Phylogenetic Diversity of Numerically Important Arctic Sea-Ice Bacteria Cultured at Subzero Temperature

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A B S T R A C T

Heterotrophic bacteria in sea ice play a key role in carbon cycling, but little is known about the predominant players at the phylogenetic level. In a study of both algal bands and clear ice habitats within summertime Arctic pack ice from the Chukchi Sea, we determined the abundance of total bacteria and actively respiring cells in melted ice samples using epifluorescence microscopy and the stains 4′, 6-diamidino-2-phenylindole 2HCl (DAPI) and 5-cyano-2,3-ditolyl tetrazolium chloride (CTC), respectively. Organic-rich and -poor culturing media were used to determine culturable members by plating (at 0°C and 5°C) and most-probable-number (MPN) analyses (at −1°C). Total bacterial counts ranged from $5.44 \times 10^3$ ml$^{-1}$ in clear ice to $2.41 \times 10^6$ ml$^{-1}$ in algal-band ice samples, with 2–27% metabolically active by CTC stain. Plating and MPN results revealed a high degree of culturability in both types of media, but greater success in oligotrophic media (to 62% of total abundance) and from clear ice samples. The bacterial enumeration anomaly, commonly held to mean ≤ 0.01% cultured, was not demonstrated in any of our samples. Denaturing gradient gel electrophoresis was used to check the purity of 44 isolates and select representatives for subsequent sequencing. Phylogenetic analyses based on 16S rRNA sequences indicated close relationships exclusively to known marine psychrophiles within two bacterial divisions, Proteobacteria (in the genera Alteromonas, Colwellia, Glaciecola, Octadecabacter, Pseudoalteromonas and Shewanella) and Cytophaga-Flexibacter-Bacteroides (Cytophaga, Flavobacterium, Gelidibacter and Polaribacter). All cultures from the clear ice sample with highest (62%) culturability were closely related to each other or to psychrophilic Cytophaga-Flexibacter-Bacteroides (94.9–99.6% sequence similarities). Overall, these findings suggest limited, heterotrophic bacterial diversity at cold temperatures and may provide insight into the recent evolution of psychrophilic bacteria.

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Introduction

Sea ice provides the coldest habitats on Earth for marine life, with temperatures ranging from 0 to −35°C [39]. Extensive microbial communities develop annually within the ice in spite of low temperatures and highly variable water activity [25, 42, 43, 60]. Heterotrophic bacteria represent a major group within these communities [78], as evidenced by measures of bacterial production [38, 40] and the microbial loop [49, 50]. Only recently, and based only on culture collections from Antarctica, has the phylogenetic diversity of sea-ice bacteria been considered [8, 29]. Those culturing efforts have yielded new genera and species within the divisions of γ- and α-Proteobacteria (e.g., [7, 10, 11, 13, 44], the Cytophaga–Flexibacter–Bacteroides (CFB) group [9, 12, 32] and the gram-positive Bacteria [46]. One new genus obtained from Arctic sea ice has been described [31], but the diversity of Arctic sea-ice bacteria is unknown. Most of the described Arctic and Antarctic sea-ice bacteria are psychrophiles with growth optima well below 15°C.

In spite of these advances, no information exists on the phylogenetic diversity of heterotrophic bacteria that may be the dominant members of their sea-ice habitats. All previous studies have addressed total community abundance or activity without examining phylogenetic diversity or else have examined diversity of selected sea-ice isolates without knowing if they are rare or common in the community. The main objective of this study was to assess the phylogenetic diversity of heterotrophic bacteria determined to be numerically abundant in sea ice. To meet this objective, we were confronted with a major unresolved issue in microbial ecology—the “bacterial enumeration anomaly” [45, 75]. In most aquatic habitats, of the total number of bacteria that can be seen microscopically, commonly less than 0.01% can be cultured using plate count techniques [1]. Counts of colony-forming units (CFU) as high or higher than 1% are usually only found in organic-rich systems such as the coastal Baltic Sea ([62; K. Junge, unpublished], some eutrophic lakes [75], or sewage sludge systems [81]. An exception may be cold oligotrophic seawater, where use of an unamended natural seawater medium has led to viable counts higher than anticipated [15].

Although most sea ice is visibly clear, some zones contain organic-rich habitats (e.g., green or brown algal bands) that might be expected to yield a higher than usual degree of culturability [40]. Even clear ice horizons, however, harbor significant bacterial populations and rates of production [e.g. 19, 30, 35, 38, 40]. To enumerate cultivable bacteria in both organic-rich (algal bands) and presumably poor (clear) sea ice, we used both organic-rich and -poor culturing media in plating (at 0°C and 5°C) and most-probable-number (MPN) analyses (at −1°C) of representative (melted) ice. We also examined methods for preparing, storing, and staining sea-ice samples to obtain accurate counts of total and metabolically active bacteria by epifluorescence microscopy. The ice samples were collected from the pack-ice region (drifting ice extending well offshore) of the Chukchi Sea in the Western Arctic, from which no prior studies of sea-ice bacteria appear to be available. The only study of Arctic sea ice that included both viable and total counts of sea-ice bacteria centered on coastal fast ice (ice that remains attached to land) near Barrow, Alaska [30]; some of those results suggested that the enumeration anomaly may not pertain to this Arctic environment.

Here we present results demonstrating that the bacterial enumeration anomaly does not apply to Arctic pack-ice samples: numerically important heterotrophic bacteria were successfully obtained in culture. To assess culture purity and select representative isolates for sequencing, we applied the technique of denaturing gradient gel electrophoresis (DGGE) of enzymatically amplified 16S-DNA encoding rRNA [55]. We then examined the phylogenetic diversity of the cultured strains based on 16S rRNA sequencing and their relationships to each other and known bacterial taxa.

Methods

Sample Collection

Samples were collected during the Arctic West Summer 1996 (AWS96) cruise of the USCGC icebreaker Polar Sea into the Chukchi Sea in June (Fig. 1). Sea-ice cores were taken at four stations along the cruise route (4, 6, 7, and 11; Fig. 1) using an ice auger of 10-cm diameter. Except for the core from station 4, which showed a mixture of columnar and platelet ice, all ice cores were composed entirely of columnar ice (A.J. Gow, personal communication). Careful attention was paid to maintaining sterile conditions as much as possible during sampling and processing; e.g., a sterile saw was used to cut the cores into 10-cm sample sections. Four sections containing algal bands and four clear ice samples (Fig. 1) were selected for processing. The ice sections were placed in sterile plastic bags, crushed mechanically, and melted at 4°C in measured volumes of unamended, prefiltered (0.2-μm pore size), autoclaved natural seawater (NSW,