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Gas Vacuolate Bacteria from the Sea Ice of Antarctica

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Gas-vacuolate heterotrophic bacteria from marine habitats are reported here for the first time. They have been isolated from Antarctic sea ice microbial communities and the underlying water column. The predominant gas-vacuolate bacterium from the sea ice is filamentous and pigmented, whereas those of the water column are unicellular and nonpigmented. The highest concentrations of bacteria in sea ice were found in conjunction with the highest algal (chlorophyll *a*) concentrations.

Gas vacuoles are produced by many freshwater procaryotic organisms. These structures are composed of small subunits, called gas vesicles, which are bound by a thin protein membrane containing the gas (17). Gas vesicles reduce cell density, thereby enabling organisms which possess them to float in a water column (9). Freshwater habitats, which are often vertically stratified, may contain different species of gas-vacuolate procaryotes at various depths, each species regulating the formation of its gas vacuoles to enable it to reside at a depth favorable for growth (10, 17).

The discovery of marine gas-vacuolate bacteria from Antarctica was made during the enumeration of chitinolytic bacteria in the water column near fast ice in the vicinity of the U.S. Palmer Station (64°S, 64°W) during the austral summer of 1986 to 1987 (4). It was hypothesized that their unique occurrence in Antarctica might be due to their association with the sea ice microbial community which develops each summer season in polar environments. However, by the time the colonies were discovered on the enumeration medium it was too late to conduct further experiments because of the deterioration of sea ice conditions that field season. Therefore, plans were made to sample for gas-vacuolate bacteria during the next austral summer. McMurdo Sound near the U.S. McMurdo Station (78°S, 167°E) was selected as the location of this study because it has been the site of previous studies of sea ice algae and sea ice microbial communities (1, 2, 11, 14, 15).

Several ice cores were obtained from the annual fast ice during the early part of December 1987. Holes were drilled with a 10-in.-diameter (25.4-cm-diameter) Jiffy ice drill (Feldman Engineering and Manufacturing Co., Sheboygan Falls, Wis.). Three-inch-diameter (7.62-cm-diameter) ice cores were taken from partially drilled holes by using a coring device (USA–Cold Regions Research and Engineering Laboratory, Hanover, N.H.) (sometimes also called a Snow Ice and Permafrost Research Establishment corer). Water samples were collected in completed holes with a Niskin bottle that had been rinsed previously with 70% ethanol. Ice cores and water samples were handled aseptically. Samples were taken to the McMurdo laboratory in an ice chest. All samples were kept refrigerated and dark before processing. Core samples were processed in the laboratory as soon as the ice had melted. Samples to be tested for chlorophyll *a* and phaeophytin pigments were collected on glass fiber filters (GF/F; Whatman, Inc., Clifton, N.J.) and stored frozen. They were extracted with 90% acetone and analyzed with a Turner 111 Fluorometer (11) within 7 to 10 days of collection. Bacteria were plated on modified Ordal marine cytophaga agar containing succinate as a carbon source (this medium contained the following [grams per liter of distilled water]: tryptone, 0.5; yeast extract, 0.4; beef extract, 0.4; sodium succinate, 0.2; full-strength artificial seawater salts [NaCl, 24; $MgCl_2 \cdot 6H_2O$, 5.2; $MgSO_4 \cdot 7H_2O$, 7.0; $CaCl_2 \cdot 2H_2O$, 1.46; KCl, 0.7; KH_2PO_4, 0.01; iron citrate, 0.001; NH_4Cl, 0.2]; vitamin solution, 10 ml [12]; SL-6 mineral salts solution [6], 1.0 ml; and agar, 12.0; pH 7.0 before autoclaving). Plates were incubated at refrigerator temperatures and examined 6 to 8 weeks after collection. Colony types containing gas-vacuolate organisms were identified by microscopic examination of material picked from colonies. Salinities of the melted ice core and water samples were determined with a Reichert refractometer. Total microscopic bacterial concentrations were determined by acridine orange staining (4).

The first ice core was taken near Armitage Point (site 1) (Fig. 1). The ice at this site was 2.2 m thick and covered by 0.6 m of snow. Beneath the surface congelation ice was a layer of platelet ice (individual pieces of ice the shape and size of potato chips that float beneath congelation ice). There was no evidence of ice algae either in the core ice or in the platelet ice at this site (heavy snow cover prevents adequate light penetration necessary for ice algal development) (3). Thus, site 1 served as a control site for the studies. All five other sites had ice algae that could be detected macroscopically and later confirmed with chlorophyll *a* analyses. The algae occurred predominantly in the lower 10 to 20 cm of the congelation ice which was chosen as one of the sample horizons. Samples were also taken from the 20 cm directly above the ice algae section. At three sites (sites 2, 6, and 7) ice algae were found, not only in the congelation ice, but also in the underlying platelet ice. At those sites the platelet layer was also sampled (Table 1). Water samples were collected at two or three depths beneath the ice from holes drilled near the ice core sites.

As the chlorophyll *a* concentrations indicate, the highest levels of algae were found in the bottom 10 to 20 cm of the congelation ice column at most sites (Table 1). However, at two of the sites the highest chlorophyll *a* concentrations were found in platelet ice (sites 2 and 6). These results are consistent with the macroscopic appearance of the sections of the cores and platelet layers from those sites, all of which had distinctly reddish colors due to the pigments of the predominant algae.

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TABLE 1. Characteristics of McMurdo Sound ice core profiles collected 5 to 12 December 1987

Source and depth[a]	Site 1 (2.2 m thick)					Site 2 (2.2 m thick)						Site 4 (2.2 m thick)					
			Viable bacteria						Viable bacteria						Viable bacteria		
	Chl *a*[b] (μg/liter)	Phaeophytin (μg/liter)	No./ml	% GV[c]	% Pigmented	Salinity (‰)	Chl *a* (μg/liter)	Phaeophytin (μg/liter)	No./ml	% GV	% Pigmented	Salinity (‰)	Chl *a* (μg/liter)	Phaeophytin (μg/liter)	No./ml	% GV	% Pigmented
Upper ice (20 cm)	0.08	0.06	7	0	33	5	17	0.38	104	15	100	5	2.91	0.24	360	83	94
Lower ice (10–20 cm)	0.11	0.08	87	0.6	36	8	117	12.9	1,520	0	94	12	1,005	97	6,280	0.3	46
Platelet ice or water (0–1 m)	0.10 (P)[d]	0.05	41	0	58	11 (P)	2,057	253	7,065	0.3	79	35	31	4.3	120	16.7	54
Water (3–6 m)	0.04	0.03	145	0.2	17	35	0.42	0.08	64	0	55	35	0.5	0.26	58	2.8	45
Water (18–19 m)	0.05	0.02	187	0	4	35	0.24	0.06	26	1.9	50	35	1.7	0.7	47	0	17

[a] For ice depth, this is the distance above the ice-water interface; thus, upper ice (20 cm) refers to a depth either 10 to 30 or 20 to 40 cm above the ice-water interface, whereas lower ice (10 to 20 cm) refers to the distance 0 to 10 or 0 to 20 cm directly above the ice-water interface. Depths in water column are measured from the ice-water interface downward.
[b] Chl *a*, Chlorophyll *a*.
[c] Percent gas-vacuolate (GV) bacteria.
[d] P, Platelet layer.

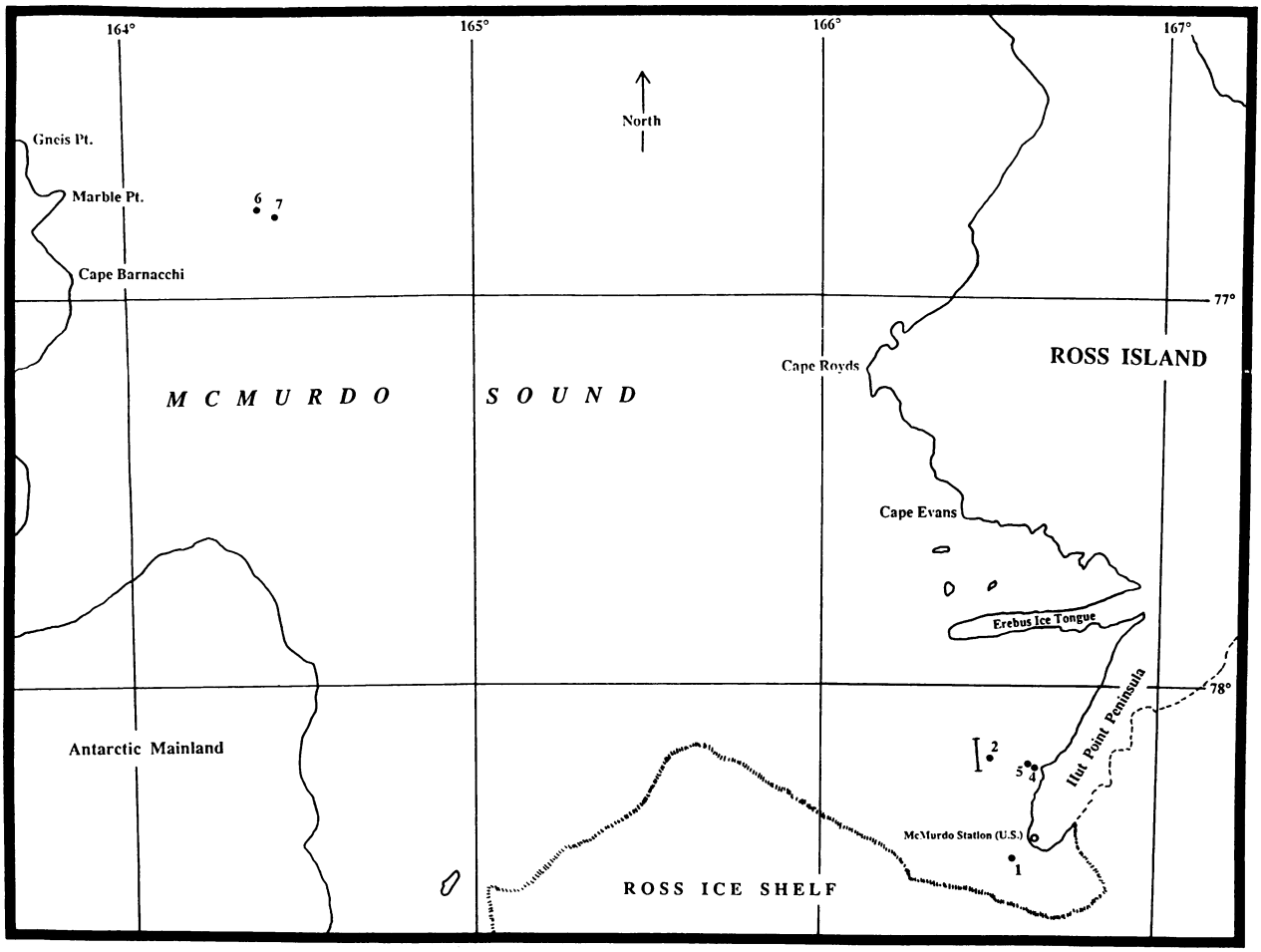

FIG. 1. Map showing McMurdo Sound, Antarctica. Sites where ice cores were collected are numbered. Line near site 2 represents the temporary ice runway during the austral summer of 1987 to 1988.

TABLE 1—*Continued*

Site 5 (2.2 m thick)						Site 6 (1.8 m thick)						Site 7 (1.8 m thick)					
			Viable bacteria						Viable bacteria						Viable bacteria		
Salinity (‰)	Chl *a* (μg/liter)	Phaeophytin (μg/liter)	No./ml	% GV	% Pigmented	Salinity (‰)	Chl *a* (μg/liter)	Phaeophytin (μg/liter)	No./ml	% GV	% Pigmented	Salinity (‰)	Chl *a* (μg/liter)	Phaeophytin (μg/liter)	No./ml	% GV	% Pigmented
5	7.3	0.23	466	91	91	5	4.6	0.2	650	0	100	6	5.9	0.3	121	0.5	95
5	99	2.5	87	11.5	92	8	497	32	5,170	0.06	88	8	1,454	150	6,000	5.4	94
35	0.87	0.19	30	4.2	73	12 (P)	626	12.8	10,120	0	0	10 (P)	108	12.7	2,300	9.6	59
35	0.55	0.16	14	3.6	43	35	1.5	0.6	144	0	49	35	0.9	0.3	64	0	39
35	0.30	0.09	22	0	23	35	0.3	0.19	30	0.18	43	35	0.6	0.3	47	1.1	20

The highest bacterial concentrations (both total microscopic counts as well as viable counts) were found in conjunction with the highest chlorophyll *a* concentrations (Table 1 and Fig. 2). The highest bacterial levels were either in the lower 10 to 20 cm of the congelation ice or, again at sites 2 and 6 (which had platelet blooms), in the platelet layer. The single exception to this was a site 5, where the highest concentrations of viable bacteria were found in the upper ice layer. The strong correlation between total ($R^2 = 0.93$) and viable ($R^2 = 0.57$) bacterial counts and chlorophyll *a* concentration (Fig. 2) clearly indicates there is a positive relationship between the numbers of bacteria and the concentration of ice algae (16). It is also interesting to note the approximately 100× difference between total and viable bacteria. This low percent recovery of heterotrophic bacteria is typical of ecosystems which are oligotrophic or mesotrophic (13). The bacteria are probably either growing heterotrophically from the released photosynthate of this community or utilizing the organic remnants of dead and dying algae.

One of the most striking results of this investigation was the high percentage of pigmented bacteria from the congelation ice samples. From 92 to 100% of the viable bacteria from at least one ice depth at each site were pigmented orange, red, or yellow (Table 1). In contrast, only 4 to 55% of those from the water column were pigmented. Previous studies have reported high percentages of pigmented bacteria from arctic sea water samples (7, 8). Despite their pigmentation, these bacteria are not obligately photosynthetic, since they were incubated completely in the dark.

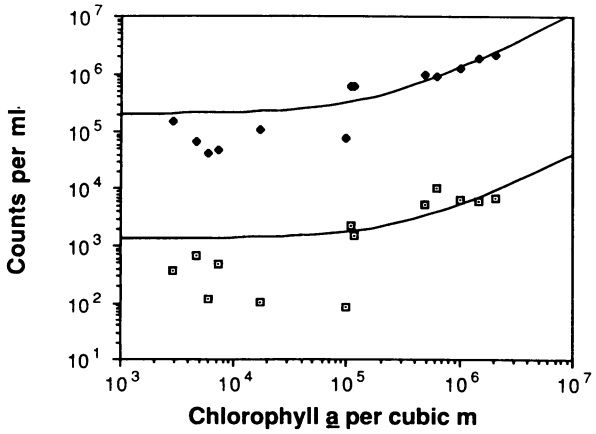

FIG. 2. Log-log plots of viable (⊡) and acridine orange (◆) counts per milliliter versus chlorophyll *a* concentration (milligrams per cubic meter) for sea ice samples collected at sites 2, 4, 5, 6, and 7. Curved lines through data points are linear regression lines.

Some of the pigmented bacteria from the congelation ice were gas vacuolate. Indeed, at sites 4 and 5, located between McMurdo Station and the ice runway, colonies of gas-vacuolate bacteria predominated. Their concentrations ranged from 299/ml of ice core melt water at site 4 to 424/ml at site 5, where they composed 83 and 91% of the total viable bacteria, respectively. The gas-vacuolate bacteria at these two sites consisted of only two or three colony types. All of them were pigmented a red-orange color and contained nonmotile, filamentous bacteria (Fig. 3). Figure 3a shows the typical high refractility of cells containing gas vacuoles, and Fig. 3b (arrow) shows cells with individual gas vesicle subunits. Although they did not dominate at any of the other sites, gas-vacuolate bacteria were also found in high concentrations in the upper ice at site 2 (near the ice runway) and in lower ice of site 7 and in its platelet bloom (site 7 was located on the west side of McMurdo Sound near Marble Point).

In addition to the group of filamentous gas-vacuolate bacteria commonly found in the congelation ice, other unicellular gas-vacuolate bacteria were found in the underlying platelet ice layer and water column. Most of these were nonpigmented. For example, in the platelet layer at site 2, where less than 1% of the total number of viable bacteria were gas vacuolate, none of these was pigmented. Both pigmented and nonpigmented gas-vacuolate types were observed sporadically in the water column, although the concentrations of all bacteria were greatly reduced (these bacteria could be dispersal forms or contaminants from other layers of the core or water column). Isolates of the various gas-vacuolate types are now being characterized.

The sea ice environment and its underlying water column constitute a vertically stratified environment in much the same sense that freshwater lakes are stratified thermally into a warm, surface epilimnion and a cold, deep hypolimnion. As in the stratified freshwater environments, gas-vacuolate bacteria are distributed in the surface layer (i.e., the filamentous, pigmented sea ice bacteria) as well as in the deeper

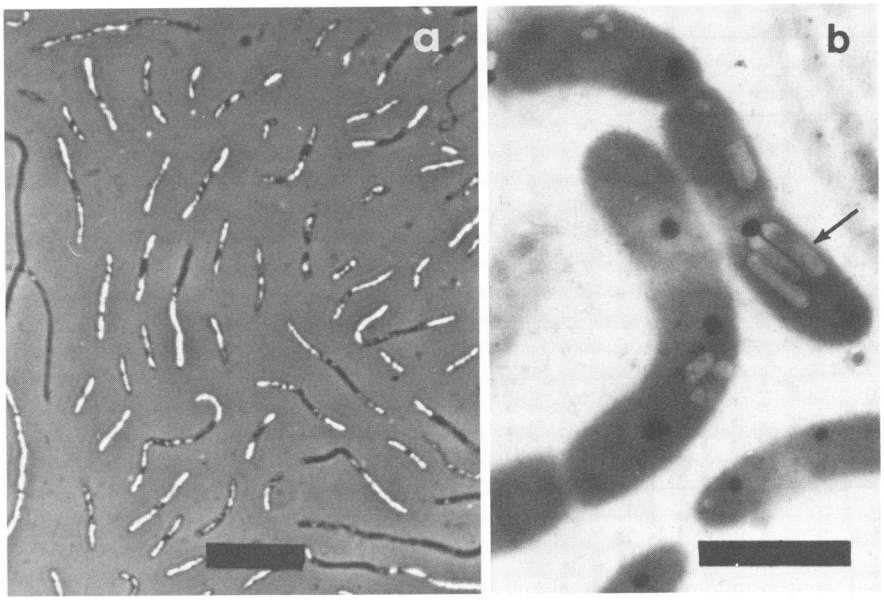

FIG. 3. Filamentous gas-vacuolate bacterium isolated from core ice at site 5 as observed by (a) phase microscopy (bar = 10 μm) and (b) transmission electron microscopy (bar = 0.5 μm). Arrow indicates cells with individual gas vesicles subunits.

layers (i.e., the nonpigmented unicellular bacteria). The sea ice community is, however, an unusual example of vertical stratification in that it consists of water in both liquid and solid states and contains an inverted temperature stratification with the colder, less dense ice floating on the warmer water.

Additional work is needed to better understand (i) the vertical distribution of these gas-vacuolate and pigmented bacteria throughout the entire ice column, not just the lower 40 cm; (ii) whether they are actually forming gas vacuoles while in the ice; and (iii) whether they are facultative phototrophs. It is now clear, however, that these gas-vacuolate bacteria make up an important, previously overlooked group. Furthermore, the results of this study suggest that gas-vacuolate bacteria may be found in other stratified marine and estuarine habitats.

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ADDENDUM IN PROOF

An in press manuscript provides preliminary characteristics of gas vacuolate bacteria isolated from Antarctic seawater (R. L. Irgens, I. Suzuki, and J. T. Staley, Curr. Microbiol., in press).

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