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Vertical distribution of bacteria in arctic sea ice

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1. SUMMARY

Heterotrophic bacteria were enumerated in north polar sea ice cores obtained near Point Barrow, Alaska. Highest concentrations of total and viable bacteria were found in the layer containing the sea ice microbial community identified by the maximum chlorophyll *a* content. Gas vacuolate bacteria were also found in the sea ice, a discovery which is consistent with their recent report from antarctic sea ice microbial communities. The gas vacuolate bacteria comprised 0.2% or less of the viable bacteria isolated from sea ice cores, lower than concentrations reported for most antarctic samples. Most gas vacuolate isolates from the sea ice cores were pigmented pink, orange, or yellow. An ice core from nearby saline Elson Lagoon contained an inverted sea ice microbial community with highest chlorophyll *a* concentrations and bacterial counts found in the top 0–20 cm of the ice. This surface layer also contained high numbers (up to 186 bacteria/ml) of a nonpigmented, gas vacuolate, elongated rod-shaped bacterium.

2. INTRODUCTION

Sea ice microbial communities (SIMCO) are important microbial communities in polar areas of the earth inasmuch as some 12% of the earth's surface is currently layered by sea ice on a seasonal basis [1]. The SIMCO is dominated by algae whose activities contribute significantly to the primary production in polar habitats. As a result most of the research on SIMCOs has concentrated on the algae and much of that work has been conducted in Antarctica. In this paper we report that, consistent with studies in antarctic sea ice, heterotrophic bacteria are found in the SIMCO of the arctic, and furthermore, some of these bacteria comprise an interesting new group of gas vacuolate bacteria.

Gas vacuolate prokaryotes, including representatives of the eubacteria, cyanobacteria, and archeabacteria, are usually found in the water

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column or sediments of aquatic environments. Gas vesicles, the intracellular subunits of a gas vacuole, are hollow, gas-containing, proteinaceous structures. They reduce cell density and thereby increase the buoyancy of the organism allowing it to float at a favorable depth in the water column. Therefore, gas vesicles are especially useful to organisms that live in freshwater or saline habitats which have vertical photic, thermal, or chemical stratification [2]. Marine environments have not been known to contain gas vacuolate prokaryotes, with the exception of cyanobacteria in the genus *Trichodesmium* [3].

Recently, however, extremely psychrophilic, pigmented, gas vacuolate bacteria of unknown classification were discovered in the sea ice of Antarctica [4,5] living in close association with the dense bottom type SIMCO. This SIMCO is a community of algae, protozoa, and prokaryotes that grows in microscopic brine pockets in the lower 20 cm of the ice. In the brine pockets temperatures typically range from 0°C to −2°C, salinities from 1 to 5 times normal sea water concentration, and light intensities are 0.02% to 0.8% of surface irradiance [6]. It has been proposed that algae are the primary producers, and heterotrophic bacteria the secondary producers in this community [7].

The discovery of psychrophilic gas vacuolate bacteria in the SIMCO of antarctic sea ice led us to examine north polar sea ice for gas vacuolate species. This paper reports similar gas vacuolate bacteria in the sea ice off Point Barrow, Alaska.

3. MATERIALS AND METHODS

Ice cores and underlying water samples were collected from near Point Barrow, Alaska in mid May of 1991 (Fig. 1). All sites were relatively free of snow cover (less than 2 cm), and all ice cores were 1.7–1.85 m thick. Sites 1, 3, and 4 (open water sites) were taken from the Chukchi Sea ice several hundred meters offshore in approximately 4 m of underlying water. The lower layer of ice containing the SIMCO is often soft and frazzled and can be partially lost in retrieving the ice cores which may explain some of the variability in the

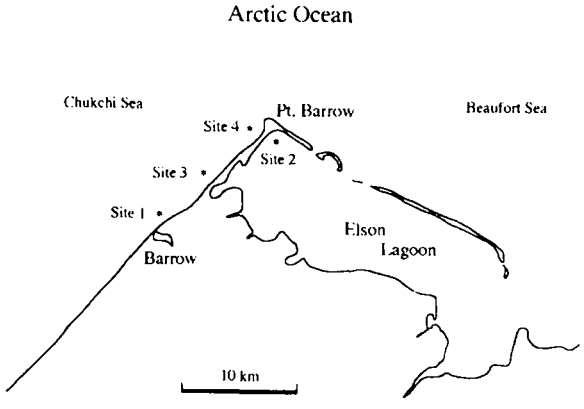

Fig. 1. Map of Point Barrow vicinity showing the three open water sites (sites 1, 3 and 4) in the Chukchi Sea and the Elson Lagoon site (site 2).

measurements. The core from Elson Lagoon had zones with sand and grit presumably derived from either wind-blown debris from land or from sediments of dislodged anchor ice. This site was shallow and frozen to within 15 cm of the bottom of the lagoon.

Ice cores were collected using a modified 14 cm ($5\frac{1}{2}$″) diameter SIPRE Corer (USA Cold Regions Research and Engineering Laboratory, Hanover, NH). Water samples were also taken at 0, 2, and 4 m (or at the sea floor) beneath the ice with a van Dorn bottle rinsed with 95% ethanol. The ice cores were sectioned into 10, 12 or 20 cm sections with a clean ice saw and thawed at room temperature in sterile plastic bags. Aliquots of melted ice or sea water were removed for salinity measurements, chlorophyll *a* measurements, acridine orange direct counts (AODC), and viable cell counts.

Salinity was measured with a Beckman Industrial Solu Bridge. For chlorophyll *a* measurements, 50–500 ml aliquots were filtered through 47 mm Whatman GF/F glass fiber filters (Whatman, Inc., Clifton, NJ) and stored at −20°C in polypropylene vials in the dark. They were extracted with 90% acetone and analyzed with a Turner Model 112 Fluorometer within 5 weeks of collection [7]. AODCs were determined by the method of Herwig et al. [8]. Viable counts were obtained by triplicate plating 0.1–1.0 ml aliquots

Fig. 2. (**A**) Phase photomicrograph of a non-pigmented rod-shaped gas vacuolate bacterium isolated from Elson Lagoon. Bright areas within cells are gas vacuoles. Bar = 5 μm. (**B**) Electron micrograph of a non-pigmented rod-shaped gas vacuolate bacterium from Elson Lagoon showing the gas vesicles in the cell. Bar = 1 μm.

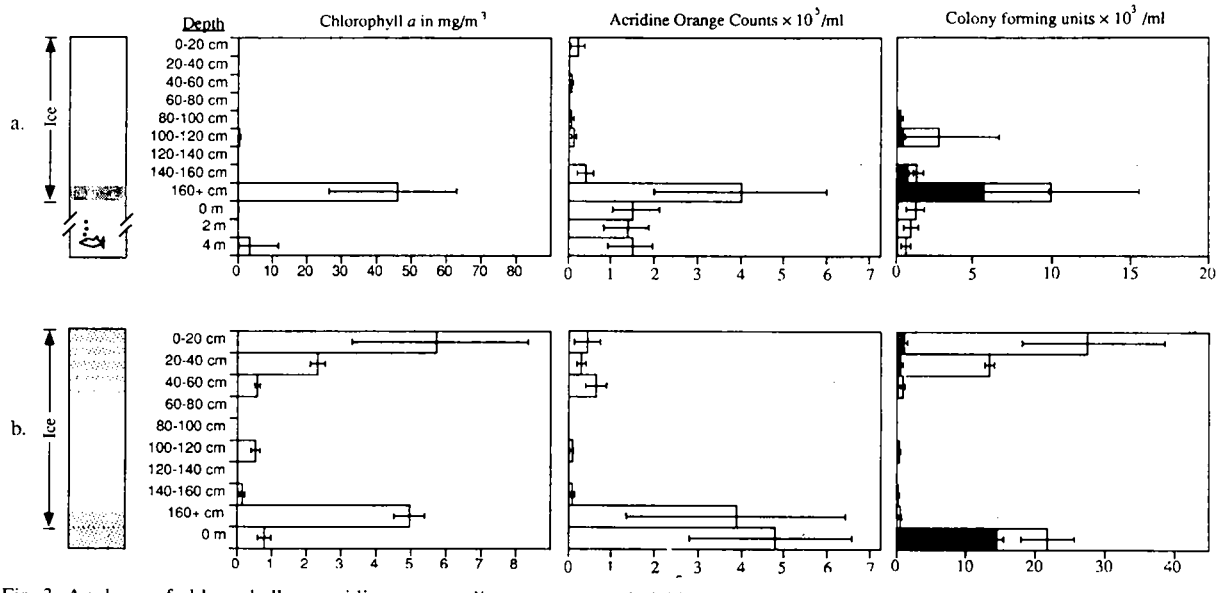

Fig. 3. Analyses of chlorophyll *a*, acridine orange direct counts, and viable counts of bacteria from the ice core and water column samples from the arctic. (**A**) Open water sites (sites 1, 3, and 4) with a diagram showing a cross section of the ice and water column, stippling indicates the sea ice microbial community (SIMCO) layer. Shaded areas in viable count column indicate concentrations of pigmented bacteria. (**B**) The Elson Lagoon (site 2) has the same legend except that stippled areas in the cross-section indicate grit. Error bars indicate 1 standard deviation.

on Ordal's modified sea water cytophaga medium (SWC) [5] at approx. 5°C (pH adjusted to 7.6 with 0.5 M Tris Base (Sigma Chemical Co., St. Louis MO.)). SWC medium is a dilute medium containing beef extract, yeast extract, and tryptone in half strength artificial sea water with succinate as the principal carbon source. Colonies were counted after 8 weeks incubation in the dark. Colonies of gas vacuolate bacteria often have a chalky, translucent appearance. All such colonies were tallied, picked and streaked for isolation. Under the phase contrast microscope cells containing gas vacuoles appear highly refractile (Fig. 2A). Final confirmation of gas vesicles in presumptive gas vacuolate strains was made by electron microscopy (Fig. 2B). Electron micrographs were obtained of unstained cells using a JEOL-100B transmision electron microscope at 60 kV.

4. RESULTS AND DISCUSSION

The highest concentrations of chlorophyll *a* in the three open water sites were found in the bottom 4 cm layer of ice (Fig. 3A). This corresponded to the visually apparent reddish-brown band of primary producers in the SIMCO layer of these ice cores. The highest acridine orange counts, averaging $4 \pm 2 \times 10^5$ counts/ml, and the greatest viable counts, averaging $1.0 \pm 0.7 \times 10^4$ bacteria/ml, were also found in this layer. For these open water sites the viable count ranged from 0.05% to 5% of the AODC, typical of results obtained from the antarctic sea ice samples [4].

The Elson Lagoon site differed from the open water sites in several ways. First, the underlying water had a higher salinity (50‰) than the open water sites (35‰). Second, the ice extended to within 15 cm of the lagoon floor, and underlying water samples that were collected were brownish in color due to suspended particulate material. Finally, areas at the top, within, and at the bottom of the ice core as well as in the underlying water column were discolored with wind-blown grit or sediments. Not surprisingly this site showed different profiles for salinity, chlorophyll *a*, total AODC and viable counts of bacteria compared to the three open water sites.

In contrast to the open water samples, the highest concentrations of chlorophyll *a* were found in the surface layers of the ice (6 mg/m^3 in the 0–20 cm layer) of Elson Lagoon (Fig. 3B). These values, however, were lower than the

Table 1

Comparison of phaeophytin versus chlorophyll *a* concentrations (mg/m^3) in the ice core and water column samples

Depth	Open water sites [a]			Elson Lagoon		
	Phaeophytin	Chlorophyll *a*	Ratio [b]	Phaeophytin	Chlorophyll *a*	Ratio
0–20 cm	0.03	0.1	0.3	3	6	0.5
20–40 cm	– [c]	–	–	1	2	0.5
40–60 cm	0.02	0.08	0.3	0.3	0.6	0.5
60–80 cm	–	–	–	–	–	–
80–100 cm	0.04	0.1	0.4	–	–	–
100–120 cm	0.2	0.7	0.3	0.1	0.6	0.2
120–140 cm	–	–	–	–	–	–
140–160 cm	0.1	0.2	0.5	0.08	0.2	0.4
160+ cm	10	50	0.2	5	5	1
0 m [d]	0.2	0.2	1	5	0.8	6
2 m	0.07	0.05	1	–	–	–
4 m	1	4	0.3	–	–	–

[a] The values for phaeophytin and chlorophyll *a* for the three open water sites (1, 3, 4) are given by the average of the results from these three sites.

[b] Ratio is the ratio of phaeophytin to chlorophyll *a*.

[c] – indicates that no data were obtained.

[d] Depth below the bottom of the ice.

SIMCO values for the open water sites (46 mg/m^3). High total and viable bacterial counts were also found in the surface layer of the ice. But, unlike the open water samples, viable counts from the surface layer of the lagoon ($2.8 \pm 1.0 \times 10^4$ bacteria/ml) were more than double that of the SIMCO from the open water samples ($1.0 \pm 0.7 \times 10^4$ bacteria/ml) and comprised about 70% ($4.3 \pm 2.7 \times 10^4$ counts/ml) of the lagoon surface AODC (Fig. 3B). The high viable counts coupled with their high proportion to total counts suggests enriched organic conditions in the surface ice [9] perhaps due to the large amounts of grit blown in from the nearby land or to sediment-loaded anchor ice that is found in these layers. The high viable bacterial count in the upper 160 cm of the ice core also correlates ($r^2 = 0.98$) with the relatively high levels of chlorophyll *a* at this site. However, it seems unlikely that algal excretion alone could account for the increased numbers of bacteria because the concentration of chlorophyll *a* in the surface layers (4.0 ± 2.5 mg/m^3 in the top 40 cm) of the Elson Lagoon core was only one tenth that of the SIMCO layer of the open water cores (46 ± 18 mg/m^3).

High concentrations of chlorophyll *a* were also found in the water column below the lagoon sea ice. However, the proportion of phaeophytin to chlorophyll *a* was very high in this sample (Table 1). This suggests that many of the algae beneath the lagoon ice were physiologically inactive or dead, perhaps due to the low levels of light that were available during the winter.

Of the approximately 100,000 colonies that were visually screened for gas vacuolate bacteria, 177 were confirmed as positive by phase microscopy. It was not possible to discern gas vacuolate bacteria directly from AODC. Table 2 shows that most (165 out of 177) of the gas vacuolate bacteria were found in the Elson Lagoon ice sample. Furthermore, most (160 of 165) of the Elson Lagoon isolates were similar looking long (5–15 μm $\times$1–2 μm), highly gas vacuolate rods which form white, raised colonies on SWC. The open water isolates, in contrast, accounted for only a small proportion (12 of 177) of the gas vacuolate isolates, and most were pigmented pink, orange, or yellow. Finally, only one gas vacuolate bacterium was found in the SIMCO layer. The others were found mostly in the ice above the SIMCO layer, or in the water column below the SIMCO layer.

Table 2

Distribution of gas vacuolate (G.V.) bacteria isolated from ice core and water samples from the Arctic

Depth	Open water sites		Elson Lagoon	
	Total G.V.	% of viable [a]	Total G.V.	% of viable
0–20 cm	0	0	32	0.4
20–40 cm	– [b]	-	112	1
40–60 cm	5	1	12	0.3
60–80 cm	–	–	–	–
80–100 cm	3	1	–	–
100–120 cm	2	0.03	1	0.09
120–140 cm	–	–	–	–
140–160 cm	0	0	7	0.7
160+ cm	0	0	1	0.06
0 m [c]	1	0.08	0	0
2 m	0	0	–	–
4 m	1	0.1	–	–

[a] The values for the % of viable cfus from the open water sites (1, 3, 4) are given by the average of the results from these three sites.

[b] – indicates that no data were obtained.

[c] Depth below the bottom of the ice.

The arctic sites contained low populations of viable gas vacuolate bacteria. Gas vacuolate bacteria accounted for no more than 1% (Table 2) of the viable colony forming units (cfus). In contrast, some of the antarctic samples contained 91% gas vacuolate cfus [4]. Most of our arctic isolates were obtained from the surface layers of Elson Lagoon, and unlike most antarctic isolates, they were not pigmented.

As in Antarctica [4], pigmented prokaryotes comprised the most numerous viable species in the lower ice layers (see Fig. 3). Orange, yellow, and red pigmented colonies made up approximately 60% of the the total cfus in the lowest layers of the open water site ice cores. In contrast, an average of only 10% of the colony forming units from the open water water column were pigmented. It is not understood why there is a higher concentration of pigmented bacteria in the SIMCO, but it has been suggested that pigmenta-

tion may play a role in protecting bacteria from ultraviolet radiation [10].

An interesting result of this study was that gas vacuolate bacteria were rarely found in or near the SIMCO layer of the arctic sea ice (Table 2). In Antarctica most gas vacuolate bacteria were found either in or near the SIMCO layer although some were found in the underlying water column [4]. Additional research is needed to assess what niche the gas vacuolate bacteria occupy and to determine the role of gas vacoules in polar environments. A priori it is difficult to understand why gas vacuoles might be useful to bacteria living in the solid ice matrix. However, it is possible that the gas vacuoles are not needed or synthesized by these bacteria during the period in which they are living in the sea ice, but are produced by them for the summer dispersal period when the annual sea ice has melted.

One of the long term objectives of our work is to determine whether any species of these sea ice bacteria are endemic in their geographic distribution. We are not aware of free-living prokaryotes endemic to specific locales on earth. There are, however, many examples of pathogens or symbionts whose distribution is dependent upon their hosts or symbiotic partners. Sea ice bacteria may be one group of prokaryotes ideally suited to the study of the biogeography of free-living prokaryotes. Many gas vacuolate sea ice bacteria isolated from Antarctica are extreme psychrophiles [5]. It seems likely that those from the north polar SIMCO are also psychrophilic. If so, it would seem that the tropical equator and the 11,000 km span of open ocean comprises a formidable barrier to dispersal or population exchange of these psychrophiles. By examining bacteria from the two polar regions we propose to address the question of whether these polar species are endemic or cosmopolitan.

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