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Diversity of phototrophic bacteria in microbial mats from Arctic hot springs (Greenland)

Guus Roeselers,[1] Tracy B. Norris,[2] Richard W. Castenholz,[2] Søren Rysgaard,[3] Ronnie N. Glud,[4] Michael Kühl[4] and Gerard Muyzer[1]*

[1]*Department of Biotechnology, Delft University of Technology, Julianalaan 67, NL-2628 BC Delft, the Netherlands.*

[2]*Center for Ecology & Evolutionary Biology, University of Oregon, Eugene, OR 97403, USA.*

[3]*Greenland Institute of Natural Resources, Kivioq 2, Box 570, 3900 Nuuk, Greenland, Denmark.*

[4]*Marine Biological Laboratory, Institute of Biology, University of Copenhagen, Strandpromenaden 5, DK-3000 Helsingør, Denmark.*

Summary

We investigated the genotypic diversity of oxygenic and anoxygenic phototrophic microorganisms in microbial mat samples collected from three hot spring localities on the east coast of Greenland. These hot springs harbour unique Arctic microbial ecosystems that have never been studied in detail before. Specific oligonucleotide primers for cyanobacteria, purple sulfur bacteria, green sulfur bacteria and *Choroflexus/Roseiflexus*-like green non-sulfur bacteria were used for the selective amplification of 16S rRNA gene fragments. Amplification products were separated by denaturing gradient gel electrophoresis (DGGE) and sequenced. In addition, several cyanobacteria were isolated from the mat samples, and classified morphologically and by 16S rRNA-based methods. The cyanobacterial 16S rRNA sequences obtained from DGGE represented a diverse, polyphyletic collection of cyanobacteria. The microbial mat communities were dominated by heterocystous and non-heterocystous filamentous cyanobacteria. Our results indicate that the cyanobacterial community composition in the samples were different for each sampling site. Different layers of the same heterogeneous mat often contained distinct and different communities of cyanobacteria. We observed a relationship between the cyanobacterial community composition and the *in situ* temperatures of different mat parts. The Greenland mats exhibited a low diversity of anoxygenic phototrophs as compared with other hot spring mats which is possibly related to the photochemical conditions within the mats resulting from the Arctic light regime.

Received 5 April, 2006; accepted 27 June, 2006. *For correspondence. E-mail g.muijzer@tudelft.nl; Tel. (+31) 15 278 1193; Fax (+31) 15 278 2355.

Introduction

Microbial mats are layered structures composed of physiologically different groups of microorganisms (van Gemerden, 1993). They are present in a variety of environments where grazing is limited, such as hot springs, shallow coastal lagoons, hypersaline ponds and permanently ice-covered lakes (Stal and Caumette, 1994). The top layer of microbial mats is typically dominated by oxygenic phototrophs, such as cyanobacteria and diatoms, with underlying or intermixed layers of anoxygenic phototrophs, i.e. green and purple sulfur bacteria (GSB and PSB) and green non-sulfur bacteria (Nübel *et al*., 2002; Martinez-Alonso *et al*., 2005). Cyanobacteria are the most important primary producers in these ecosystems (Stal, 1995). Their photosynthetic activity fuels a variety of mineralization processes and conversions catalysed by heterotrophic and lithotrophic bacteria in the mat community (Ward *et al*., 1998).

Geothermal springs with homeothermic source water temperatures above 0°C can be found all over Greenland, especially around the island of Disko on Western Greenland, where the occurrence of several thousands of such springs have been estimated (Heide-Jørgensen and Kristensen, 1998). However, the hottest springs, with source water temperatures of 55–62°C are found on Greenland's east coast at a number of locations north and south of Scoresbysund. In these springs, temperatures are high enough to allow significant growth of thermophilic microorganisms and most springs are characterized by the presence of thick gelatinous microbial mats (Kühl *et al*., 2004). These Arctic hot springs are all located close to 70°N latitude, and the phototrophic organisms are thus subject to extreme seasonal variations in the supply of photosynthetic active radiation (PAR); ranging from continuous daylight during the Arctic summer, to almost complete darkness during the winter when the sun does not rise above the horizon for several months (Cockell and Rothschild, 1997; 1999). Within the Arctic environment,

Table 1. Source water characteristics at the three sampling sites.

Spring	Source temperature (°C)	Salinity (‰)	pH	Oxygen (μM)
Kap Tobin	60–62	13–16	7.0–7.5	5–8
Nørrefjord	53–57	9–13	8.2–8.6	6–8
Rømerfjord	56–61	5–7	9.1–9.5	0

hot spring microbial mats form isolated and disconnected habitats, which raises the question whether endemic taxa or species exist in such microbial communities (Castenholz, 1996). Some of the East Greenland springs have been known since the early explorers visited Greenland, and the presence of coloured slimy coatings in the hot springs was mentioned in several early expedition journals (e.g. Nordenskjöld, 1907). Presence of cyanobacteria was also mentioned in the few botanical and geological surveys of the springs that have been reported (Halliday *et al*., 1974). However, the microbiology of the East Greenland hot springs was never explored in detail.

In July 2003 an expedition was carried out to perform the first microbiological field studies in hot springs at three localities (i.e. Nørrefjord, Rømerfjord and Kap Tobin) on the East Greenland coast (Kühl *et al*., 2004). In these localities, over 70 springs with source water temperatures of > 40°C were found. All springs, irrespective of pronounced differences in water chemistry and temperature, contained characteristic orange, green and brownish coloured gelatinous biofilms covering the sides and the bottom of the springs. Microscopic observation of these biofilms revealed that they were mainly composed of a dense network of filamentous cyanobacteria embedded in a slimy matrix of exopolymers. The biogeochemistry of these microbial mats will be presented elsewhere (M. Kühl, S. Rysgaard and R. N. Glud, unpubl. data).

In this study we provide an inventory of the oxygenic and anoxygenic photoautotrophic microorganisms in the microbial mat samples collected during the expedition. The microbial diversity of these unique Arctic ecosystems was studied by genetic fingerprinting analysis of samples and pure cultures of cyanobacteria isolated from various mat samples. This approach enabled identification of the dominant cyanobacteria, PSB, GSB and green non-sulfur bacteria belonging to the *Chloroflexaceae* in the microbial mat communities.

Results

Mat structure and spring water chemistry

The Nørrefjord and Rømerfjord localities harboured numerous hot springs with source water temperatures > 50°C, while only one spring, with several sources, was found at Kap Tobin. The water chemistry differed among the three localities (Table 1). The Kap Tobin spring reached the highest source temperature of ~62°C, was almost pH neutral (pH 7–7.5) and had a relatively high salinity of ~15‰. A slight smell of sulfide was detected close to the source, where yellow-whitish material indicated sulfur precipitation. The springs at Nørrefjord were alkaline (pH 8.2–8.6) and had a slightly lower and more variable salinity of 9–13‰. The springs at Rømerfjord were highly alkaline (pH 9–9.5) with a low salinity of 5–7‰. Whitish material and streamers indicating sulfur precipitation covered the sediment closest to the source in several springs at Rømerfjord. The source water had a low oxygen content (< 10 μM) at Kap Tobin and Nørrefjord, while the source water in hot springs at Rømerfjord was anoxic.

Despite the differences in water chemistry, all springs harboured massive growth of several cm thick microbial mats with a very gelatinous and translucent structure on top of the sediment and gravel in the springs (Fig. 1). Around fast flowing sources, green streamers were also frequently observed. Microscopic investigation of the mats showed the presence of a dense network of cyanobacteria embedded in copious amounts of exopolymers. Most mats had a thick orange-greenish surface layer on top of a darker green layer (Fig. 1E, Table 2). In a few mats at Nørrefjord, a reddish layer was sometimes observed below the surface layers. Some springs at Nørrefjord also harboured floating mats with a dark brownish surface colour of the air-exposed parts.

Diversity of oxygenic phototrophs

Polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) analysis with primers specific for cyanobacteria and chloroplasts revealed differences in community composition among the three locations as well as among the individual samples of each location. Most of the DGGE profiles from the Kap Tobin mat samples showed three or four dominant bands, while the DGGE profiles from Nørrefjord and, Rømerfjord samples showed only one or two dominant bands (Fig. 2). All DNA sequences except for three (i.e. band R0-1, R8-9 and band R8-10, Fig. 2B) were related to cyanobacteria. A phylogenetic tree was constructed with the sequences derived from the DNA fragments (Fig. 3). The Nørrefjord mat samples were characterized by the presence of sequences related to filamentous cyanobacteria of the genera *Chlorogloeopsis*, *Fischerella* and, *Leptolyngbya* (Figs 2A and 3). The mat samples from Kap Tobin were

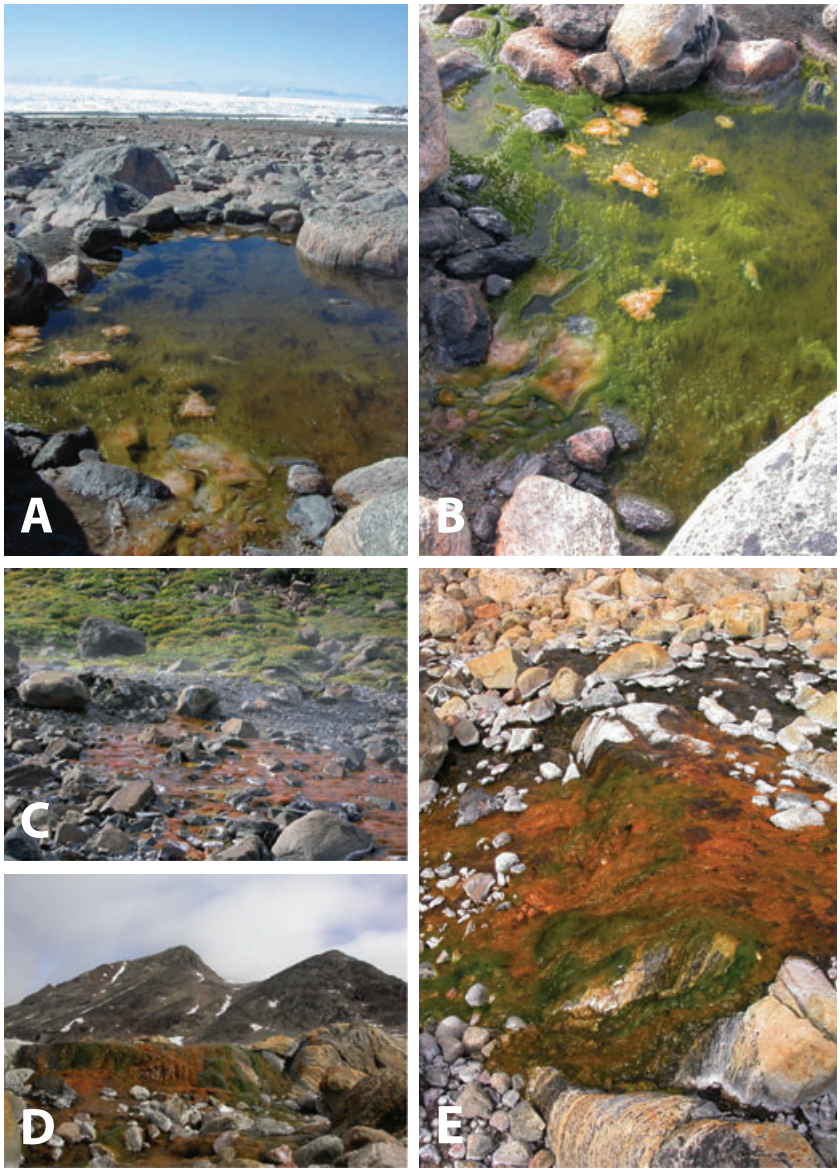

Fig. 1. Pictures showing some of the field sites at Kap Tobin (A, B), Rømerfjord (C), and Nørrefjord (D, E), where thick gelatinous microbial mats covered the bottom of the hot springs.

characterized by the presence of sequences related to *Leptolyngbya* and *Scytonema* species (Figs 2C and 3). Rømerfjord was characterized by the presence of *Phormidium* species (Figs 2B and 3).

The sequences derived from band R0-1, band R8-8, and band R8-9 are affiliated to uncultured members of the genus *Nitrospira*, suggesting partial non-specificity of the cyanobacterial primers. These sequences were not incorporated in the phylogenetic tree. Out of the 10 samples collected from Nørrefjord, samples N2, N5, N8 and N10 showed one dominant band at identical position on the DGGE gel (Fig. 2A). The identical sequences derived from these bands were affiliated to *Leptolyngbya* species (Fig. 3). All 12 samples obtained from the Kap Tobin spring, except for sample K7, K11 and K12, showed a dominant band at identical positions on the DGGE gel. The sequences derived from these bands were 99% similar to a slightly higher positioned band that was present in K3, K4, K5, K6 and K9. All bands clustered closely together with the *Leptolyngbya* affiliated sequences found in the Nørrefjord springs (Fig. 3) Nevertheless, within this cluster the Nørrefjord sequences exhibited only 95% similarity with the Kap Tobin sequences. Samples N6 and N9 from Nørrefjord and sample K12 from Kap Tobin all showed a dominant band positioned high on the denaturing gradient gel (Fig. 2A and C) with high sequence similarities (> 99%). All of them exhibited at least 8% dissimilarity with the closest related *Anabaena* species from the database. The identical sequences from band R1-2 and band R2-4 (Fig. 2B) showed 15% dissimilarity to their closest relative in the database, an uncultured Antarctic cyanobacterium

Table 2. Temperature, morphology and colour of the collected mat samples.

Sample	T (°C)	Mat morphology and colour
K1	60–61	Dark green mat and streamers
K2	45–50	Gelatinous mat, top layer
K3	45–50	Gelatinous mat, underlying layer
K4	45–50	Gelatinous mat, orange top layer
K5	45–50	Gelatinous mat, green under layer
K6	50	Cushion forming mat
K7	< 40	Greenish-brown mat
K8	61,5	Sample from source
K9	50	Thick greenish gelatinous mat
K10	40–45	Orange cushion mat
K11	40–42	Brown mat
K12	~35	Green-brown mat
R0	56–58	White streamer material with sediment close to source
R1	42	Orange reddish mat, reddish top layer
R2	42	Orange reddish mat, green underneath top layer
R3	50–53	Rigid gelatinous orange mat
R4	45–48	Surface layer of thick gelatinous greenish mat
R5	20	Dense dark green mat (receives splashes of hot water from source)
R6	50	Orange gelatinous mat
R7	40	Orange-green gelatinous mat
R8	~58	Black sediment below and between white streamers
N1	~50	Green streamers near source
N2	49	Orange mat, top layer
N3	49	Orange mat, green layer
N4	49	Orange mat, red layer
N5	45	Green gelatinous mat
N6	42	Cohesive brown surface layer on top of a pale yellow-greenish layer
N7	46	Orange mat
N8	46	Orange gelatinous mat
N9	~20	Brown mat, surface layer
N10	46	Orange mat

K1-K12 were collected from Kap Tobin, R0-R8 were collected from Rømerfjord and, N1-N10 were collected from Nørrefjord.

derived from the ice cover of Lake Bonney (Antarctica) (Gordon *et al*., 2000).

Photomicrographs of cyanobacterial isolates GRN-1, GRN-7, GRN-8 and, GRN-11 show, respectively, *Chlorogloeopsis*-like trichomes, *Leptolyngbya*-like filaments, *Mastigocladus* (*Fischerella*) *laminosus*-like filaments and, typical *Synechococcus*-like unicells (Fig. 4). The 16S rRNA sequences obtained from these pure cultures of cyanobacteria, which were isolated from the mat samples, clustered together with DGGE derived sequences (Fig. 3). The sequences of isolates GRN-1 to GRN-5 as well as the DGGE band sequences N1-1 and K1-1 were all more than 99% similar to the sequence of *Chlorogloeopsis* sp. PCC 7518, which was originally isolated from an Icelandic hot spring (Fig. 3). Sequences of isolates GRN-6 and GRN-7 clustered together with the sequences K1-2, K4-3, K4-4, K6-6, and, K8-10, which are all affiliated to *Leptolyngbya*.

Diversity of anoxygenic phototrophs

Polymerase chain reaction with primers specific for GSB only yielded significant amounts of PCR products from samples N3, N4, N8, R5, K1 and K9. Subsequent DGGE analysis showed a dominant band at the same position for each sample (Fig. 5). A total of six bands were excised, re-amplified and sequenced. The obtained sequences had an identical position in the phylogenic tree and are related to an uncultured *Chlorobium* species (Fig. 6A).

Amplification with primers specific for PSB resulted in significant PCR products from eight of the 31 samples. All *Chromatium* affiliated sequences were derived from samples from the Kap Tobin mats, except one that came from a Rømerfjord sample (Fig. 6B). Purple sulfur bacteria were absent or below detection levels in the other samples. The DGGE with the PSB 16S rRNA gene fragments was characterized by the presence of a single band for each sample with the exception of K11, which showed three bands (Fig. 5). The phylogenetic tree (Fig. 6B) with the sequences obtained from the excised DGGE bands showed that bands K7-4, K10-6, K11-7 and K12-10 were similar to *Isochromatium buderi* (Imhoff *et al*., 1998) and K119 was similar to *Thiocapsa roseopersicina,* both are known microbial mat inhabitants. The other bands were related to uncultured *Chromatiaceae*.

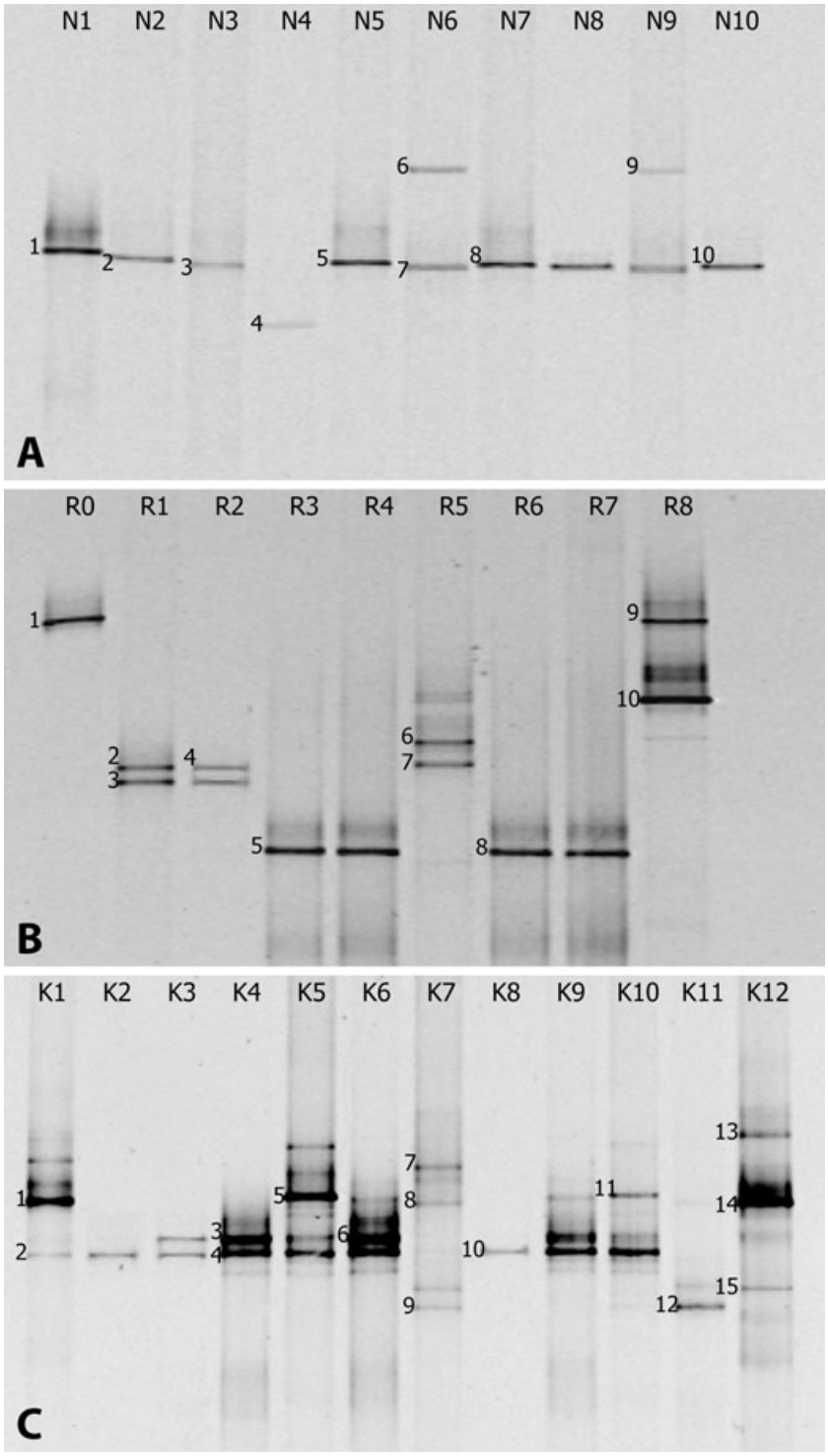

Fig. 2. Denaturing gradient gel electrophoresis patterns of 16S rRNA gene fragments obtained after enzymatic amplification using primers specific for oxygenic phototrophs and genomic DNA from various microbial mat samples.
A. Mat samples from Nørrefjord hot spring (samples N1 to N10).
B. Mat samples from Rømerfjord hot spring (samples R0 to R8).
C. Mats samples from Kap Tobin hot spring (samples K1 to K12).
Numbers at the left of each lane correspond to bands that were excised, PCR-amplified and sequenced.

Amplification with primers specific for photosynthetic green non-sulfur bacteria within the *Chloroflexaceae* resulted in a significant amount of PCR product in 15 out of 31 samples. However, only four samples resulted in a clear DGGE pattern from which DNA sequences could be derived successfully (Figs 6C and 7). The DGGE profile of Rømerfjord sample R5 contained two bands. One DGGE band (Band R5-6) was closely related to *Roseiflexus castenholzii*, a red filamentous green non-sulfur bacterium that was first isolated from microbial mats in alkaline hot springs in Japan (Hanada *et al*., 2002). The other band from R5 clustered together with band 1 from Nørrefjord

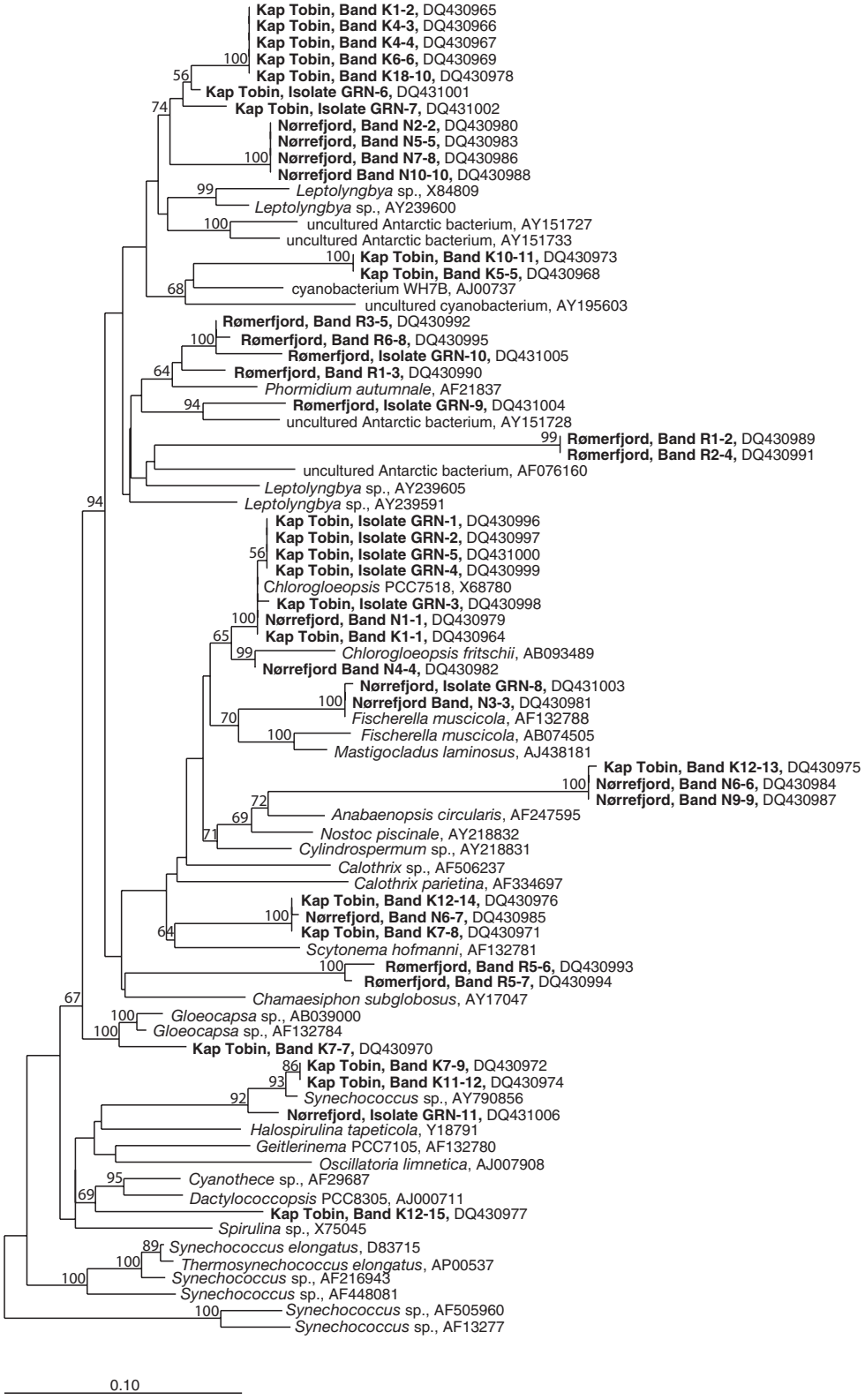

Fig. 3. Evolutionary tree showing the phylogenetic affiliations of the cyanobacterial 16S rRNA gene sequences derived from the DGGE gels shown in Fig. 2. The sequences obtained in this study are printed bold. *Escherichia coli* (AJ567606) was used as an out-group, but was pruned from the tree. Scale bar indicates 10% sequence divergence. Bootstrap values (1000 replicates) that were > 50 are placed at the nodes of the branches.

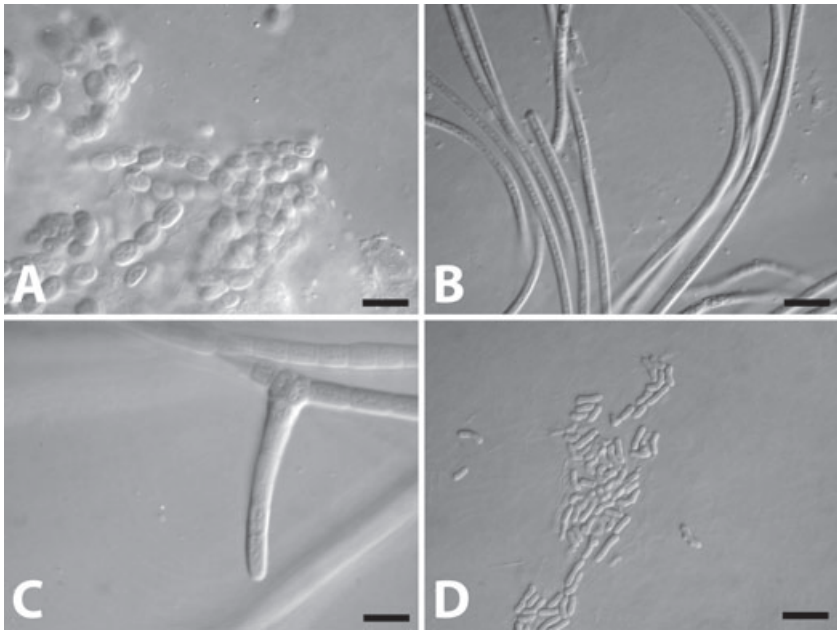

Fig. 4. Nomarski DIC photomicrographs of cyanobacterial isolates. All cyanobacteria are shown at the same magnification and the scale bar in all panels indicates 10 μM.
A. 'High temperature form' *Chlorogloeopsis* trichomes isolated from mat sample Kap Tobin 9B (57–61°C).
B. *Leptolyngbya*-like filaments (~2.0–2.2 μM wide) isolated from mat sample Kap Tobin 16 (40°C).
C. *Mastigocladus* (*Fischerella) laminosus*-like filaments isolated from mat sample Nørrefjord 11 (green layer, 45°C). It should be noted that the Pasteur Culture Collection (PCC) classifies all *Mastigocladus* a*s Fischerella*.
D. Typical *Synechococcus*-like unicells, about 1.5 μM wide, isolated from mat sample Nørrefjord 11 (green layer 45°C).

sample N3 and an uncultured *Chloroflexus*-like bacterium. Bands 2 and 3 from Nørrefjord sample N3 clustered together with the freshwater planktonic species *Chloronema giganteum* (Dubinina and Gorlenko, 1975) and the hot spring inhabiting *Chloroflexus aggregans* and *Chloroflexus aurantiacus* (Hanada *et al*., 1995).

Discussion

Our survey of phototrophic genotypes revealed that cyanobacteria were the predominant oxygenic phototrophs. The differences in the cyanobacterial community structure among the three locations were more prevalent than the differences among the individual samples from one location. Most of the samples from the Kap Tobin mats showed several dominant bands, while the samples from Nørrefjord and, Rømerfjord showed only one or two dominant bands suggesting a higher number of dominant cyanobacterial strains in the Kap Tobin mats.

The analysed mat samples were morphologically very diverse and were sampled from different *in situ* temperatures (Table 2), and our inventory of the microbial phototrophs did not reveal a clear-cut correspondence between the genotypic diversity and the morphological characteristics of the mats. However, the phylogenetic analysis showed that the 16S rRNA gene sequences affiliated with *Chlorogloeopsis* PCC7518, *Chlorogloeopsis fritschii* and *Fischerella muscicola* were all derived from

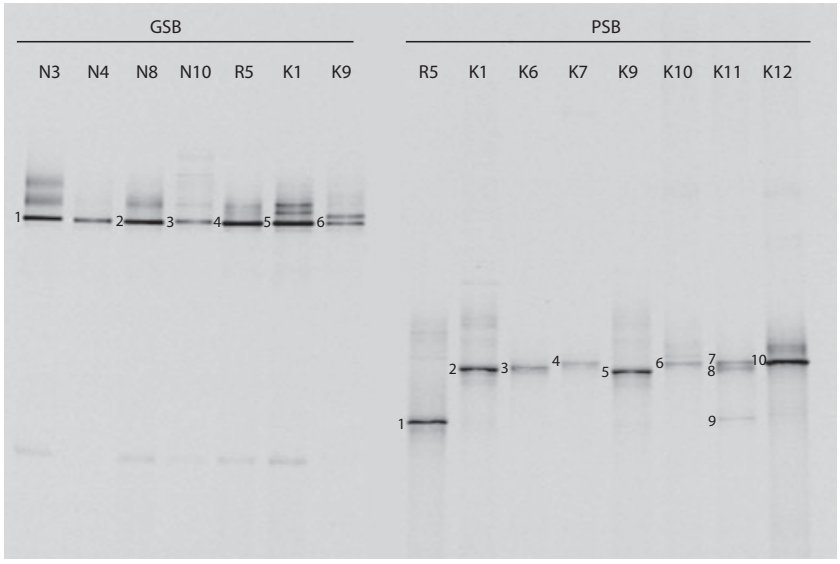

Fig. 5. Denaturing gradient gel electrophoresis patterns of 16S rRNA gene fragments obtained after enzymatic amplification using primers specific for green sulfur bacteria (GSB) and purple sulfur bacteria (PSB) and DNA samples from microbial mats of Rømerfjord hot spring (R5), Nørrefjord hot spring (N3, N4, N8 and N10), and Kap Tobin hot spring (K1, K6, K7 K9, K10, K11 and K12). Numbers at the left of each lane correspond to bands that were excised, PCR-amplified and sequenced.

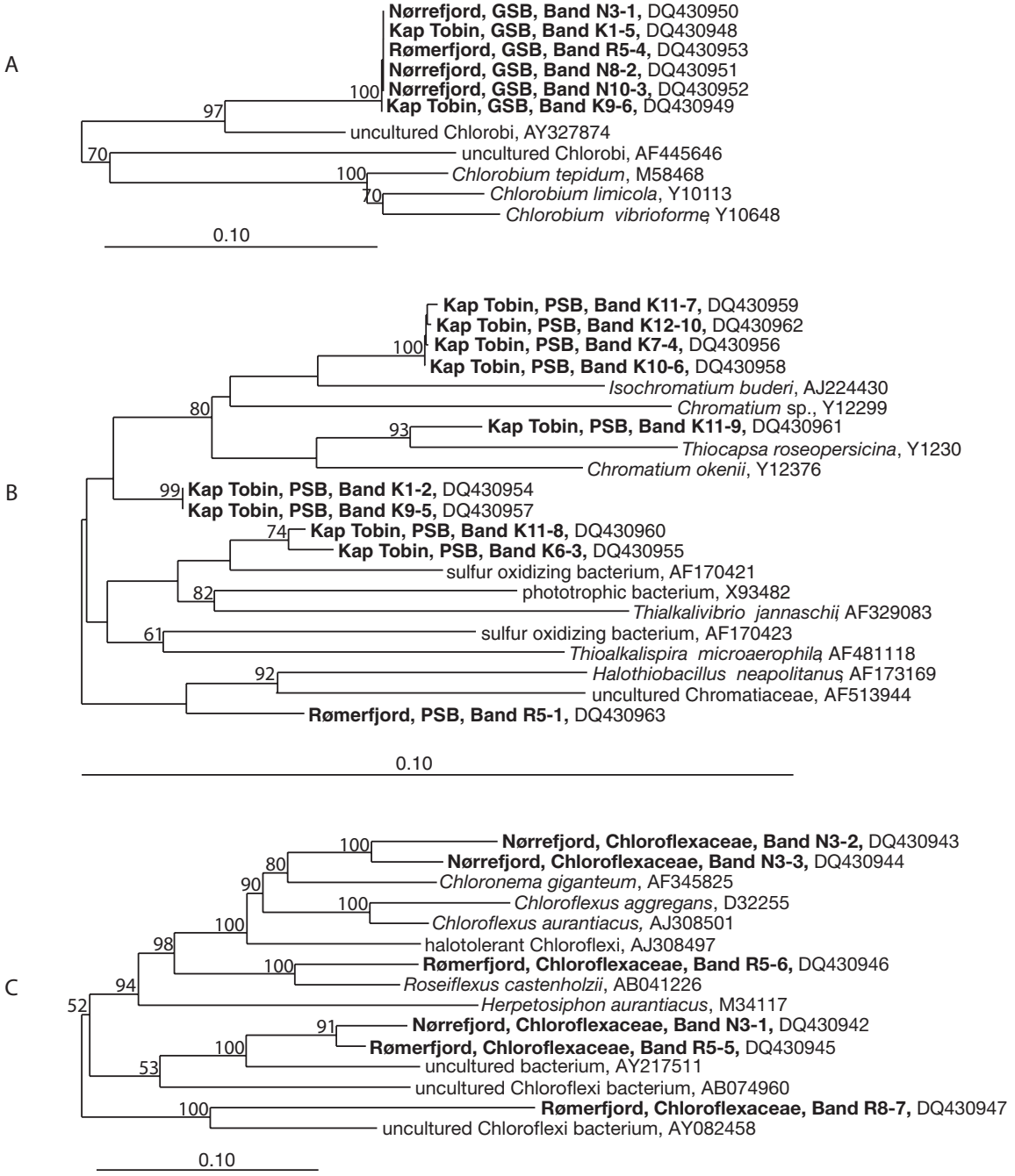

Fig. 6. Evolutionary trees showing the phylogenetic affiliations of the green sulfur bacteria (A) purple sulfur bacteria (B) and Chloroflexaceae types (C). The 16S rRNA gene sequences used to generate the trees were obtained from DNA fragments excised from the denaturing gradient gel shown in Fig. 5 and 7. *Escherichia coli* (AJ567606) was used as an out-group, but pruned from the trees. Scale bar indicates 10% sequence divergence. Bootstrap values (1000 replicates) that were > 50 are placed at the nodes of the branches.

microbial mats growing at ≥ 45°C, like the clusters of sequences affiliated with *Leptolyngbya* species found in the Kap Tobin and Nørrefjord mats. *Chlorogloeopsis* PCC7518 [(formerly referred to as 'HTF' *Mastigocladus* (Castenholz, 1969)] and *Leptolyngbya* species have been described to inhabit high temperature environments (Castenholz, 1996; 2001). However, the morphotypes *C. fritschii* and *F. muscicola* are not known as thermophiles. The sequences affiliated with *Anabaenopsis* sp. and *Scytonema* sp. originated from samples with temperatures below 45°C (Table 1, Fig. 3).

The cyanobacterial 16S rRNA gene sequences obtained from the DGGE gels and from the isolates were related to a diverse, polyphyletic group of cyanobacteria.

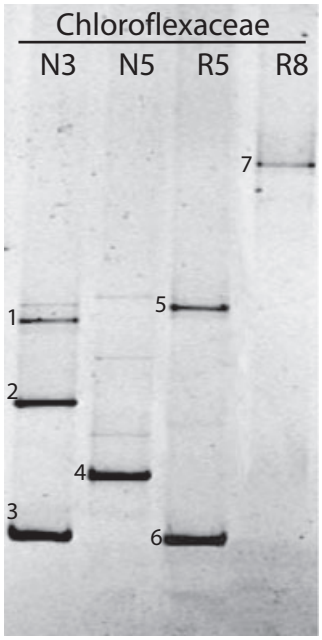

Fig. 7. Denaturing gradient gel electrophoresis patterns of 16S rRNA gene fragments obtained after enzymatic amplification using primers specific for *Chloroflexus* spp. and phylogenetic relatives and DNA samples from microbial mats of Rømerfjord hot springs (R5 and R8), and Nørrefjord hot springs (N3 and N5). Numbers at the left of each lane correspond to bands that could be excised, PCR-amplified and sequenced.

The presence of long branching sequences in the cyanobacterial phylogenetic tree could indicate that some of the phylotypes obtained in this study are restricted to this specific environment. The Arctic light regime clearly distinguishes the Greenland mats from microbial mats found in geothermal springs under more temperate climates, although Icelandic hot springs at 66°N latitude are only on a ~4 degrees lower latitude than those near Scoresbysund. Sperling (1975) found that hot spring mats persisted during the winter in some Icelandic hot springs. The mats also persisted during winter in Kap Tobin and Nørrefjord and had the same morphology and colour as the summer mats (H. C. Scoresby Hammeken, pers. comm.). We speculate that some cyanobacteria inhabiting the Greenland springs may have special ecophysiological adaptations to the high Arctic light regime and the resulting photochemical effects. For example, it has been shown that phylogenetically distinct Antarctic oscillatorians show a high level of adaptive flexibility in pigmentation in response to changes in PAR supply (Quesada and Vincent, 1997).

Recently, it was shown that unicellular cyanobacteria growing at 60–65°C in some geothermal springs employ a complex network of metabolic switching during a diel cycle (Steunou *et al*., 2006), during which the mat undergoes a change from highly supersaturated oxygen conditions in daylight to almost complete anoxia during nighttime. Expression of genes coding for enzymes involved in nitrogen fixation, and the N_2-fixing activity were closely coupled to the diminishing light and oxygen levels thereby minimizing inhibition of nitrogenase by oxygen in the mat. Such a mechanism would not work in the Greenland hot springs, which are constantly supersaturated with oxygen throughout the Artic summer months (Kühl *et al*., 2004). This may explain the abundance of sequences affiliated with heterocystous cyanobacteria like *Chlorogloeopsis*, *Fischerella* and *Scytonema* species in our samples. The fact that microbial mats can become highly saturated with oxygen during daytime would probably only allow diazotrophy by heterocystous cyanobacteria, because non-heterocystous types usually require a dark period for this anaerobic process (Stal, 2000). We speculate that the persistence of oxic conditions in the mats for several months during summer may select for heterocystous cyanobacteria. The temporal and spatial distribution of diazotrophic activity within the mats could be an interesting subject for further investigation.

Recent molecular ecology studies support the hypothesis of Antarctic cyanobacterial endemism (Priscu *et al*., 1998; Vincent, 2000; Taton *et al*., 2003). There is, however, no consensus concerning the extent of endemism in Arctic cyanobacteria. The fact that many of the identified cyanobacterial sequences exhibit high dissimilarity with the sequences available in the databases may be an indication of the existence of endemic or at least specially adapted cyanobacteria in these environments. On the one hand, the dominant, mat building primary producers in these ecosystems might have unique adaptations to the Arctic light regime, and the resulting shift between long periods of continuous oxic or anoxic conditions in the mats. On the other hand, the island-like characteristics of the hot springs and the resulting geographical isolation of their inhabitants could be considered as one of the possible factors of speciation (Papke *et al*., 2003).

Thermophilic cyanobacteria of the genus *Synechococcus* are absent in Iceland and Alaska (Castenholz, 1996) while in the western United States thermophilic *Synechococcus* species of this morphotype are found in chemically diverse hot springs (Ferris *et al*., 1996a). Because all thermophilic forms of *Synechococcus* appear to be absent in Iceland, the presence of members of this genus (i.e. isolate GRN-11) (Figs 3 and 4D) in the Greenland springs is quite surprising, and may reflect the fact that these springs are found in a geologically much older setting than the springs on Iceland (the oldest crust of Iceland is only 20 million years old, while Greenland is *c*. 3.7 billion years old). In contrast, the thermophilic cyanobacterium *Chlorogloeopsis* PCC7518, found in hot springs world-wide (Castenholz, 1996), also occurs in the

Greenland hot springs. Several of the other cyanobacterial isolates and genotypes we found in the Greenland hot springs are representative of genera and species that occur commonly in geographically close hot springs on Iceland. A spreading of microbes from Iceland to East Greenland and vice versa may be facilitated, e.g. by migrating birds, which are known to use hot springs on both Iceland and the East Greenland coast as resting places; a similar dispersal mechanism has been proposed for the unique flora found surrounding the East Greenland hot springs (Halliday *et al*., 1974). Evidently, this type of dispersal works only for thermophilic microbes that can survive exposure to much lower temperatures and desiccation, and the fact that thermophilic *Synechococcus* have not spread from Greenland to Iceland could thus indicate that these thermophiles have a much lower tolerance to conditions involved in dispersal, such as freezing or desiccation. Although, it has been shown that widely varying chemical environments do not a priori restrict *Synechococcus* colonization (Papke *et al*., 2003), possible unidentified abiotic effects on the distribution of this genotype cannot be ruled out.

No sequences were found that are affiliated with algal chloroplasts indicating that eukaryotic algae are very low in number or absent in the hot spring mats. This was also evident from microscopic investigations of many mat samples.

The DGGEs and the derived sequence affiliations revealed a lower diversity among the GSB and PSB compared with other mat ecosystems (Ferris *et al*., 1996b; Elshahed *et al*., 2003). Green sulfur bacteria are a taxonomically very distinct group of anoxyphototrophic bacteria. They require strictly anoxic conditions, and they need only about one-fourth of the light intensity of the PSB to attain comparable growth rates. The low diversity of GSB could again be related to the continuous oxic state of the mats throughout the Arctic summer (Kühl *et al*., 2004).

Although pigment synthesis in phototrophic PSB is inhibited by continuous exposure to oxygen most species of the *Chromatiaceae* are relatively tolerant to periodic exposure. The DGGE profile of sample K11 showed one band (Fig. 5, Band K11-9) affiliated to *T. roseopersicina,* which is ranked among the most oxygen tolerant members of the *Chromatiaceae.* Nevertheless, the diversity of PSB was also low. Green and purple sulfur bacteria could not be detected in any of the Rømerfjord samples except for sample R5 with a relatively low *in situ* temperature of 20°C. The source water of Rømerfjord has a higher average pH (9.1–9.5) than the source water from Kap Tobin and Nørrefjord. The majority of the GSB and PSB show optimal growth rates ranging from pH 6.5 to 8, but some halophilic PSB are true alkaliphiles with optimal growth at pH 10 (Sorokin and Kuenen, 2005). The only PSB sequence derived from sample R5 (Figs 5 and 7B) shows distant affiliation to a *Halothiobacillus* strain (a non-photosynthetic bacterium), which is not known as akaliphilic. It must be noted, however, that only two-thirds of the presently known 16S rRNA gene sequences of Chromatiaceae will be amplified with primer Chr986f (Overmann *et al*., 1999). Hence, we cannot exclude the possibility that the diversity of Chromatiaceae is higher than encountered by our approach.

Mat communities dominated by *Chloroflexus* spp. are well represented in geothermal springs where elevated sulfide concentrations inhibit cyanobacteria and promote anoxygenic photoautotrophy (Giovannoni *et al*., 1987). We could only obtain *Chloroflexaceae* DGGE profiles for a few mat samples and from some DGGE bands we were unable to derive DNA sequences. We conclude that *Chloroflexaceae* are not very abundant in the studied mat systems especially compared with similar hot spring mats, e.g. in Iceland (Skirnisdottir *et al*., 2000). Samples from Nørrefjord contained a few sequences that cluster loosely with *C. aurantiacus* and *C. aggregans* (Hanada *et al*. (1995). *Roseiflexus* spp., another group of green non-sulfur bacteria, appears to predominate in non-sulfidic hot springs (van der Meer *et al*., 2005). Only one sequence affiliated with *R. castenholzii* was derived from Rømerfjord sample R5.

In conclusion, our survey of the phototrophic inhabitants of the hot springs of Greenland shows the presence of phylogenetically diverse populations of filamentous and unicellular cyanobacteria and a few representatives of anoxygenic phototrophs. Cyanobacteria appear to be the dominant mat inhabitants and their diversity includes cyanobacteria with a cosmopolitan distribution in hot springs. We detected a thermophilic *Synechococcus* sp., which was not found in Alaskan or in Icelandic hot springs. The physiology and geographical distribution of thermophilic cyanobacteria should be the subject of future investigations. This will enhance our understanding of the role and the dispersal of cyanobacteria as the dominant primary-producing organisms in geothermal environments and their ability to survive in high latitudes. In addition, the described hot springs offer a unique opportunity to study the effect of the Arctic light regime on microbial ecosystems without the persistent low temperatures normally associated with polar environments.

Experimental procedures

Environmental parameters of the springs

The mat samples used in this study were obtained from East Greenland hot springs at Kap Tobin (70°24.9′N; 21°57.04′W) and Nørrefjord (71°08.3′N; 22°2.1′W) on Jameson Land north of Scoresbysund, and at Rømerfjord (69°43.7′N; 23°41.9′W), on Blosseville's Coast south of Scoresbysund. The source water in the springs was characterized by tempera-

ture measurements with an electronic thermometer (Omnitherm, Germany), salinity determination with a calibrated refractometer (Atago, USA), pH determination with a portable pH-meter (Radiometer, Denmark) and oxygen determination by Winkler titration (Strickland and Parsons, 1972).

Sample collection

Samples were taken from a number of springs at each locality harbouring microbial mats growing at different temperatures and exhibiting different coloration and structure (Tables 1 and 2). Most samples were cut out with a sharp knife and carefully transferred into 25 mm diameter Petri-dishes that were sealed *in situ* with air- and water-proof tape (3M, USA). Additional samples were taken with small Perspex cores and cut 50 ml syringes that were sealed *in situ*. Samples were kept cool (< 5–10°C) during transport back to Denmark, where they were frozen and kept at −80°C. Samples for molecular analysis were shipped on dry ice to the laboratory in Delft. Fresh samples for isolation attempts were kept in darkness at 4–6°C.

Isolation of cyanobacteria

The cultures were isolated from most of the samples by various methods previously described in Castenholz (1988). Small pieces of inoculum were placed on BG-11 and D medium agar plates, incubated at 45°C at approximately 20 Wm^{-2} of coolwhite fluorescent illumination, and trichomes were allowed to migrate out (or grow out) from the source. Single trichomes (or filaments) were then picked off (including the small agar piece supporting the trichome) with a watchmaker's forceps and inoculated into liquid medium. In addition, especially for unicellular species, small pieces of inoculum were broken up and vigorously dispersed with a syringe using a large needle (17 gauge) or cannula. This was then processed through a dilution to extinction series and the final enrichment at 45° and 50°C was streak diluted on D medium agar plates to produce clonal colonies. Some of these colonies were then resuspended and replated to obtain new colonies, which were then transferred to liquid medium (BG-11 and D). Unfortunately, the original material from the springs had been stored for several months at 4–6°C, rather than at room temperature, which is recommended for hot spring collections (Castenholz, 1988). Because of this, some of the collected material may not have resulted in viable cells. Therefore, the cultures isolated probably represent an incomplete picture of the species present. Photomicrographs were taken with a Zeiss Axioplan microscope (Carl Zeiss, Jena, Germany), using a 100 × plan-neofluar objective with Nomarski differential interference contrast (DIC) optics. The cultures are kept in the *Culture Collection of Microorganisms from Extreme Environments* (CCMEE) at the University of Oregon (http://cultures.uoregon.edu) both as growing batch cultures at 45° and at −80°C.

DNA extraction

Genomic DNA was extracted from the mat samples by applying approximately 500 mg frozen biomass to the UltraClean Soil DNA Isolation Kit™ (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. Cell lysis was confirmed by phase-contrast microscopy. DNA dilutions were stored at −20°C.

Genomic DNA extraction from culture isolates was performed using a modification of the method of More *et al*. (1994). Culture aliquots were pelleted and approximately 100 μl of pelleted cells was transferred to 2-ml screw-cap microcentrifuge tubes. Next, 0.75 g of 0.1 mm diameter zirconia/silica beads (BioSpec Products, Bartlesville, OK) were added to each tube along with 600 μl of 120 mM sodium phosphate (pH 8.0) and 400 μl of lysis buffer (10% (wt/vol) sodium dodecyl sulfate, 0.5 M Tris-HCl (pH 8.0) and 0.1 M NaCl). Cells were lysed by shaking for 3 min at high speed on a Mini-Beadbeater (BioSpec Products, Bartlesville, OK) and then centrifuged for 3 min at 13 000 *g*. Supernatant (700 μl) was collected and DNA was precipitated on ice using 2:5 (v/v) of 7.5 M ammonium acetate and then centrifuged again. The supernatant was collected and the DNA was isopropanol precipitated. Finally, the pellet was washed with ice-cold 70% (v/v) ethanol; air-dried and resuspended in 100 μl of 10 mM Tris (pH 8.0).

Polymerase chain reaction amplification of rRNA gene fragments

Extreme care was taken to prevent any DNA contamination of solutions and plastic disposables used for PCR. All heat sterilized plastic tubes were exposed to UV light for 30 min before use. Only DNA and RNA-free water (Sigma-Aldrich, St. Louis, MO, USA) was used to prepare PCR reagent stock solutions and reaction mixtures. To amplify the 16S rRNA encoding gene fragments of cyanobacteria, The DNA dilutions were used as template DNA in 100 μl of PCR reactions using the universal primer 359F-GC and an equimolar mixture of the reverse primers 781R(a) and 781R(b), and PCR conditions as described by Nübel *et al*. (1997). Primer pairs 341F + GC (Schäfer and Muyzer, 2001)/GSB822R (Overmann *et al*., 1999), and Chr986F (Overmann *et al*., 1999)/1392R + GC (Schäfer and Muyzer, 2001) were specific for anoxygenic phototrophic proteobacteria belonging to genera of Chromatiaceae (i.e. the PSB) and for the members of the Chlorobi (GSB) respectively. The PCR amplification was carried out as described by Overmann *et al*. (1999). The primers CCR-344-F and CCR-1338-R + GC were used to amplify 16S rRNA gene encoding sequences affiliated to filamentous phototrophic bacteria within the kingdom of 'green non-sulfur bacteria' or '*Chloroflexus* relatives' (Nübel *et al*., 2001). This PCR was carried out with a denaturation step of 2 min at 94°C, followed by 30 cycles of denaturation for 45 s at 94°C, annealing for 45 s at 60°C, and extension for 4 s at 72°C, followed by a final extension step of 7 min at 72°C. All amplification reactions were performed in a T1 Thermocycler (Biometra, Westburg, the Netherlands).

The primers 8F and 1492R (Amann *et al*., 1995) were used to amplify 16S-rRNA gene sequences from the cyanobacterial isolates, resulting in a product of approximately 1485 bp. The PCR amplification cycle was as follows: initial denaturation for 2 min at 95°C, then 34 cycles of 1 min of denaturation at 95°C, 1 min annealing at 45°C, and 1 min extension at 72°C followed by a final extension of 7 min at 72°C.

Denaturing gradient gel electrophoresis

Denaturing gradient gel electrophoresis (DGGE) was performed as described by Schäfer and Muyzer (2001). Briefly, one mm thick 6% acrylamide gels with a urea-formamide (UF) gradient of 20–70% were used for cyanobacterial 16S rRNA gene fragments. Gradients of 20–80% were used for 16S rRNA gene fragments of GSB and PSB. Gradients of 30–80% UF were used for *Chloroflexus* relatives.

Gels were run for 16 h at 100 V and at a constant temperature of 60°C. The gels with *Chloroflexus* amplicons were run for 20 h at 72 V and at 60°C. Gels were stained in an ethidium bromide solution and analysed and photographed using the GelDoc UV Transilluminator (Bio-Rad, Hercules, CA, USA). The dominant bands were excised from the DGGE gels with a sterile surgical scalpel. Each gel slice was placed in 15 μl of sterile water for 24 h at 4°C. Subsequently, the solution was used as template DNA for re-amplification as described above. The PCR products were again subjected to DGGE analysis to confirm the purity and their position relative to the bands from which they were originally excised. The PCR products were purified using the QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany).

The purified PCR products were sequenced by a commercial company (BaseClear, Leiden, the Netherlands), using the appropriate specific forward primers without GC clamp.

Comparative sequence analysis

Partial 16S rRNA gene sequences were first compared with the sequences stored in the GenBank nucleotide database using the BLAST algorithm (Altschul *et al*., 1990) in order to obtain a first identification of the mat community members. Subsequently, the sequences were imported into the ARB SSU rRNA database (available at http://www.arb-home.de) (Ludwig *et al*., 2004) and aligned based on the secondary structure of the small subunit (SSU) rRNA. The dissimilarity values were used to calculate distance matrices. Distance matrix trees were generated by the Neighbour-Joining (NJ) method with the Felsenstein correction as implemented in the PAUP 4.0B software (Sinauer, Sunderland, MA, USA). The NJ calculation was subjected to bootstrap analysis (1000 replicates). The partial 16S rRNA gene sequences were deposited in the GenBank database. The sequences were deposited under Accession numbers: DQ430942 to DQ431006.

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