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Keywords:	Cyanobacteria, microbial mats, Antarctic lakes, polyphasic characterization, rRNA operon, bioactive compounds
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Polyphasic study of Antarctic cyanobacterial strains

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Abstract

We isolated 59 strains of cyanobacteria from the benthic microbial mats of 23 Antarctic lakes, from 5 locations in 2 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 4 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 found concerning the diversity in terms of species richness, novelty and geographical distribution. For the 56 strains, 21 OTUs (Operational Taxonomic Unit, defined as groups of partial 16S rRNA gene sequences with more than 97.5% similarity) were found, including 9 novel and 3 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 found 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 specific to certain strains.

Introduction

Cyanobacteria are a major component of Antarctic ecosystems (Vincent 2000). Classical taxonomic studies have described Antarctic species compositions based on morphological and ecological features (e.g. 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 (Casamatta et al.

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2005, Nadeau et al. 2001, Rudi et al. 1997, Smith et al. 2000, Vincent et al. 2000) and 144 16S rRNA gene sequences are available from uncultured Antarctic cyanobacteria (Bowman et al. 2000, Christner et al. 2003, De la Torre et al. 2003, Jungblut et al. 2005, Priscu et al. 1998, Smith et al. 2000, Taton et al. 2003). These studies have shown that many sequences from Antarctic cyanobacteria are grouped together in distinct clusters (Nadeau et al. 2001, Priscu et al. 1998, Smith et al. 2000, Taton et al. 2003). 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 remains extremely important for studies of the cyanobacterial diversity, even where culture independent techniques based on the rRNA operon have successfully been used (e.g. Ward et al. 1998). Indeed, they permit to make a link between genotypic and phenotypic features to allow a better understanding of their 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 (e.g. Sabbe et al. 2004, Taton et al. 2003, Van Trappen et al. 2002) has made mass cultivation and biotechnological exploration of isolated Antarctic strains particularly interesting (Marinelli et al. 2004). Although the search for bioactive

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products from cyanobacteria, i.e. antitumor, antifungal, antibacterial and antiviral molecules, has intensified during recent decades (e.g. Burja et al. 2001, Namikoshi and Rinehart 1996), until this study, there has not been a pharmaceutical screening of a significant number of Antarctic cyanobacteria.

The aims of the present study were: 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. 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 km^2 and 400 km^2, respectively (Hodgson et al. 2001b). 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. 2001a). 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 (http://www.cip.ulg.ac.be/AppendixesStr.pdf).

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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. 3 - maintained in liquid culture media and 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, 6 new media (1NP, 2NP, 3NP and 1, 2, 3 with and without nitrogen, respectively - Appendix 2 - http://www.cip.ulg.ac.be/AppendixesStr.pdf) were created based on water chemical data from the LH and RI lakes (Hodgson et al. 2001a, 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.

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Unialgal cultures were obtained by picking material from the edge of discrete colonies that had been growing for about 3 weeks on solid media. Cultures were cleaned of eukaryotic contaminants, by one transfer to solid media containing 50 mg/l 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 then kept in their isolation media as well as in BG11 and BG11$_0$ for non-heterocystous 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. 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, of necridic cells, of a

sheath, color of the sheath, number of trichomes per filament, presence or absence of false branching, of heterocysts, and the width and length of heterocysts. For each biometrical character, thirty to fifty 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 (e.g. 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, 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.), 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 primers 16S378F or 16S784R. Complete sequences (*E. coli* positions 27 to 1542) were determined (on one DNA strand) for at least one representative strain selected at random from each OTU. An OTU was defined as a group of sequences that exhibited more than 97.5% similarity with each others, using the *E. coli* positions 405 – 780, not taking into account indels and ambiguous bases (Stackebrandt and Göbel 1994, 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) as well as with the sequencing primers 16S1514F (5' - GTC GTA ACA AGG TAG CCG TAC – 3') (Wilmotte et al. 2002) and/or Ile23F (5' - ATT AGC TCA GGT GGT TAG – 3') (Wilmotte et al. 1993). Sequencing was carried out by Genome Express (Meylan, France) on an ABI PRISM system

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377 (PE Applied Biosystems, USA) and contig sequences were obtained using the software Sequencher (Gene Codes Corporation, USA). The sequences (*E. coli* positions: 100-1450 and 405-780) were initially analyzed by similarity search using the 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 database of the ARB software package (Ludwig et al. 2004) at [http://www.arb-home.de] and aligned with the cyanobacterial sequences available from GenBank. Phylogenetic trees were constructed using the maximum likelihood of fastDNAml (Olsen et al. 1994) implemented in ARB, the Wagner parsimony of PHYLIP 3.63 (Felsenstein 1989) and the neighor-joining (Saitou and Nei 1987) on the Jukes and Cantor distances matrix (Jukes and Cantor 1969) 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 looked for the sequences of the two closest cultured strains and added them. 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 between 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 in the tree. Furthermore, the OTUs were divided in 3 categories: 1 - the new OTUs only composed of our sequences, none exhibiting more than 97.5% similarity with GenBank sequences. 2 - the Antarctic OTUs, in which our sequences were grouped with GenBank Antarctic sequences with a minimal threshold of 97.5% similarity. 3 - the cosmopolitan OTUs in which our sequences were grouped with GenBank sequences that originated from non-Antarctic samples.

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 - http://www.cip.ulg.ac.be/AppendixesStr.pdf).

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Screening for antimicrobial and cytotoxic activities

Strains were axenically mass-cultivated in the inorganic media BG11 and $BG11_0$ in 500 to 1100 ml glass tubes bubbled with air/CO_2 (98/2, v/v) at 30 µmol photons m^{-2} s^{-1} irradiance, provided by daylight fluorescent tubes (Osram Lumilux L 58W). PAR (Photosynthetic Available Radiation) irradiance was measured with a Li-Cor Li185A quantum meter equipped with a Li190SB cosine quantum sensor. Temperature was maintained at 20 ± 2 °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 the sucking of a saline solution under vacuum. This would further reduce bacterial contaminants. Biomass was then frozen, thawed and extracted overnight with ethyl acetate (50 ml per 1 g of dry biomass) or with methanol (50 ml per 1 g of dry biomass). After filtration on paper, the solvent was evaporated under vacuum and the dry residue was dissolved in 40 µl 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

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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; *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 Instituto 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 micro-dilution method was used to confirm positive broths and to assay their potency (Gaspari et al. 2005). For the cytotoxic assay, HeLa cells were used 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 of at least the 40% the cellular thymidine uptake, set as 100 % in the control condition when only DMSO/ H_2O was added to the Hela cells.

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 to AY493600, AY493601 to AY493627 and AY493628 to 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 6 were newly created, and 3 incubation temperatures were used for the strain isolation. In total, 59 clonal unialgal strains

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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 - http://www.cip.ulg.ac.be/AppendixesStr.pdf). 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 (http://www.cip.ulg.ac.be/AppendixesStr.pdf).

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Morphology

Fifteen strains belonged to the Nostocales, one strain to the Chroococcales and 43 strains to the Oscillatoriales (Figures 1 and 2 – Table 1). Within the Nostocales order, 8 strains belonged to the genus *Nostoc*, 5 strains to the genus *Calothrix*, 1 strain to the genus *Petalonema* and 1 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, of necrids, of a sheath, of false branching and the number of trichomes per filament allowed 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 - http://www.cip.ulg.ac.be/AppendixesStr.pdf).

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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 *L. 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 to 780). Fifteen belonged to the Oscillatoriales, 5 to the Nostocales and 1 to the Chroococcales (Table 2). Complete 16S rRNA gene sequences were obtained for at least 1 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 *ca.* 400 bp. In total, 29 complete sequences were obtained.

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The new sequences have 89.6 to 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 databases. Three OTUs were related to sequences found only in Antarctica, and in two cases, they were from clone libraries. The remaining 9 OTUs exhibited more than 97.5% similarity with polar and/or non-polar sequences and were considered as cosmopolitan OTUs.

The phylogenetic analyses (Figure 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 3 groups: new, Antarctic and cosmopolitan OTUs.

New OTUs. The sequences belonging to these new OTUs exhibited 2.5 to 10.4% dissimilarity with all sequences available in the databases. 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. **16ST09**[New] comprised 2 sequences of *L. frigida,* identical to each other but isolated from different lakes. The sequence of *L. frigida* ANT.L52.2, belonged to **16ST08**[New]. Three sequences of *P. priestleyi* belonged to **16ST03**[New]. 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. **16ST01**[New] comprised 5 sequences of *Pseudophormidium* sp. / *Schizothrix* sp. Four of them were identical to each other even though they originated from 3 different lakes. The minimum level of similarity within this OTU was 99.4%. Seven sequences of *L. frigida* belonged to **16ST07**[New]. Six of them fell into two groups of identical sequences, one group with 4 sequences originating from 3 lakes, and one group with two sequences from 2 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 OTU **16STO7**[New] The sequence of *P. priestleyi* ANT.LACV5.1 belonged to **16ST06**[New].

Antarctic OTUs. Eight identical sequences of *L. antarctica* belonged to **16ST11**[Ant] and came from 5 different lakes in 3 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 Antarctic DV (Taton et al. 2003) and

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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 3 *Nostoc* strains originating from Lake L52B (LH) possessed 16S rRNA sequences that exhibited 1.4 to 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 databases. 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) that is considered as moderately thermotolerant (Dillon and Castenholz 2003). **16ST14** comprised 3 identical sequences of *P. murrayi* from two lakes of the VH. These sequences were identical to these of *Microcoleus glaciei* Johansen & 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 2 lakes in the LH and assigned to *L. frigida* and *P. priestleyi*,

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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). **16ST02** comprised the identical sequences of *L.* cf. *fragilis* ANT.L52.1 and ANT.RI8.1 that were isolated from 2 lakes of 2 neighboring regions (LH and RI). 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. **16ST04** comprised 2 sequences of *P. priestleyi* strains isolated from Progress 2 pond that were 99.4% similar to each other 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 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 that 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. Alltogether, eighteen 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 3 ITS-types, database sequences could be meaningfully

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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 6 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 2 and 3 different types were obtained for the strains belonging to 16ST02 and 16ST07^{New}, respectively.

Bioactivity

A total of 126 samples were 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, http://www.cip.ulg.ac.be/AppendixesStr.pdf). The frequency of antibacterial activity against the Gram-positive *S. aureus* was 29%. No activities were detected *vs.* 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. The bioactivities were compared to the evolutionary relationships of the strains.

Among the 19 isolates assigned to the new OTUs, 6 strains of *Pseudophormidium sp. / Schizothrix sp. and P. priestleyi* belonging to **16ST01^{New}** and **16ST03^{New}**, respectively, produced antimicrobial activities, coupled for 4 strains with a significant cytotoxicity. None

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Deleted: **16ST03^{New}**. Strains of *Pseudophormidium sp. / Schizothrix sp.* belonging to **16ST01^{New}** showed similar antimicrobial profiles against *S. aureus* and *C. neoformans*, coupled with a significant cytotoxicity in the *in vitro* assay, except strain ANT.L52B.4, which was inactive against pathogens and not cytotoxic. Two out of the three *P. priestleyi* strains belonging to **16ST03^{New}**, inhibited *A. fumigatus* growth. Though

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of the 9 isolates of *L. frigida* belonging to novel OTUs (**16ST07^{New}, 16ST08^{New}** and **16ST09^{New}**) showed any antibacterial activity. However, 4 of them, with identical 16S rRNA gene sequences produced cellular toxins. Among the Antarctic OTUs, 3 out of the 7 strains screened were microbiologically active and specifically inhibited *S. aureus* growth. These 3 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, 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**): 5 out of 6 strains were active. Furthermore, 5 strains exhibited a high-level cytotoxicity. In contrast, the screened strains of the genus *Calothrix* were microbiologically inactive but 3 out of 5 were cytotoxic.

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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, their physiology and their 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 7 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 found 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 9 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 novel culture media, designed on the basis of the lake water chemical composition, and the strain selection procedure should have permitted to obtain 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. Though the ITS was used successfully in several studies to discriminate cyanobacterial strains at the intra- or interspecific level (e.g. Ernst et al. 2003, Otsuka et al. 1999), this is not the case

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here, except for 2 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 6 clones sequences from Lake Fryxell are more similar to each other than to the 4 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 (7 morphospecies). Moreover, the Antarctic oscillatorian strains belonging to different OTUs fell into paraphyletic lineages. This confirms the polyphily of the Oscillatoriales order (e.g. Ishida et al. 2001, 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 suggests multiple origins for the same morphospecies and makes the phylogenetic interpretation of morphological criteria difficult. As often suggested (e.g. Wilmotte 1994), this confirms that cyanobacterial taxonomy cannot be based solely on morphology.

Nevertheless, besides these divergences, a one-way correlation between morphological and molecular results was found. 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 out 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 such as the cell shape, the cell width, the presence or absence of cross wall constrictions and the number of trichomes per filament. Though this latter character depends

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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, what 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 databases. 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 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. (2005). Indeed, 22 strains corresponding to 9 OTUs did not have relatives in the databases, and 11 strains corresponding to 3 OTUs were closely related only (more than 97.5% similarity) to other Antarctic sequences from uncultivated organisms (Priscu et al. 1998, Jungblut et al. 2005, Taton et al. 2003). 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 9

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cosmopolitan OTUs (23 strains) were related to non-polar database sequences, of which 2 were obtained for the first time from Antarctic biotopes and the remaining 7 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.

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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, that correspond to *ca.* 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 (1 out of 2 strains), 16ST10 (1 out of 2 strains), 16ST11^{Ant} (3 out of 6 strains), 16ST14 (1 out of 3 strains) and 16ST16 (1 out of 6 strains) whereas the strains exhibiting only antifungal or both antibacterial and antifungal activities belonged to 16ST01^{New} (4 out of 5 strains), 16ST03^{New} (2 out of 3 strains) and 16ST16 (5 out of 6 strains). Furthermore, all the oscillatorian strains belonging to 16ST02, 16ST05^{Ant}, 16ST06^{New}, 16ST07^{New}, 16ST08^{New}, 16ST09^{New}, 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-

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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 term 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 found, 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 3 OTUs that previously comprised only uncultivated sequences from Antarctic biotopes (Priscu et al. 1998, Jungblut et al. 2003, Taton et al. 2003). 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

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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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References

Bowman, J. P., Rea, S. M., McCammon, S. A. & McMeekin, T. A. 2000. Diversity and community structure within anoxic sediment from marine salinity meromictic lakes and a coastal meromictic marine basin, Vestfold Hills, eastern Antarctica. *Environ. Microbiol.* 2:227-37.

Deleted: Baurain, D., Renquin, L., Grubisic, S. & Scheldeman, P. 2002. Remarkable conservation of internally transcribed spacer sequences of *Arthrospira* ('*Spirulina*') (cyanophyceae, cyanobacteria) strains from four continents and of recent and 30-year-old dried samples from Africa. *J. Phycol.* 38:384-93.¶

Broady, P. A. & Kibblewhite, A. L. 1991. Morphological characterization of Oscillatoriales (Cyanobacteria) from Ross Island and southern Victoria Land, Antarctica. *Antarct. Sci.* 3:35-45.

Burja, A. M., Banaigs, B., Abou-Mansour, E., Burgess, J. G. & Wright, P. C. 2001. Marine cyanobacteria - a prolific source of natural products. *Tetrahedron* 57:9347-77.

Burja, A. M., Dhamwichukorn S., Wright P. C. 2003. Cyanobacterial postgenomic research and systems biology. *Trends in Biotechnol.* 21:504-11.

Casamatta, D. A., Johansen, J. R., Vis, M. L. & Broadwater, S. T. 2005. Molecular and morphological characterization of ten polar and near-polar strains within the oscillatoriales (cyanobacteria). *J. Phycol.* 41:421-38.

Christner, B. C., Kvitko, I. I. & Reeve, J. N. 2003. Molecular identification of bacteria and eukarya inhabiting an Antarctic cryoconite hole. *Extremophiles* 7:177-83.

De la Torre, J. R., Goebel, B. M., Friedmann, E. I. & Pace, N. R. 2003. Microbial diversity of cryptoendolithic communities from the McMurdo Dry Valleys, Antarctica. *Appl. Environ. Microbiol.* 69:3858-67.

Dillon, J. G. & Castenholz, R. W. 2003. The synthesis of the UV-screening pigment, scytonemin, and photosynthetic performance in isolates from closely related natural populations of cyanobacteria (*Calothrix* sp.). *Environ. Microbiol.* 5:484-91.

Ernst, A., Becker, S., Wollenzien, U. I. A. & Postius, C. 2003. Ecosystem-dependent adaptive radiations of picocyanobacteria inferred from 16S rRNA and ITS-1 sequence analysis. *Microbiol.* 149:217-28.

Felsenstein, J. 1989. PHYLIP - Phylogeny Inference Package (Version 3.2). *Cladistics* 5:164.

Deleted: Boyer, S. L., Johansen, J. R., Flechtner, V. R. & Howard, G. L. 2002. Phylogeny and genetic variance in terrestrial *Microcoleus* (cyanophyceae) species based on sequence analysis of the 16S rRNA gene and associated 16S-23S ITS region. *J. Phycol.* 38:1222-35.¶
Brambilla, E., Hippe, H., Hagelstein, A., Tindall, B. J. & Stackebrandt, E. 2001. 16S rDNA diversity of cultured and uncultured prokaryotes of a mat sample from Lake Fryxell, McMurdo Dry Valleys, Antarctica. *Extremophiles* 5:23-33.¶

Formatted

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Fumanti, B., Cavacini, P. & Alfinito, S. 1997. Benthic algal mats of some lakes of Inexpressible Island (northern Victoria Land, Antarctica). *Polar Biol.* 17:25-30.

Flechtner, V. R., Boyer, S. L. , Jeffrey, S. W. & DeNoble M. L. 2002. *Spirirestis rafaelensis* gen. et sp. nov. (Cyanophyceae), a new cyanobacterial genus from arid soils. *Nova Hedwigia* 74: 1-24.

Fuhrman, J. A. & Campbell, L. 1998. Microbial microdiversity. *Nature* 393:410-1.

Gaspari, F., Paitan, Y., Mainini, M., Losi, D., Ron, E. Z. & Marinelli, F. 2005. Myxobacteria isolated in Israel as potential source of new anti-infectives. *J. Appl. Microbiol.* 98:429-39.

Geitler, L. 1932. Cyanophyceae. *In* Kolkwitz, R. [Ed.] *Rabenhorst's Kryptogamenflora von Deutschland, Osterreich und der Schweiz*. Akademische Verlagsgesellschaft, Leipzig, pp.

Gordon, D. A., Priscu, J. & Giovannoni, S. 2000. Origin and phylogeny of microbes living in permanent Antarctic lake ice. *Microbial. Ecol.* 39:197-202.

Hodgson, D. A., Vyverman, W. & Sabbe, K. 2001a. Limnology and biology of saline lakes in the Rauer Islands, eastern Antarctica. *Antarct. Sci.* 13:255-70.

Hodgson, D. A., Noon, P. E., Vyverman, W., Bryant, C. L., Gore, D. B., Appleby, P., Gilmour, M., Verleyen, E., Sabbe, K., Jones, V. J., Ellis-Evans, J. C. & Wood, P. B. 2001b. Were the Larsemann Hills ice free through the Last Glacial Maximum?' *Antarct. Sci.* 13:440-54.

Ishida, T., Watanabe, M. M., Sugiyama, J. & Yokota, A. 2001. Evidence for polyphyletic origin of the members of Oscillatoriales and Pleurocapsales as determined by 16S rDNA analysis. *FEMS Microbiol. Lett.* 201:79-82.

Iteman, I., Rippka, R., Tandeau de Marsac, N. & Heussner, S. 2000. Comparison of conserved structural and regulatory domains within divergent 16S rRNA-23S rRNA spacer sequences of cyanobacteria. *Microbiol.* 146:1275-86.

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Deleted: Garcia-Pichel, F., López-Cortés, A. & Nübel, U. 2001. Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado Plateau. *Appl. Environ. Microbiol.* 67:1902-10.¶

Deleted: Gerwick, W. H., Roberts, M. A., Proteau, P. J. & Chen, J.-L. 1994. Screening cultured marine microalgae for anticancer-type activity. *J. Appl. Phycol.* 6:143-9.¶

Deleted: Göttlich, E., de Hoog, G. S., Genilloud, O., Jones, B. E. & Marinelli, F. 2003. MICROMAT: Culturable fungal diversity in microbial mats of Antarctic lakes. *In* Huiskes, A. H. L., Gieskes, W. W. C., Rozema, J., Schorno, R. M. L., van der Vies, S. M. & Wolff, W. J. [Eds.] *VIII SCAR International Biology Symposium - Antarctic biology in a global context.* Amsterdam, The Netherlands, pp. 251–4.¶

Jukes, T. H. & Cantor, C. R. 1969. Evolution of protein molecules. *In* Munro, H. N. [Ed.] *Mammalian protein metabolism*. Academic Press, New York, pp. 21-132.

Jungblut, A.D., Hawes, I., Mountfort, D., Hitzfeld, B., Dietrich, D.R., Burns, B.P. & Neilan B.A. (2005) Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. *Env. Microbiol.*, 7 : 519-29.

Komárek, J. 1999. Diversity of cyanoprokaryotes (cyanobacteria) of King George Island, maritime Antarctica - a survey. *Arch. Hydrobiol.* 94:181-93.

Komárek, J. & Anagnostidis, K. 1989. Modern approach to the classification system of cyanophytes 4. Nostocales. *Arch. Hydrobiol.* 82:247-345.

Komárek, J. & Watanabe, M. 1990. Morphology and taxonomy of the genus *Coleodesmium* (Cyanophyceae/Cyanobacteria). *In* Watanabe, M. & Malla, S. B. [Eds.] *Cryptogams of the Himalayas*. National Science Museum, Tsukuba, Japan, pp. 1-22.

Komárek, J. & Anagnostidis, K. 1998. Cyanoprokaryota 1. Teil Chroococcales. Gustav Fischer Verlag, Stuttgart.

Komárek, J. & Anagnostidis, K. 2005. Cyanoprokaryota 2. Teil Oscillatoriales. Elsevier GmbH, Spektrum Akademischer Verlag, Heidelberg.

Kreitlow, S., Mund, S. & Lindequist, U. 1999. Cyanobacteria - a potential source of new biologically active substances. *J. Biotechnol.* 70:61-3.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, X., Buchner, A., Lai, T., Steppi, S., Jobb, G., Förster, W., Brettske, I., Gerber, S., Ginhart, A. W., Gross, O., Grumann, S., Hermann, S., Jost, R., König, A., Liss, T., Lüßmann, R., May, M., Nonhoff, B., Reichel, B., Strehlow, R., Stamatakis, A., Stuckmann, N., Vilbig, A., Lenke, M., Ludwig, T.,

Formatted

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Bode, A. & Schleifer, K. H. 2004. ARB: a software environment for sequence data. *Nucleic Acids Res.* 32:1363-71.

Maidak, B. L., Cole, J. R., Lilburn, T. G., Parker, C. T. J., Saxman, P. R., Farris, R. J., Garrity, G. M., Olsen, G. J., Schmidt, T. M. & Tiedje, J. M. 2001. The RDP-II (Ribosomal Database Project). *Nucleic Acids Res.* 29:173-4.

Marinelli, F., Brunati, M., Sponga, F., Ciciliato, I., Losi, D., Van Trappen, S., Mergaert, J., Swings, J., Göttlich, E., de Hoog, S., Rojas, J. L. & Genilloud, O. 2004. Biotechnological exploitation of heterotrophic bacteria and filamentous fungi isolated from benthic mats of Antarctic lakes. *In* Kurtböke, I. & Swings, J. [Eds.] *Microbial genetic resources and biodiscovery*. Queensland complete printing service, Queensland, Australia, pp. 163-4.

Martiny, J. B. H., Bohannan, B. J. M., Brown, J. H., Colwell, R. K., Fuhrman, J. A., Green, J. L., Horner-Devine, M. C., Kane, M., Krumins, J. A., Kuske, C. R., Morin, P. J., Naeem, S., Ovreas, L., Reysenbach, A.-L., Smith, V. H. & Staley, J. T. 2006. Microbial biogeography: putting the microorganisms on the map. *Nat. Rev.* 4:102-4

Miller, S. R. & Castenholz, R. W. 2001. Ecological physiology of *Synechococcus* sp. strain SH-94-5, a naturally occurring cyanobacterium deficient in nitrate assimilation. *Appl. Environ. Microbiol.* 67:3002-9.

Nadeau, T. L., Milbrandt, E. C. & Castenholz, R. W. 2001. Evolutionary relationships of cultivated Antarctic Oscillatoriaceans (cyanobacteria). *J. Phycol.* 37:650-4.

Namikoshi, M. & Rinehart, K. L. 1996. Bioactive compounds produced by cyanobacteria. *Microbiol.* 17:373-84.

Deleted: B

Formatted

Deleted: McKnight, D. M., Alger, A., Tate, C. M., Shupe, G. & Spaulding, S. A. 1998. Longitudinal Patterns in algal abundance and species distribution in meltwater streams in Taylor Valley, southern Victoria Land, Antarctica. *In* Priscu, J. C. [Ed.] *Ecosystem dynamics in a polar desert: the McMurdo Dry Valleys, Antarctica*. American Geophysical Union, Washington, D.C, pp. 109-27.¶

Nelissen, B., De Baere, R., Wilmotte, A. & De Wachter, R. 1996. Phylogenetic relationships of non axenic filamentous cyanobacterial strains based on 16S rRNA sequence analysis. *J. Mol. Evol.* 42:194-200.

Olsen, G. J., Matsuda, H., Hagstrom, R. & Overbeek, R. 1994. FastDNAml: A tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. *Comput. Applic. Biosci.* 10:41-8.

Otsuka, S., Suda, S., Li, R., Watanabe, M., Oyaizu, H., Matsumoto, S. & Watanabe, M. M. 1999. Phylogenetic relationships between toxic and non-toxic strains of the genus *Microcystis* based on 16S to 23S internal transcribed spacer sequence. *FEMS Microbiol. Lett.* 172:15-21.

Priscu, J. C., Fritsen, C. H., Adams, E. E., Giovannoni, S. J., Paerl, H. W., McKay, C. P., Doran, P. T., Gordon, D. A., Lanoil, B. D. & Pinckney, J. L. 1998. Perennial Antarctic lake ice: an oasis for life in a polar desert. *Science* 280:2095-8.

Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M. & Stanier, R. Y. 1979. Generic assignment, strains histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology* 111:1-61.

Rudi, K., Skulberg, O. M., Larsen, F. & Jakobsen, K. S. 1997. Strain characterization and classification of oxyphotobacteria in clone cultures on the basis of 16S rRNA sequences from the region V6, V7, and V8. *Appl. Environ. Microbiol.* 63:2593-9.

Sabbe, K., Hodgson, D. A., Verleyen, E., Taton, A., Wilmotte, A., Vanhoutte, K. & Vyverman, W. 2004. Effects of physical disturbance, salinity and light regime on microbial mat structure and composition in continental Antarctic lakes (Larsemann Hills and Bølingen Islands). *Freshwater Biol.* 49:296-319.

Deleted: Nübel, U., Garcia-Pichel, F., Clavero, E. & Muyzer, G. 2000. Matching molecular diversity and ecophysiology of benthic cyanobacteria and diatoms in communities along a salinity gradient. *Environ. Microbiol.* 2:217-26.¶
O'Sullivan, L. A., Weightman, A. J. & Fry, J. C. 2002. New degenerate *Cytophaga-flexibacter-Bacteroides*-Specific 16S ribosomal DNA-targeted oligonucleotide probes reveal high bacterial diversity in river Taff Epilithon. *Appl. Environ. Microbiol.* 68:201-10.¶

Deleted: f

Deleted: Patterson, G. M. L., Larsen, L. K. & Moore, R. E. 1994. Bioactive natural products from blue-green algae. *J. Appl. Phycol.* 6:151-7.¶

Deleted: Qiu, X., Wu, L., Huang, H., McDonel, P. E., Palumbo, A. V., Tiedje, J. M. & Zou, J. 2001. Evaluation of PCR-generated chimeras, mutations, and heteroduplexes with 16S rRNA gene-based cloning. *Appl. Environ. Microbiol.* 67:880-7.¶
Redfield, E., Barns, S. M., Belnap, J., Daane, L. L. & Kuske, C. R. 2002. Comparative diversity and composition of cyanobacteria in three predominant soil crusts of the Colorado Plateau. *FEMS Microbiol. Ecol.* 40:55-63.¶

Saitou, N. & Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4:406-25.

Smith, M. C., Bowman, J. P., Scott, F. J. & Line, M. A. 2000. Sublithic bacteria associated with Antarctic quartz stones. *Antarct. Sci.* 12:177-84.

Speksnijder, A. G. C. L., Kowalchuk, G. A., De Jong, S., Kline, E., Stephen, J. R. & Laanbroek, H. J. 2001. Microvariation artefacts introduced by PCR and cloning of closely related 16S rRNA gene sequences. *Appl. Environ. Microbiol.* 67:469-72.

Stackebrandt, E. & Göbel, B. M. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* 44:846-9.

Taton, A., Grubisic, S., Brambilla, E., de Wit, R. & Wilmotte, A. 2003. Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (Dry Valleys, Antarctica): A morphological and molecular approach. *Appl. Environ. Microbiol.* 69:5157-69 Turner, S. 1997. Molecular systematics of oxygenic photosynthetic bacteria. *Pl. Syst. Evol.* Suppl. 11:13-52.

Turner, S., Pryer, K. M., Miao, V. P. & Palmer, J. D. 1999. Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. *J. Eukaryot. Microbiol.* 1:327-38.

Van de Peer, Y. & De Wachter, R. 1997. Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. *Comput. Applic. Biosci.* 13:227-30.

Deleted: oe

Deleted: ¶
Tindall, B. J., Brambilla, E., Steffen, M., Neumann, R., Pukall, R., Kroppenstedt, R. M. & Stackebrandt, E. 2000. Cultivable microbial biodiversity: gnawing at the Gordian knot. *Environ. Microbiol.* 2:310-8.¶

Van Trappen, S., Mergaert, J., Van Eygen, S., Dawyndt, P., Cnockaert, M. C. & Swings, J. 2002. Diversity of 746 heterotrophic bacteria isolated from microbial mats in Antarctic lakes. *Syst. Appl. Microbiol.* 25:603-10.

Vincent, W. F. 2000. Cyanobacterial dominance in the polar regions. *In* Whitton, B. A. & Potts, M. [Eds.] *The ecology of cyanobacteria*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 321-40.

Vincent, W. F., Bowman, J. P., Rankin, L. M. & McMeekin, T. A. 2000. Phylogenetic diversity of picocyanobacteria in Arctic and Antarctic ecosystems. *In* Bell, C., Brylinsky, M. & Johnson-Green, M. [Eds.] *8th International Symposium on Microbial Ecology - Microbial Biosystems: New Frontiers.* Atlantic Canada Society for Microbial Ecology, Halifax, pp. 317-22.

Ward, D. M., Ferris, M. J., Nold, S. C. & Bateson, M. M. 1998. A natural view of microbial diversity within hot spring cyanobacterial mat communities. *Microbiol. Mol. Biol. Rev.* 62:1353-70.

Waterbury, J. B. & Stanier, R. Y. 1981. Isolation and growth of cyanobacteria from marine and hypersaline environments. *In* Starr, M. P., Stolp, H., Truper, H. G., Balows, A. & Schlegel, H. G. [Eds.] *The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria*. Springer-Verlag, Berlin, pp. 221-3.

Wilmotte, A. 1994. Molecular evolution and taxonomy of cyanobacteria. *In* Bryant, D. A. [Ed.] *The molecular biology of cyanobacteria*. Kluwer Academic publishers, Dordrecht, The Netherlands, pp. 1-25.

Deleted: Vincent, W. F. 2000a. Evolutionary origins of Antarctic microbiota: invasion, selection and endemism. *Antarct. Sci.* 12:374-85.¶

Deleted: b

Deleted: Wilmotte, A. 1991. Taxonomic study of marine oscillatoriacean strains (cyanophyceae, cyanobacteria) wih narrow trichomes. I. Morphological variability and autoecological features. *Arch. Hydrobiol.* Suppl. 92, Algological Studies 64:215-48.¶

Wilmotte, A. & Herdman, M. 2001. Phylogenetic relationships among cyanobacteria based on 16S rRNA sequences. *In* Boone, D. R. & Castenholz, R. W. [Eds.] *Bergey's manual of systematic bacteriology*. Springer, New York, pp. 487-93.

Wilmotte, A., Van der Auwera, G. & De Wachter, R. 1993. Structure of the 16S ribosomal RNA of the thermophilic cyanobacterium *Chlorogloeopsis* HTF '*Mastigocladus laminosus* HTF' strain PCC7518, and phylogenetic analysis. *FEBS Lett.* 317:96-100.

Wilmotte, A., Demonceau, C., Goffart, A., Hecq, J.-H., Demoulin, V. & Crossley, A. C. 2002. Molecular and pigment studies of the picophytoplankton in a region of Southern Ocean (42-54°S, 141-144°E) in March 1998. *Deep-Sea Res.* 49:3351-63.

Yeager, C. M., Kornosky, J. L., Housman, D. C., Grote, E. E., Belnap, J. & Kuske, C. R. 2004. Diazotrophic community structure and function in two successional stages of biological soil crusts from the Colorado Plateau and Chihuahuan Desert. *Appl. Environ. Microbiol.* 70:973-83.

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); 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. & 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. & 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	*Leptolyngbya*	Filamentous; trichomes ensheathed, slightly	11

antarcticum West & West	*antarctica* (West & West) Anagn. & Kom.[a]	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.	
Lyngbya murrayi West & West	*Phormidium murrayi* (West & West) Anagn. & 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 & West	*Phormidium pseudopriestleyi* Anagn. & 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; colourless 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 ±	5

		2.36 (1.98 – 11.51) µm long; lamellated yellow-brown sheath.	
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 divergeant 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 sheat; 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

1 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[Ne w]	*Leptolyngbya* sp. PCC73110 (Nelissen et al. 1996) (95.8 – 96.3%)	ITS05
ANT.LPR.3	16ST01[Ne w]	id.	ITS05
ANT.LG2.1	16ST01[Ne w]	id.	ITS05
ANT.LG2.2	16ST01[Ne w]	id.	ITS05
ANT.L52B.4	16ST01[Ne w]	id.	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	id.	ITS01
ANT.RI8.1	16ST02	id.	ITS01
ANT.L52.4	16ST03[Ne w]	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[Ne w]	id.	ITS03
ANT.L52.6	16ST03[Ne w]	id.	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	id.	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]	id.	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 & Casamatta sp. nov. UTCC471 (Casamatta et al. 2005) (97.2 - 97.5%)	ITS04
ANT.L52.3	16ST07[New]	id.	ITS04
ANT.L8.1	16ST07[New]	id.	ITS04
ANT.L53B.2	16ST07[New]	id.	ITS04
ANT.L52B.3	16ST07[New]	id.	ITS13
ANT.L64B.1	16ST07[New]	*Phormidium autumnale* UTCC471, reassignated to	ITS14

	w	*Pseudanabaena tremula* Johansen & Casamatta sp. nov. UTCC471 (Casamatta et al. 2005) (96.6 - 97.2%)	
ANT.L70J.1	16ST07^{Ne} w	id.	nd.
ANT.L52.2	16ST08^{Ne} w	*Leptolyngbya* sp. CNP1-B3-C9 (M. C. Payne and J. R. Johansen, unpublished data) (90.9%)	ITS12
ANT.LMA.1	16ST09^{Ne} w	*Leptolyngbya* sp. VRUC135 (Nelissen et al. 1996) (89.6%)	ITS06
ANT.L70.1	16ST09^{Ne} w		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	id.	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}	id.	nd.
ANT.L18.1	16ST11^{Ant}	id.	ITS08
ANT.LG2.5	16ST11^{Ant}	id.	ITS08
ANT.LWA.1	16ST11^{Ant}	id.	nd.
ANT.L18.2	16ST11^{Ant}	id.	ITS08
ANT.LFR.1	16ST11^{Ant}	id.	nd.
ANT.LWAV6.1	16ST11^{Ant}	id.	nd.
ANT.LAC.1	16ST12	*Oscillatoria* sp. Ant-SOS (Nadeau et al. 2001) (98.0%)	ITS02
ANT.LACV6.1	16ST12	id.	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	id.	nd.
ANT.LPE.2	16ST14	id.	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	id.	nd.
ANT.L61.1	16ST16	*Nostoc* sp. NIVA-CYA 124 (Rudi et al. 1997) (99.4 - 100%)	nd.
ANT.LG2.6	16ST16	id.	nd.
ANT.L34.1	16ST16	id.	nd.
ANT.L36.1	16ST16	id.	nd.
ANT.L52B.7	16ST16	id.	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 {Flechtner2002} (97.7%)	ITS11
ANT.LG2.8	16ST20[Ne]	*Anabaena* sp. NIVA-CYA 267/4 (Rudi et al. 1997) (96.1%)	nd.

	w	
ANT.L59B.1	16ST21[Ne] w	*Chroococcus submarinus* kopara-BM (L. Richert, S. Golubic, nd. A. Herve, R. Le Guedes, J. Guezennec and C. Payri, unpublished data) (94.9%)

Abbreviation: nd., not determined ; id., idem.

[a] When the first hit indicated by BLAST was an uncultivated cyanobacteria, the first strain indicated by BLAST was added.

Table 3. Antimicrobial activities and cytotoxicity of the seventeen bioactive strains ordered in function of their OTU and the morphospecies to which they belonged

OTU	Morphospecies	Strain name	Activities[a] on *S. aureus*	Activities[a] on *A. fumigatus*	Activities[a] on *C. neoformans*	Cytotoxicity[b]
16ST01[Ne w]	*Pseudophormidium* sp. / *Schizothrix* sp.	ANT.LPR.2	64	0	512	640
16ST01[Ne w]	id.	ANT.LPR.3	64	0	64	320
16ST01[Ne w]	id.	ANT.LG2.1	0	0	64	160
16ST01[Ne w]	id.	ANT.LG2.2	32	0	256	280
16ST03[Ne w]	*P. priestleyi*	ANT.L52.4	0	512	1024	0
16ST03[Ne w]	id.	ANT.L52.6	8	512	512	160
16ST04	id.	ANT.LPR.6	32	0	0	320
16ST10	*P. priestleyi*	ANT.L61.2	8	0	0	0
16ST11[Ant]	*L. antarctica*	ANT.LG2.3	64	0	0	640
16ST11[Ant]	id.	ANT.LG2.5	8	0	0	0
16ST11[Ant]	id.	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

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16ST16	id.	ANT.LPR.1	0	0	8	1280
16ST16	id.	ANT.LG2.6	8	0	16	160
16ST16	id.	ANT.L34.1	8	0	0	0
16ST16	id.	ANT.L36.1	8	0	8	160

Abbreviation: id., idem

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

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

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Deleted: [a] The values reported are the upper limit¶

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Figure Legends

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*; 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*; E - *Chondrocystis* sp. The photomicrograph C was taken from the sample from which the strain was isolated (scale bars = 10 µm).

FIG. 3. Phylogenetic tree inferred from 16S rRNA gene sequences (*E. coli* positions 100 to 1450) by maximum likelihood (likelihood = -25328.76). In the windows, neighbor-joining tree inferred from partial 16S rRNA gene sequences (*E. coli* positions 405 to 780) for the OTUs. Bootstrap values obtained using the neighbor joining and the parsimony (only for near-complete sequences) methods 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 outgroup. 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).

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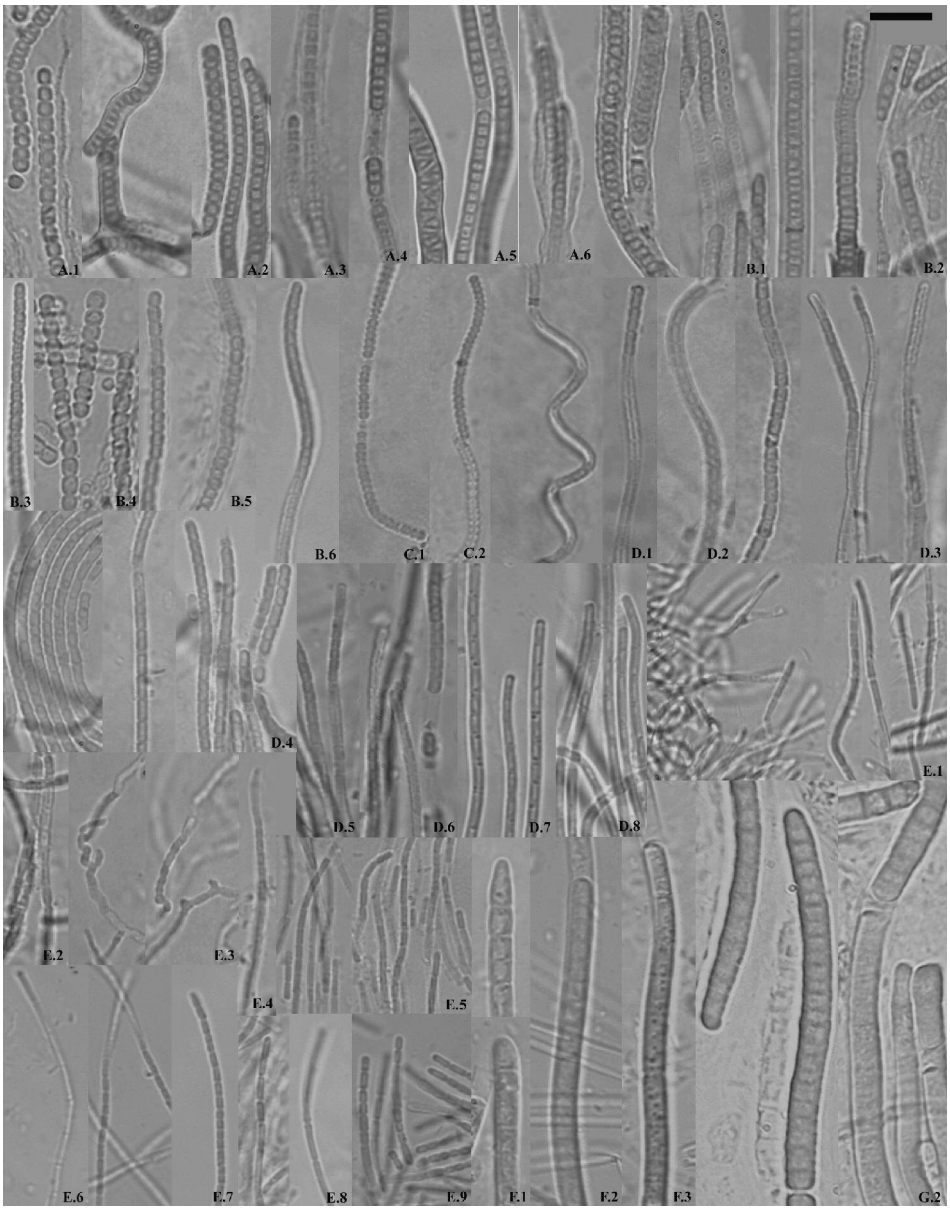
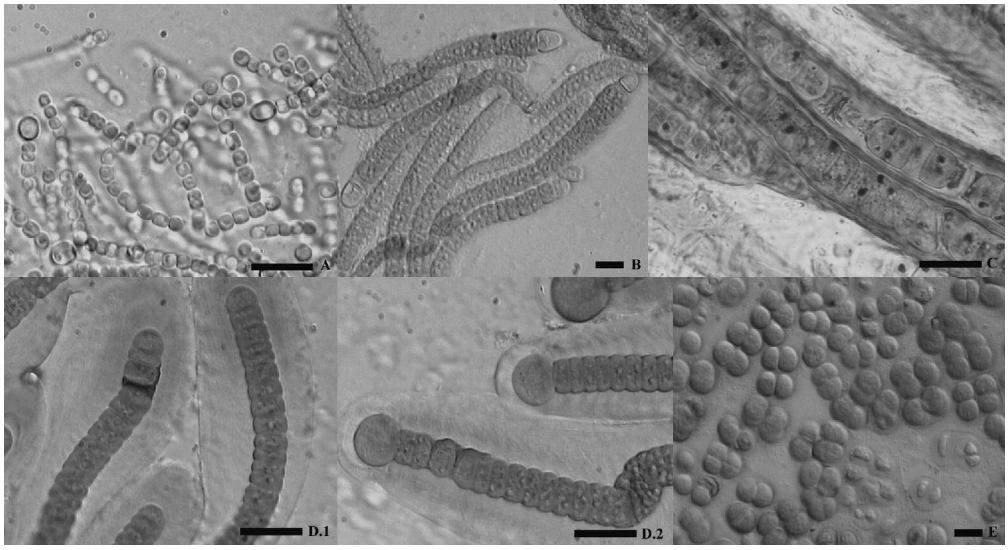
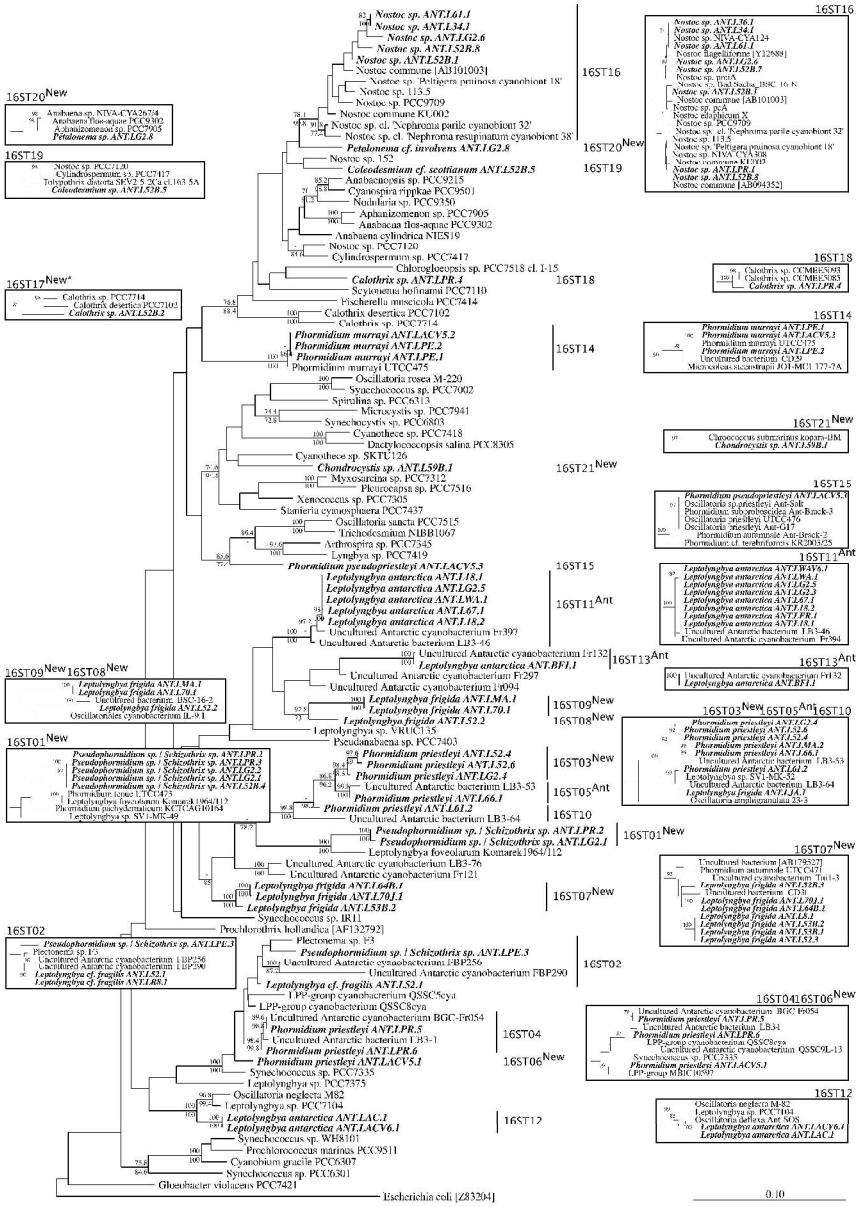


Photomicrographs of the morphospecies belonging to the Oscillatoriales order: A - Pseudophormidium sp. / Schizothrix sp.; B - Phormidium priestleyi; C - Leptolyngbya cf. fragilis; D - Leptolyngbya frigida; E - Leptolyngbya antarctica; F - Phormidium murrayi; G - Phormidium pseudopriestleyi (scale bar = 10 µm).



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; E - Chondrocystis sp. The photomicrograph C was taken from the sample from which the strain was isolated (scale bars = 10 µm).



Phylogenetic tree inferred from 16S rRNA gene sequences (E. coli positions 100 to 1450) by maximum likelihood (likelihood = -25328.76). In the windows, neighbor-joining tree inferred from partial 16S rRNA gene sequences (E. coli positions 405 to 780) for the OTUs. Bootstrap values obtained using the neighbor joining and the parsimony (only for near-complete sequences) methods 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 outgroup. 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).

APPENDIXES

Polyphasic study of Antarctic cyanobacterial strains

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

Region	Lake Name	Lake No	Location	Grid Ref	Alt. (m)	Area (ha)	Z max (m)	pH	Conductivity (mS/cm^{-1})	Salinity (ppt)	Strain name	Reference
DV	Fryxell	LFR	Southern Victoria Land	163°07'E 77°37'S	18	700	19.5	-	0.5 – 8.6	-	ANT.LFR.1	(Spigel and Priscu 1998)
LH	-	L61	W. Broknes	76°19'E 69°22'S	50	0.5	0.5	6.29	0.713	0.3	ANT.L61.1	(Sabbe et al. 2004)
	-	L64b	W. Broknes	76°18'E 69°23'S	50	0.1	1.0	7.16	0.552	0.3	ANT.L64B.1	(Sabbe et al. 2004)
	-	L66	W. Broknes	76°2°'E 69°24'S	25	2.5	2.3	7.43	0.795	0.4	ANT.L66.1	(Sabbe et al. 2004)
	"Gentner 2"	LG2	W. Broknes	76°19'E 69°23'S	65	0.3	1.0	6.92	0.219	0.1	ANT.LG2.1 - 8	(Sabbe et al. 2004)
	Bruehwiler	L52	Broknes	76°21'E 69°24'S	80	1	0.7	6.75	0.223	0.1	ANT.L52.1 - 6	(Sabbe et al. 2004)
	-	L52b	Broknes	76°21'E 69°24'S	80	0.5	1.0	7.02	0.237	0.1	ANT.L52B.1 - 8	(Sabbe et al. 2004)
	-	L53b	Broknes	76°23'E 69°24'S	40	0.5	0.5	6.68	0.139	0.1	ANT.L53B.1 -2	(Sabbe et al. 2004)
	-	L59b	Broknes	76°21'E 69°24'S	20	0.3	0.8	7.6	1 304	0.7	ANT.L59B.1	(Sabbe et al. 2004)
	-	L67	Broknes	76°21'E 69°23'S	45	4.5	5	6.68	1 761	0.9	ANT.L67.1	(Sabbe et al. 2004)
	"Reid / Big"	L70	Broknes	76°23'E 69°23'S	30	5.5	3.8	7.06	7.38	4.1	ANT.L70.1, ANT.L70J.1	(Sabbe et al. 2004)
	"Spate" / Oskar"	L18	Central Stornes	76°07'E 69°25'S	85	9	11	6.97	0.376	0.2	ANT.L18.1 - 2	(Sabbe et al. 2004)
	Jack	LJA	Central Stornes	76°23'E 69°23'S	5	5.0	4.5	6.79	0.111	0.1	ANT.LJA.1	(Sabbe et al. 2004)
	-	L8	N. Stornes	76°05'E 69°09'S	5	4.8	4.8	6.34	0.355	0.2	ANT.L8.1	(Sabbe et al. 2004)
	-	L36	Grovnes	76°13'E 69°25'S	60	5.5	15	6.6	0.23	0.1	ANT.L36.1	(Sabbe et al. 2004)
	"Progress 2 Pond"	LPR	Mirror Peninsula	76°23'E 69°23'S	10	0.3	0.8	7.04	0.807	0.4	ANT.LPR.1 - 6	(Sabbe et al. 2004)
	"Manning"	LMA	Manning / "Vikoy" Island	76°19'E 69°21'S	30	0.4	1.0	6.88	0.406	0.2	ANT.LMA.1 - 2	(Sabbe et al. 2004)
	Kirisjes Pond	L34	McLeod / "Kolloy" Island	76°09'E 69°22'S	5	12	9	6.42	0.387	0.2	ANT.L34.1	(Sabbe et al. 2004)
VH	Pendant	LPE	Long Peninsula	78°14'E 66°28'S	2.75	12.4	23	8.3	-	13.53-36.6	ANT.LPE.1 - 3	(Dartnall 2000, Gibson 1999, Roberts and McMinn 1999)
	Watts	LWA	E. end of Ellis Fjord	78°11'E 68°36'S	-5	-	29.5	7.6 – 8.6	0.47 – 4.14	2.24-2.40	ANT.LWA.1, ANT.LWAV6.1	(Dartnall 2000, Gibson 1999, Roberts and McMinn 1999)
	Ace	LAC	Long Peninsula	78°11'E 68°28'S	8.91	18	25	8.5	-	16.19-40.35	ANT.LAC.1, ANT.LACV5.1 - 3, ANT.LACV6.1	(Dartnall 2000, Gibson 1999, Roberts and McMinn 1999)
RI	"Rauer 8"	RI8	Shcherbinina Island	77°56'E 68°50'S	18	1 094	1	7.86	6.26	4.6	ANT.RI8.1	(Hodgson et al. 2001)
BI	Firelight	BFI	Sydney Island	75°45'E 69°31'S	30	0.9	1.5	9.38	3 927	2.1	ANT.BFI.1	(Sabbe et al. 2004)

Dartnall, H. J. G. 2000. A limnological reconnaissance of the Vestfold Hills. *In* Australian Antarctic Division, pp. 57.
Gibson, J. A. E. 1999. The meromictic lakes and stratified marine basins of the Vestfold Hills, East Antarctica. *Antarct. Sci.* 11:175 - 92.
Hodgson, D. A., Vyverman, W. & Sabbe, K. 2001. Limnology and biology of saline lakes in the Rauer Islands, eastern Antarctica. *Antarct. Sci.* 13:255-70.
Roberts, D. & McMinn, A. 1999. Diatoms of the saline lakes of the Vestflold Hills, Antarctica.

Sabbe, K., Hodgson, D. A., Verleyen, E., Taton, A., Wilmotte, A., Vanhoutte, K. & Vyverman, W. 2004. Effects of physical disturbance, salinity and light regime on microbial mat structure and composition in continental Antarctic lakes (Larsemann Hills and Bølingen Islands). *Freshwater Biol.* 49:296-319.
Spigel, R. H. & Priscu, J. C. 1998. Physical limnology of the McMurdo Dry Valleys lakes. *In* Priscu, J. C. [Ed.] *Ecosystem Dynamics in a Polar Desert: The McMurdo Dry Valleys, Antarctica*. American Geophysical Union, Washington, D.C, pp. 153-88.

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

	1	1NP	2	2NP	3	3NP
$CaCl_2.2H_2O$	3.7 mg	3.7 mg	18.4 mg	18.4 mg	125.7 mg	125.7 mg
EDTA	1 mg	1 mg	1 mg	1 mg	1 mg	1 mg
$K_2HPO_4.3H2O$	0.015 mg	1.6 mg	0.015 mg	4.5 mg	0.015 mg	4.5 mg
KCl	2.2 mg	1.15 mg	5.5 mg	1.5 mg	51.8 mg	48.8 mg
$MgCl_2.6H_2O$	/	10 mg	35.9 mg	35.9 mg	344.8 mg	344.8 mg
$MgSO_4.7H_2O$	18 mg	6.15 mg	32.75 mg	32.75 mg	489 mg	489 mg
Na_2CO_3	5.7 mg	5.7 mg	22.4 mg	22.4 mg	195.7 mg	195.7 mg
NaCl	37.9 mg	/	148.7 mg	/	1.96 g	1.96 g
$NaNO_3$	/	59.3 mg	/	170 mg	/	170 mg
NH_4Cl	/	22.7 mg	/	108 mg	/	108 mg
Cyano Trace Metal[a]	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
Distilled water	*ad* 1000 ml	*ad* 1000 ml	*ad* 1000 ml	*ad* 1000 ml	*ad* 1000 ml	*ad* 1000 ml

[a] Cyano trace metal (Waterbury and Stanier 1981)

Citric Acid H_2O	6.25 g
$Co(NO_3)2{\cdot}6H_2O$	0.025 g
Ferric Ammonium Citrate	6.0 g
$MnCl_2{\cdot}4H_2O$	1.4 g
$Na_2MoO_4{\cdot}2H_2O$	0.39 g
$ZnSO_4{\cdot}7H_2O$	0.222 g
Distilled water	*ad* 1000 ml

Remarks: Media were adjusted to pH 7; for solid media, 1,4% agar was added to the culture solution.

Waterbury, J. B. & Stanier, R. Y. 1981. Isolation and growth of cyanobacteria from marine and hypersaline environments. *In* Starr, M. P., Stolp, H., Truper, H. G., Balows, A. & Schlegel, H. G. [Eds.] *The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria*. Springer-Verlag, Berlin, pp. 221-3.

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

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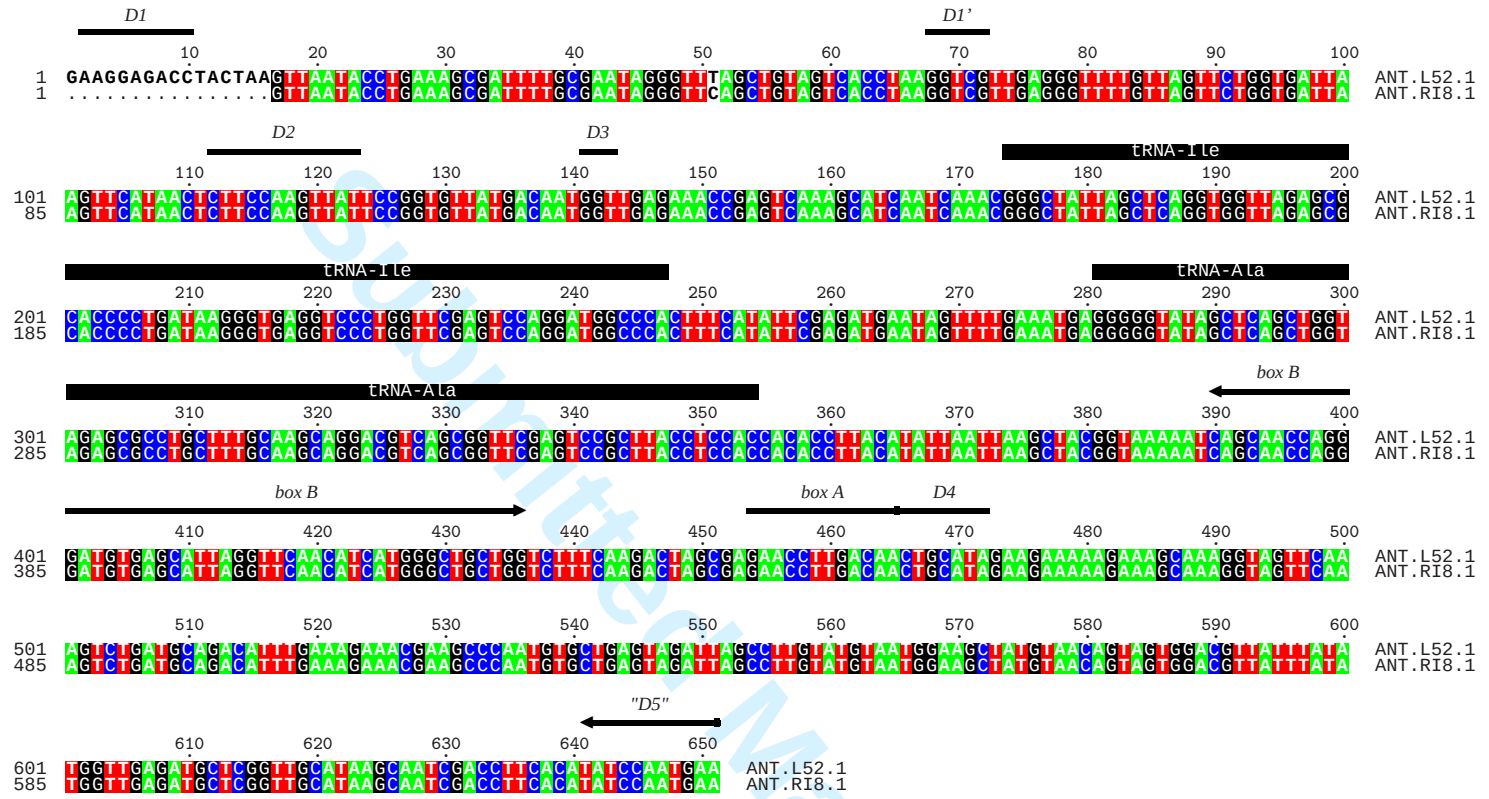

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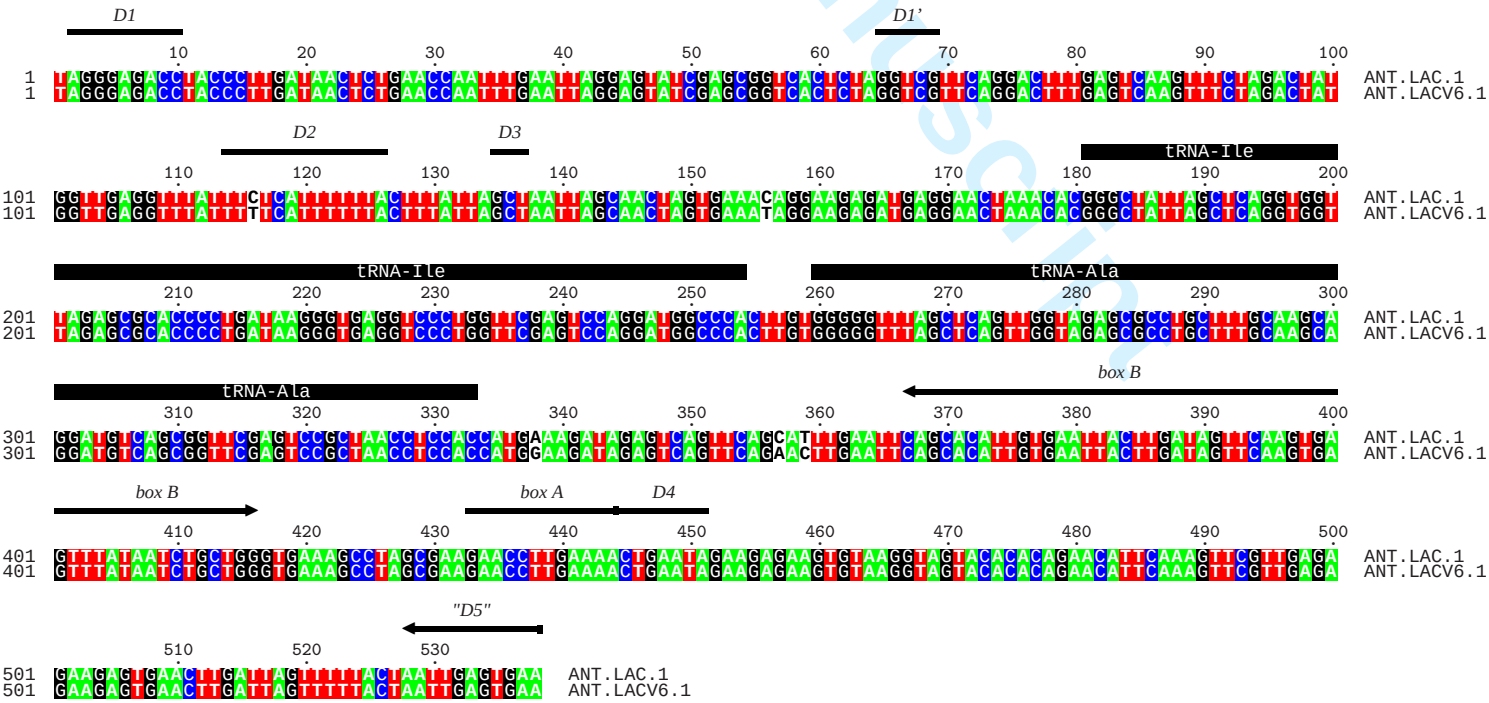

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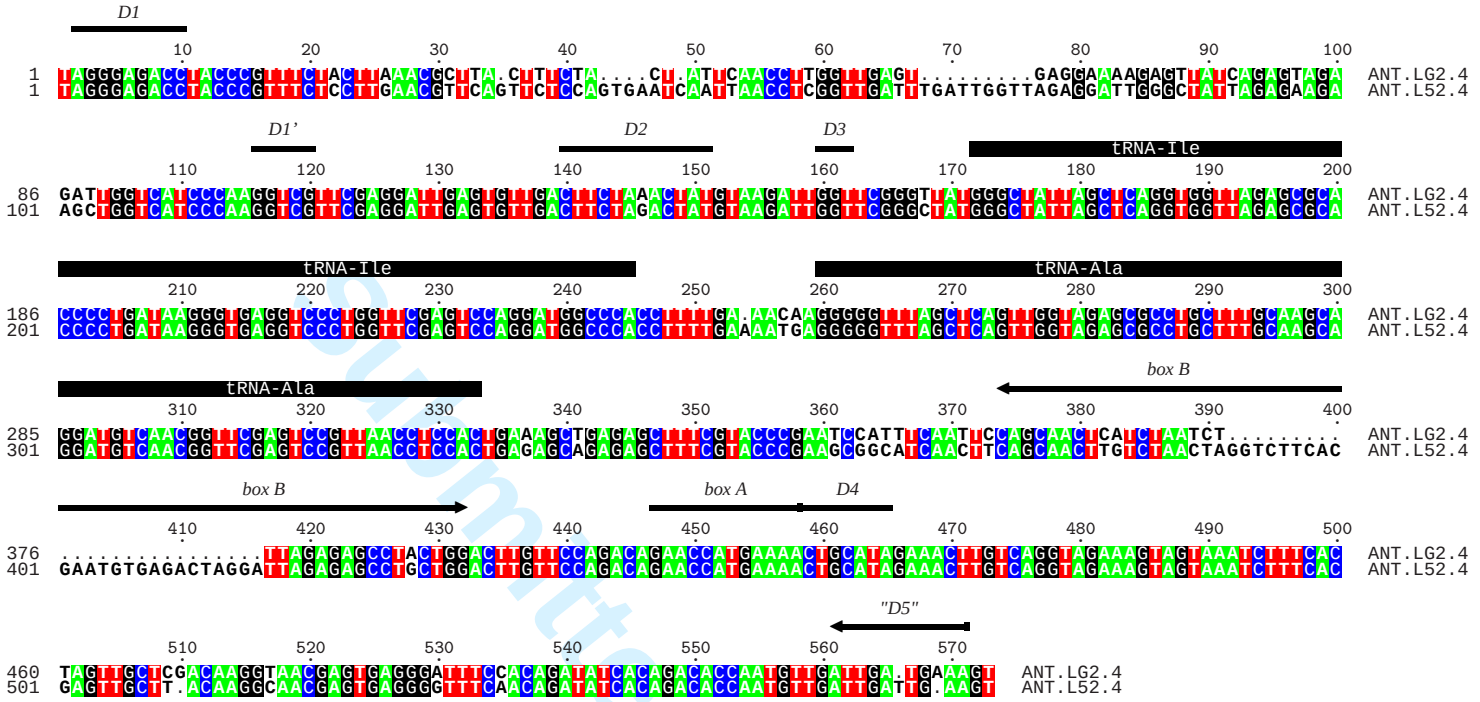

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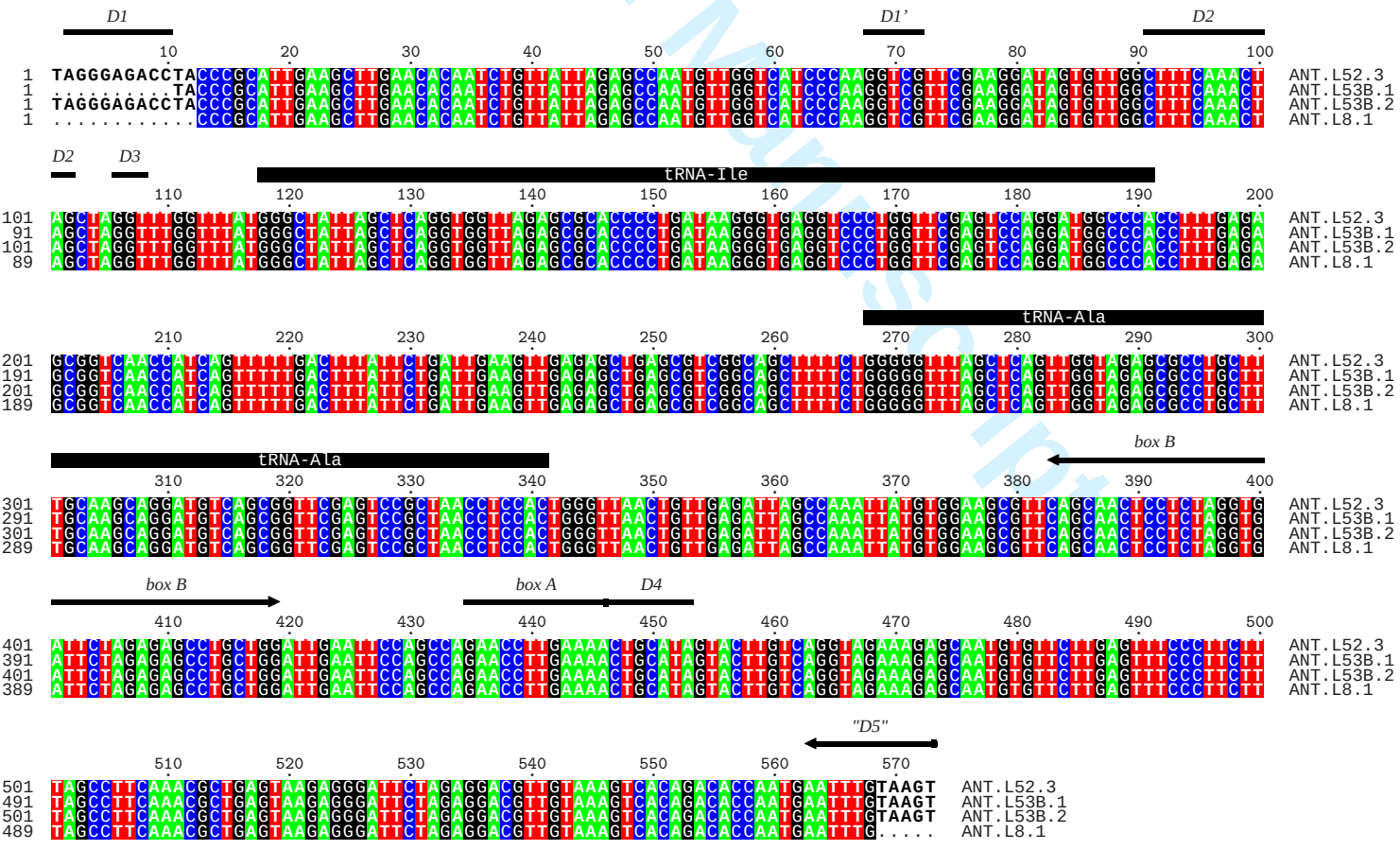

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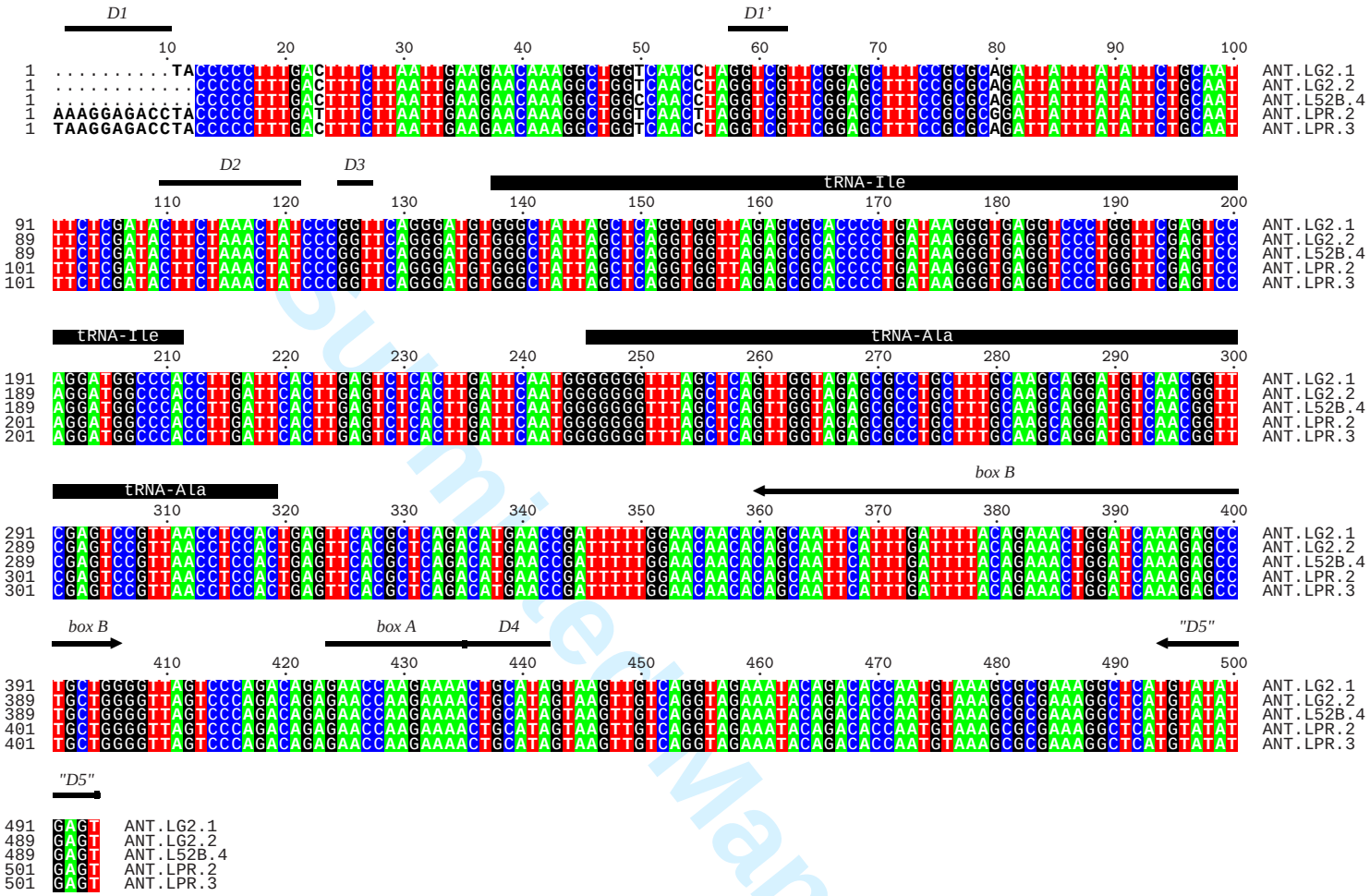

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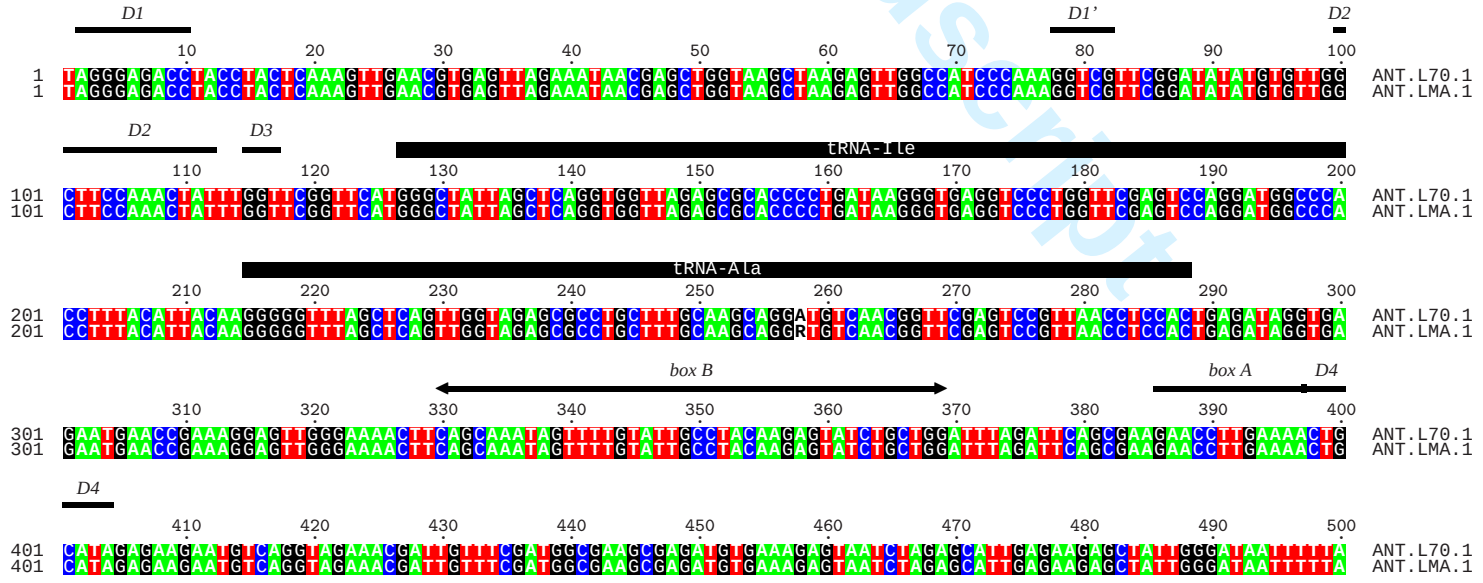

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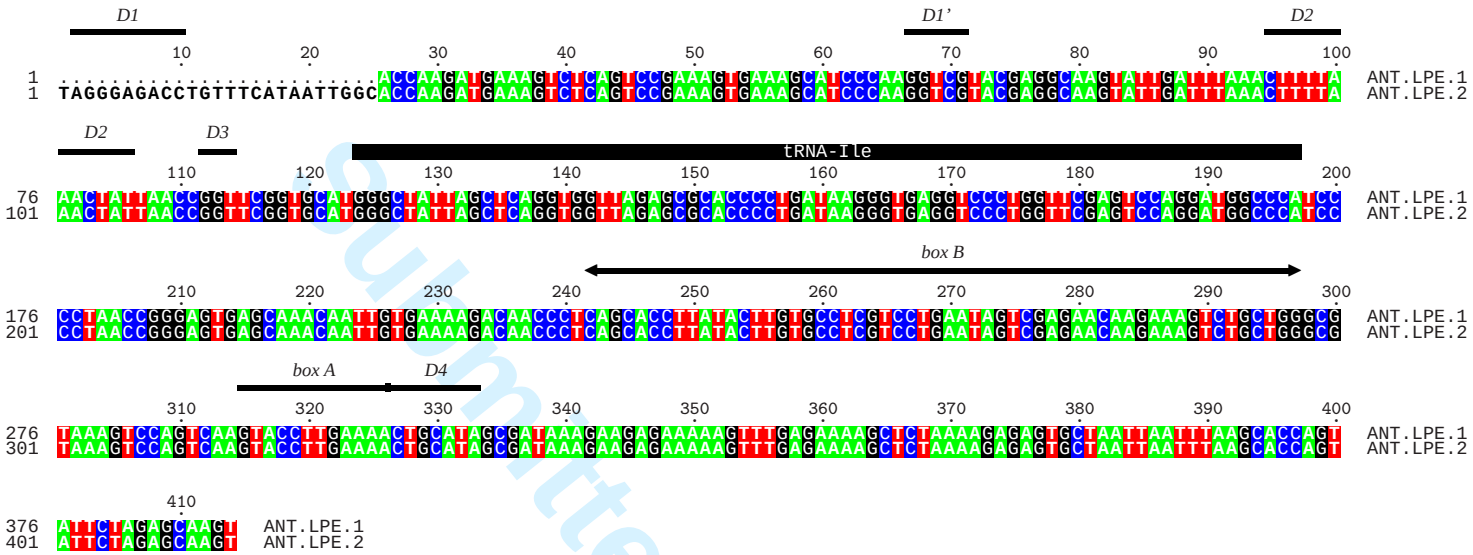

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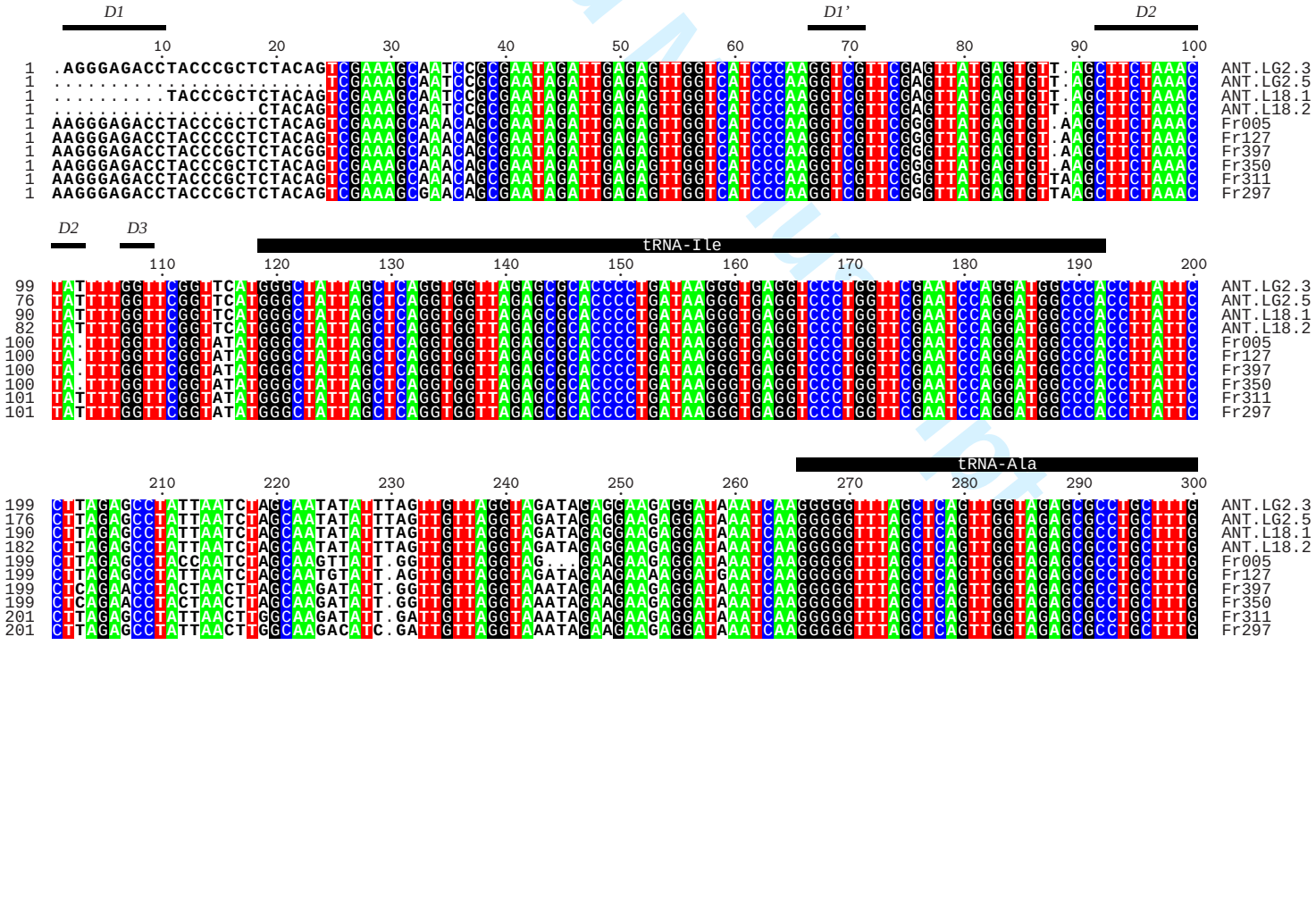

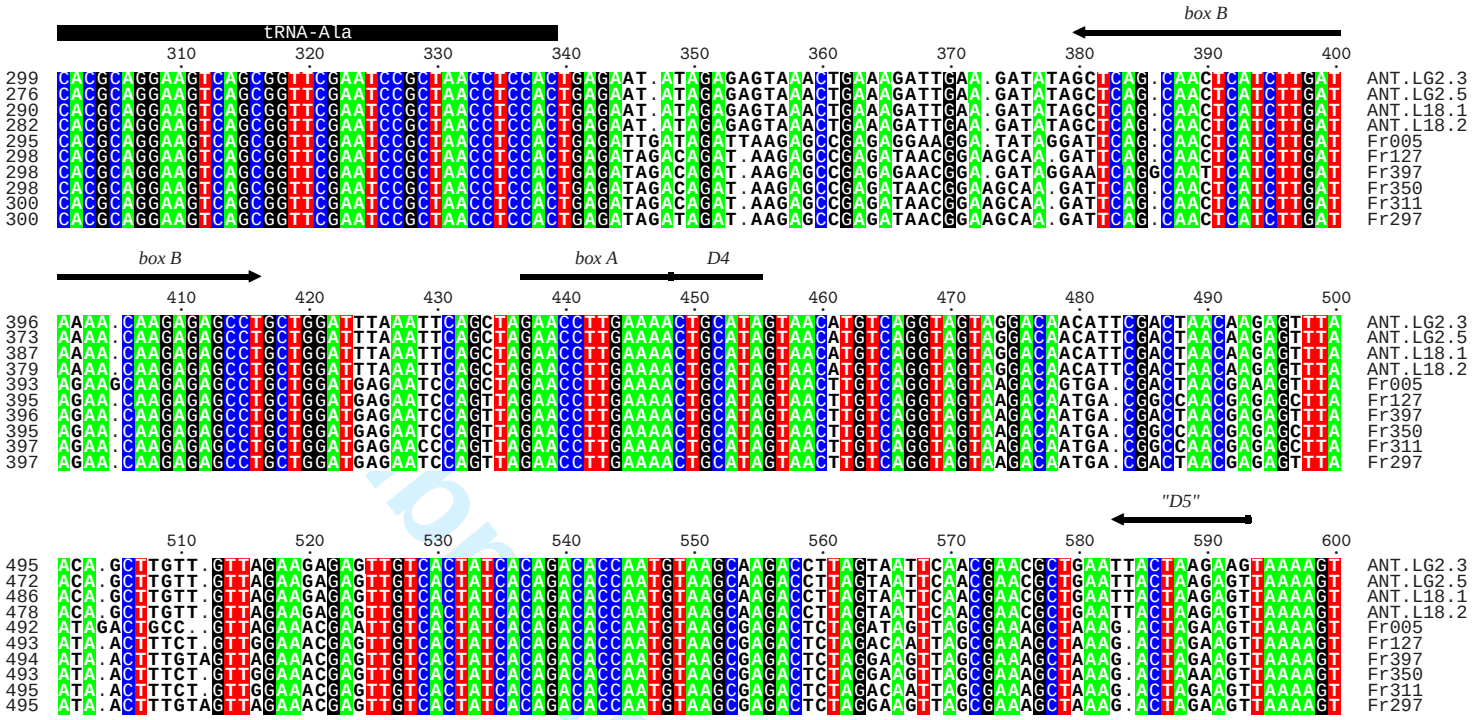

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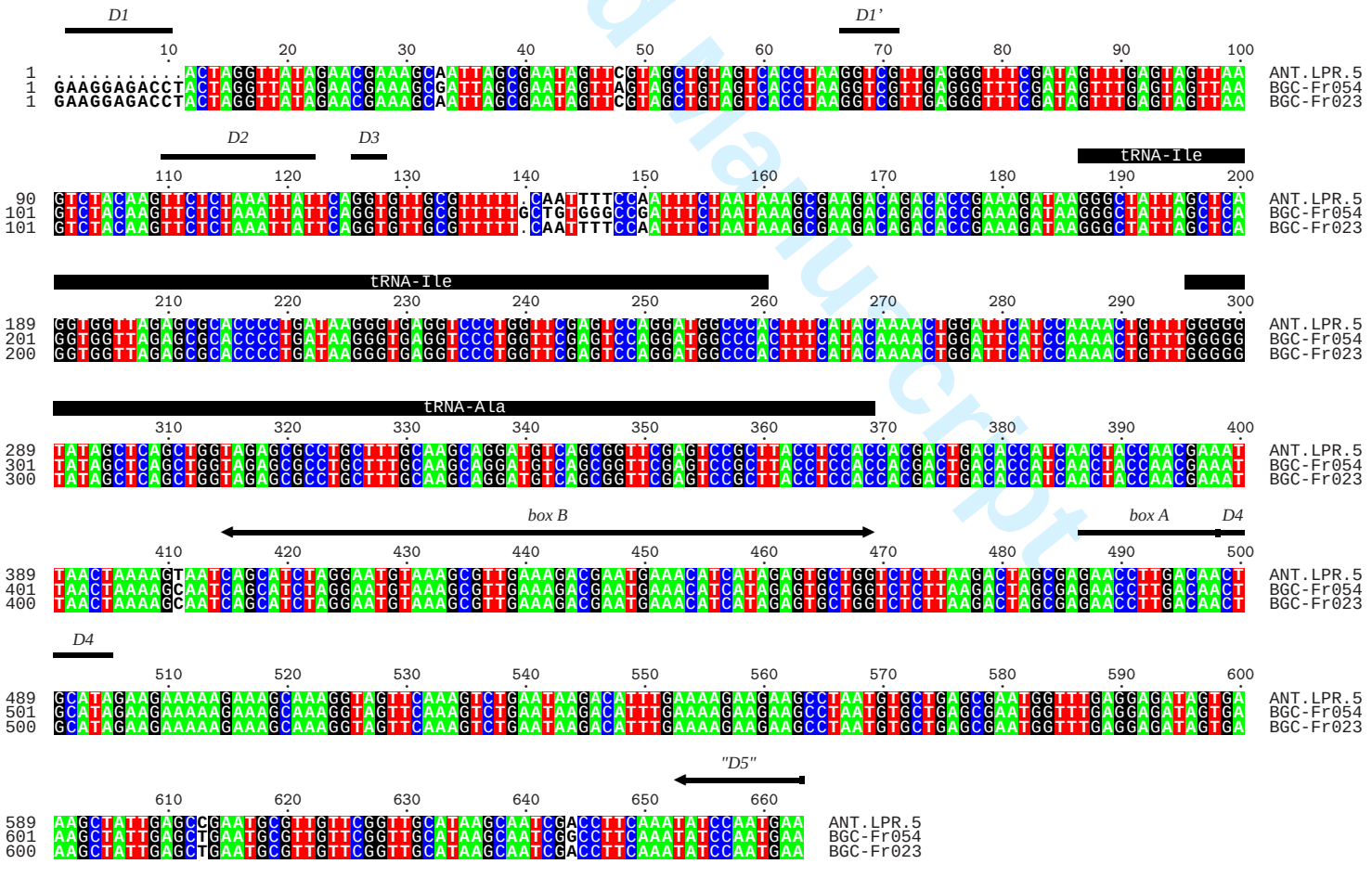
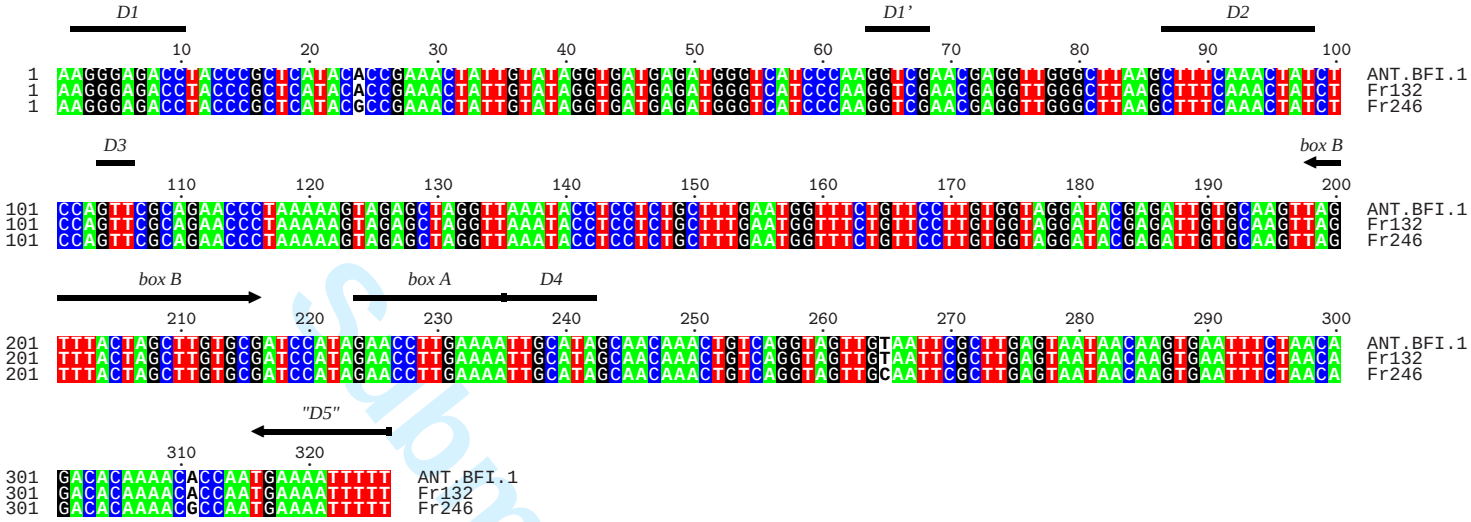


ITS-Type 10



ITS-Types 11 to 18 (ITS sequences that are too different to be meaningfully aligned with any other ITS sequences)

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GAGAGGG

Iteman, I., Rippka, R., Tandeau de Marsac, N., and Heussner, S. 2000. Comparison of conserved structural and regulatory domains within divergent 16S rRNA-23S rRNA spacer sequences of cyanobacteria. *Microbiol.* 146 :1275-86.

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

Morphospecies	Strain name	Isolation media	Sheath	Number of trichomes	False branching	Cross-wall constriction	Cross-wall granulation	Necridic cell	Cell shape	Cell width M. ± S.D. (Min. - Max.)	Cell length M. ± S.D. (Min. - Max.)	End-cell shape	Comment	OTU
Pseudophormidium sp. / *Schizothrix* sp.			+	+	(+)	++	-	+	</=	2.06 ± 0.33 (1.33 - 2.86)	1.81 ± 0.53(0.83 - 3.42)	r		
	ANT.LPR.2	3NP	+	+	(+)	++	-	+	=	2.18 ± 0.27 (1.46 - 2.66)	2.42 ± 0.54 (1.19 - 3.42)	r		16ST01^{New}
	ANT.LPR.3	3NP	+	(+)	+	++	-	+	=	2.02 ± 0.29 (1.43 - 2.57)	1.64 ± 0.51 (0.83 - 2.59)	r		16ST01^{New}
	ANT.LG2.1	2NP	+	+	(+)	++	-	+	=	1.93 ± 0.26 (1.46 - 2.43)	1.60 ± 0.32 (1.03 - 3.08)	r		16ST01^{New}
	ANT.LG2.2	2NP	+	+	(+)	++	-	+	=	2.06 ± 0.28 (1.44 - 2.66)	1.83 ± 0.58 (1.01 - 3.42)	r		16ST01^{New}
	ANT.L52B.4	BG11	+	+	(+)	++	-	+	=	1.78 ± 0.18 (1.33 - 2.11)	1.53 ± 0.23 (1.18 - 2.09)	r		16ST01^{New}
	ANT.LPE.3	2	+	+	(+)	++	-	+	<	2.49 ± 0.25 (2.07 - 2.86)	1.80 ± 0.30 (1.08 - 2.52)	r		16ST02
Phormidium priestleyi			+	+	((+))	++	-	+	</=	1.98 ± 0.40 (1.14 - 3.15)	1.82 ± 0.65 (0.65 - 3.80)	r		
	ANT.L52.4	3	+	(+)	(+)	++	-	+	=	2.33 ± 0.36 (1.66 - 3.15)	2.46 ± 0.61 (1.56 - 3.80)	r		16ST03^{New}
	ANT.LG2.4	2NP	(+)	1	-	++	-	+	=	1.84 ± 0.16 (1.44 - 2.22)	1.58 ± 0.32 (1.18 - 3.08)	r		16ST03^{New}
	ANT.L52.6	2NP	?	1	-	++	-	+	=	2.44 ± 0.30 (1.94 - 3.08)	2.55 ± 0.6 (1.67 - 3.69)	r		16ST03^{New}
	ANT.LPR.5	3NP	(+)	1	-	++	-	?	<	1.89 ± 0.22 (1.23 - 2.20)	1.23 ± 0.27 (0.84 - 2.26)	r		16ST04
	ANT.LPR.6	3NP	(+)	((+))	((+))	++	-	+	=	1.80 ± 0.14 (1.40 - 2.05)	1.66 ± 0.36 (1.11 - 2.50)	r		16ST04
	ANT.L66.1	GANX	+	1	-	++	-	+	<	2.11 ± 0.51 (1.48 - 3.08)	1.82 ± 0.31 (1.23 - 2.94)	r		16ST05^{Ant}
	ANT.LMA.2	3NP	+	1	-	++	-	+	=	1.89 ± 0.32 (1.41 - 2.74)	1.74 ± 0.21 (1.14 - 2.10)	r		16ST05^{Ant}
	ANT.LACV5.1	ASNIII$_0$/2	-	1	-	++	-	?	<	1.42 ± 0.19 (1.14 - 1.90)	0.99 ± 0.32 (0.65 - 2.01)	r		16ST06^{New}
	ANT.L61.2	3	+	1	(+)	++	-	+	=	2.12 ± 0.23 (1.55 - 2.58)	2.31 ± 0.51 (1.25 - 3.38)	r-c		16ST10
Leptolyngbya cf. *fragilis*			+	1	(+)	++	-	+	</=	1.42 ± 0.17 (1.14 - 1.90)	1.23 ± 0.27(0.76 - 2.09)	r		
	ANT.L52.1	3NP	+	1	(+)	++	-	+	=	1.35 ± 0.16 (1.14 - 1.72)	1.40 ± 0.27 (0.95 - 2.09)	r		16ST02
	ANT.RI8.1	3	+	1	(+)	++	-	+	<	1.50 ± 0.15 (1.20 - 1.90)	1.07 ± 0.15 (0.76 - 1.38)	r		16ST02
Leptolyngbya frigida			+	1	-	+	-	(+)	>	1.44 ± 0.34 (0.72 - 2.96)	2.78 ± 0.92(1.16 - 7.79)	r		
	ANT.L53B.1	3	+	1	-	++	-	+	>	1.39 ± 0.17 (1.03 - 1.73)	2.67 ± 0.55 (1.79 - 3.80)	r		16ST07^{New}
	ANT.L53B.2	1	+	1	-	++	-	+	>	1.27 ± 0.25 (0.72 - 1.85)	2.22 ± 0.50 (1.16 - 3.50)	r		16ST07^{New}
	ANT.L52.3	3	+	1	-	++	-	+/(+)	>	1.42 ± 0.27 (0.93 - 1.98)	3.23 ± 1.12 (1.35 - 6.04)	r		16ST07^{New}
	ANT.L8.1	1NP	+	1	((+))	++	-	(+)	>	1.47 ± 0.19 (1.10 - 1.94)	2.62 ± 0.74 (1.52 - 5.06)	r		16ST07^{New}
	ANT.L52B.3	3	-	1	-	+	-	+	>	1.39 ± 0.07 (1.25 - 1.52)	3.31 ± 1.03 (2.13 - 7.79)	r		16ST07^{New}

ANT.L64B.1	GANX	+	1	-	++	-	+	>	1.49 ± 0.29 (1.02 - 2.21)	2.79 ± 0.92 (1.74 - 4.92)	r		16ST07^{New}
ANT.L70J.1	3NP	(-)	1	-	++	-	?	>	1.30 ± 0.30 (1.84 - 2.20)	2.61 ± 0.37 (1.85 - 3.42)	r		16ST07^{New}
ANT.L52.2	3	+	1	-	++	-	?	>	2.15 ± 0.38 (1.48 - 2.96)	3.09 ± 0.92 (1.52 - 5.05)	r		16ST08^{New}
ANT.LMA.1	3NP	+	1	-	+	-	-	>	1.54 ± 0.21 (1.22 - 2.01)	3.46 ± 1.04 (1.71 - 6.55)	r		16ST09^{New}
ANT.L70.1[a]	2NP	+	1	((+))	+	-	-	=	1.73 ± 0.23 (1.29 - 2.24)	1.76 ± 0.94 (0,46 - 3.91)			16ST09^{New}
ANT.LJA.1	3	+	1	-	++	-	+	>	1.15 ± 0.11 (0.91 - 1.38)	2.06 ± 0.44 (1.27 - 3.19)	r		16ST10
Leptolyngbya antarctica		-	1	-	(+)	-	-	>	0.91 ± 0.16 (0.65 - 1.75)	2.47 ± 0.87(0.95 - 7.37)	r		
ANT.LG2.3	2NP	-	1	-	+	-	-	>	0.88 ± 0.22 (0.65 - 1.40)	2.09 ± 0.61 (1.14 - 3.90)	r		16ST11^{Ant}
ANT.LG2.5	3	-	1	-	(+)	-	-	>	0.94 ± 0.07 (0.84 - 1.11)	2.06 ± 0.44 (1.44 - 3.10)	r-c		16ST11^{Ant}
ANT.L67.1	2	-	1	-	(+)	-	-	>	0.87 ± 0.12 (0.68 - 1.29)	2.89 ± 0.62 (1.67 - 4.22)	r		16ST11^{Ant}
ANT.L18.1	3NP	-	1	-	+	-	-	>	0.96 ± 0.08 (0.84 - 1.23)	1.69 ± 0.33 (0.95 - 2.32)	r	True B	16ST11^{Ant}
ANT.L18.2	3	-	1	-	(+)	-	-	>	0.86 ± 0.04 (0.76 - 0.91)	2.89 ± 1.09 (1.63 - 5.84)	r		16ST11^{Ant}
ANT.LWA.1	3	-	1	-	(+)	-	-	>	0.90 ± 0.14 (0.72 - 1.18)	2.66 ± 0.78 (1.37 - 4.37)	r		16ST11^{Ant}
ANT.LWAV6.1	3	-	1	-	(+)	(+)	-	>	0.89 ± 0.07 (0.76 - 1.10)	2.32 ± 0.72 (1.37 - 4.51)	r	rs.	16ST11^{Ant}
ANT.LFR.1	3	-	1	-	++			>	0.85 ± 0.15 (0.68 - 1.23)	2.10 ± 0.42 (1.32 - 3.75)	r		16ST11^{Ant}
ANT.LAC.1	GANX	-	1	-	(+)	-	-	>	1.17 ± 0.20 (0.84 - 1.75)	3.80 ± 1.11 (2.32 - 7.37)	r	rs.	16ST12
ANT.LACV6.1	ASNIII/2	-	1	-	(+)	-	-	>	0.90 ± 0.16 (0.68 - 1.25)	2.09 ± 0.49 (1.18 - 3.23)	r		16ST12
ANT.BFI.1	3NP	-	1	-	(+)	-	-	>	0.82 ± 0.05 (0.68 - 0.91)	2.81 ± 0.44 (2.13 - 3.80)	r	rs.	16ST13^{Ant}
Phormidium murrayi		+	1	-	-	-	-	>	3.09 ± 0.38 (2.43 - 4.29)	5.33 ± 1.26(2.70 - 9.04)	r	-	
ANT.LPE.1	ASNIII$_0$/2	+	1	-	-	-	-	>	2.89 ± 0.24 (2.43 - 3.39)	5.09 ± 1.02 (2.70 - 6.61)	r		16ST14
ANT.LPE.2	ASNIII/2	+	1	-	-	-	-	>	2.95 ± 0.33 (2.55 - 3.94)	5.05 ± 0.83 (3.42 - 6.80)	r-c	gran.	16ST14
ANT.LACV5.2	ASNIII/2	+	1	-	-	-	-	>	3.45 ± 0.28 (3.00 - 4.29)	5.88 ± 1.65 (3.02 - 9.04)	r		16ST14
Phormidium pseudopriestleyi		+	1	-	-	-	-	<	5.86 ± 0.73 (4.02 - 7.22)	3.24 ± 0.70(1.87 - 4.52)	r		
ANT.LACV5.3	ASNIII/2	+	1	-	-	-	+	<	5.86 ± 0.73 (4.02 - 7.22)	3.24 ± 0.70 (1.87 - 4.52)	r		16ST15

Abbreviation: r, rounded; c, conical; rs. refracting structure; gran., granule ; True B, true branching

[a] cell measurements of this strain were not considered in the average value of the morphospecies

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

Morphospecies	Strain name	Isolation media	Cell width Moy. ± S.D. (Min. - Max.)	Cell length Moy. ± S.D. (Min. - Max.)	Heterocyst width M. ± S.D. (Min. - Max.)	Heterocyst length M. ± S.D. (Min. - Max.)	Filament width M. ± S.D. (Min. - Max.)	OTU
Nostoc sp.			3.67 ± 0.62 (2.22 – 5.97)	3.64 ± 0.96 (1.41 – 6.69)	4.60 ± 0.88 (2.85 – 7.6)	4.81 ± 0.95 (2.54 – 8.32)		
	ANT.L52B.1	2	3.02 ± 0.29 (2.51 - 3.59)	2.88 ± 0.55 (1.62 - 4.14)	3.32 ± 0.28 (2.85 - 3.97)	3.58 ± 0.61 (2.54 - 5.26)		16ST16
	ANT.LPR.1	3	3.95 ± 0.49 (3.11 - 5.97)	4.00 ± 0.70 (1.99 - 5.97)	5.10 ± 0.55 (4.41 - 6.80)	5.74 ± 0.56 (4.86 - 7.03)		16ST16
	ANT.L52B.8	3	3.11 ± 0.47 (2.22 - 4.12)	3.15 ± 0.77 (2.22 - 5.33)	4.34 ± 0.47 (3.53 - 6.11)	4.34 ± 0.54 (3.46 - 5.36)		16ST16
	ANT.L61.1	3	4.58 ± 0.27 (3.80 - 5.13)	5.02 ± 0.78 (3.57 - 6.69)	5.07 ± 0.66 (3.57 - 5.97)	5.01 ± 0.71 (3.57 - 5.89)		16ST16
	ANT.L36.1	2	3.44 ± 0.49 (2.70 - 4.90)	3.27 ± 0.49 (1.91 - 4.14)	3.97 ± 0.78 (2.96 - 6.14)	4.62 ± 1.05 (3.15 - 8.03)		16ST16
	ANT.LG2.6	$BG11_0$	3.68 ± 0.27 (3.23 - 4.29)	2.98 ± 0.55 (1.41 - 3.80)	5.25 ± 0.51 (4.33 - 6.84)	5.11 ± 0.66 (3.63 - 6.46)		16ST16
	ANT.L34.1	GOX	3.71 ± 0.25 (3.34 - 4.29)	4.17 ± 0.84 (2.96 - 6.16)	5.24 ± 0.87 (3.95 - 7.60)	5.61 ± 0.99 (3.95 - 8.32)		16ST16
	ANT.L52B.7	GOX	4.02 ± 0.36 (3.34 - 4.79)	4.03 ± 0.44 (3.23 - 4.75)	4.52 ± 0.61 (3.65 - 6.31)	4.64 ± 0.55 (3.80 - 6.23)		16ST16
Calothrix sp.			6.91 ± 1.19 (4.26 – 10.87)	4.88 ± 2.36 (1.98 – 11.51)	6.63 ± 2.14 (2.55 – 13.49)	5.66 ± 2.19 (1.56 – 11.40)	10.27 ± 2.68 (3.08 – 15.01)	
	ANT.L52B.2	2	7.04 ± 1.30 (4.26 - 9.99)	3.63 ± 0.74 (2.47 - 4.90)	6.86 ± 1.46 (2.66 - 8.89)	3.47 ± 1.17 (1.56 - 5.78)	11.36 ± 1.60 (8.36 – 13.95)	16ST17[New]
	ANT.L52.5	3	6.42 ± 1.24 (4.48 - 10.30)	5.00 ± 1.25 (2.66 - 7.60)	6.74 ± 1.39 (4.37 - 9.42)	3.55 ± 0.81 (2.01 - 5.36)	11.11 ± 1.35 (8.63 – 14.44)	
	ANT.LPR.4	3	7.29 ± 0.88 (5.66 - 9.27)	3.30 ± 0.88 (1.98 - 5.81)	8.65 ± 2.03 (4.48 - 13.49)	7.47 ± 1.59 (4.83 - 11.40)	12.67 ± 1.15 (9.96 – 15.01)	16ST18
	ANT.L52B.6	3NP	7.04 ± 0.79 (5.81 - 8.51)	9.93 ± 0.81 (8.74 - 11.51)	3.43 ± 0.61 (2.55 - 4.48)	7.04 ± 0.87 (5.36 - 8.21)	5.39 ± 1.18 (3.80 – 7.03)	
	ANT.LG2.7	3	6.73 ± 1.39 (4.64 - 10.87)	4.23 ± 0.81 (2.66 - 5.59)	7.80 ± 1.05 (6.42 - 11.40)	7.23 ± 1.08 (6.08 - 10.26)	9.20 ± 1.49 (6.31 – 13.07)	
Coleodesmium cf. *scottianum*								
	ANT.L52B.5	3	5.59 ± 0.76 (4.37 - 7.22)	4.49 ± 0.76 (3.08 - 6.08)	7.09 ± 0.88 (5.32 - 9.12)	7.13 ± 1.04 (5.36 - 9.54)	10.14 ± 2.04 (6.73 – 14.82)	16ST19
Petalonema cf. *involvens*								
	ANT.LG2.8	2	5.62 ± 0.41 (4.84 - 6.52)	3.40 ± 0.75 (1.92 - 5.08)	6.29 ± 0.56 (5.40 - 7.60)	4.90 ± 0.87 (3.36 - 6.92)	11.57 ± 2.24 (8.60 - 17.48)	16ST20[New]
Chondrocystis sp.								
	ANT.L59B.1	3	4.00 ± 0.81 (2.36 - 5.81)	5.53 ± 0.80 (4.26 - 7.07)			6.82 ± 0.63 (5.36 – 7.98)	16ST21[New]

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

OTU	Morphospecies	Strain name	Activities[a] on *S. aureus*	Activities[a] on *A. fumigatus*	Activities[a] on *C. neoformans*	Cytotoxicity[b]
16ST01[New]	*Pseudophormidium* sp. / *Schizothrix* sp.	ANT.LPR.2	64	0	512	640
16ST01[New]	id.	ANT.LPR.3	64	0	64	320
16ST01[New]	id.	ANT.LG2.1	0	0	64	160
16ST01[New]	id.	ANT.LG2.2	32	0	256	280
16ST01[New]	id.	ANT.L52B.4	0	0	0	0
16ST02	id.	ANT.LPE.3	0	0	0	0
16ST02	*L.* cf. *fragilis*	ANT.L52.1	0	0	0	160
16ST02	id.	ANT.RI8.1	0	0	0	0
16ST03[New]	*P. priestleyi*	ANT.L52.4	0	512	1024	0
16ST03[New]	id.	ANT.LG2.4	0	0	0	160
16ST03[New]	id.	ANT.L52.6	8	512	512	160
16ST04	id.	ANT.LPR.5	0	0	0	160
16ST04	id.	ANT.LPR.6	32	0	0	320
16ST05[Ant]	id.	ANT.L66.1	0	0	0	0
16ST05[Ant]	id.	ANT.LMA.2	nd.	nd.	nd.	nd.
16ST06[New]	id.	ANT.LACV5.1	0	0	0	0
16ST07[New]	*L. frigida*	ANT.L53B.1	0	0	0	0
16ST07[New]	id.	ANT.L52.3	0	0	0	80
16ST07[New]	id.	ANT.L8.1	0	0	0	80
16ST07[New]	id.	ANT.L53B.2	0	0	0	80
16ST07[New]	id.	ANT.L52B.3	0	0	0	1280
16ST07[New]	id.	ANT.L70J.1	0	0	0	0
16ST07[New]	id.	ANT.L64B.1	nd.	nd.	nd.	nd.
16ST08[New]	id.	ANT.L52.2	0	0	0	0
16ST09[New]	id.	ANT.LMA.1	0	0	0	0
16ST09[New]	id.	ANT.L70.1	0	0	0	0
16ST10	id.	ANT.LJA.1	0	0	0	0
16ST10	*P. priestleyi*	ANT.L61.2	8	0	0	0
16ST11[Ant]	*L. antarctica*	ANT.LG2.3	64	0	0	640
16ST11[Ant]	id.	ANT.L67.1	0	0	0	0
16ST11[Ant]	id.	ANT.L18.1	0	0	0	0
16ST11[Ant]	id.	ANT.LG2.5	8	0	0	0
16ST11[Ant]	id.	ANT.L18.2	8	0	0	0
16ST11[Ant]	id.	ANT.LFR.1	0	0	0	0
16ST11[Ant]	id.	ANT.LWA.1	nd.	nd.	nd.	nd.
16ST11[Ant]	id.	ANT.LWAV6.1	nd.	nd.	nd.	nd.
16ST12	id.	ANT.LAC.1	0	0	0	1280
16ST12	id.	ANT.LACV6.1	0	0	0	160
16ST13[Ant]	id.	ANT.BFI.1	nd.	nd.	nd.	nd.
16ST14	*P. murrayi*	ANT.LPE.1	8	0	0	160
16ST14	id.	ANT.LACV5.2	0	0	0	0
16ST14	id.	ANT.LPE.2	0	0	0	0
16ST15	*P. pseudopriestleyi*	ANT.LACV5.3	nd.	nd.	nd.	nd.
16ST16	*Nostoc* sp.	ANT.L52B.1	32	0	32	640
16ST16	id.	ANT.LPR.1	0	0	8	1280
16ST16	id.	ANT.L61.1	0	0	0	640
16ST16	id.	ANT.LG2.6	8	0	16	160
16ST16	id.	ANT.L34.1	8	0	0	0
16ST16	id.	ANT.L36.1	8	0	8	160
16ST16	id.	ANT.L52B.7	nd	nd	nd	nd
16ST16	id.	ANT.L52B.8	nd.	nd.	nd.	nd.
16ST17[New]	*Calothrix* sp.	ANT.L52B.2	0	0	0	0
16ST18	id.	ANT.LPR.4	0	0	0	80.
nd.	id.	ANT.L52.5	0	0	0	80
nd.	id.	ANT.L52B.6	0	0	0	320
nd.	id.	ANT.LG2.7	0	0	0	0
16ST19	*C.* cf. *scottianum*	ANT.L52B.5	nd.	nd.	nd.	nd.
16ST20[New]	*P.* cf. *involvens*	ANT.LG2.8	nd.	nd.	nd.	nd.
16ST21[New]	*Chondrocystis* sp.	ANT.L59B.1	nd.	nd.	nd.	nd.

Abbreviation: id., idem; nd., not determined

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

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