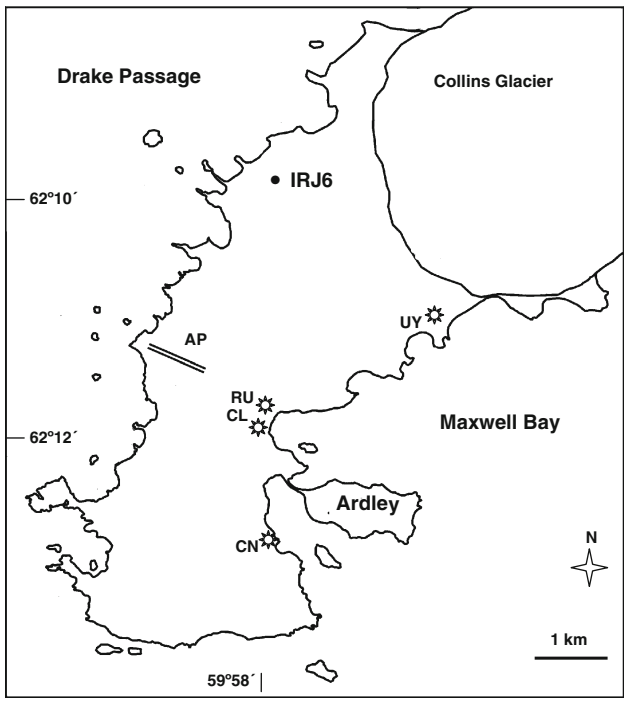

Fig. 1 Map of the Fildes Peninsula showing the location where sample IRJ6 was collected. Locations: UY, Artigas Base (Uruguay); AP, Airport Teniente Marsh; RU, Bellinhausen Base (Russia); CL, Pte. Frei Base (Chile); CN, Great Wall Base (China)

Sample IRJ6 (*ca.* 10 ml of mat material) was collected and placed in a sterile tube (January 2005). The sample was stored on ice for transport to the lab (IIBCE, Montevideo, Uruguay), after which it was frozen until processed. Genomic DNA from the sample was purified using a combination of both physical (zirconium beads) and chemical lysis methods previously described by Griffiths et al. (2000). Integrity of DNA preparations was qualitatively evaluated by agarose gel electrophoresis. Although the sample had become black in appearance, presumably due to oxidation of pigments, this did not affect the ability to prepare, PCR amplify and analyze DNA present in the sample.

Amplification of cyanobacterial 16S rRNA gene fragments

PCR amplification of cyanobacterial 16S rRNA gene fragments was done in a 25 µl reaction mixture

containing genomic DNA prepared as described above, 1× Hot Start PCR buffer, 0.8 mM of each dNTP, 4.0 μM each of cyanobacterial-specific 16S rDNA gene primers, 359f (5′-GGGGAATYTTCCGCAATGGG-3′) and 781ra (5′-GACTACTGGGGTATCTAATCCCATT-3′) (Nübel et al. 1997; see also Taton et al. 2003), and 1U Hot Start *Taq* DNA polymerase (Fermentas, Ontario, Canada). Amplification was done in a Perkin-Elmer GeneAmp PCR System 2400 thermocycler programmed as follows: initially the samples were heated for 5 min at 94°C; then 5 cycles of 30 s at 94°C, 30 s at 56°C and 30 s at 72°C; and 25 cycles of 30 s at 94°C, 30 s at 55°C and 30 s at 72°C, and a final elongation step of 4 min at 72°C. PCR products were separated by electrophoresis on 1% agarose gels then purified from the agarose using a Sephaglas™ BandPrep kit (Amersham Biosciences AB, Vienna, Austria).

Clone library construction and DNA sequencing

Cloning of PCR products was done using the InsTAclone™ PCR Product Cloning Kit #K1213 (Fermentas, Ontario, Canada) following manufacturer instructions. Aliquots of ligation mixtures were used to transform *Escherichia coli* TOP10 (Invitrogen, Carlsbad, CA) using a chemical transformation protocol described by Ausubel et al. (1992). Transformants were screened for loss of (α-complementation on LB agar plates supplemented with ampicillin (50 μg ml^{-1}) and 5-bromo-4-chloro-3-indolyl *β*-galactopyranoside, X-Gal (10 μg ml^{-1}).

Plasmid DNA was purified by an alkaline lysis method described previously (Ausubel et al. 1992). DNA fragment inserts were re-amplified by PCR as described above, and classified into different groups using amplified ribosomal DNA restriction analysis (ARDRA). Briefly, re-amplified fragments from individual colonies were independently digested with: *Alu*I, *Hinf*I or *Msp*I (Fermentas, Ontario, Canada). The resulting ARDRA profiles, restriction enzyme digestion profiles, were analyzed by electrophoresis on 6% native polyacrylamide gels and using a silver stain detection method (Ausubel et al. 1992). Clones having the same digestion pattern or fragment size profile were assigned to a given ARDRA group. The ARDRA analysis thus served as a guide to select clones representative of each group for subsequent DNA sequence and phylogenetic analysis. PCR cycle sequencing using the pUC/M13 reverse primer (Promega Corp., Madison, WI) was done using a model ABI Prism 377 sequencer (Applied Biosystems, Foster City, CA). DNA sequencing was done at the CTAG sequencing facility in the Faculty of Sciences, University of the Republic, Montevideo, Uruguay.

Phylogenetic analysis

DNA sequence data was analysed by BLASTn (www.ncbi.nlm.nih.gov). Multiple alignments were generated using the CLUSTALW program (Thompson et al. 1994). Phylogenetic distance trees were inferred by Maximum-Parsimony (MP, heuristic search factor of 2) and Neighbour-Joining (NJ, p-distance matrix) analyses, using MEGA4.1 (Tamura et al. 2007). Confidence in topologies was assessed using bootstrapping (1,000 replicates).

Accession numbers

GenBank Accession Numbers are: c19(DQ533823), c20(DQ533824), c24(DQ533825), c30(DQ533826), c46 (DQ533827), c47(DQ533828), c48(DQ533829).

Results

DNA sequence and phylogenetic analysis

Total environmental DNA was isolated from the mat and degenerate primers were used to specifically amplify cyanobacterial 16S rDNA gene fragments. A clone library was then prepared using this family of fragments. Fifty-three clones having an insert of the expected size were selected for analysis. Forty-four of the clones (83%) had the same ARDRA pattern, and the remaining nine clones had six other patterns using *Alu*I and *Hinf*I. All clones had the same ARDRA pattern when *Msp*I was used. Representative clones from each ARDRA group were selected for DNA sequence analysis. BLASTn analysis of sequences obtained for the seven groups showed highest similarity to previously cultured cyanobacteria, *i.e.*, *Tychonema bourrellyi*, *T. bornetii*, *Phormidium autumnale*, *Phormidium* sp., *Oscillatoria* sp., *Microcoleus* sp. and to uncultured Antarctic and Artic cyanobacterial clones. Notably, all the cultivable cyanobacteria with highest similarity belonged to the order Oscillatoriales. As such the NJ distance tree shown in Fig. 2 includes reference sequences with highest similarity from cultured Oscillatoriales and sequences from uncultured clones in the GenBank database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Both NJ and MP algorithms gave similar topologies (Figures 2 and 1S in supplemental material).

Clone c47 represented the ARDRA group accounting for 83% (44/53) of the clones, and over the sequences compared was found to be identical (100% identity for 100% query coverage) with phylotypes of cultivated cyanobacteria isolated from environmental samples from southern Norway (*ca.* 60°N), Northern Ireland and uncultured cyanobacterial clones discussed below. Isolates from

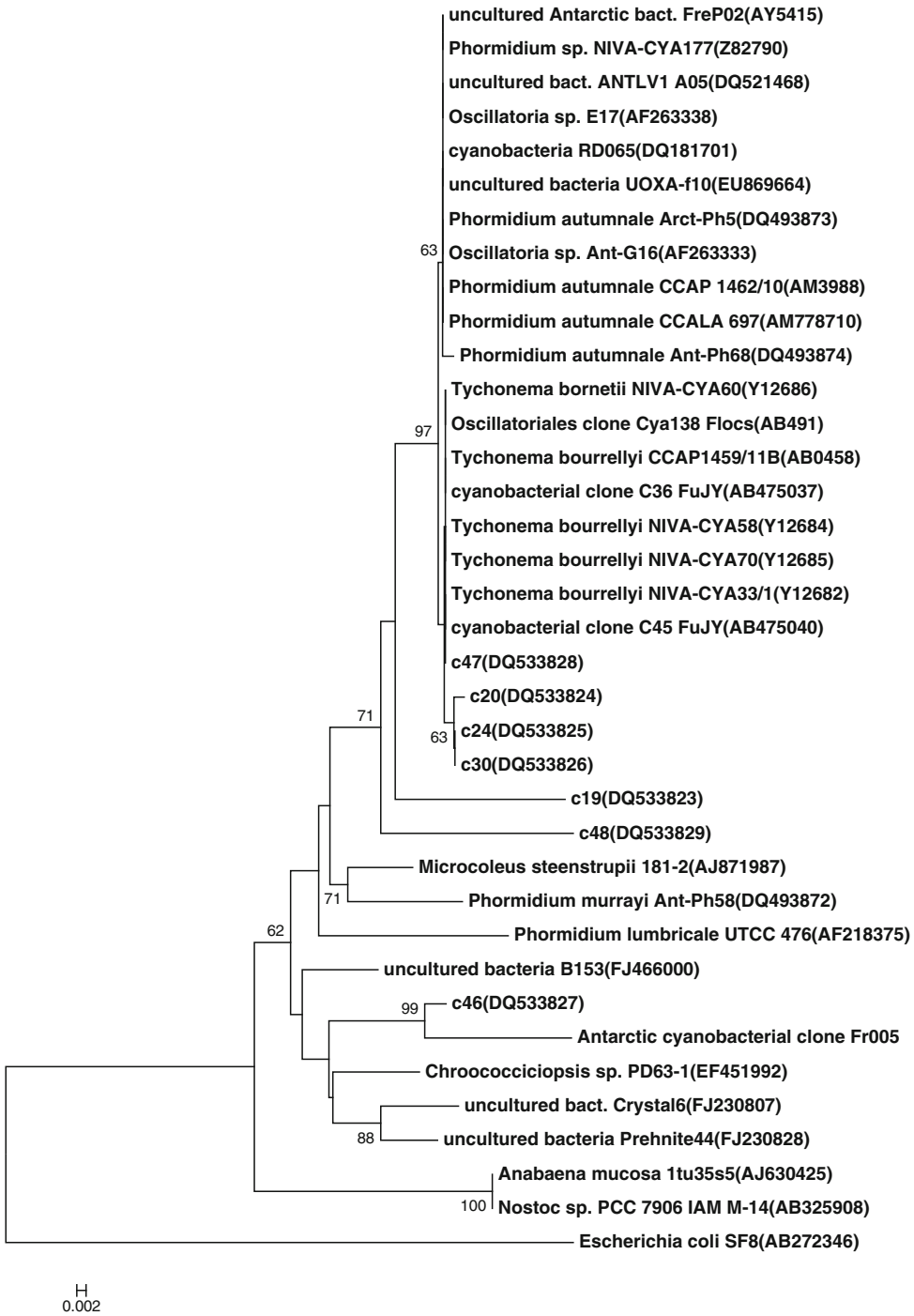

Fig. 2 Rooted phylogenetic tree showing sequences with highest similarity from the GenBank database of the NCBI and the 37 operational taxonomic units that were used in this analysis. The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees (>50%) in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. All positions containing gaps and missing data were eliminated from the dataset. *Escherichia coli* was used as an outgroup taxon. GenBank/EMBL accession numbers are given between parentheses

Lake Mjøsa, Norway included *T. bourrellyi* (Y12685) and *T. bornetii* (Y12686) (Rudi et al. 1998), and from Loughgall, Northern Ireland, *T. bourrellyi* (AB045897) (Suda et al. 2002). Phylotypes c20, c24, c30, represented by five clones (9.4% of the library), clustered with the *Tychonema* group of clone c47. These clones exhibited 99% maximum identity for 100% query coverage with the same sequences noted for phylotype c47.

The remaining three phylotypes represented by only four clones (7.4%), in general, had highest similarity with uncultured cyanobacterial clones of Antarctic origin, e.g., Lake Fryxell and fresh meltwater on the McMurdo Ice Shelf. Clone c48 had highest identity (96% for 90% query coverage) with BGC-Fr018 which was cultivated from material collected at Lake Fryxell. Clone c48 also exhibited some similarity (94% identity for 97% coverage) with both FreP19, from Fresh Pond (meltwater pond) on the McMurdo Ice Shelf reported by Jungblut et al. (2005), as well as with the *Tychonema* groups discussed above. Clone c46 had highest similarity (98% identity for 100% query

coverage) with uncultured cyanobacteria clones from Lake Fryxell (Fr147 and Fr005) (Taton et al. 2003). Clone c19 had highest similarity (maximum identity, 93% for 100% coverage) with uncultured cyanobacteria clones of Antarctic and Artic origin (uncultured bacterium UOXA-f10 from Onyx River, Wright Valley, Victoria Land), cultured *P. autumnale* Arct-Ph5, CCALA697 (Arctic Canada, Ellesmere Island, Sverdrup pass) and *P. autumnale* CCAP 1462/10, Antarctica, South Orkney Islands soil. Although clone c19 would appear to be of polar origin, it was very distantly related to the other uncultured clones or cultivated bacteria. Phylogenetic analyses also indicated that it might be related to the *Tychonema* groups as well.

Discussion

Traditional methods for the classification of cyanobacteria as Cyanophyceae are based on the International Code of Botanical Nomenclature using morphological criteria (e.g., *Oscillatoriaceae*, http://www.bgbm.org/IAPT/). Guidelines for their classification as oxygenic phototrophic bacteria using biochemical, physiological, ecological, genetic and molecular criteria have been described and discussed in detail (Castenholz and Waterbury 1989, Anagnostidis and Komárek 1988, Suda et al. 2002, Komárek 2006). It is well-established that morphology and other phenotypic characteristics of cyanobacteria can be dramatically influenced by both environmental factors as well as the stage of development (Suda et al. 2002). Unfortunately, there is very little information available regarding what influences the morphological appearance of cyanobacteria grown as part of a consortium in its natural environment.

Using a molecular strategy to investigate cyanobacterial populations in a microbial mat on King George Island, our results were consistent with studies using the same basic molecular strategy for investigation of sites in the McMurdo region. The minor representatives (four clones representing 7.6% of the library) generated from sample IRJ6 exhibited highest overall similarity with phylotypes identified in Lake Fryxell (Taton et al. 2003) and the McMurdo Ice Shelf (Jungblut et al. 2005). In addition, these three groups had similarity with other uncultured Antarctic bacterial or cyanobacterial clones and cultivated *Phormidium* of high Artic origin. We found, however, that clone c47, representing 44 clones (83%), had sequence identity with the corresponding 16S rDNA gene sequences of organisms classified as members of the genus *Tychonema,* whose geographic origins were southern Norway and Northern Ireland. Also, morphotypes of *Tychonema* have previously been reported in environmental samples from Ward Hunt Lake (83°N) (Villeneuve et al. 2001) and western Europe, e.g., (52°8′N 4°19′E, see strain collection, http://sagdb.uni-goettingen.de/, strain number: 23.89). Recently, the c47 phylotype was found in environmental samples from Switzerland (AB475037) as well as in microbial flocs floating in a pond of a shrimp hatchery in Banyuwangi, Java Island, Indonesia (8°14.18′S) (AB491884).

Cyanobacterial morphotypes or ribotypes corresponding to *Tychonema* sp., to our knowledge, have not been reported in Antarctica or elsewhere in the Southern Hemisphere except on Java Island which can be considered equatorial. Three of the ARDRA groups represented by 5 clones (9.4%) had highest similarity with the apparently dominant *Tychonema* group (c47) together accounting for 92.4% of the clones. In conclusion, our study provides one of the first molecular characterizations of microbial mats in maritime Antarctica. Similar analysis of cyanobacterial mat community genotypic structure at sites between King George Island and the McMurdo region could help provide a detailed perspective on the impact of global warming on biological systems.

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