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POLYPHASIC STUDY OF ANTARCTIC CYANOBACTERIAL STRAINS[1]

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We isolated 59 strains of cyanobacteria from the benthic microbial mats of 23 Antarctic lakes, from five locations in two regions, in order to characterize their morphological and genotypic diversity. On the basis of their morphology, the cyanobacteria were assigned to 12 species that included four Antarctic endemic taxa. Sequences of the ribosomal RNA gene were determined for 56 strains. In general, the strains closely related at the 16S rRNA gene level belonged to the same morphospecies. Nevertheless, divergences were observed concerning the diversity in terms of species richness, novelty, and geographical distribution. For the 56 strains, 21 operational taxonomic units (OTUs, defined as groups of partial 16S rRNA gene sequences with more than 97.5% similarity) were found, including nine novel and three exclusively Antarctic OTUs. Sequences of *Petalonema* cf. *involvens* and *Chondrocystis* sp. were determined for the first time. The internally transcribed spacer (ITS) between the 16S and the 23S rRNA genes was sequenced for 33 strains, and similar groupings were observed with the 16S rRNA gene and the ITS, even when the strains were derived from different lakes and regions. In addition, 48 strains were screened for antimicrobial and cytotoxic activities, and 17 strains were bioactive against the gram-positive *Staphylococcus aureus*, or the fungi *Aspergillus fumigatus* and *Cryptococcus neoformans*. The bioactivities were not in coincidence with the phylogenetic relationships, but rather were specific to certain strains.

Key index words: **Antarctic lakes; bioactive compounds; cyanobacteria; microbial mats; polyphasic characterization; rRNA operon**

Abbreviations: **ITS, internally transcribed spacer; OTU, operational taxonomic unit**

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Cyanobacteria are a major component of Antarctic ecosystems (Vincent 2000). Classical taxonomic studies have described Antarctic species compositions based on morphological and ecological features (Broady and Kibblewhite 1991) in several types of habitat, including lacustrine benthic microbial mats. However, morphological features do not necessarily reflect the real genetic and physiological divergences, which can be revealed using molecular data (Nadeau et al. 2001). To date, only 22 strain sequences (e.g. Casamatta et al. 2005 and Nadeau et al. 2001) and 144 16S rRNA gene sequences are available from uncultured Antarctic cyanobacteria (e.g. Jungblut et al. 2005 and Taton et al. 2003). These studies have shown that many sequences from Antarctic cyanobacteria are grouped together in distinct clusters. In contrast, morphological studies have identified an apparently cosmopolitan distribution (Vincent 2000), principally due to a lack of morphological diacritical traits for certain groups, the use of taxonomic keys written for temperate and tropical floras, and a lack of consideration for ecological information (Komárek 1999).

The isolation and characterization of cyanobacterial strains from diverse biotopes remain extremely important for studies of the cyanobacterial diversity, even where culture-independent techniques based on the rRNA operon have successfully been used (Ward et al. 1998). Strain results provide a link between genotypic and phenotypic features to allow a better understanding of cyanobacteria physiology, autoecology, and biotechnological potential. In addition, by using clonal strains instead of environmental clone libraries, artifacts such as the formation of chimeras and other cloning biases are avoided. Finally, characterizations based on polyphasic studies improve the resolution of cyanobacterial taxonomy (Wilmotte 1994) and currently constitute the best-defined baseline for biodiversity and ecological studies. The taxonomy of cyanobacteria is still under revision, and too few studies have investigated cyanobacterial morphotypes and genotypes in parallel.

The discovery of novel and endemic bacterial, fungal, and algal genotypes using a multidisciplinary approach (Van Trappen et al. 2002, Taton et al. 2003, Sabbe et al. 2004) has made mass cultivation and biotechnological exploration of isolated Antarctic strains particularly interesting (Marinelli et al. 2004). Although the search for bioactive products from cyanobacteria (i.e. antitumor, antifungal, antibacterial, and antiviral molecules) has intensified during recent decades (Burja et al. 2001), until this study, there has not been a pharmaceutical screening of a significant number of Antarctic cyanobacteria.

The aims of the present study were as follows: (1) to obtain a wide variety of strains from different Antarctic lakes by experimenting with isolation methods, growth conditions, and novel culture media; (2) to characterize the isolated strains using a polyphasic approach and assign them to new, endemic, or known organisms; (3) to compare this new diversity with culture and environmental sequences already available for Antarctica and to examine the geographical distribution of genotypes; and (4) to compare the patterns of antimicrobial and cytotoxic activities with the evolutionary relationships.

MATERIALS AND METHODS

Sampling. Twenty-seven benthic microbial mat samples were collected during the Antarctic summers 1997–1998 and 1998–1999 from 23 lakes and ponds in the Larsemann Hills (LH), Bølingen Islands (BI), Vestfold Hills (VH), Rauer Islands (RI), and the McMurdo Dry Valleys (DV). The LH and VH, located in the Prydz Bay region, constitute two major ice-free areas in continental east Antarctica of around 50 and 400 km^2, respectively (Sabbe et al. 2004). The Bølingen Islands form a smaller, though significant, ice-free archipelago 25 km to the west south west of the LH. The RI are a coastal archipelago of ice-free islands situated in the southeastern Prydz Bay (Hodgson et al. 2001). The DV, the largest ice-free area in Antarctica (4800 km^2), are located in southern Victoria Land between the polar plateau and McMurdo Sound (Gordon et al. 2000). These locations and the main abiotic characteristics of the lakes are listed in Appendix 1.

Isolation of strains. Strains were isolated using three different methods: small subsamples of microbial mats were (1) spread out on solid media using a dissecting needle under a binocular microscope, (2) homogenized with a Potter tube and 500 μL of the suspension spread out on solid media, and (3) maintained in liquid culture media and the resulting cyanobacterial biofilm spread out on solid media. The media ASNIII/2, GANX, BG11 and $ASNIII_0/2$, GOX, $BG11_0$ (Rippka et al. 1979, Waterbury and Stanier 1981) were used with and without nitrogen. In addition, six new media (1NP, 2NP, 3NP and 1, 2, 3, with and without nitrogen, respectively—Appendix 2) were created based on chemical data from the LH and RI lakes (Hodgson et al. 2001 and Sabbe et al. 2004). Incubation temperatures were 5, 12, and 22° C. When several strains from the same sample with similar morphologies were isolated in the same conditions, isolation was pursued for only one.

Unialgal cultures were obtained by picking material from the edge of discrete colonies that had been growing for ~3 weeks on solid media. Cultures were cleaned of eukaryotic contaminants by one transfer to solid media containing 50 $mg \cdot L^{-1}$ of cycloheximide. Clonal isolates were obtained by subculturing one filament or some cells originating from the same colony twice (Rippka et al. 1979).

All the strains were maintained in their isolation media and in BG11 and $BG11_0$ for nonheterocystous and heterocystous cyanobacteria, respectively. The strains were named after the lakes from which they originated.

Morphological characterization. The strains were observed with a Wild MS-20 microscope equipped with a screw micrometer (Wild, Heerbrugg, Switzerland). The diacritical morphological traits used in botanical species descriptions were considered, including cell shape for intercalary and terminal cells; width and length of intercalary cells; presence or absence of constrictions at the cross-wall, necridic cells, and a sheath; color of the sheath; number of trichomes per filament; presence or absence of false branching and heterocysts; and the width and length of heterocysts. For each biometrical character, 30–50 measurements were taken of cells and heterocysts, and filaments were sampled at random. Taxonomy was based on Geitler (1932), Komárek and Anagnostidis (1989, 1998, 2005), and Antarctic literature (Broady and Kibblewhite 1991).

Molecular characterization. The method used for the nucleic acid extraction was described by Taton et al. (2003), but glass beads had a diameter of 0.1 mm (BioSpec, Bartlesville,

OK, USA), and the shaking was performed by vigorous vortexing for 10 min. The crude DNA preparations were purified using the Prep-A-Gene® DNA Purification Systems (Bio-Rad U.S.A., Baltimore, MD, USA), following the manufacturer's instructions. The PCR amplification of cyanobacterial 16S rRNA gene plus ITS using the primer pair 16S27F/23S30R is described in Taton et al. (2003).

Partial 16S rRNA gene sequences with a minimum length corresponding to *Escherichia coli* positions 405–780 were determined for 56 strains using the sequencing primer 16S378F or 16S784R. Complete sequences (*E. coli* positions 27–1542) were determined (on one DNA strand) for at least one representative strain selected at random from each operational taxonomic unit (OTU). An OTU was defined as a group of sequences that exhibited more than 97.5% similarity with each other, using the *E. coli* positions 405–780, not taking into account indels and ambiguous bases (Taton et al. 2003).

In addition, complete ITS sequences were determined for 32 Oscillatoriales and 1 Nostocales. Sequencing was carried out with the primers used by Taton et al. (2003), the sequencing primers 16S1514F (5′-GTC GTA ACA AGG TAG CCG TAC-3′) and/or Ile23F (5′-ATT AGC TCA GGT GGT TAG-3′). Sequencing was carried out by Genome Express (Meylan, France) on an ABI PRISM system 377 (PE Applied Biosystems, Foster City, CA, USA) and contiguous sequences were obtained using the software Sequencher (Gene Codes Corporation, Ann Arbor, MI, USA). The sequences (*E. coli* positions: 100–1450 and 405–780) were initially analyzed by similarity search using the basic local alignment search tool (BLAST) software (widely available on Internet), and chimera detection was performed using "Check Chimera" from the Ribosomal Database Project (Maidak et al. 2001). The 16S rRNA gene sequences determined in this study were included in the data base of the ARB software package (Ludwig et al. 2004) and aligned with the cyanobacterial sequences available from GenBank. Phylogenetic trees were constructed using the maximum likelihood of fastDNAml implemented in ARB, the Wagner parsimony of PHYLIP 3.63 (Felsenstein 1989), and the neighbor-joining on the Jukes and Cantor distances matrix of TREECON 1.3b (Van de Peer and De Wachter 1997). Bootstrap analyses involving the construction of 500 resampled trees were performed for the parsimony and neighbor-joining methods. Aligned 16S rRNA gene sequences corresponding to *E. coli* sequence positions 100–1450 were used, but indels were not taken into account in the distance matrix calculation. The trees comprised the sequences determined in this study together with their two nearest neighbors, indicated by BLAST, that contained the same positions. If these hits were from uncultured clones, we added the sequences of the two closest cultured strains. Furthermore, we included at least one sequence of each of the clusters previously defined by Wilmotte and Herdman (2001).

Because we generated more partial than complete sequences, and there are a lot of short sequences in GenBank, we also constructed a neighbor-joining tree, as described above, with all our partial and complete Antarctic strain sequences plus the sequences indicated by BLAST, but using *E. coli* sequence positions 405–780 for the procedure. This allows us to show the relationships among all our sequences in all OTUs, and complements the tree based on near-complete sequences. The OTUs, as defined above, were used to delineate the clusters. Furthermore, the OTUs were divided into three categories: (1) new OTUs only composed of our sequences, none exhibiting more than 97.5% similarity with GenBank sequences; (2) Antarctic OTUs, in which our sequences were grouped with GenBank Antarctic sequences with a minimal threshold of 97.5% similarity; and (3) cosmopolitan OTUs in which our sequences were grouped with GenBank sequences that originated from non-Antarctic samples.

The ITS sequences were aligned on the basis of conserved domains (Iteman et al. 2000) and tRNAs. Among their closest relatives available from GenBank, those for which the alignment with our ITS sequences seemed meaningful were included in the alignments (Appendix 3).

Screening for antimicrobial and cytotoxic activities. Strains were axenically cultivated in the inorganic media BG11 and $BG11_0$ in 500–1100 mL glass tubes bubbled with air/CO_2 (98/2, v/v) at 30 μmol photons $\cdot m^{-2} \cdot s^{-1}$ irradiance, provided by daylight fluorescent tubes (Osram Lumilux L 58W, Danvers, MA, USA). PAR irradiance was measured with a Li-Cor Li185A quantum meter (Li-Cor, Lincoln, NE, USA) equipped with a Li190SB cosine quantum sensor. Temperature was maintained at $20 \pm 2^{\circ}$ C. The cultures were routinely and carefully screened by microscopic observations; they were healthy and dense so that the bacterial contamination would be very small. Furthermore, the cyanobacterial biomass was harvested and washed with stirring on a nylon net of 12 μm mesh by sucking a saline solution under vacuum. This would further reduce bacterial contaminants. Biomass was then frozen, thawed, and extracted overnight with ethyl acetate (50 mL/g of dry biomass) or with methanol (50 mL/g of dry biomass). After filtration on paper, the solvent was evaporated under vacuum, and the dry residue was dissolved in 40 μL dimethylsulfoxide (DMSO)/H_2O (1/9, v/v). In addition, for the same cultures, the thawing water was recovered, filtered, frozen, lyophilized, and finally extracted overnight with methanol. Media and procedures used for the antimicrobial screening in liquid microtiter assay have been previously described in Gaspari et al. (2005).

The following human pathogens were used: *Staphylococcus aureus* ATCC 6538 *Escherichia coli* L47, *Candida albicans* L145, *Aspergillus fumigatus* ATCC 90112, and *Cryptococcus neoformans* IUM 94698. They originated from the American Type Culture Collection (ATCC) or from the Lepetit Culture Collection (L; c/o Vicuron Pharmaceuticals, Gerenzano, Varese, Italy) or from the Istituto di Igiene, Università di Milano, Italy (IUM). Optical density at 620 nm was checked to detect pathogen growth inhibition by cyanobacterial extracts. One point test was used to select the "active" strains, i.e. those inhibiting more than 80% of the pathogen growth in comparison with the control growth set as 100%, when only DMSO/H_2O was added to the pathogen inoculum. The broth microdilution method was used to confirm positive broths and to assay their potency (Gaspari et al. 2005). For the cytotoxic assay, HeLa cells were used for screening in an *in vitro* test, previously developed for a rapid identification of extracts active on mammalian cells (Marinelli et al. 2004). Those cyanobacterial extracts able to inhibit at least 40% of the cellular thymidine uptake, set as 100% in the control condition when only DMSO/H_2O was added to the Hela cells, were considered cytotoxic.

Nucleotide sequence accession numbers. Twenty-nine almost complete, 27 partial 16S rRNA gene, and 33 ITS sequences were deposited under the following accession numbers; AY493572–AY493600, AY493601–AY493627, and AY493628–AY493660, respectively.

RESULTS

Strain isolation. In order to reduce the selection of opportunistic cyanobacteria and to promote diversity among the isolated strains, 12 culture media, of which six were newly created, and three incubation temperatures were used for the strain isolation. In total, 59 clonal unialgal strains from 26 samples derived from 23 lakes were isolated. Even though the relative efficiency of the different media cannot be rigorously compared, 76% of the strains were

isolated with the media 2, 2NP, 3, or 3NP (Appendixes 2 and 4). Furthermore, 34 strains were isolated at 22° C, 23 at 12° C, and 2 at 5° C. This reflected the slower growth at lower temperature. The origin of these strains and a short description of the main abiotic parameters of the lakes are summarized in Appendix 1.

Morphology. Fifteen strains belonged to the Nostocales, one strain to the Chroococcales, and 43 strains to the Oscillatoriales (Figs. 1 and 2; Table 1). Within the Nostocales order, eight strains belonged to the genus *Nostoc*, five strains to the genus *Calothrix*, one strain to the genus *Petalonema*, and one strain to the genus *Coleodesmium*. The only Chroococcales isolated belonged to the genus *Chondrocystis*. Ten morphological criteria were used to describe the oscillatorian strains. Of these, trichome width; cell shape; presence or absence of cross-wall constrictions, necrids, a sheath, and false branching; and the number of trichomes per filament allowed us to distinguish seven morphospecies (Table 1).

A description of the morphospecies and the corresponding number of isolated strains is presented in Table 1. In addition, biometrical and other morphological criteria are given for each strain (Appendix 4).

The strain ANT.L70.1 did not clearly belong to any of these morphospecies, mainly because of the large variations in cell length observed in culture. Two types of trichomes were observed, one with cells longer than wide and one with cells shorter than wide. Nevertheless, no evidence was found of the coexistence of two distinct strains in the culture. We suspected that the presence of shorter cells could be due to the cultivation conditions. Therefore, this strain was considered as belonging to *Leptolyngbya frigida*, but the cell dimensions were not used in the average value of the morphospecies description. Four of our morphospecies were considered as endemic to Antarctica by Komárek (1999) (Table 1).

16S rRNA gene analysis. For 56 strain sequences, 21 OTUs were defined using a threshold of 97.5% similarity and partial 16S rRNA gene (*E. coli* positions 405–780). Fifteen belonged to the Oscillatoriales, five to the Nostocales, and one to the Chroococcales (Table 2). Complete 16S rRNA gene sequences were obtained for at least one strain per OTU, except for *Calothrix* sp. ANT.L52B.2, the only strain belonging to 16ST17^{New}. The PCR did not work when we tried to obtain PCR products longer than ~400 base pairs (bp). In total, 29 complete sequences were obtained.

The new sequences have 89.6%–100% 16S rRNA gene similarities with their closest relatives, currently deposited in GenBank (Table 2). Nine out of 21 OTUs had at least 2.5% dissimilarity with the sequences in the data bases. Three OTUs were related to sequences found only in Antarctica and obtained from clone libraries. The remaining nine OTUs also included non-Antarctic sequences and were considered as cosmopolitan OTUs.

The phylogenetic analyses (Fig. 3) based on near-complete sequences and using several methods for tree construction, showed that the OTUs constituted monophyletic clades that were usually well supported by the bootstrap values. Therefore, as many sequences in GenBank are partial, but generally contain EC positions 405–780, the analyses of evolutionary relationships were based on the comparisons of partial sequences. The partial sequences recently obtained by Jungblut et al. (2005) were not indicated in the tree, but were included in the analysis. Here, we distinguish three groups: new, Antarctic, and cosmopolitan OTUs.

New OTUs. The sequences belonging to these new OTUs exhibited 2.5%–10.4% dissimilarity with all sequences available in the data bases. The sequence of *P.* cf. *involvens* ANT.LG2.8 was the first sequence determined for this genus and belonged to **16ST20^{New}**. The sequence of *Calothrix* sp. ANT.L52B.2 belonged to **16ST17^{New}**. Its closest relative belonged to the genus *Calothrix,* although with less than 97.5% binary similarity. The sequence of *Chondrocystis* sp. ANT.L59B.1 belonged to **16ST21^{New}** and was loosely associated with other unicellular cyanobacteria. Two sequences of *L. frigida,* constituted **16ST09^{New}** and were identical to each other but isolated from different lakes. The sequence of *L. frigida* ANT.L52.2 belonged to **16ST08^{New}**. Three sequences of *Phormidium priestleyi* belonged to **16ST03^{New}**, and two of them, isolated from Lake Bruehwiler (VH), were identical to each other and 1.4% dissimilar to the third strain isolated from another lake. Five sequences of *Pseudophormidium* sp./*Schizothrix* sp. constituted **16ST01^{New}**, and four of these sequences were identical to each other even though they originated from three different lakes. The minimum level of similarity within this OTU was 99.4%. Seven sequences of *L. frigida* belonged to **16ST07^{New}**, and six of these sequences fell into two groups of identical sequences, one group with four sequences originating from three lakes and one group with two sequences from two different lakes. A seventh sequence (*L. frigida* ANT.L52B.3) shared more than 97.5% similarity with the first group but only 96.9% similarity with the second group. However, to avoid adding one new OTU on the basis of a sequence that was only slightly divergent, we included this strain in **16STO7^{New}**. The sequence of *P. priestleyi* ANT.LACV5.1 belonged to **16ST06^{New}**.

Antarctic OTUs. Eight identical sequences of *Leptolyngbya antarctica* belonged to **16ST11^{Ant}** and came from five different lakes in three distinct regions: the LH and VH in eastern Antarctica and the DV in the Ross Sea region. In addition, these sequences appear related (at least 99.1%) to clones of the DV, Fr397 (Taton et al. 2003), LB3-46 (Priscu et al. 1998), and clones from Fresh and Orange ponds on Bratina Island (Antarctica) (Jungblut et al. 2005). Within **16ST13^{Ant}**, the sequence of *L. antarctica* ANT.BFI.1 (BI) was identical to the sequence of clone Fr132 isolated from microbial mats of Lake Fryxell in the

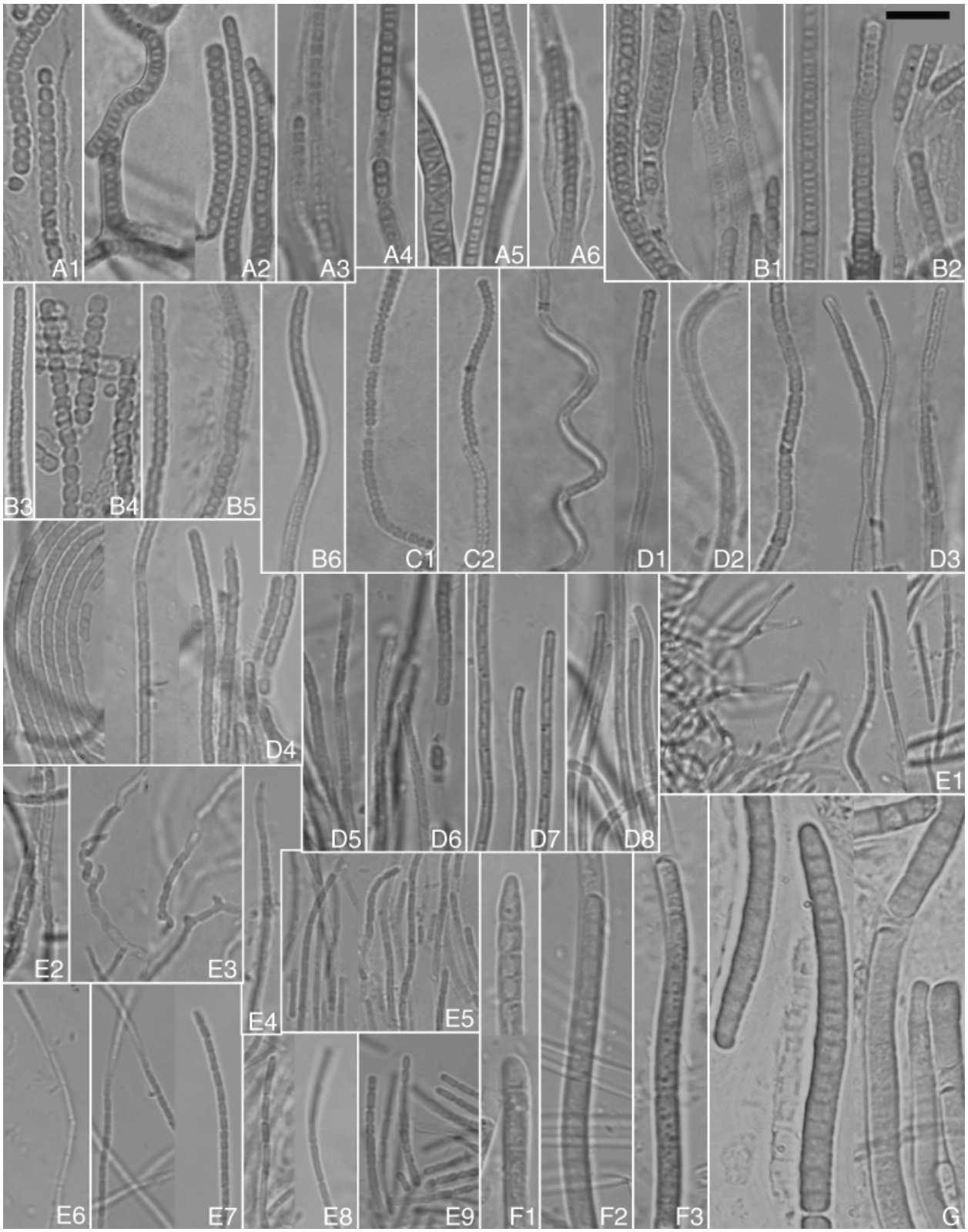

FIG. 1. Photomicrographs of the morphospecies belonging to the Oscillatoriales order. (A) *Pseudophormidium* sp./*Schizothrix* sp., (B) *Phormidium priestleyi*, (C) *Leptolyngbya* cf. *fragilis*, (D) *Leptolyngbya frigida*, (E) *Leptolyngbya antarctica*, (F) *Phormidium murrayi*, and (G) *Phormidium pseudopriestleyi*. Scale bar, 10 μm.

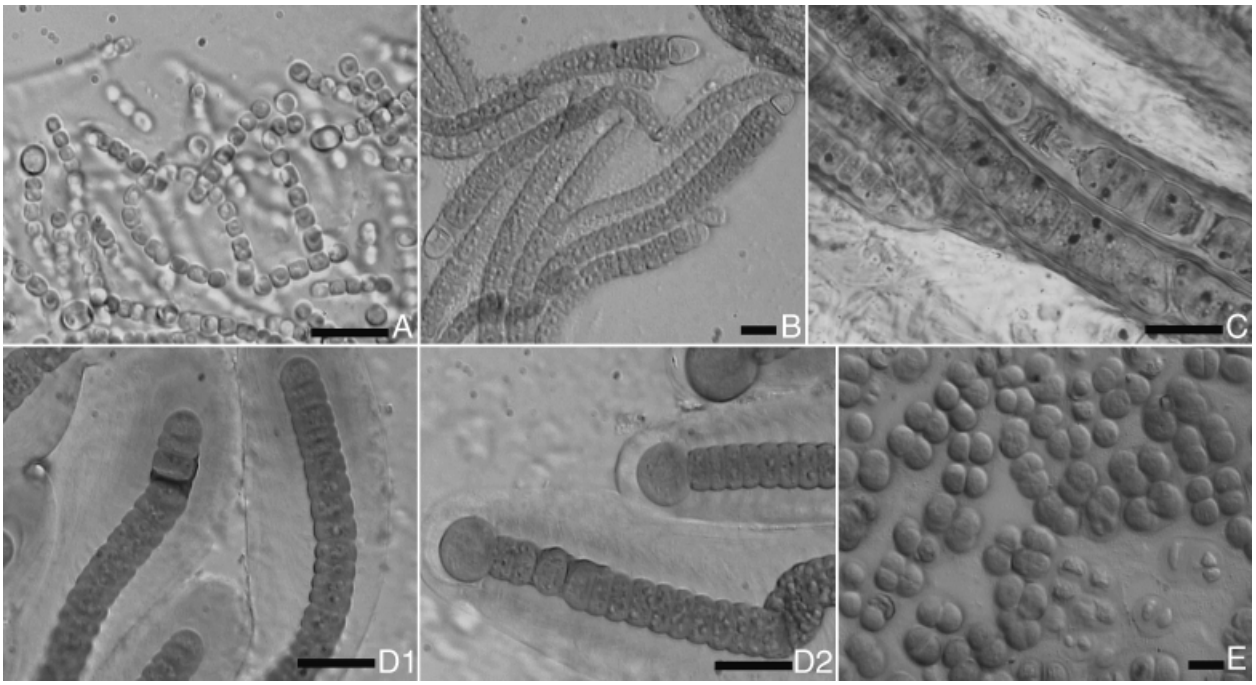

FIG. 2. Photomicrographs of the morphospecies belonging to the Nostocales and the Chroococcales orders. (A) *Nostoc* sp., (B) *Calothrix* sp., (C) *Coleodesmium* cf. *scottianum*, (D) *Petalonema* cf. *involvens*, and (E) *Chondrocystis* sp. The photomicrograph C was taken from the sample from which the strain was isolated. Scale bar, 10 μm.

Antarctic DV (Taton et al. 2003) and clone sequences from Fresh and Orange ponds on Bratina Island (Jungblut et al. 2005). Two identical sequences of *P. priestleyi* belonged to **16ST05^{Ant}** and were isolated from different lakes in the LH. These sequences exhibited 99.4% similarity with the sequence of clone LB3-53 from the Antarctic DV (Priscu et al. 1998).

Cosmopolitan OTUs. Nostoc sequences were grouped in **16ST16**. The minimum similarity within this cluster was 96.0%, but distinct OTUs within this OTU could not be easily distinguished. Interestingly, the three *Nostoc* strains originating from Lake L52B (LH) possessed 16S rRNA sequences that exhibited 1.4%–3.1% dissimilarity. The Antarctic clone OraP15 (Jungblut et al. 2005) also fell in this OTU. *C.* cf. *scottianum* ANT.L52B.5 belonging to **16ST19** clustered together (97.7% similarity) with *Tolypothrix distorta* SEV2-5-2-Ca, isolated from arid soil in New Mexico (USA) (Flechtner et al. 2002). Furthermore, it exhibited a maximum of 96.9% similarity with the other sequences of the genus *Coleodesmium* available in the data bases. The *Calothrix* sp. ANT.LPR.4 sequence belonging to **16ST18** exhibited 98.3% similarity with the sequence of *Calothrix* sp. CCMEE 5085, from hot spring microbial mats in Yellowstone (USA) and considered as moderately thermotolerant (Dillon and Castenholz 2003). Three identical sequences of *Phormidium murrayi* from two lakes of the VH were included in **16ST14**. These sequences were identical to those of *Microcoleus glaciei* Johansen and Casamatta UTCC475 (Casamatta et al. 2005), previously assigned to *P. murrayi* UTCC475 and isolated from a pond on Bratina Island. Furthermore, this OTU comprised clones from Fresh Pond (Jungblut et al. 2005) and the clone CD29 from a soil crust on the Colorado Plateau (USA) (Yeager et al. 2004). The sequence of *P. pseudopriestleyi* ANT.LACV5.3 from Ace Lake (VH) and belonging to **16ST15** was identical to clone and strain sequences from mats in ponds on Bratina Island (Nadeau et al. 2001 and Jungblut et al. 2005). The strains ANT.LJA.1 and ANT.L61.2 belonged to **16ST10**. Both strains were isolated from two lakes in the LH and assigned to *L. frigida* and *P. priestleyi*, respectively. Their sequences exhibited levels of similarity ranging from 98.9% to 99.4% with those of clone LB3-64 (Priscu et al. 1998) and of *Leptolyngbya* sp. SV1-MK-52 from a soil crust in the Silurian Valley (USA) (M. C. Payne and J. R. Johansen, unpublished data). The identical sequences of *L.* cf. *fragilis* ANT.L52.1 and ANT.RI8.1 that were isolated from two lakes of two neighboring regions (LH and RI) were included in **16ST02**. They exhibited 98.9% similarity with the clone FBP256 from a cryptoendolithic community in DV (De la Torre et al. 2003) and with the sequence of the marine non-polar strain of *Plectonema* sp. F3 (Turner 1997). Furthermore, this OTU also included *Pseudophormidium* sp./*Schizothrix* sp. ANT.LPE.3. Two sequences of *P. priestleyi* strains isolated from Progress 2 pond that were 99.4% similar to each other belonged to **16ST04** and clustered together with the clones Fr-BGC054 and LB3-1 from DV (Priscu et al. 1998, Taton et al. 2003) with levels of similarity ranging from 98.6% to 100%. The identical sequences of *L. antarctica* ANT.LAC.1

TABLE 1. Description of the morphospecies, taxonomical identifications, and possible endemic species.

Taxonomic assignment (Geitler 1932)	Taxonomic assignment (Komárek and Anagnostidis 1998, 2005)	Description	Number of strains
Plectonema sp./*Schizothrix* sp.	*Pseudophormidium* sp./*Schizothrix* sp.	Filamentous; false branching; sometimes several trichomes in the same sheath; trichomes constricted at the cross-walls; necridic cells; cells shorter than wide to quadratic of 2.06 ± 0.33 (1.33–2.86) μm wide, 1.81 ± 0.53 (0.83–3.82) μm long; end cells rounded	6
Phormidium priestleyi Fritsch	*Phormidium priestleyi* Fritsch[a]	Filamentous; trichomes ensheathed, constricted at the cross-walls; necridic cells; cells shorter than wide to quadratic of 1.98 ± 0.40 (1.14–3.15) μm wide, 1.82 ± 0.65 (0.65–3.80) μm long; end cells rounded	9
Phormidium cf. *fragile* Gomont	*Leptolyngbya* cf. *fragilis* (Gomont) Anagn. and Kom.	Filamentous; trichomes ensheathed, constricted at the cross-walls; necridic cells; cells shorter than wide to isodiametric of 1.42 ± 0.17 (1.14–1.90) μm wide, 1.23 ± 0.27 (0.76–2.09) μm long; end cells rounded	2
Phormidium frigidum Fritsch	*Leptolyngbya frigida* (Fritsch) Anagn. and Kom.[a]	Filamentous; trichomes ensheathed, constricted at the cross-walls; necridic cells; cells longer than wide of 1.44 ± 0.34 (0.72–2.96) μm wide, 2.78 ± 0.92 (1.16–7.79) μm long; end cells rounded	11
Phormidium antarcticum West and West	*Leptolyngbya antarctica* (West and West) Anagn. and Kom.[a]	Filamentous; trichomes ensheathed, slightly constricted at the cross-walls; 0.91 ± 0.16 (0.65–1.75) μm wide, 2.47 ± 0.87 (0.95–7.37) μm long; end cells rounded	11
Lyngbya murrayi West and West	*Phormidium murrayi* (West and West) Anagn. and Kom.	Filamentous; trichomes ensheathed, without constriction at the cross-wall, sometimes slightly curved at the end; cells 3.09 ± 0.38 (2.43–4.29) μm wide, 5.33 ± 1.26 (2.70–9.04) μm long; calyptra present or not, but in this case end cells rounded	3
Oscillatoria priestleyi West and West	*Phormidium pseudopriestleyi* Anagn. and Kom.[a]	Filamentous; trichomes ensheathed, not constricted to slightly constricted at the cross-walls, briefly attenuated at the end; necridic cells; cells disk-shaped, 5.86 ± 0.73 (4.02–7.22) μm wide, 3.24 ± 0.70 (1.87–4.52) μm long; necridic cells present; end cells rounded	1
Nostoc sp.		Heterocystous filamentous; cells subspherical, 3.67 ± 0.62 (2.22–5.97) μm wide, 3.64 ± 0.96 (1.41–6.69) μm long; heterocysts 4.60 ± 0.88 (2.85–7.6) μm wide, 3.58 ± 0.61 (2.54–5.26) μm long; confluent gel holds trichome masses in spherical hyaline or brown colonies	8
Calothrix sp.		Heterocystous filamentous; heteropolar; heterocysts basal cylindrical, 6.63 ± 2.14 (2.55–13.49) μm wide, 5.66 ± 2.19 (1.56–11.40) μm long; colorless hair; filaments 10.27 ± 2.68 (3.08–15.01) μm at the base; basal cells 6.91 ± 1.19 (4.26–10.87) μm wide, 4.88 ± 2.36 (1.98–11.51) μm long; lamellated yellow-brown sheath	5
Coleodesmium cf. *scottianum*		Heterocystous filamentous; several trichomes in one common yellow-brown sheath; false branching; filaments 10.14 ± 2.04 (6.73–14.82) μm wide per trichome; basal and intercalary heterocysts of 7.09 ± 0.88 (5.32–9.12) μm wide, 7.13 ± 1.04 (5.36–9.54) μm long; cells 5.59 ± 0.76 (4.37–7.22) μm wide, 4.49 ± 0.76 (3.08–6.08) μm long	1
Petalonema cf. *involvens*		Heterocystous filamentous; false branching; very thick yellow-brown sheath with divergent lamelation; filaments 11.57 ± 2.24 (8.60–17.48) μm wide; basal and intercalary heterocysts 6.29 ± 0.56 (5.40–7.60) μm wide, 4.90 ± 0.87 (3.36–6.92) μm long; cells 5.62 ± 0.41 (4.84–6.52) μm wide, 3.40 ± 0.75 (1.92–5.08) μm long	1
Gloeocapsa sp.	*Chondrocystis* sp.	Colonies composed of densely packed subcolonies; slightly lamellate yellow-brown sheath; cells almost spherical, 4.00 ± 0.81 (2.36–5.81) μm wide and 5.53 ± 0.80 (4.26–7.07) μm long; sheath 6.82 ± 0.63 (5.36–7.98) μm thick	1

[a]Possible Antarctic endemic species.

TABLE 2. Summary of the molecular data analysis.

Strain name	OTU (EC: 405–780)	First hit indicated by BLAST (EC: 405–780)[a]	ITS-type
ANT.LPR.2	16ST01^{New}	*Leptolyngbya* sp. PCC73110 (Nelissen et al. 1996) (95.8%–96.3%)	ITS05
ANT.LPR.3	16ST01^{New}	Idem	ITS05
ANT.LG2.1	16ST01^{New}	Idem	ITS05
ANT.LG2.2	16ST01^{New}	Idem	ITS05
ANT.L52B.4	16ST01^{New}	Idem	ITS05
ANT.LPE.3	16ST02	Uncultured cyanobacterium clone FBP403 (De la Torre et al. 2003) (97.5–98.9%)/ *Plectonema* sp. F3 (Turner 1997) (97.5%–98.9%)	ITS18
ANT.L52.1	16ST02	Idem	ITS01
ANT.RI8.1	16ST02	Idem	ITS01
ANT.L52.4	16ST03^{New}	Uncultured Antarctic bacterium LB3-53 (Priscu et al. 1998) (95.8%–97.2%)/*Leptolyngbya* sp. SV1-MK-52 (M. C. Payne and J. R. Johansen, unpublished data) (96.3%–96.9%)	ITS03
ANT.LG2.4	16ST03^{New}	Idem	ITS03
ANT.L52.6	16ST03^{New}	Idem	ND
ANT.LPR.5	16ST04	Uncultured Antarctic cyanobacterium BGC-Fr054 (Taton et al. 2003) (99.4%–100%)/ LPP-group cyanobacterium QSSC8cya (Smith et al. 2000) (97.7%)	ITS09
ANT.LPR.6	16ST04	Idem	ND
ANT.L66.1	16ST05^{Ant}	Uncultured Antarctic bacterium LB3-53 (Priscu et al. 1998) (99.4%)/*Leptolyngbya* sp. SV1-MK-52 (M. C. Payne and J. R. Johansen, unpublished data) (94.9%)	ND
ANT.LMA.2	16ST05^{Ant}	Idem	ND
ANT.LACV5.1	16ST06^{New}	LPP-group MBIC10597 (S. Suda, M. Atsumi, H. Miyashita, M. Kawachi, D. Honda, K. Watanabe, N. Kurano, S. Miyachi and S. Harayama, unpublished data) (97.2%)	ITS15
ANT.L53B.1	16ST07^{New}	Uncultured bacterium Tui1-3 (R. Howarth, D. J. Saul, V. Lane, P. Swedlund and J. G. Webster, unpublished data) (95.2%–97.2%)/*Phormidium autumnale* UTCC471, reassignated to *Pseudanabaena tremula* Johansen and Casamatta sp. nov. UTCC471 (Casamatta et al. 2005) (97.2%–97.5%)	ITS04
ANT.L52.3	16ST07^{New}	Idem	ITS04
ANT.L8.1	16ST07^{New}	Idem	ITS04
ANT.L53B.2	16ST07^{New}	Idem	ITS04
ANT.L52B.3	16ST07^{New}	Idem	ITS13
ANT.L64B.1	16ST07^{New}	*Phormidium autumnale* UTCC471, reassignated to *Pseudanabaena tremula* Johansen and Casamatta sp. nov. UTCC471 (Casamatta et al. 2005) (96.6%–97.2%)	ITS14
ANT.L70J.1	16ST07^{New}	Idem	ND
ANT.L52.2	16ST08^{New}	*Leptolyngbya* sp. CNP1-B3-C9 (M. C. Payne and J. R. Johansen, unpublished data) (90.9%)	ITS12
ANT.LMA.1	16ST09^{New}	*Leptolyngbya* sp. VRUC135 (Nelissen et al. 1996) (89.6%)	ITS06
ANT.L70.1	16ST09^{New}	Idem	ITS06
ANT.L61.2	16ST10	*Leptolyngbya* sp. SV1-MK-52 (M. C. Payne and J. R. Johansen, unpublished data) (98.9%–99.4%)	ITS16
ANT.LJA.1	16ST10	Idem	ND
ANT.LG2.3	16ST11^{Ant}	Uncultured Antarctic cyanobacterium Fr397 (Taton et al. 2003) (99.2%–99.4%)/*Leptolyngbya* sp. CNP1-B3-C9 (M. C. Payne and J. R. Johansen, unpublished data) (90.9%–91.1 %)	ITS08
ANT.L67.1	16ST11^{Ant}	Idem	ND
ANT.L18.1	16ST11^{Ant}	Idem	ITS08
ANT.LG2.5	16ST11^{Ant}	Idem	ITS08
ANT.LWA.1	16ST11^{Ant}	Idem	ND
ANT.L18.2	16ST11^{Ant}	Idem	ITS08
ANT.LFR.1	16ST11^{Ant}	Idem	ND
ANT.LWAV6.1	16ST11^{Ant}	Idem	ND
ANT.LAC.1	16ST12	*Oscillatoria* sp. Ant-SOS (Nadeau et al. 2001) (98.0%)	ITS02
ANT.LACV6.1	16ST12	Idem	ITS02
ANT.BFI.1	16ST13^{Ant}	Uncultured Antarctic cyanobacterium clone Fr132 (Taton et al. 2003) (100%)/*Leptolyngbya* sp. PCC 9221 (Miller and Castenholz 2001) (91.6%)	ITS10
ANT.LPE.1	16ST14	*Phormidium murrayi* UTCC 475 (M. C. Payne and J. R. Johansen, unpublished data) (100.0%)	ITS07
ANT.LACV5.2	16ST14	Idem	ND
ANT.LPE.2	16ST14	Idem	ITS07
ANT.LACV5.3	16ST15	*Oscillatoria* sp. Ant-Salt (Nadeau et al. 2001) (100.0%)	ITS17
ANT.L52B.1	16ST16	*Nostoc* sp. pcA (T. C. Summerfield, D. J. Galloway and J. J. Eaton-Rye, unpublished data) (99.4%)	ND
ANT.LPR.1	16ST16	*Nostoc commune* (T. Sakamoto, N. Horiguchi, M. Nakajima and K. Wada, unpublished data) (100%)	ND
ANT.L52B.8	16ST16	Idem	ND
ANT.L61.1	16ST16	*Nostoc* sp. NIVA-CYA 124 (Rudi et al. 1997) (99.4%–100%)	ND
ANT.LG2.6	16ST16	Idem	ND
ANT.L34.1	16ST16	Idem	ND
ANT.L36.1	16ST16	Idem	ND
ANT.L52B.7	16ST16	Idem	ND
ANT.L52B.2	16ST17^{New}	*Calothrix desertica* PCC7102 (Turner et al. 1999) (92.5%)	ND
ANT.LPR.4	16ST18	*Calothrix* sp. CCMEE 5085 (Dillon and Castenholz 2003) (98.3%)	ND
ANT.L52B.5	16ST19	*Tolypothrix distorta* Sev2-5-Ca clone 163-5B + 163-8 (Flechtner et al. 2002) (97.7%)	ITS11
ANT.LG2.8	16ST20^{New}	*Anabaena* sp. NIVA-CYA 267/4 (Rudi et al. 1997) (96.1%)	ND
ANT.L59B.1	16ST21^{New}	*Chroococcus submarinus* kopara-BM (L. Richert, S. Golubic, A. Herve, R. Le Guedes, J. Guezennec and C. Payri, unpublished data) (94.9%)	ND

ND, not determined.

[a]When the first hit indicated by BLAST was an uncultivated cyanobacteria, the first strain indicated by BLAST was added. Levels of similarity were determined by the computation of similarity matrixes; indels and ambiguous bases were not taken into account. Ranges of similarities were given when several of our strains shared the same hit (indicated by "Idem").

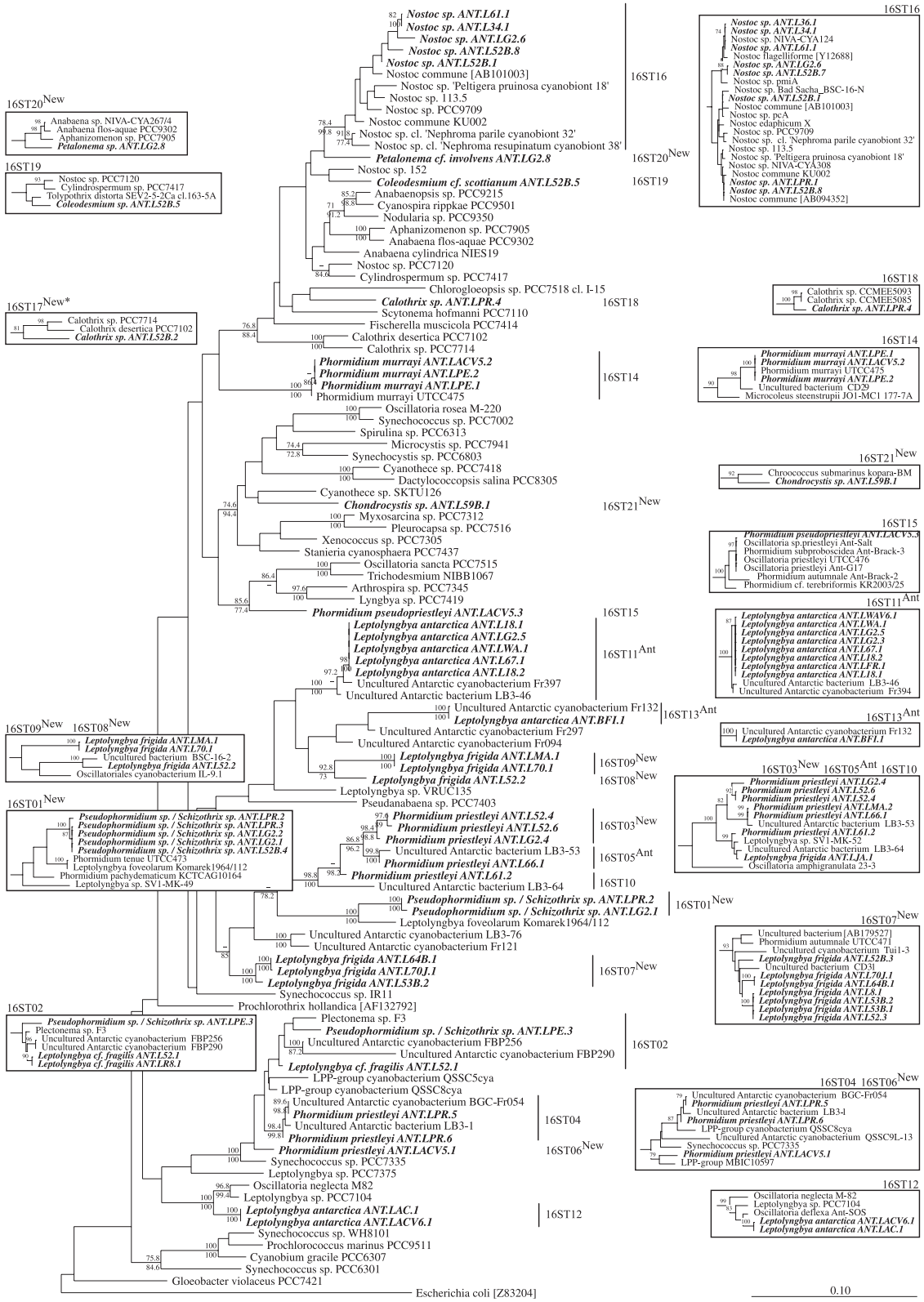

FIG. 3. Phylogenetic tree inferred from 16S rRNA gene sequences (*Escherichia coli* positions 100–1450) by maximum likelihood (likelihood = −25,328.76). In the windows, neighbor-joining tree inferred from partial 16S rRNA gene sequences (*E. coli* positions 405–780) for the OTUs. Bootstrap values obtained using the neighbor-joining and the parsimony (only for near-complete sequences) are indicated at the nodes when equal to or greater than 70%. The sequences determined in the present study are in bold italic. The *E. coli* sequence was used as out-group. The evolutionary distance between two sequences is obtained by adding the lengths of the horizontal branches connecting them and using the scale bars (0.1 mutation per position).

TABLE 3. Antimicrobial activities and cytotoxicity of the 17 bioactive strains ordered in function of their OTU and morphospecies.

OUT	Morphospecies	Strain name	Activities[a] on *Staphylococcus aureus*	Activities[a] on *Aspergillus fumigatus*	Activities[a] on *Cryptococcus neoformans*	Cytotoxicity[b]
16ST01^{New}	*Pseudophormidium* sp./*Schizothrix* sp.	ANT.LPR.2	64	0	512	640
16ST01^{New}	Idem	ANT.LPR.3	64	0	64	320
16ST01^{New}	Idem	ANT.LG2.1	0	0	64	160
16ST01^{New}	Idem	ANT.LG2.2	32	0	256	280
16ST03^{New}	*Phormidium priestleyi*	ANT.L52.4	0	512	1024	0
16ST03^{New}	Idem	ANT.L52.6	8	512	512	160
16ST04	Idem	ANT.LPR.6	32	0	0	320
16ST10	*P. priestleyi*	ANT.L61.2	8	0	0	0
16ST11^{Ant}	*Leptolyngbya antarctica*	ANT.LG2.3	64	0	0	640
16ST11^{Ant}	Idem	ANT.LG2.5	8	0	0	0
16ST11^{Ant}	Idem	ANT.L18.2	8	0	0	0
16ST14	*P. murrayi*	ANT.LPE.1	8	0	0	160
16ST16	*Nostoc* sp.	ANT.L52B.1	32	0	32	640
16ST16	Idem	ANT.LPR.1	0	0	8	1280
16ST16	Idem	ANT.LG2.6	8	0	16	160
16ST16	Idem	ANT.L34.1	8	0	0	0
16ST16	Idem	ANT.L36.1	8	0	8	160

[a]Antibacterial and antifungal activities are measured as endpoints in microdilution method, i.e. the highest dilution that inhibits 80% of test strain growth.

[b]Cytotoxicity is measured as endpoint in microdilution method, i.e. the highest dilution that inhibits 40% of HeLa cell thymidine uptake.

and ANT.LACV6.1 belonged to **16ST12** and exhibited 98.6% similarity with the sequence of *Oscillatoria* sp. ANT.SOS (Nadeau et al. 2001). The clone SalP05 (Jungblut et al. 2005) also fell in this OTU. Interestingly, Ace Lake and both ponds on Bratina Island are saline.

ITS analysis. For 32 oscillatorian strains, and *C.* cf. *scottianum* ANT.L52B.5, both tRNA Ile and Ala genes were found, except for strains ANT.BFI.1 and ANT.LACV5.3, which did not possess any tRNA genes in the amplified rRNA operon. Eight groups of ITS sequences where the alignment seemed meaningful (Wilmotte 1994) were defined as ITS types (ITS01–ITS08) (Table 2). In addition, 10 sequences did not belong to any groups and were the unique representatives of their ITS types. Altogether, 18 ITS types were defined. Levels of sequence similarities within these ITS types ranged from 98.8% to 100% (indels taken into account) with the exception of ITS-type ITS03, in which sequences ANT.L52.4 and ANT.LG2.4 only exhibited 83.8% similarity. Furthermore, for three ITS types, data base sequences could be meaningfully included in the alignment, but all came from Lake Fryxell in the Antarctic DV (Taton et al. 2003). The ITS sequences of clones Fr005, Fr127, Fr297, Fr311, Fr350, and Fr397 obtained from Lake Fryxell could be aligned with the sequences belonging to ITS-type ITS08. However, the six clone sequences from Lake Fryxell (DV) were more similar to each other than to the strain sequences from LH lakes. The clone sequences BGC-Fr023 and BGC-Fr054 (Taton et al. 2003) were aligned with the sequence of *P. priestleyi* ANT.LPR.5 (ITS09), and the clone sequences of Fr132 and Fr246 (Taton et al. 2003) were aligned with the sequence of *L. antarctica* ANT.BFI.1 (ITS10). Table 2 lists the different ITS types in relation to the OTUs based on 16S rRNA data. The strains that possessed the same ITS types belonged also to same OTUs. However, ITS sequences of two and three different types were obtained for the strains belonging to 16ST02 and 16ST07^{New}, respectively.

Bioactivity. A total of 126 samples was prepared from the culture of the 48 cyanobacterial strains and tested against the panel of human pathogens used at Vicuron Pharmaceuticals. Seventeen strains were active, and among them, 14 produced antibacterial activities, and 12 showed inhibition of fungal strains (Table 3—Appendix 5 reports the results for all the tested strains). The frequency of antibacterial activity against the gram-positive *S. aureus* was 29%. No activity was detected against the gram-negative *E. coli* and the yeast *C. albicans,* whereas 4% and 20% of the tested strains inhibited the growth of *A. fumigatus* and *C. neoformans,* respectively. Half of the tested isolates were cytotoxic to the mammalian cell line.

Among the 19 isolates assigned to the new OTUs, six strains of *Pseudophormidium* sp./*Schizothrix* sp. *and P. priestleyi* belonging to 16ST01^{New} and 16ST03^{New}, respectively, produced antimicrobial activities, and four strains had a significant cytotoxicity. None of the nine isolates of *L. frigida* belonging to novel OTUs (16ST07^{New}, 16ST08^{New}, and 16ST09^{New}) showed any antibacterial activity. However, four of them with identical 16S rRNA gene sequences produced cellular toxins. Among the Antarctic OTUs, three out of the seven strains screened were microbiologically active and specifically inhibited *S. aureus* growth. These three

strains assigned to *L. antarctica*, belonged to 16ST11^{Ant} and exhibited identical 16S rRNA gene sequences. One of them was cytotoxic. A similar absence of correlation of the metabolic profiles with the geographical origin and genetic/morphological characteristics was observed within 16ST02, 16ST04, 16ST10, 16ST12, and 16ST14. Indeed, diverse patterns of antimicrobial/cytotoxic activities were often observed among the strains of *L.* cf. *fragilis, P. priestleyi, L. antarctica*, and *P. murrayi* having identical sequences, isolated from different regions or even from the same lake. The frequency of antimicrobial activities against *S. aureus* and *C. neoformans* was particularly high in the *Nostoc* group (16ST16): five of six strains were active. Furthermore, five strains exhibited a high-level cytotoxicity. In contrast, the screened strains of the genus *Calothrix* were microbiologically inactive, but three of five were cytotoxic.

DISCUSSION

Several studies have focused on the cyanobacterial diversity of microbial mats in Antarctic lakes, mainly based on species morphology. Nevertheless, the number of Antarctic cyanobacterial strains available in culture collections is limited. Furthermore, little is known concerning their phylogenetic affiliations, geographic distribution, physiology, and bioactive metabolites. To our knowledge, this is the first study in which a concentrated effort has been carried out to obtain a wide variety of cyanobacterial strains from this biota from different regions, and where a combined microscopic analysis with 16S rRNA gene and ITS analyses, as well as an evaluation of bioactivities, has been performed.

Diversity and geographical distribution. The genotypic diversity (21 OTUs) appeared higher than the morphological diversity (12 morphospecies). In addition, each OTU might correspond to more than one species following the bacteriological standards, but is likely to be distinct from other OTUs at the specific level (Stackebrandt and Göbel 1994). In seven cases (16ST01^{New}, 16ST02, 16ST03^{New}, 16ST04, 16ST07^{New}, 16ST10, and 16ST16), slightly different sequences (levels of similarity ranging from 97.5% to 99.9%) within the same OTUs were observed and were reminiscent of the microdiversity observed in molecular ecology studies using clone libraries (Fuhrman and Campbell 1998). If we consider such microheterogeneities as a real feature of the 16S rRNA gene that could be explained, for example, by the presence of different ecotypes (Fuhrman and Campbell 1998), these divergences would increase the genotypic diversity. This hypothesis is even more probable, given that the PCR and cloning biases that are well known in molecular ecology (Speksnijder et al. 2001), are not relevant here. In contrast, identical strain sequences isolated in different lakes were found for nine OTUs (16ST01^{New}, 16ST02, 16ST05^{Ant}, 16ST07^{New}, 16ST09^{New}, 16ST11^{Ant}, 16ST12, 16ST14, and 16ST16). The cultivation conditions may have selected identical ecotypes, or direct sequencing of the PCR products without cloning may have hidden microheterogeneities between different operons of the same strain. However, the wide range of culture conditions, including the use of culture media designed on the basis of the lake chemical composition, and the strain selection procedure should have facilitated growth of different ecotypes, if they were present.

With the exception of strains belonging to 16ST02 and 16ST07^{New}, similar groupings were found with the ITS and the 16S rRNA gene. The levels of similarity were lower between ITS-types than between 16S types, giving a more clear-cut distinction of the groups. Although the ITS was used successfully in several studies to discriminate cyanobacterial strains at the intra- or interspecific level (Ernst et al. 2003), this is not the case here, except for two ITS sequences of type ITS03. The high levels of similarity within ITS types in this study seemed to reflect a remarkable conservation of sequences from different lakes/regions. Interestingly, in ITS-type ITS08, the six clone sequences from Lake Fryxell are more similar to each other than to the four LH strain sequences, giving a hint of a better geographical resolution for the ITS marker than 16S rRNA gene.

The divergence between the morphological and molecular results was particularly evident in the Oscillatoriales that concealed a high degree of genotypic diversity (15 OTUs) despite a very simple morphology (seven morphospecies). Moreover, the Antarctic oscillatorian strains belonging to different OTUs fell into paraphyletic lineages. This finding confirms the polyphily of the Oscillatoriales order (Wilmotte 1994) and implies that psychrotolerance has arisen several times among the Antarctic oscillatorians (Nadeau et al. 2001).

The strains belonging to the same morphospecies may possess sequences belonging to paraphyletic OTUs. This trend suggests multiple origins for the same morphospecies and makes the phylogenetic interpretation of morphological criteria difficult. As often suggested, this confirms that cyanobacterial taxonomy cannot be based solely on morphology (Wilmotte 1994).

Nevertheless, besides these divergences, a one-way correlation between morphological and molecular results was determined. Indeed, most strains closely related at the 16S rRNA gene level belonged to the same morphospecies, and most strains that belonged to different morphospecies were different at the 16S rRNA gene level. This was the case for 51 of the 56 sequenced strains. Consequently (although they cannot be used alone), several morphological characters used for the oscillatorian morphospecies description were of taxonomic value, including the cell shape, the cell width, the presence or absence of cross-wall constrictions, and the number of trichomes per filament. Although this latter character depends on the culture age and the sheath structure, it appeared to be a good diacritical trait if frequently displayed by the culture.

In *Nostoc* strains, the 16S rRNA gene sequences belonged to 16ST16 but exhibited a minimal internal similarity of 96.6% only (*E. coli* positions: 405–780). The morphological criteria did not permit a clear-cut distinction between the different strains. The sequences of *Calothrix* sp. belonged to two *Calothrix* clusters and exhibited 8.4% dissimilarity, which hints to a large genetic diversity of this morphogenus. The two strains differed in the length of the heterocysts. As already mentioned, the 16S rRNA gene sequence of *P.* cf. *involvens* ANT.LG2.8 was the first available for this genus. Interestingly, this sequence exhibited 94.6% to 96.6% similarity with strain sequences of *Scytonema* sp. available in the data bases. Both genera are morphologically very similar. However, Komárek and Anagnostidis (1989) place the genus *Scytonema* into the family Scytonemataceae and the genus *Petalonema* into the family Microchaetaceae. Interestingly, *C.* cf. *scottianum* ANT.L52B.5 was grouped in the tree with *Tolypothrix distorta* SEV2-5-2-Ca (97.7% similarity) and exhibited more than 3.1% dissimilarity with other *Coleodesmium* sequences in GenBank. The genera *Coleodesmium* and *Tolypothrix* have basically the same structure but with different branching processes (Komárek and Watanabe 1990).

This study contributes to the interesting and debatable topic of microbial biogeography recently reviewed by Martiny et al. (2006). Indeed, 22 strains corresponding to nine OTUs did not have relatives in the data bases, and 11 strains corresponding to three OTUs were closely (more than 97.5% similarity) related only to other Antarctic sequences from uncultivated organisms (Priscu et al. 1998, Taton et al. 2003, Jungblut et al. 2005). In contrast, the taxonomic assignments based on morphology showed a majority of known cosmopolitan taxa. Hence, molecular studies show that endemism in Antarctic cyanobacteria is likely to be more common than has been previously estimated on the basis of morphology alone. The nine cosmopolitan OTUs (23 strains) were related to nonpolar data base sequences, of which two were obtained for the first time from Antarctic biotopes and the remaining seven OTUs had previously been found in southern Victoria Land and/or Bratina Island, as well as in Dronning Maud Land (only one strain). This supports the idea that cosmopolitan OTUs are well adapted to transport and colonization and thus were quite successful in their dispersal and occupation of new habitats in different regions of Antarctica.

Bioactivities. Strains isolated from the same lakes and belonging to the same OTUs showed different patterns of activity in antimicrobial and cytotoxic assays. This finding confirms that the strain isolation procedures described above permitted us to obtain different ecotypes with diverse metabolic profiles. As in the case of morphology or cyanotoxin production (Otsuka et al. 1999), differences in secondary metabolism do not correspond to genetic differences as indicated by rRNA and ITS analysis. These results suggest that a complementary way to screen cyanobacterial diversity may be to directly look for secondary metabolic operons such as polyketide synthase (PKS) and nonribosomal peptide synthase (NRPS) clusters, which correspond to ~1% of all cyanobacterial sequences submitted to GenBank (Burja et al. 2003). Nevertheless, we observed a certain clustering of activities, as the oscillatorian and *Nostoc* strains that exhibited only antibacterial activities belonged to 16ST04, 16ST10, 16ST11^{Ant}, 16ST14, and 16ST16, whereas the strains exhibiting only antifungal or both antibacterial and antifungal activities belonged to 16ST01^{New} (four of five strains), 16ST03^{New}, and 16ST16. Furthermore, all the oscillatorian strains belonging to 16ST02, 16ST05^{Ant}, 16ST06^{New}, 16ST07^{New}, 16ST08^{New}, 16ST09^{New}, and 16ST12, as well as *Calothrix* strains 16ST17^{New} and 16ST18, were microbiologically inactive. In the course of our screening, the majority of the microbiologically active strains inhibited a gram-positive pathogen, whereas no activities were found against gram-negative and yeast representatives. This is in agreement with the few data available in the literature about antimicrobial frequencies in cyanobacterial screening programs (Kreitlow et al. 1999). Promising results from our screenings were the demonstrated activities against filamentous fungi, which are worthy of further investigation. As previously reported (Burja et al. 2001), cyanobacteria constitute a major source of toxins. Indeed, the alkaloid neurotoxins and the cyclic peptide hepatotoxins are responsible for toxic cyanobacterial blooms in waterbodies worldwide. More than half of the Antarctic isolates of this study produced a cytotoxic activity, and at a first screening level, it was not possible to differentiate between cytotoxic and antibacterial/antifungal activities because the crude extracts contained a variety of different compounds. Further work, including HPLC fractionation and mass spectrometry of the active fractions, is in progress on the characterization of these cyanobacterial metabolites.

CONCLUSIONS

Molecular and morphological approaches revealed different diversity patterns in terms of species richness but also novelty and geographical distribution (endemism). Divergences were particularly evident for the oscillatorian strains for which a very simple morphology can hide a considerable genotypic diversity. A previously unknown molecular diversity was observed, not only for the oscillatorian strains, but also for strains of the genera *Petalonema*, *Calothrix*, and *Chondrocystis*. In addition, several new strain sequences have allowed us to assign morphology to three OTUs that previously comprised only uncultivated sequences from Antarctic biotopes. This study also showed that morphologically and genotypically identical strains were isolated from widely separated Antarctic regions. Genotypically identical strains isolated either from the same lake or from different lakes may produce different patterns of

bioactivity. Cultivation and screening of novel and/or endemic species of Antarctic cyanobacteria holds promise for the discovery of new biotechnologically valuable antifungal and antibacterial metabolites.

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Supplementary Material

The following supplementary material is available for this article:

Appendix 1. Location and main abiotic characteristics of the lakes from which the strains were isolated.

Appendix 2. Chemical composition of the culture media created in the frame of the present study.

Appendix 3. Alignments of the spacers between the 16S and 23S rRNA genes (including tRNA-Ile and tRNA-Ala genes) of Antarctic cyanobacterial strains. Conserved domains (Iteman et al. 2000) are indicated above.

Appendix 4a. Isolation media, morphological features and OTU assignments of the strains belonging to the Oscillatoriales order.

Appendix 4b. Isolation media, morphological features and OTU assignments of the strains belonging to the Nostocales and Chroococcales orders.

Appendix 5. Antimicrobial activities and cytotoxicity of the strains ordered in function of their OTU and the morphospecies to which they belonged.

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