

Biogeography of terrestrial cyanobacteria from Antarctic ice-free areas

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ABSTRACT. Cyanobacteria inhabit the Antarctic continent and have even been observed in the most southerly ice-free areas of Antarctica (86–87°S). The highest molecular diversity of cyanobacterial communities was found in the areas located between 70°S and 80°S. Further south and further north from this zone, the diversity abruptly decreased. Seventy-nine per cent (33 of 42 operational taxonomic units) of Antarctic terrestrial cyanobacteria have a cosmopolitan distribution. Analysis of the sampling efforts shows that only three regions (southern Victoria Land, the Sør Rondane Mountains and Alexander Island) have been particularly well studied, while other areas did not receive enough attention. Although cyanobacteria possess a capacity for long-range transport, regional populations in Antarctic ice-free areas seem to exist. The cyanobacterial communities of the three most intensively studied regions, separated from each other by a distance of 3000–3400 km, had a low degree of similarity with each other. Further development of microbial biogeography demands a standardized approach. For this purpose, as a minimal standard, we suggest using the sequence of cyanobacterial 16S rRNA gene between *Escherichia coli* positions 405 and 780.

INTRODUCTION

Ice-free areas of Antarctica cover a tiny proportion (0.34%) of the continent. They provide one of the most remote and harsh environments for terrestrial life. Antarctica is the only continent that is dominated by microbial (cyanobacteria and algae) and lower plant (predominantly mosses and lichens) communities. Only two vascular plants are known from the Antarctic, both restricted to coastal regions of the Antarctic Peninsula (Convey, 2006).

Cyanobacteria are photosynthetic bacteria that require solar light, liquid water, air and some mineral nutrients for growth. They serve as primary colonizers of soils newly exposed by glacial retreat. Some taxa can fix atmospheric nitrogen, which can locally enrich the predominantly oligotrophic biotopes. Cyanobacteria are widespread in all geographical zones of Antarctica, where they can form macroscopically visible crusts or thin biofilms on the surface of soils and rocks, or occupy endolithic niches (Friedmann, 1982).

Microbial biogeography is currently at the center of a scientific debate. Some authors propose that local geographical populations of microorganisms exist, as is the case of the biogeography of higher organisms. Others propose that bacteria have such enormous dispersal capabilities, due to their small size and large populations, that only different environmental conditions permit the development of distinctive microbial populations (Martiny and others, 2006).

Attempts to describe the biogeography of Antarctic cyanobacteria pose additional questions. Firstly, what are the means of transportation of cyanobacteria to and from Antarctica over the Southern Ocean? Secondly, do regional populations of cyanobacteria exist in Antarctica? Ice-free areas are separated from each other. However, the existence of a long-range cyanobacterial transport to Antarctica also implies their potential ability for intra-continental transport and mixing of different Antarctic populations. Thirdly, what is the history of species endemic to Antarctica? Endemism at

species, genus and even family level is observed in Antarctic biota and it seems unlikely that such levels of endemism could have evolved after the last glaciation ~18 000 years ago (Taton and others, 2006a; Convey and others, 2008). Indeed, studies have proposed the existence of relict taxa that have evolved before glacial maxima (Lawley and others, 2004; De Wever and others, 2009). Identification of the refugia of these taxa is important for understanding Antarctic biogeography (Convey and others, 2008).

The comparison of cyanobacterial sequences found in different geographical areas allows us to estimate their degree of similarity and draw conclusions about their distribution. In this paper, we gathered all available information on the diversity of Antarctic terrestrial cyanobacteria into a single database in order to identify whether any broad-scale patterns in biogeography exist.

Terrestrial habitats were selected according to Broady (1996) and include lithophytic ('on or within rock substrata' including epilithic, endolithic, chasmoendolithic, crypto-endolithic and hypolithic), edaphic ('on the surface of and within soils') and epiphytic ('on the living surfaces of mosses and liverworts'), but we exclude the cryophilic ('between ice crystals in surface layers of melting snow') communities. In addition, limnetic (occurring in waters of lakes), supralittoral (occurring on the coastline) and fluvial (associated with streams and rivers) cyanobacteria were not taken into account. Historically, two approaches (floristic and molecular) were used to identify the Antarctic taxa, and they are treated separately below.

Floristic studies

Before the introduction of molecular techniques, the Antarctic continent was intensively studied by traditional methods, which included microscopic observations of the field samples, isolations of cyanobacteria in cultures and identification based on morphological characteristics. The morphological approach has clear weaknesses compared

with the molecular approach. Morphological features do not necessarily reflect the real genetic and physiological divergences, which can only be revealed using molecular data (Nadeau and others, 2001).

Cyanobacteria occurred in nearly all ice-free localities so far investigated (Broady and Weinstein, 1998). The Antarctic terrestrial microflora was dominated by cyanobacteria and chlorophytes (green algae), with xanthophytes (yellow-green algae) and bacillariophytes (diatoms) as frequent associates. Cyanobacteria and algae were more significant in the cold and arid coastal and slope provinces of continental Antarctica. In contrast, bryophytes and lichens dominated the vegetation in the milder and moister maritime Antarctica (Broady, 1996).

The most isolated and climatically extreme terrestrial habitats are found in inland mountain ranges and nunataks. No cyanobacteria were detected in the most southerly outcrop, Mount Howe (87°21' S, 149°18' W), which is located ~330 km from the South Pole (Cameron and others, 1971). In the La Gorce Mountains (86°30' S, 147° W), located 90 km north of Mount Howe at ~1750 m a.s.l., Cameron (1972) found two morphotypes of cyanobacteria: *Schizothrix calcicola* (Ag.) Gom and *Porphyrosyphon notarisii* (Menegh.). In the same location, five morphotypes of cyanobacteria were observed by Broady and Weinstein (1998). These include: *Gloeocapsa* cf. *kuetzingiana*, *Gloeocapsa* cf. *ralfsiana*, cf. *Aphanocapsa* cf., *Phormidium autumnale* and *Ammatoidea normanni* (syn. *Hammatoida normanni*). The authors noted that in full sun, soils could reach high surface temperatures (up to 12°C when ambient air was -7.5°C) and were moist due to ice melting. In cultures isolated from the La Gorce Mountains, the growth started after 7 weeks at 8°C and all isolates grew at 15°C (Broady and Weinstein, 1998).

Compared with these most southerly ice-free areas, the nunataks closer to the coast, in Dronning Maud Land and on the Edward VII Peninsula, support a richer flora. The diversity increases even more at coastal locations such as the Vestfold Hills. Broady and Weinstein (1998) concluded that the 'abundance and diversity of organisms decreases with progression south from the Maritime to the Continental Antarctic Zone, and within the latter with increasing altitude and latitude from the Coast to the Ice Slope Region due to increasingly severe conditions and isolation from rich propagule sources'.

Molecular methods

We included in our database all cyanobacterial sequences recorded from terrestrial habitats in the sub-Antarctic islands, maritime and continental Antarctica (Convey, 2006). The cyanobacterial 16S rRNA gene sequences were downloaded from the GenBank-NIH (US National Institutes of Health) database (<http://www.ncbi.nlm.nih.gov/genbank/>). In addition, a database of literature records was constructed to find cyanobacterial sequences deposited in databases as 'uncultured bacterium', but not marked as cyanobacterial. In total, 274 sequences were collected and analysed by similarity search using BLAST software. The sequences of chloroplasts of eukaryotic phototrophs misinterpreted as cyanobacterial sequences were excluded from the database. Chimera detection was performed using Pintail software (Ashelford and others, 2005). The sequences were included in the database of the ARB software package (Ludwig and others, 2004) and aligned with the cyanobacterial sequences

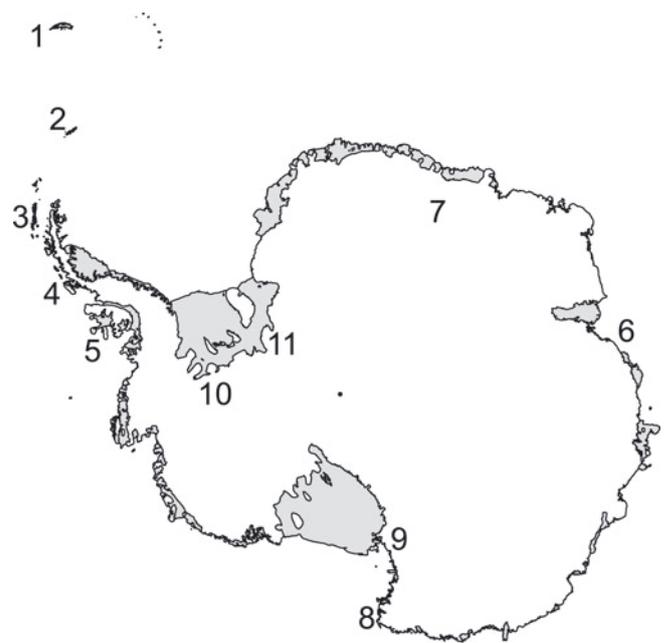


Fig. 1. Map of Antarctica showing localities where terrestrial molecular studies were performed: 1. South Georgia Island, 2. Signy Island, 3. King George Island, 4. Anchorage Island, 5. Alexander Island, 6. Vestfold Hills, 7. Sør Rondane Mountains, 8. northern Victoria Land, 9. southern Victoria Land, 10. Ellsworth Mountains, 11. Dufek Massif.

available from GenBank. The sequences in alignment were sorted in clusters according to the highly variable region corresponding to *E. coli* positions 463–468, and the resulting alignment was manually verified (Taton and others, 2003). The partial sequences that did not overlap with the *E. coli* positions 405–780 (V3 and V4 regions) or had <75% overlap with this region were excluded from the analysis. This step excluded some sequences from the Vestfold Hills (Princess Elizabeth Land) (Smith and others, 2000) and part of the sequences from southern Victoria Land (Wood and others, 2008). However, this did not change the final conclusions. The remaining 173 sequences were grouped into 42 operational taxonomic units (OTUs) with DOTUR software using the average neighbour method (Schloss and Handelsman, 2005; Taton and others, 2006a). The OTUs were defined as groups of sequences that exhibit >97.5% similarity with each other, using the *E. coli* positions 405–780. Each OTU might correspond to more than one species, following the bacteriological standards (Stackebrandt and Goebel, 1994).

The OTUs that contained sequences from Antarctica and temperate environments from other continents were considered 'cosmopolitan'. The OTUs with sequences from Antarctica and 'cold' environments (Arctic, glaciers and deglaciated zones at low latitudes (Andes, Tibet, etc.)) were considered 'cosmopolitan-cold'. The OTUs with sequences from different regions of Antarctica, but limited to this continent, were considered 'Antarctic'. The Antarctic OTUs with sequences found only in one region were considered 'Antarctic-regional'. OTU records were classified into areas in two ways: firstly by latitudinal zone and secondly into predefined areas based on the EBA Bioregions version 1.0 maps produced by the Australian Government Antarctic Division (Fig. 1). The OTU numbers were calculated for defined bioregions and latitudinal zones. Abundance information was not used because of the non-quantitative

Table 1. Locations and the number of cyanobacterial OTUs at the study sites

No.*	Location	Lat.	Long.	Number of OTUs	Source
	<i>Sub-Antarctic Islands</i>				
1	South Georgia Island	54°15' S	36°30' W	0	Yergeau and others (2007)
	<i>Maritime Antarctica</i>				
2	South Orkney Islands, Signy Island	60°43' S	45°38' W	0	Yergeau and others (2007)
3	South Shetlands Archipelago, King George Island, Admiralty Bay	62°04' S	58°21' W	0	Chong and others (2009, 2010) Teixeira and others (2010)
4	Anchorage Island	67°34' S	68°08' W	1	Yergeau and others (2007)
5	Alexander Island			9	C.W. Chong (GenBank)
	Fossil Bluff	71°19' S	68°18' W	0	Yergeau and others (2007)
	Mars Oasis	71°53' S	68°15' W	8	Yergeau and others (2007)
	Coal Nunatak	72°03' S	68°31' W	0	Yergeau and others (2007)
	<i>Continental Antarctica</i>				
6	Princess Elizabeth Land, Vestfold Hills	68° S	78° E	0	Smith and others (2000)
7	East Dronning Maud Land, Sør Rondane Mountains	72° S	23° E	16	R. Fernandez-Cazaro and others (unpublished information)
8	Northern Victoria Land, Admiralty Range, Luther Vale	72°22' S	169°53' E	2	Niederberger and others (2008)
9	Southern Victoria Land, Battleship Promontory	76°55' S	160°55' E	1	De la Torre and others (2003)
	Granite Harbour	77°00' S	162°34' E	1	De los Rios and others (2007)
	Marble Point	77°25' S	163°41' E	1	Aislabie and others (2006)
	McKelvey Valley	77°26' S	161°33' E	7	Pointing and others (2009)
	Ross Island, Mount Erebus	77°31' S	167°06' E	1	Soo and others (2009)
	Beacon Valley	77°48' S	160°48' E	0	Wood and others (2008)
	Bratina Island and Miers Valley	78° S	165° E	0	Smith and others (2006)
	Miers Valley	78°06' S	164°00' E	3	Wood and others (2008)
10	Ellsworth Land, Ellsworth Mountains, Sentinel Range	78°26' S	85°60' W	2	Yergeau and others (2007)
11	Pensacola Mountains, Dufek Massif	82°27' S	51°16' W	4	Hodgson and others (2010)

*See Figure 1.

nature of sampling at all locations. Only 'presence' data were used in the analyses.

RESULTS

Geographic coverage

The spread of localities where cyanobacterial sequences were recorded (Fig. 1; Table 1) indicates a large geographic coverage. The latitudinal range extends from the South Orkney Islands (Signy Island, 60° S, 45° W) to the Dufek Massif (82° S, 51° W) located in the Pensacola Mountains (part of the Transantarctic Mountain Range). Around the periphery of the continent, observations have been made at widely spaced localities (Table 1). Moreover, the sampling intensity varied widely in different geographical areas. Only three regions, southern Victoria Land, the Sør Rondane Mountains and Alexander Island, have detailed data on cyanobacteria, while the other localities have much sparser datasets.

Latitudinal gradient

Only one study of Antarctic terrestrial communities along a latitudinal gradient that used a uniform molecular methodology exists (Yergeau and others, 2007). The transect ranged from the Falkland Islands (51° S, cool temperate zone) through South Georgia (54° S, sub-Antarctic), Signy (60° S, maritime Antarctica), Anchorage (67° S) and Alexander Island (71–72° S) to the Ellsworth Mountains (78° S, continental Antarctica). The study showed that, with the exception of Mars Oasis (Alexander Island), the number of

bacterial OTUs decreases with increasing latitude. However, within the transect, cyanobacterial sequences were found in significantly higher proportions at the most southerly locations: Alexander Island and the Ellsworth Mountains (Yergeau and others, 2007).

We have plotted the number of OTUs of Antarctic terrestrial cyanobacterial communities on the latitudinal scale. Our results were in line with the data of Yergeau and others (2007) and showed a sharp increase in diversity of cyanobacteria in the areas located between 70° S and 80° S (Fig. 2). Further south and further north from this zone, the diversity abruptly decreases.

Composition of communities

Fifty-seven per cent (24 of 42 OTUs) of the cyanobacterial OTUs found in Antarctic ice-free areas do not have cultured representatives. The sequences in these OTUs have 87–90% to 97% similarity with sequences of cultured cyanobacteria deposited in GenBank. According to bacteriological standards, it means that potentially new taxa at the species and genus level are present (Tindall and others, 2010).

The OTUs that have cultured representatives are assigned to the genera *Phormidium* (five OTUs), *Leptolyngbya* (five OTUs) and *Nostoc* (two OTUs). The genera *Coleodesmium*, *Cyanothece*, *Geitlerinema*, *Anabaena*, *Fischerella* and *Limnothrix* are each represented by one OTU. The majority of these genera belong to the order Oscillatoriales which includes filamentous cyanobacteria without heterocytes and akinetes: *Phormidium*, *Leptolyngbya*, *Geitlerinema* and *Limnothrix*. The genera *Nostoc*, *Coleodesmium*, *Anabaena*

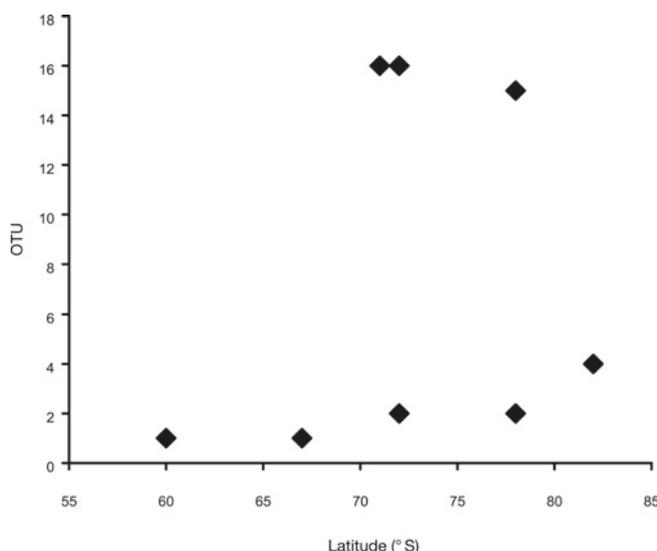


Fig. 2. The number of OTUs found in geographical areas plotted on the latitudinal scale.

and *Fischerella* belong to the order Nostocales which includes filamentous cyanobacteria with heterocysts. The genus *Cyanothece* belongs to the order Chroococcales which includes unicellular cyanobacteria.

Antarctic endemism versus cosmopolitanism

Of the 42 OTUs obtained in Antarctica, the majority had a cosmopolitan distribution (79%, 33 out of 42 OTUs), with a fraction of 'cosmopolitan-cold' OTUs of 12% (5/42). Only 21% (10/42) belonged to potential Antarctic endemic taxa, which included potential regional endemics (14%, 6/42). At the regional scale, the highest number of endemic OTUs was found in the richest terrestrial communities of the Sør Rondane Mountains and Alexander Island located between 70° S and 80° S (Fig. 3).

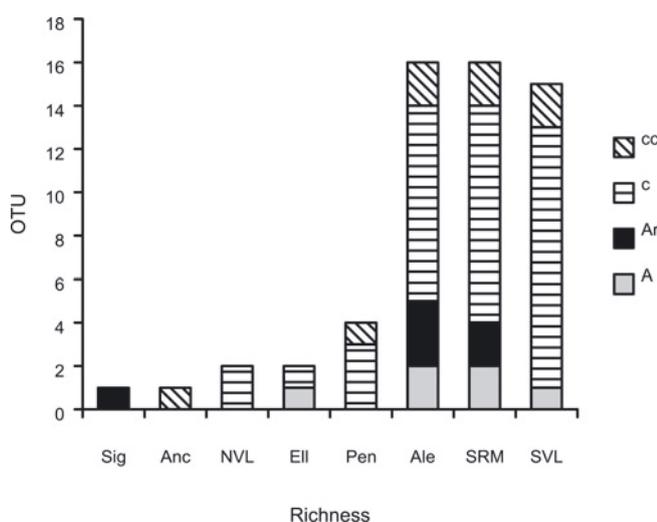


Fig. 3. The number of cosmopolitan ('c'), cosmopolitan-cold ('cc'), Antarctic-regional ('Ar') and Antarctic ('A') OTUs in the geographical areas. Sig: Signy Island; Anc: Anchorage Island; NVL: northern Victoria Land; Ell: Ellsworth Mountains; Pen: Pensacola Mountains; Ale: Alexander Island; SRM: Sør Rondane Mountains; SVL: southern Victoria Land.

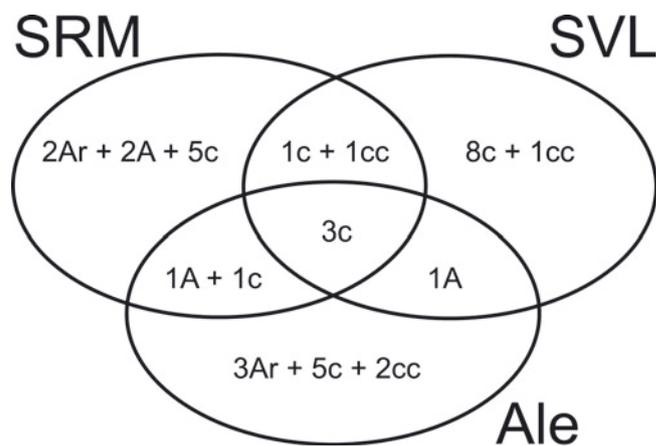


Fig. 4. Comparison of the composition of cyanobacterial communities. Ale: Alexander Island; SRM: Sør Rondane Mountains; SVL: southern Victoria Land; c: cosmopolitan OTU; cc: cosmopolitan-cold OTU; Ar: Antarctic-regional; A: Antarctic OTU.

Comparisons between Antarctic regions

We have compared the composition of cyanobacterial communities of the three richest and most intensively studied regions, separated from each other by a distance of 3000–3400 km. Interestingly, they had a very low degree of similarity with each other (Fig. 4). These results suggest that regional populations in Antarctic ice-free areas probably exist.

Of the 36 OTUs found in these three areas, 78% (28 of 36 OTUs) were found only in one of the regions. Five OTUs (14%) were shared between two regions. Three of them were Antarctic OTUs (**16ST21**, **16ST42**, **16ST109**), one was cosmopolitan (**16ST36**) and one 'cosmopolitan-cold' (**16ST11**). Only three OTUs (8%) were present in all three regions. All three are cosmopolitan: **16ST44** which belongs to 'lineage B' (Siegesmund and others, 2008) that contains the filamentous cyanobacteria *Microcoleus vaginatus* and *Phormidium autumnale*, **16ST73** which includes the strain *Leptolyngbya* sp. ANT.LH52.1 (Taton and others, 2006b) and **16ST88** which does not include any cultured representatives.

DISCUSSION

The data suggest that favourable microclimatic conditions (northward exposition of slopes, availability of meltwater and increase of the soil temperature in full sun, stable substrate for growth and protection from wind) and the ability of cyanobacteria to withstand freezing and desiccation allow the development of terrestrial cyanobacteria in all ice-free areas of Antarctica (Hawes and others, 1992; Elster and Benson, 2004). Cyanobacteria were found even in the most southerly ice-free areas of Antarctica (86–87° S) (Broady and Weinstein, 1998).

Latitudinal distribution pattern

The latitudinal analysis of the molecular diversity of cyanobacterial communities shows a sharp increase in diversity in the areas located between 70° S and 80° S. Further south and further north from this zone the diversity decreases.

We cannot exclude that this distribution is biased by a more intensive sampling in the three richest regions

(southern Victoria Land, the Sør Rondane Mountains and Alexander Island) located within this latitudinal range. However, our observation is supported by other studies. Yergeau and others (2007) showed the increase in cyanobacterial diversity along a transect from 50° S to 70–80° S. These authors have used a standardized molecular method for all samples. Hodgson and others (2010) showed that cyanobacteria were the dominant life forms in lakes and terrestrial habitats of Dufek Massif (82° S), but that their diversity was much lower than in the regions north to 80° S. However, this is the only study of molecular cyanobacterial diversity south of 80° S.

Assuming our conclusions are correct, we propose the following hypothesis. The increase in biodiversity of cyanobacteria from the sub-Antarctic to continental Antarctica could be explained by disappearance of the vegetation cover (Peat and others, 2007). Plants and mosses limit the amount of nutrients available for cyanobacteria, reduce the amount of light reaching the cyanobacterial crusts and replace cyanobacteria in the last stages of succession (Dunne, 1989; Büdel, 2005). The cyanobacteria may have lower rates of photosynthesis than eukaryotic organisms, but cyanobacteria are particularly well adapted to low temperatures and freezing (Elster and Benson, 2004). When plants and mosses are absent from an ecosystem, the cyanobacteria may have an advantage. This observation is also supported by the fact that the majority of sequences obtained north of 70° S were misinterpreted as cyanobacterial, but in fact they belonged to chloroplasts of green algae and mosses (Yergeau and others, 2007; Chong and others, 2010). Further south of 80° S, the diversity of cyanobacteria decreases, probably due to harsher environmental conditions (Hodgson and others, 2010). This is supported by the low cyanobacterial diversity found in the La Gorce Mountains using a floristic approach (Broady and Weinstein, 1998).

Comparison with limnetic communities

Direct comparison between terrestrial and limnetic molecular cyanobacterial diversity is not possible because there are no data along a latitudinal transect in the Antarctic Peninsula. The only available data come from the three regions located in continental Antarctica, but not from maritime Antarctica. These are the southern Victoria Land, Pensacola Mountains/Shackleton Range and Prydz Bay oases (Priscu and others, 1998; Vincent, 2000; Nadeau and others, 2001; Taton and others, 2003, 2006a,b; Jungblut and others, 2005; Mosier and others, 2007).

In the past, many cyanobacterial OTUs from Antarctic lakes have been identified as possibly endemic (Taton and others, 2003, 2006a,b). However, when new sequences from different geographic areas were added to GenBank, the patterns changed. New studies revealed that many OTUs, earlier identified as endemic to Antarctica, in fact have a global distribution (Dorador and others, 2008; Jungblut and others, 2010). At present, when the 97.5% 16S rRNA similarity value is used for the OTU definition, Antarctic limnetic environments appear dominated by cosmopolitan cyanobacteria.

Dominance of cosmopolitan cyanobacteria

The majority of OTUs of Antarctic terrestrial cyanobacteria have a cosmopolitan distribution (79%, 33 out of 42 OTUs). These data are in agreement with earlier observations that used a floristic approach. Indeed, according to Broady

(1996), the Antarctic terrestrial cyanobacteria are mostly represented by cosmopolitan taxa.

There are two possibilities to explain the origin of cosmopolitan cyanobacteria in Antarctica. They could have colonized Antarctica (1) after the Last Glacial Maximum (~18 000 years ago) or (2) before.

1. An example of a recent colonization of the Antarctic continent by cyanobacteria from other continents was suggested by the finding of a cosmopolitan thermophilic cyanobacterium on the fumarolic ground of Mount Erebus (Melick and others, 1991; Broady, 1993). According to Broady (1996), 'eruptions of the Antarctic volcanoes in recent centuries would probably have destroyed any earlier biota and recolonization by propagules from outside the region would then be required'. Soo and others (2009) found chimeric sequences of the thermophilic cyanobacterium *Fischerella* sp. in the fumarolic ground of Mount Erebus. After analysis of these sequences, we found that the first 600–630 BP have up to 98% similarity with the cosmopolitan thermophile *Fischerella muscicola* PCC 7414 (OTU **16ST101**). The closest geothermal habitats are located in New Zealand, >4000 km away. Several studies have investigated possible means of transportation of bacteria to Antarctica (Schlichting and others, 1978; Marshall, 1996; Vincent, 2000; Pearce and others, 2009). Viable bacteria could be transported by wind, oceanic currents or by other organisms (e.g. migrating birds and humans). More information would be needed on the aerial dispersal of cyanobacteria, as well as by other living organisms.
2. Another hypothesis suggests that cosmopolitan cyanobacteria were present in Antarctica before the glaciations and survived in refugia (mountain ranges and nunataks, subglacial lakes, etc.). Taking into account a molecular clock rate estimation of 1% 16S rRNA gene divergence per 50×10^6 years (Ma), the Antarctic cyanobacteria could have been present on the continent even before the Gondwana supercontinent broke up (>100–65 Ma) (Ochman and Wilson, 1987). At this time, Antarctica had a tropical to subtropical climate as the first ice began to appear only around 40 Ma (Convey and others, 2008). Further glaciations could have served as a 'filter', selecting freeze-tolerant species that later recolonized Antarctic ice-free areas

Regional populations of cyanobacteria

Regional populations of Antarctic cyanobacteria seem to exist, as shown by the low degree of similarity between communities from southern Victoria Land, the Sør Rondane Mountains and Alexander Island located in the same latitudinal range. The observed distribution of cyanobacteria may indicate different environmental conditions in these areas or the existence of obstacles to the dissemination processes within the continent (Martiny and others, 2006). A possible regional endemicity of eukaryotes in Antarctic soils was suggested earlier by Lawley and others (2004).

However, we cannot exclude the possibility of some biases introduced by the existing molecular approach (von Wintzingerode and others, 1997). Certain OTUs could be present in low abundance in the original environment, but not detected. This could affect the degree of similarity between different regional communities. The use of

high-throughput sequencing techniques could potentially solve this problem as they should also detect the less abundant sequences (Reeder and Knight, 2009).

SUGGESTIONS FOR FUTURE RESEARCH

Wider geographical coverage

Long transects from Signy Island to the most southerly ice-free areas like the La Gorce Mountains and similar transects along the Victoria Land mountains and the Transantarctic Mountains are necessary. In addition, more coastal ice-free areas (i.e. Schirmacher and Syowa Oases, the Framnes, Scott and Prince Charles Mountains, Bunger Hills, Ford Ranges, Ahlmannryggen, etc.) should be studied by molecular methods.

Standardized molecular approach

The comparison of different microbial communities is possible only when the same genes and parts of genes are analysed. For this purpose, we suggest using as a minimal standard the sequence of the 16S rRNA gene between *E. coli* positions 405 and 780 which includes the V3 and V4 variable regions. Longer sequences are better for phylogenetic analyses, but if only partial sequences can be obtained, they should encompass the same regions. This is especially important for high-throughput sequencing that only generates short reads.

More variable markers and importance of culture isolation

Study of the evolutionary history of each species of cyanobacteria requires molecular markers that are much more variable than the 16S rRNA gene. The use of ITS sequences was already proposed by Wilmotte (1994) to investigate intra-species variability. Multi-locus sequence typing (MLST) could also be used for this purpose (Maiden and others, 1998). This approach requires the isolation of cyanobacterial cultures from geographically distinct areas and analysis of several loci from each isolate. The overall sensitivity of this approach can be higher than use of a single locus.

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