Ultrastructural and genetic characteristics of endolithic cyanobacterial biofilms colonizing Antarctic granite rocks

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Abstract

The precise identification of the cyanobacteria that comprise an endolithic biofilm is hindered by difficulties in culturing the organisms found in these biofilms and a lack of previous molecular and ultrastructural data. This study characterizes, both at the ultrastructural and molecular level, two different cyanobacterial biofilms found in fissures of granite from continental Antarctica. Electron microscopy revealed structural differences between the two biofilms. One was only loosely adhered to the substrate, while the other biofilm showed a closer association between cells and rock minerals and was tightly attached to the substrate. Cells from both biofilms where ultrastructurally distinct, displaying, for instance, clear differences in their sheaths. The amounts of EPS and their organization associated with the cyanobacteria may determine the differences in adhesion and effects on the lithic substrate observed in the biofilms. By sequencing part of the 16S rRNA gene, the two cyanobacteria were also genetically characterized. The gene sequence of the cells comprising the biofilm that was tightly attached to the lithic substrate showed most homology with that of an endolithic cyanobacterium from Switzerland (AY153458), and the cyanobacterial type loosely adhered to the rock, clustered with *Acaryochloris marina,* the only organism unequivocally known to contain chlorophyll *d*. This study reveals the presence of at least two different types of endolithic biofilm, dominated each by a single type of cyanobacterium, able to withstand the harsh conditions of the Antarctic climate.

Introduction

Since the first description of endolithic algae in dolomite rock (Diels, 1914), endolithic organisms are known to inhabit a variety of rock types, from hard granite (Schultz *et al*., 2000; De los Ríos *et al*., 2005) to porous rocks, such as limestone and sandstone (Friedmann, 1982; Tschermak-Woess & Friedmann, 1984; Gross *et al*., 1998; Danin, 1999; Matthes *et al*., 2001; Wierzchos & Ascaso, 2001), in numerous terrestrial ecosystems that vary tremendously in terms of depth, biomass, species richness, and species composition. The lack of light is a limiting factor for photosynthetic microorganisms. However, cyanobacteria have been detected in significant abundance in almost every endolithic ecosystem (Friedmann *et al*., 1988; Matthes-Sears *et al*., 1997; Hughes & Lawley, 2003; Büdel *et al*., 2004; Cockell *et al*., 2005), and their presence is especially relevant in desert environments (Wynn-Williams, 2000). For photosynthetic microorganisms to live in dark habitats, certain adjustments to the surroundings could be necessary, e.g., higher cellular pigment concentrations or the presence of certain biliproteins (Vincent, 1988; Samsonoff & MacColl, 2001).

The microbial colonization of rock fissures and cavities typically leads to the formation of endolithic biofilms. These films support life under a relatively broad range of environmental conditions, and are often the dominant or even the only biological components of extreme terrestrial environments, such as those of Antarctica (De los Ríos *et al*., 2003). The study of endolithic communities from extreme environments is gaining interest, given their role as carbon reservoirs across large areas.

Antarctica is a continent whose severe life conditions differ substantially from those of almost all other parts of the Earth's biosphere, but where different cyanobacterial types regularly appear (Komárek, 1999). Cyanobacterial life in the Antarctic desert is threatened by a scarcity of water and the enzymes of these cyanobacteria are known to be at their

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structural and physiological limits (Wynn-Williams, 2000). Yet, the resilience of cyanobacteria in the extremes of polar climate, including their ability to survive freezing and desiccation, makes this group, the single largest contributor to the biomass on the Antarctic continent (Vincent *et al.*, 1999).

Identifying endolithic cyanobacteria is problematic since they are typically difficult to culture *in vitro* and the morphology of laboratory cultures may not always represent their native form (Tschermak-Woess & Friedmann, 1984; Sigler *et al.*, 2003). Essential phylogenetic work remains to be performed on most of these organisms (Van Thielen & Garbary, 1999). Thus, most of the information available on the systematics of Antarctic endolithic cyanobacteria has emerged from laboratory cultures and/or identifications made only on the basis of morphology (Broady, 1981; Friedmann *et al.*, 1988; Siebert & Hirsch, 1988; Siebert *et al.*, 1996), a trait that generally does not correlate well with the phylogeny of cyanobacteria (Turner, 1997; Turner *et al.*, 2001). Culture independent molecular approaches to the study of endolithic communities are being consequently pursued, and these have provided interesting results in the last few years (Smith *et al.*, 2000; De la Torre *et al.*, 2003; Sigler *et al.*, 2003; McNamara *et al.*, 2006). For a more holistic picture of cyanobacterial diversity, combined molecular/ultrastructural studies are needed but such combined studies are still rare. In this study, we use both molecular and ultrastructural approaches to characterize two different types of cyanobacterial biofilms found in granite rocks collected in Antarctica.

Materials and methods

Materials

Pieces of granite rock taken from different orientations (three for each orientation) were split off granite boulders across a range of altitudes, from the coast to the summit of Discovery Bluff (5 m a.s.l., 100 m a.s.l. and 500 m a.s.l.), Ross Sea cost, Granite Harbour (77°00′-S, 162°34′-E). The granite could be easily broken along existing fissures. Endolithic colonization was detected in the field with a magnifying glass after breaking the rock surfaces. Fragments of granite, 0.5–1 cm thick, with visible signs of endolithic microbial growth on their underside were placed in sterile polyethylene bags, transported by air to our laboratory and stored dry at −20 °C until processing for microscopy or molecular analysis.

Transmission electron microscopy

Endolithic cyanobacterial biofilms from the different samples collected were removed from the rock under the stereomicroscope using a sterile needle, and embedded in 2% (w/v) agar. The small pieces of agar were then processed according to the procedure described by De los Ríos & Ascaso (2002). In brief, these fragments of agar containing cyanobacteria were fixed in glutaraldehyde and then in osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in Spurr's resin. Ultrathin sections were poststained with lead citrate (Reynolds, 1963) and observed in a Zeiss EM910 transmission electron microscope.

Scanning electron microscopy with backscattered electron imaging and energy dispersive spectroscopy (SEM-BSE)

Rock fragments with cyanobacterial biofilms on the underside were prepared according to a procedure developed for observing the rock-microorganism interface by SEM-BSE (Wierzchos & Ascaso, 1994). The pieces of rock were fixed in glutaraldehyde and osmium tetroxide solutions, dehydrated in a graded ethanol series and embedded in LR-White resin. Blocks of resin-embedded rock samples were finely polished, carbon coated and observed using a DMS 940 SEM microscope. Microprobe analyses were performed using an energy dispersive spectroscopy (EDS) Link ISIS microanalytical system during SEM observation.

Low temperature scanning electron microscopy (LTSEM)

The cyanobacterial biofilms were also examined by LTSEM. Small fragments were mechanically fixed onto the specimen holder of a cryotransfer system (Oxford CT1500), plunged into subcooled liquid nitrogen, and then transferred to the microscope's preparation unit via an air-lock transfer device. The frozen specimens were cryofractured and etched for 2 min at −90 °C. After ice sublimation, the etched surfaces were gold sputter coated and the specimens then placed on the cold stage of the SEM chamber. Fractured surfaces were observed under a DSM960 Zeiss SEM microscope at −135 °C.

DNA extraction, amplification and sequencing

Total DNA was extracted from the cyanobacteria biofilms previously examined by microscopy according to a modified CTAB method (Cubero *et al.*, 1999). DNA extracts were used for PCR-amplification of the 16S rRNA gene sequence using the primers CYA359F and CYA781R, as described by Nübel *et al.* (1997). Fifty microliters of the PCR mix (10 mM Tris pH 8.3/50 mM KCl 1.5 mM-1 $MgCl_2$/50 μg gelatine) contained 1.25 U of Taq polymerase, 0.2 mM of each of the four dNTPs, 0.5 μM of each primer and *ca* 10–50 ng genomic DNA. Annealing conditions were 55 °C. Products were cleaned on a QIAGEN quick spin column (Qiagen). Both complementary strands were sequenced using the Dye

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Terminator Cycle Sequencing Ready Reaction Kit (ABI, Vienna) according to the manufacturer's instructions. Sequences were run on an ABI310 automated sequencer (ABI). The sequences were submitted to EMBL/GenBank.

Phylogenetic analysis

DNA sequence alignments were phylogenetically analysed using MrBayes version 3 (http://morphbank.ebc.uu.se/mrbayes). For the Bayesian analysis, the general time reversible model was used, including site-specific rate heterogeneity and a fraction of invariant characters (GTR+I+G). This model was suggested both by hierarchical likelihood rate tests and the Akaike information criterion as implemented in Modeltest (Posada & Crandall, 1998). Two million generations were run, every 100th tree was sampled, and the first 1000 generations were discarded as burn-in. A consensus phylogram showing mean branch lengths was calculated using the sumt command in MrBayes. We used *Escherichia coli* as outgroup for the phylogenetic tree.

Results

Ultrastructural characterization of the endolithic biofilms

SEM-BSE examination of the different samples revealed the presence of two different endolithic biofilms harbouring cyanobacteria in nine Antarctic granite fragments collected at 500 m a.s.l. from southeast, horizontal and vertical orientations. The biofilms were adhered to the underside of rock fragments 0.5–1 cm-thick, split-off from granite boulders. The cyanobacterial biofilms were obtained from rock specimens where lichen growth was not visible. One of the biofilms was only loosely adhered to the substrate (Fig. 1a); the other was tightly attached to the substrate and showed a closer association with the rock minerals (Fig. 1b). The loosely adhered biofilm was observed in samples collected from horizontal and southeast orientations. The tightly attached biofilm was observed, in pieces of rock collected from southeast and vertical orientations. Our LTSEM analysis also revealed structural differences between these two types of biofilm. Thus, the loosely adhered biofilm could be observed as a layer composed of cell clusters covering the surface examined (arrows in Fig. 1c) that was attached to the substrate by only a thin film of extracellular polymeric substances (EPS). In contrast, the tightly attached biofilm appeared as a dense matrix in which cells, EPS and mineral fragments were intermixed (Fig. 1d). Cells (arrows in Fig. 1d) and mineral fragments (arrow-heads in Fig. 1d) were completely embedded in the EPS network (asterisks in Fig. 1d).

TEM analysis of the cells comprising the loosely adhered biofilm revealed the presence of only one type of cyanobacterium (Fig. 2a). These cells were spherical to oblong and measured 1.1–1.3 × 0.85–0.95 μm (Table 1). The cytoplasm

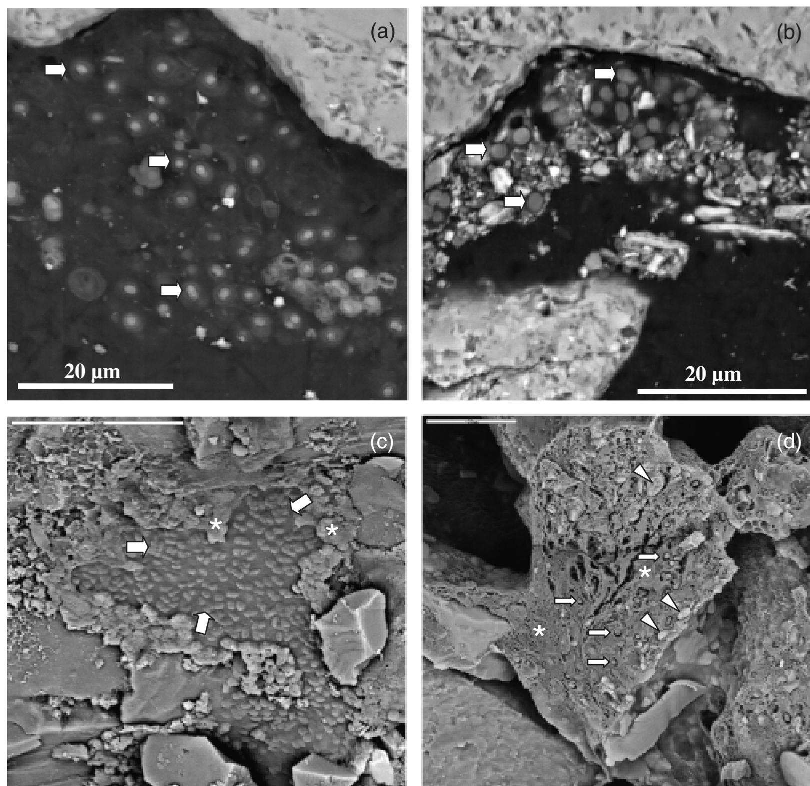

Fig. 1. (a–d) SEM-BSE images of a loosely adhered to the substrate cyanobacteria-rich biofilm found on the underside of granite fragments collected from horizontal orientation (a) and of a tightly attached to the substrate collected from southeast orientation (b) (c,d): LTSEM images of the loosely adhered (c) and tightly attached (d) biofilms. Arrows indicate the cells, head-arrows the mineral fragments and asterisks the EPS network.

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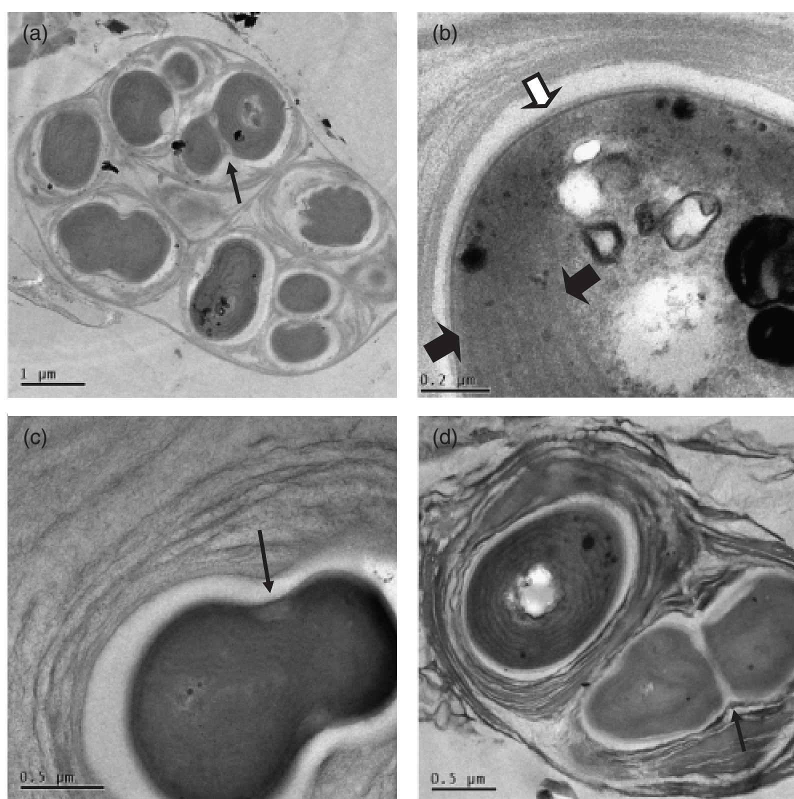

Fig. 2. (a–d) TEM images of the cyanobacteria cells composing the loosely adhered biofilm, (a) Group of dividing cells within a polymeric matrix; arrow indicates the septum formed in cell division, (b) Detailed image of a cell wall (white arrow), and thylakoids (black arrows), (c, d) dividing cells show invagination of the cytoplasmic membrane (c) and connect after the division process (arrow in d).

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Table 1. Features of the dominant cyanobacteria in the biofilms that were either closely related to *Acaryochloris* species (loosely attached biofilm) or to *Gloeocapsa*-like cells (firmly attached biofilm)

	Cell shape	Thylakoids	Binary fission	Cell division	Sheaths	Cell size
Loosely attached biofilm	Spherical to oblong	Parallel parietal thylakoids	Pinching-cleavage	In more than one plane	Thin multilayered	$1.1–1.3 \times 0.85–0.95$
Firmly attached biofilm	Oval	Curved parietal thylakoids	Pinching	In three planes	Thick, dense multilayered	$1–1.5 \times 0.75–1.25$

was enveloped by a cell wall containing an electron-dense layer (*c.* 10 nm), generally attributed to peptidoglycans, and an outer membrane of similar thickness (white arrow in Fig. 2b). Narrow electron-transparent spaces separated the peptidoglycan cell wall layer from the outer and cytoplasmic membranes. Each cell exhibited a mucilaginous sheath of concentric electron-dense layers surrounded by older sheath material (Fig. 2a). Thylakoids showed a parietal arrangement and seven to eight of their layers filled the cell periphery (black arrows in Fig. 2b). Cell division starts with invagination of the cytoplasmic membrane and local thickening of the peptidoglycan layer (arrow in Fig. 2c). A septum forms, followed by the constriction of the outer membrane (arrow in Fig. 2a). After division, the cells remained connected (Fig. 2d). No carboxysomes were observed.

The tightly attached biofilm appeared on the lower surface of the rock fragments (Fig. 3a) but also grew between mica layers (arrow in Fig. 3b), always closely associated with the rock's mineral components. In this last circumstance, exfoliation and separation of mica layers was observed (Fig. 3b). The cyanobacterial cells were covered by a matrix of microdivided mineral fragments detached from the lithic substrate and iron-rich mineral fragments (arrows in Fig. 3c). These biofilms were composed of multicellular cyanobacterial aggregates enveloped by a common layered gelatinous sheath but each cell also had its own layered spheroidal concentric sheath (Fig. 3d). These aggregates were composed of only one type of cyanobacterial cell, which were oval and $1–1.5 \times 0.75–1.25\ \mu m$ in size (Table 1). The main components of the sheath were highly electron-dense fibres, running parallel to the cell surface (Fig. 3e). Five curved parietally-arranged thylakoids were observed in these cells (white arrows in Fig. 3f). Large electron-dense globules appeared close to the nucleoplasm and in the

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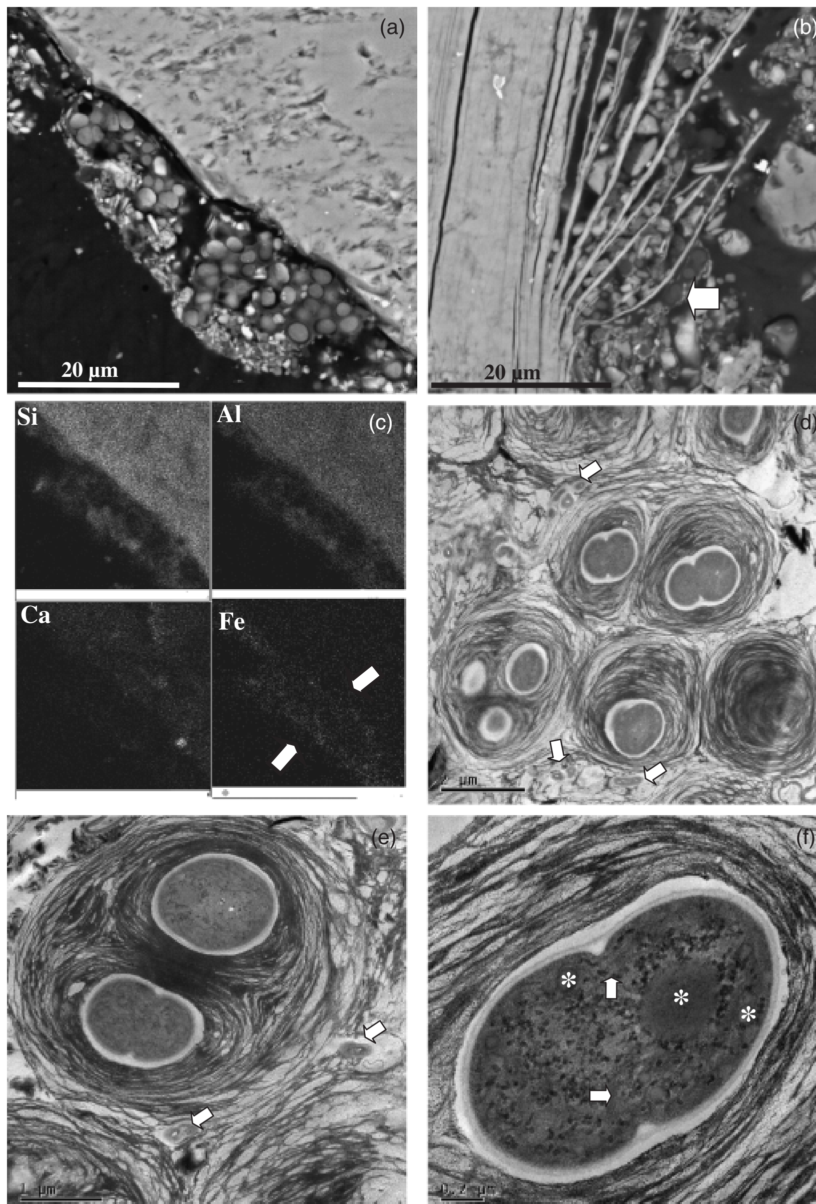

Fig. 3. (a–d) SEM-BSE images of tightly attached cyanobacteria-rich biofilms obtained from the lower part of a southeast facing rock (a) or from between mica layers (arrow in b). Figure 3c: EDS element distribution maps for a zone colonized by *Gloeocapsa*-like cells corresponding to Fig. 3a. White arrows indicate the accumulation of iron-rich minerals associated with cyanobacterial cells. (d–f): TEM images of *Gloeocapsa*-like cells comprising the tightly attached biofilm; arrows indicate presence of heterotrophic bacteria (d–e), (d) Group of cells enveloped by a dense polymeric matrix composed of concentric layers, (e) Details of the spheroidal concentric sheath around each cell and the network of fibres extending beyond the cells, (f) Cell showing thylakoids (arrows) and the presence of electro-dense globules in the cytoplasm (asterisks).

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spaces between thylakoids (white asterisks in Fig. 3f). The cells divide by binary fission in three planes. Based on these features, we identified this isolate as a *Gloeocapsa*-like strain. Closely associated with these cyanobacterial cells, it was common to find small bacteria fully embedded in the gelatinous cyanobacterial sheath (arrows in Fig. 3d and 3e).

Characterizing organisms by sequencing the 16S rRNA gene segment

DNA (coding for 16S rRNA gene) from genomic extracts of the cyanobacterial biofilms recovered from rock fragments (collected at 500 m a.s.l. from the three different orientations) was amplified to yield PCR products of *c.* 420 bp. Unfortunately, we could not obtain longer fragments using other primers (data not shown). Analysis of the sequences obtained from the samples collected at different orientations revealed two distinct cyanobacterial sequences. Each sequence corresponds to samples showing the same type of biofilm.

Phylogenetic analysis based on partial 16S rRNA gene sequences was performed to identify the taxonomic position of the Antarctic endolithic cyanobacteria (Fig. 4). Our tree indicated that both Antarctic cyanobacterial sequences clustered in two different groups. The sequence of the cells comprising the biofilm that was tightly attached to the lithic substrate corresponded to a group formed by two sequences

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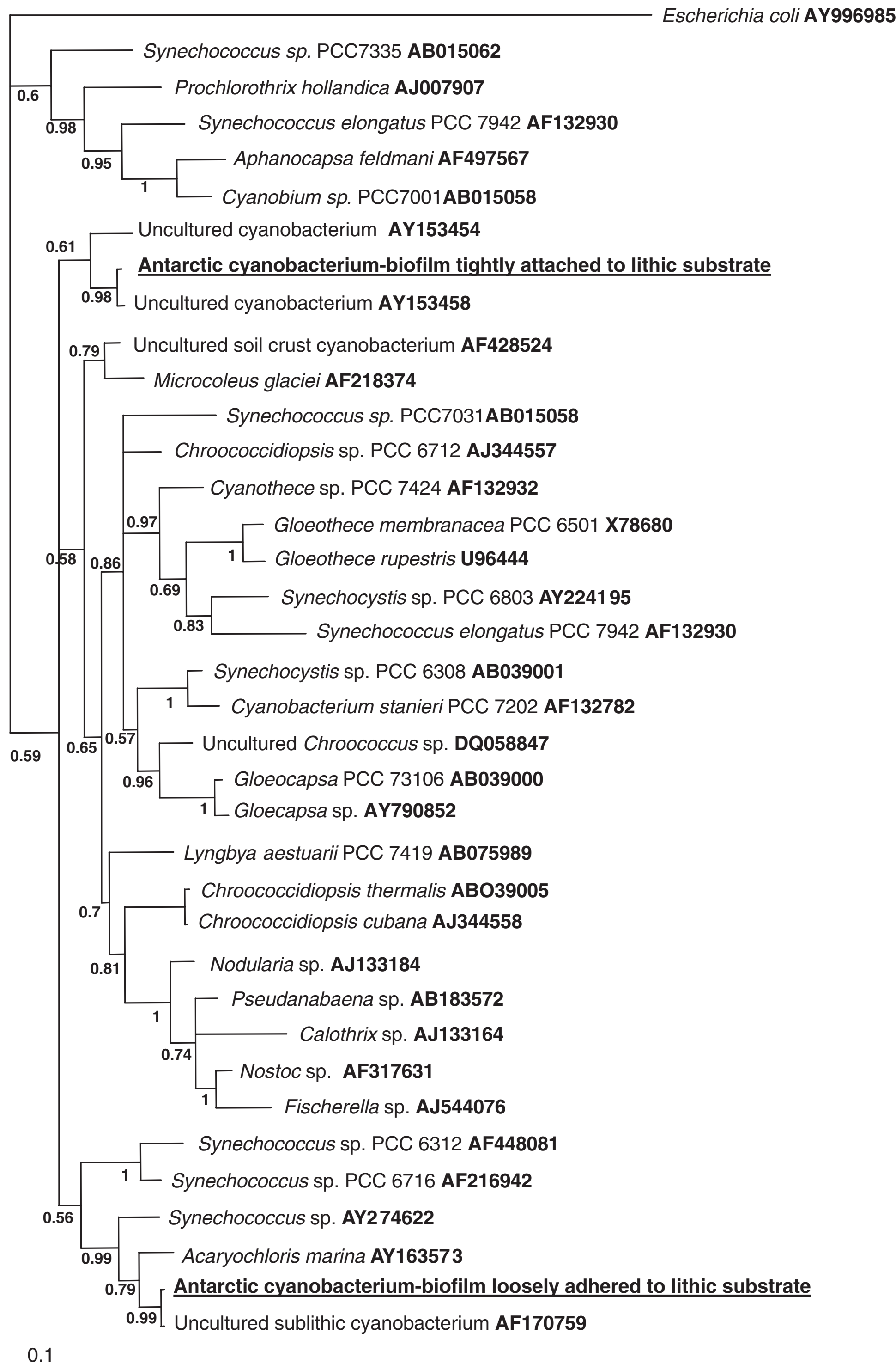

Fig. 4. Consensus Bayesian phylogram for the analysis of 390 nucleotides showing relationships between the cyanobacterial 16S rRNA gene sequences of our Antarctic isolates and those of known cyanobacteria retrieved from GenBank (including several reference strains according to Bergey's *Manual of Systematic Bacteriology*, 2001).

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from isolates of endolithic communities inhabiting exposed dolomites (accession nos. AY153454 and AY153458; Sigler *et al.*, 2003). The cyanobacterial type loosely adhered to the rock clustered with the sequence of a sublithic Antarctic cyanobacterium (accession no. AF170759), an *Acaryochloris marina* sequence (accession no. AY163573) and sequences of different *Synechococcus* species (accession nos. AF448081, AF216942 and AY274622). The likelihood parameters in the tree sample showed the following average values and variances (in brackets): rate matrix $r(GT) = 1.000$ (± 0), $r(CT) = 4.38$ ($\pm 0,88$), $r(CG) = 0.5$ (± 0.02), $r(AT) = 1.03$ (± 0.07), $r(AG) = 2.04$ ($\pm 0,2$), $r(AC) = 1.32$ (± 0.13), base frequencies $\pi(A) = 0.26$ (0.00), $\pi(C) = 0.2$ (0.00), $\pi(G) = 0.31$ (0.00), $\pi(T) = 0.22$ (0.00), gamma shape parameter $\alpha = 0.55$ (0.00), and the proportion of invariable sites p(invar) = 0.32 (0.00). Since our phylogenetic analysis based on partial 16S rRNA gene sequences revealed that one of the Antarctic cyanobacterial sequences grouped with *Acaryochloris marina*, we considered our cyanobacterial strain comprising the biofilm that was loosely adhered to the rock could be closely related to the species *Acaryochloris*. On the other hand, this strain sequence also showed 100% identity with a published sequence of a microorganism from sublithic communities in Antarctic quartz (accession no. AF170759, Smith *et al.*, 2000). Although only 165 nucleotides of this published sequence could be compared, the high similarity indicates the presence of this cyanobacterium or a closely related strain also in sublithic Antarctic environments. The authors showed that within the cyanobacteria, this isolate clustered with *Lyngbia* sp. PCC 7419 as an isolated lineage (accession no. AJ000714; Smith *et al.*, 2000).

The present cyanobacterial sequence corresponding to the cells comprising the biofilm that was tightly attached to the lithic substrate showed highest identity (98%) with an endolithic cyanobacterium that inhabits dolomite rocks in central Switzerland (accession no. AY153458). These authors found a *Chroococcidiopsis* sp. sequence as the closest identified relative (91% identity; Sigler *et al.*, 2003), but their study lacked morphological characterization. In our phylogenetic analysis, the sequences corresponding to *Chroococcidiopsis* species appeared in different positions in the tree.

Discussion

Two structurally different cyanobacterial-rich biofilms, each dominated by only one type of ultrastructurally distinct cyanobacterium, were found to colonize inner zones of the granite rocks examined. Sequencing part of the 16S rRNA gene confirmed our ultrastructural observations and revealed the presence of two different species – one dominant in each biofilm. These biofilms were able to live under the conditions of climate and light intensity reaching the granite rock fissures in this Antarctic region.

The dominating cyanobacterium in the loosely attached biofilm, shown to be genetically closely related to *Acaryochloris marina*, shared numerous ultrastructural features with those observed in cultures of this species (Marquardt *et al.*, 2000), although the cells were smaller than those of *A. marina*. It is, nevertheless, known that phenotypic characters evident in nature are often not equally manifested in culture (Tschermak-Woess & Friedmann, 1984). The natural environmental conditions of Antarctica could limit the growing capacity of these cells. The presence of an external envelope of concentric layers could also be related to the special conditions of Antarctic growth. Thick envelopes have frequently been described for cyanobacteria from severe environments (Grilli-Caiola *et al.*, 1993; De los Ríos *et al.*, 2004). The cell division process observed in our isolates was similar to that described for *Acaryochloris marina* by Marquardt *et al.* (2000) and is considered intermediate between the constrictive and the septum type (Drews & Weckesser, 1982). The features observed in our *Gloeocapsa*-like cells resemble those attributed to *Gloeocapsa* species (Komárek, 2003). However, the gene sequence of our Antarctic *Gloeocapsa*-like isolates did not cluster with sequences from Gen-Bank attributed to *Gloeocapsa* species. Anyway, extensive taxonomic revision of several *Gloeocapsa* species is pending (Komárek, 2003). The phylogenetic proximity of the partial 16S rRNA gene sequences of the present two Antarctic cyanobacteria to Swiss endolithic isolates could mean that endolithic microbiota from different locations could be fairly similar such as, for instance, the closely related *Chroococcidiopsis* inhabiting the deserts of Antarctica and Israel (Fewer *et al.*, 2002).

Gloeocapsa species have been also described in other continental Antarctic areas (Friedmann *et al.*, 1988; Broady, 1996; Banerjee *et al.*, 2000; de la Torre *et al.*, 2003). However, *Acaryochloris*-like cells have not been previously cited for Antarctic environments, nor endolithic habitats. *Acaryochloris marina* contains high Chl *d* levels rather than the Chl *a* present in other cyanobacteria (Miyashita *et al.*, 1996; Chen *et al.*, 2005). Cyanobacteria containing chlorophyll *d* may be fairly widespread, although little is known about their habitats and ecology (Kühl *et al.*, 2005). *Acaryochloris marina* has been reported in marine environments (Miyashita *et al.*, 1996; Murakami *et al.*, 2005) and in an epilithic microbial mat from a moderately hypersaline lake (Miller *et al.*, 2005). The organization as biofilms of *Acaryochloris*-like cyanobacteria colonizing the underside of didemid ascidians and their existence as free-living microorganisms have been also recently reported (Kühl *et al.*, 2005; Miller *et al.*, 2005). The presence of chlorophyll *d* would be a useful feature for thriving in habitats with little visible light (Samsonoff & MacColl, 2001) such as granite fissures but further analytical studies designed to detect Chl *d* would be needed to test this new style of adaptation of endolithic microorganism.

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EPS contribute significantly to the structure of these biofilms and could be involved in interactions between these cyanobacteria and the lithic substrate (Barker & Banfield, 1996). Although cells of both species bore a thick layered EPS sheath, greater amounts of EPS were observed in the biofilms containing *Gloeocapsa*-like cells. The extracellular polymeric substances not only occurred around each cell, but formed dense networks around groups of cells including other microorganisms such as heterotrophic bacteria. The differences in the amount and organization of the EPS observed could explain both why the biofilms composed of *Gloeocapsa*-like cells were more closely associated with the lithic substrate and why these cells interacted more intensely with the mineral components than the other type of biofilm. In effect, while interactions with the lithic substrate were not obvious in the loosely adhered biofilm, exfoliation and separation of mica sheaths and a build up of Fe-rich minerals in the biofilms, have been related to the presence of *Gloecapsa*-like cells. The presence of cyanobacterial biofilms in crevices generates two cleavage forces inside the rock causing biogeophysical damage in which EPS could be involved. EPS matrices imbibe water, causing rocks to crack (Ascaso *et al*., 2002). The second cleavage-inducing force is linked to the ability of EPS to trap particles facilitating the accumulation of sediments in the proximity of the microorganisms (Wolfaardt *et al*., 1999; Gorbushina & Krumbein, 2000). This second action could be the predominant effect observed for the *Gloeocapsa*-like cells. Biogeochemical weathering has been also attributed to endolithic cyanobacterial colonies colonizing different rock types (Ascaso *et al*., 2002; Büdel *et al*., 2004) and cannot therefore be ruled out. EPS have been proposed to significantly contribute to mineral bioweathering by participating in certain biomobilization and biomineralization processes (Barker & Banfield, 1996; Barker *et al*., 1998; De los Ríos *et al*., 2005).

The presence and distribution of photosynthetic microorganisms in these granite rocks could be the result of interplay between the incoming light intensity and its attenuation by the rock substrate, the influence of local features of rock composition of being special relevance (Matthes *et al*., 2001; Sigler *et al*., 2003). The cyanobacteria analysed in this study could be considered chasmoendolithic (Golubic *et al*., 1981), because they occurred in thin flakes where fissures developed more or less parallel to the rock surface. In this ecological niche, cyanobacteria might prefer to occupy deeper positions in the rock, since fissures increase the amount of light available. The endolithic colonization of the granite by these Antarctic cyanobacteria could have important ecological implications not only for the survival of these cells, but also for other microorganisms, in that increasing the size of the colonized fissure could facilitate colonization by other cryptoendolithic and chasmoendolithic microorganisms (Blackhurst *et al*., 2005). In effect, heterotrophic bacteria associated with both types of biofilms were observed.

Endolithic cyanobacteria-rich biofilms have also been observed in other types of continental Antarctic rocks such as sandstones (Friedmann & Ocampo, 1976; Vincent, 1988; De la Torre *et al*., 2003; De los Ríos *et al*., 2004). These films consist predominantly of *Gloeocapsa*-like and *Chroococcidiopsis*-like cyanobacteria. Eucaryotic microorganisms also form diverse biofilms in fissures and cavities of continental Antarctic rock, especially lichen dominated biofilms (Friedmann *et al*., 1988; Green *et al*., 1999; De los Ríos *et al*., 2005) but also a large number of free-living fungi such as meristematic fungi with melanised cell walls (Selbmann *et al*., 2005) and nonlichenized green-algae (Tschermak-Woess & Friedmann, 1984; Friedmann *et al*., 1988; Broady, 1996). However, biofilms dominated by eucaryotic microorganisms seem to occupy different sites within rock fissures to those preferred by cyanobacteria-rich biofilms (De la Torre *et al*., 2003; De los Ríos *et al*., 2004; Selbmann *et al*., 2005; Tschermak-Woess *et al*., 2006). Moreover, lichen-dominated and cyanobacteria-rich biofilms were both present in the granite rocks of the area examined here, although the cyanobacteria-rich biofilms were recovered from samples collected at an altitude of 500 m, whereas endolithic lichen colonization was more frequent at lower altitudes (De los Ríos *et al*., 2005). This presumably reflects their different requirements for growth. It was shown in this study that the properties and features of a biofilm depend not only on the physico-chemical properties but also on the nature of its biological components. The two cyanobacteria-rich biofilms examined here composed of different cyanobacteria species differed from each other and also differed clearly from eucaryotic biofilms colonizing granite. The endolithic environment always appears to be less altered when comparing cyanobacteria-rich biofilms to, for example, biofilms dominated by fungi (De los Ríos *et al*., 2005).

In conclusion, our study reveals an undescribed type of biofilm composed of cyanobacteria closely related to *Acaryochloris* species, and compares its ultrastructural and structural features to those of the already known biofilm composed of *Gloeocapsa*-like cells. To date, it is unclear if these two types are exclusive or if other types of cyanobacterial biofilms occur. However, since Antarctica is currently subjected to the intense effects of rapid climate change and increased solar UVB radiation, further knowledge about its present biodiversity and functional roles is needed (Vincent, 2000; Wall, 2005) to assess the qualitative and quantitative biological manifestations of this changing Antarctic ecosystem.

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