

Phylogenetic diversity of picocyanobacteria in Arctic and Antarctic ecosystems

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ABSTRACT

Oxyphotobacteria ascribed to the genera *Synechococcus* and *Synechocystis* are widely distributed in Arctic and Antarctic lakes, yet they are poorly represented in the marine polar environment. Arctic lakes contain phycoerythrin-rich as well as phycocyanin-rich picoplankton, implying a level of genetic diversity within this group that is poorly resolved by classic taxonomic criteria. Our culture studies of isolates from ponds on Bylot Island in the Canadian Arctic (lat. 74 °N, long. 135 °W) show that strains with similar pigment and morphological characteristics differ genetically in terms of their growth versus irradiance parameters. Application of 16S rDNA analysis to these picocyanobacterial isolates shows that they are most closely related to "*Microcystis elabens*" (98-99% similarity), and more distantly related to *Synechococcus* sp. PCC6301 (95%), *Prochlorococcus marinus* (93%) and *Synechocystis* sp. PCC6803 (90%). Picocyanobacteria have been observed among the water column dominants in a variety of Antarctic lakes, for example in the deep Chl a maximum of Lake Vanda (lat. 78 °S, long. 105 °E). In the saline lakes of the Vestfold Hills region (lat. 68 °S, long. 100 °E) their concentrations attain up to 8×10^6 cells/ml. Analysis of the 16S rDNA of phycoerythrin-rich isolates from lakes in this region show that they form a distinct cluster, most closely related (97% similar) to *P. marinus*.

Introduction

There is a striking dichotomy in the distribution of cyanobacteria among environments of the polar regions. In the coastal and offshore waters of the Arctic Ocean and the Southern Ocean, cyanobacteria are typically present in only low abundance, or are conspicuously absent. In marked contrast, many of the microbial communities in non-marine ecosystems of the Arctic and Antarctic are dominated by cyanobacteria, with populations that are sometimes amongst the highest recorded in any natural environment.

Previous work in the polar regions has tended to emphasize filamentous species such as *Nostoc* and oscillatorians. These organisms often produce conspicuous crusts, mats and biofilms, up to several mm or cm thick on a great variety of substrates including snow, ice, soil, rock, and the benthic substrata of streams, ponds and lakes [17]. By comparison, coccoid and rod-shaped species have received relatively little attention. Cyanobacteria with maximum dimensions less than 2 μm are referred to as picocyanobacteria, and are generally identified as *Synechococcus*, although many of these may be members of the genus *Cyanobium* [5]. In non-marine environments, several other genera fall within

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this size range including *Synechocystis*, *Chroococciopsis* and cells detached from colonies such as *Microcystis* and *Aphanothece*.

In this article we first briefly review the distribution of picocyanobacteria in marine and non-marine habitats of the polar regions. We then present new information on the biodiversity of this group, firstly in terms of their pigment, photosynthesis and growth characteristics, and then by way of phylogenetic analyses based on 16S rDNA sequencing.

Picocyanobacteria in the polar oceans

In both the north as well as south polar regions of the ocean there is a general trend of decreasing concentration and proportional abundance of picocyanobacteria with increasing latitude. In a detailed series of transects between Australia and East Antarctica, Marchant *et al.* [7] showed there was a close inverse correlation between log cell concentration of *Synechococcus* and latitude south. A similar trend of decreasing abundance of phycoerythrin-containing cells with increasing latitude has been observed in the North Atlantic [9] and the Greenland Sea [6].

Many studies have drawn attention to the conspicuously low concentrations of picocyanobacteria at the highest latitudes. For example, epifluorescence analyses showed that *Synechococcus* was absent from communities in the Ross Sea despite the abundance of small (2-3 μm) eukaryotic picoplankton which contributed up to 35% of the total planktonic Chl *a* [14]. Picocyanobacteria were also absent from samples from the Southern Ocean on the other side of the continent, in the Weddell Sea region [4]. In a comparison of Arctic sites, PE-rich cyanobacteria averaged 3.1×10^3 cells/ml in Resolute Passage, 70 cells/ml in the Northeast Water Polynya and 20 cells/ml in Hudson Bay [13]. These values lie at the lower end or below the usual range for offshore temperate waters of 10^3 to 5×10^5 cells/ml [19].

Moderate concentrations of picocyanobacteria have sometimes been observed at some freshwater-influenced sites in the Arctic Ocean. For example, flow cytometric analysis of samples from the Lara Sea revealed the presence of picophytoplankton at concentrations from 10^3 to 5×10^4 cells/ml containing a mixture of *Synechococcus* and eukaryotic cells [8]. Picocyanobacteria were recorded by Gradinger & Lenz [3] in the Greenland Sea up to 5.5×10^3 cells/ml in Arctic Intermediate Water; however, they were virtually absent from water collected inside the central Arctic basin, leading these authors to conclude that picocyanobacteria have little impact on the pelagic carbon and energy flow of the Arctic Ocean.

Non-marine environments

Picocyanobacteria have been recorded in many high latitude lakes in the northern and southern hemispheres. In subarctic lakes, Chl *a* in the $< 2 \mu\text{m}$ fraction accounts for 20-80 % of the total planktonic Chl *a* and is dominated by *Synechococcus*-like cells [1]. Similarly, in the oligotrophic lakes in the high Arctic, picocyanobacteria are the numerically most abundant components of the phytoplankton; for example in the thermokarst lakes in the tundra on Bylot Island [15,16], the polar desert lakes in the vicinity of Resolute Bay (e.g., 5×10^3 cells/ml in Char Lake, mid August 1994 [Vincent and Quesada, unpublished]), and in Lake Hazen, a deep ice-covered lake on Ellesmere Island (10^3 - 10^4 cells/ml, mid-August [Vincent and Quesada, unpublished]).

Picocyanobacteria have been observed among the water column dominants in a variety of Antarctic lakes, for example in the deep Chl *a* maximum of Lake Vanda (McMurdo Dry Valleys, lat. 78 °S, long. 105 °E), although there appears to be considerable year-to-year variability in their proportional abundance in lakes of the Dry Valley region and in the maritime zone, for example in the lakes on Signy Island [18].

In the saline lakes of the Vestfold Hills region of East Antarctica (lat. 68 °S, long. 100 °E) phycoerythrin-rich picocyanobacteria achieve extremely high concentrations, up to 8×10^6 cells/ml, that render the lake water samples pink in colour [11,12]. The populations increase in abundance over the period August-December, and then decline through late summer and winter possibly as a result of microzooplankton grazing. Highest concentrations occur at the depth of the chemocline, towards the bottom of the euphotic zone.

Genetic diversity

The relative cell concentrations of phycocyanin (PC) and phycoerythrin (PE) in the high latitude picocyanobacteria provides the first indication that there is considerable genetic diversity within this group. Samples from a subarctic lake and river contained a mixture of PE-rich and PC-rich cells, as distinguished by their emission characteristics under fluorescence microscopy, with dominance by the PC-rich strains [10]. The picocyanobacteria brought into culture from Bylot Island ponds were also PC-rich (Table 1, details of isolation and culture techniques are given in ref [16]) while high Arctic lakes were primarily dominated by PE-rich forms. The oceanic communities examined to date in

Table 1. Cellular characteristics of two picocyanobacterial isolates from ponds on Bylot Island in the Canadian high Arctic. E_{opt} is the irradiance at which maximum growth rates (μ_{max}) were measured. The pigment data are values for cultures grown in BG-11 liquid culture medium under dim ($20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) cool white fluorescent light and are expressed in terms of fg pigment per cell. Based on the data given in [15] and [16].

Characteristic	Picocyanobacterial isolate	
	P211	P214
General		
Fluorescence	Phycocyanin	Phycocyanin
Growth form in culture	Solitary cells	Loose flocs
Growth parameters		
μ_{max} (d^{-1})	0.41	0.61
Maximum generation time (d)	1.7	1.1
E_{opt} ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)	60	200
Pigment characteristics		
C-phycocyanin	21	54
Allophycocyanin	12	19
Chlorophyll <i>a</i>	14	20
Carotenoids	4	6

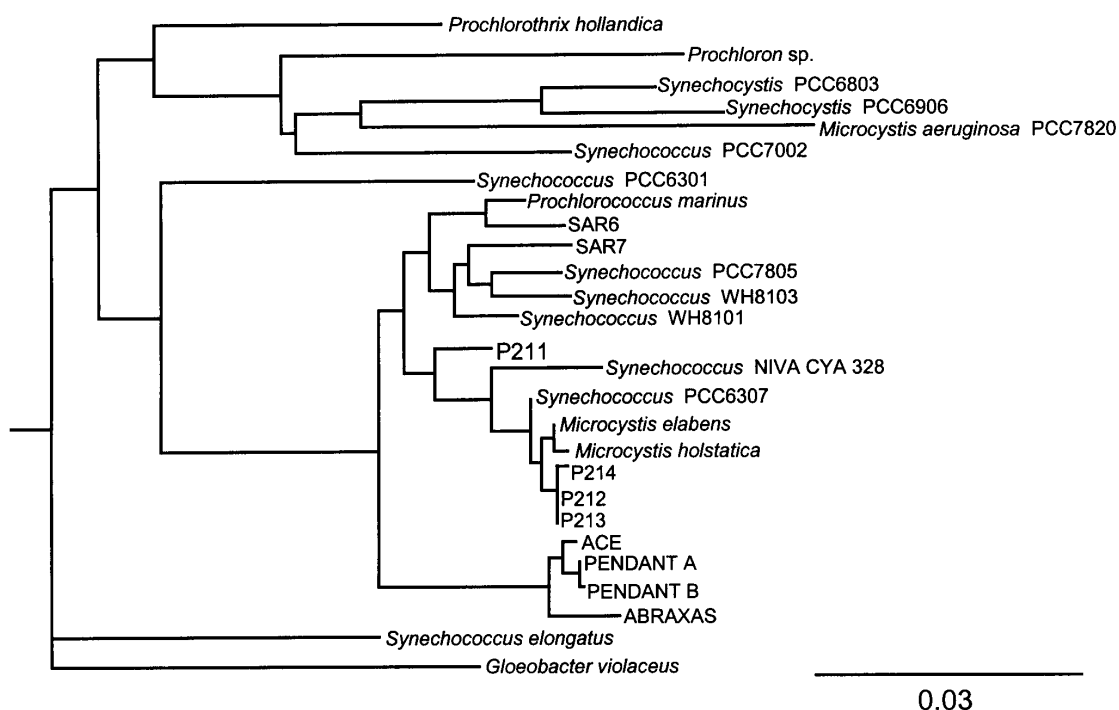


Fig. 1. 16S rRNA phylogenetic tree showing the position of the Arctic (P211, P212, P213 and P214) and Antarctic (ACE, PENDANT A/B and ABRAXAS) isolates of picocyanobacteria. Near complete 16S rDNA sequences were obtained using previously described methods [2]. The sequences were aligned manually to the Ribosomal Database alignment and thus secondary structure has been accounted for. Trees were generated using Maximum Likelihood and Neighbor-Joining methods and bootstrap analysis indicated that the branching of the isolates was robust for their given groups. The outgroup was *Gloeobacter violaceus*. The new sequences have been deposited in the GenBank database under accession numbers: AF098370 (ACE); AF098371 (PENDANT A); AF098372 (ABRAXAS); AF098373 (P211); and AF098374 (P212; the sequences for P213 and P214 were almost identical). The bar equals a sequence dissimilarity of 3%.

the polar regions, and also the isolates from Vestfold Hill lakes, are mostly PE-rich cells [6, 11, 12], although PC-rich strains co-dominated in the brackish waters of Hudson Bay [13].

A comparison of the growth and pigment characteristics of two PC-rich isolates from the Bylot Island lakes further indicates the degree of genetic variability between strains (Table 1). P214 differed considerably from P211 by having a 50% higher maximum growth rate, a threefold higher light requirement for maximum growth, and up to two-fold greater pigment concentrations per cell when grown under dim, light-limiting conditions.

The genetic diversity expressed in terms of pigments and physiological characteristics is further supported by 16S rRNA analysis (Fig. 1). For this analysis we sequenced P211, P214 and two additional isolates (P212, P213) from the Bylot Island lakes (Arctic) and four isolates from saline lakes in the Vestfold Hills (Pendant, Ace and Abraxas Lakes). The Arctic strains appeared to be most closely related to "*Microcystis elabens*" (98-99% similarity), and more distantly related to *Synechococcus* sp. PCC 6301 (95%), *Prochlorococcus marinus* (93%) and *Synechocystis* sp. PCC 6803 (90%). It is interesting that *M. elabens* is now reclassified as a species of *Aphanothece* in the same family as *Synechococcus* [5]. All of these strains differ greatly from those obtained from the Vestfold

Hill lakes which have a greater affinity to marine species. Analysis of the 16S rRNA genes of the Antarctic isolates show that they are closely related (96% similar) to *P. marinus*, but form a distinct cluster relative to all other known picocyanobacteria.

Conclusions

These preliminary studies imply a high level of genetic diversity within the picocyanobacteria at high latitudes. These micro-organisms can reach extremely high population densities in certain lake environments, but are poorly represented in the Arctic Ocean and Southern Ocean. The 16S rDNA analyses presented here underscore the diversity within this group, and the need for more detailed circumpolar and bipolar comparisons, including analysis of the dilute marine communities.

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