Proteorhodopsin-Bearing Bacteria in Antarctic Sea Ice

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Proteorhodopsins (PRs) are widespread bacterial integral membrane proteins that function as light-driven proton pumps. Antarctic sea ice supports a complex community of autotrophic algae, heterotrophic bacteria, viruses, and protists that are an important food source for higher trophic levels in ice-covered regions of the Southern Ocean. Here, we present the first report of PR-bearing bacteria, both dormant and active, in Antarctic sea ice from a series of sites in the Ross Sea using gene-specific primers. Positive PR sequences were generated from genomic DNA at all depths in sea ice, and these sequences aligned with the classes Alphaproteobacteria, Gammaproteobacteria, and Flavobacteria. The sequences showed some similarity to previously reported PR sequences, although most of the sequences were generally distinct. Positive PR sequences were also observed from cDNA reverse transcribed from RNA isolated from sea ice samples. This finding indicates that these sequences were generated from metabolically active cells and suggests that the PR gene is functional within sea ice. Both blue-absorbing and green-absorbing forms of PRs were detected, and only a limited number of blue-absorbing forms were found and were in the midsection of the sea ice profile in this study. Questions still remain regarding the protein’s ecological functions, and ultimately, field experiments will be needed to establish the ecological and functional role of PRs in the sea ice ecosystem.

Proteorhodopsins (PRs) are retinal binding bacterial integral membrane proteins that function as light-driven proton pumps (9, 10) and belong to the microbial rhodopsin superfamily of proteins (54). Since the first reported PR sequence from members of SAR86 clade marine (class Gammaproteobacteria) in 2000 (9), many other PR-bearing bacteria have been identified in a range of marine habitats (5, 18, 20, 24, 25, 46, 62). In the recent Global Ocean Sampling (GOS) expedition, almost 4,000 PR sequences from 41 distinct surface marine environments were acquired, demonstrating that these PR genes are extremely abundant in the genomes of ocean bacteria (46). In fact, PR-containing bacteria account for 13% of the community in the Mediterranean Sea and Red Sea and 70% of the community in the Sargasso Sea (18, 46, 49, 60). These light-harvesting bacteria are present in three major marine classes of bacteria: the Alphaproteobacteria, Gammaproteobacteria, and Flavobacteria. In addition, two distinct PR genes encode pigments with “blue-absorbing” and “green-absorbing” properties, which is achieved by a substitution at a single amino acid position, which thereby functions as a spectral tuning switch (10, 37, 48).

Sea ice represents a complex physicochemical environment in polar regions and covers up to 13% of the Earth’s surface (59). Although extreme gradients of temperature, salinity, nutrient availability, and light stratify the ice matrix from the surface to the ice-water interface (41), the sea ice habitat nevertheless supports a diverse microbial community of phytoplankton, Bacteria, Archaea, viruses, and protists that grow in liquid brine channels within the ice (14, 35, 56). This sea ice microbial community (SIMCO) is highly metabolically active despite being unable to avoid the extreme environmental conditions that they experience (39). In fact, very-high-standing stocks of the SIMCO exist in many regions of the Southern Ocean. For example, the concentration of chlorophyll a, a proxy for microalgal biomass, typically reaches 200 mg m⁻² in the Ross Sea, while the concentration of chlorophyll a in the water column below is approximately 2 orders of magnitude less (47), and the percentage of metabolically active bacteria (32% [39]) is significantly higher than the 10% observed for temperate marine systems (36). The SIMCO is thus a major source of biomass in ice-covered regions of the Southern Ocean (59), providing a critical food source for grazing zooplankton (and, consequently, also for higher trophic levels) for much of the year (3, 59). This biomass is of particular importance during the darkness of the polar winter, when the bottom-ice community is the only available food source for juvenile krill. These grazers absolutely rely on the sea ice microbial community to survive, as the water lacks other food sources (6, 28).

In the past decade, reports of the widespread occurrence of bacteriochlorophyll and PR pigments in planktonic marine bacteria have challenged the assumption that chlorophyll a is the only principal light-capturing pigment in ocean surface waters. These alternative pigments may in fact play a critical role in light energy harvesting for microbial metabolism in various aquatic ecosystems (5, 10, 25, 40, 49). It has been proposed that energy, rather than nutrient conservation, is important for the regulation of productivity (7). PR-containing phototrophic eubacteria could play a significant role in the energy budget of cells in the photic zone in marine environ-
ments (15). PR sequences have been detected in the Southern Ocean (9), but to our knowledge, there have been no reports of PR-bearing bacteria within the sea ice matrix.

The majority of the microbial rhodopsin genes found in oceanic samples have been detected by environmental sequencing (30, 46, 48, 60). We have used degenerate PR gene primers (5) in this study to positively identify PR-bearing operational taxonomic units (OTUs) from sea ice. Also, specific bacterial mRNA can now be detected from extracted nucleic acids and used to examine gene expression and, thus, infer metabolic activity (8). With this in mind, we have generated cDNA from RNA extracted from sea ice samples. From these observations, we deduce that PR-bearing bacteria are present in sea ice and may be actively contributing to the ecosystem within this extreme microenvironment.

**MATERIALS AND METHODS**

**Sampling strategy.** Environmental samples consisting of sea ice and brine were collected from five locations in the Ross Sea Region of Antarctica (Fig. 1), Terra Nova Bay (TNB) (74°38′S, 164°13′E), Granite Harbor (GH) (77°1′S, 162°52′E), Cape Roberts (CR) (77°1′S, 163°10′E), Cape Evans (CE) (77°38′S, 166°24′E), and McMurdo Sound (MCM) (77°51′S, 166°40′E), during the austral summers of 2007 and 2008. The sites span 300 km along the western coast of the Ross Sea.

A series of cores 180 to 240 cm long with a 13-cm diameter (Fig. 2B) were extracted at each site with a powered ice corer (Kovacs) according to previously described techniques (47). To obtain bacterial cells, 20- to 30-cm sections of sea ice were cut from the top, middle, and bottom of each core and transferred directly into black plastic bags to minimize light exposure. The center of each core section was removed using sterile techniques and subsequently melted overnight in a sterile black plastic box with added autoclaved 0.22-μm-filtered seawater. Brine was collected at TNB and GH by drilling holes to three different depths corresponding to the season's ice thickness of one-quarter, one-third, and one-half with the powered corer. The holes were covered with a dark sheet to reduce light exposure during brine drainage (~15 to 30 min). A sterile 50-ml Falcon tube (BD Biosciences) was used to manually collect liquid brine from each hole.

All melted sea ice and brine samples were filtered onto 0.22-μm mixed-cellulose acetate filters (Pall Life Sciences) using a diaphragm vacuum pump. A 1-ml aliquot was taken from the concentrated liquid above each filter, stored in 25% glycerol, and kept in liquid nitrogen until further analysis. Samples for RNA studies were melted and filtered through 0.2-μm polycarbonate filters (Whatman PLC), which were then stored in RNAlater (Ambion Inc.) and kept at –20°C.

**Nucleic acid extraction of PR genes and transcripts.** Genomic DNA was extracted from the glycerol stock using phenol-chloroform. Briefly, each glycerol sample was centrifuged in a table-top microcentrifuge at maximum speed, and the supernatant was decanted. Tris-EDTA (TE) buffer was used to resuspend the pellet, and 2 mg/ml lysozyme was then added. The mixture was mixed before incubation in a water bath maintained at 37°C for at least 30 min. Sodium dodecyl sulfate (1%) and proteinase K (0.2 mg/ml) were added, and the mixture was further incubated at 65°C for 15 min. TE-buffered phenol was added prior to centrifugation at 10,000 × g for 10 min. The upper aqueous layer was transferred aseptically into a sterile 1.5-ml centrifuge tube, and chloroform was added. Following centrifugation for 10 min at 10,000 × g, the upper aqueous layer was transferred into a clean, sterile tube with 2.5 volumes of 99% ethanol and 0.1 M sodium acetate. This mixture was then spun at 10,000 × g for 20 min, and the pellet was washed with 70% ethanol and centrifugation at 10,000 × g for 15 min. Finally, the supernatant was removed, and the pellet was allowed to air dry before being resuspended in autoclaved double-distilled water. The extracted genomic DNA was quantified with a NanoDrop spectrophotometer (Thermo Fisher Scientific Inc.) and kept at –20°C until analysis.

cDNA was prepared from samples stored in RNAlater. One half of the filter paper was cut aseptically, and RNA was extracted by using the Qiagen RNeasy minikit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. At the manufacturer's recommendation, DNase digestion was not necessary, as the purification process has a higher affinity for RNA, with most of the DNA being efficiently excluded. All purified RNA was quantified either using the NanoDrop spectrophotometer or with Ribogreen (Molecular Probes, OR) before being used as a template for subsequent cDNA synthesis with SuperScript III first-strand synthesis supermix and random hexamers as primers (Invitrogen, Carlsbad, CA). Control samples without reverse transcriptase were included in the preparation to rule out DNA contamination. The NanoDrop spectropho-
mometer was also used to quantify cDNA at the end of the synthesis. An aliquot from each extracted RNA and cDNA synthesis reaction was checked for end-process integrity by using gel electrophoresis.

**PR PCR amplification and diversity analysis.** PRs were amplified from both genomic DNA and cDNA extracts by using combinations of primers (six sets in total) as reported previously by Atamna-Ismaeel et al. (5). PCRs were performed by using a high-fidelity proofreading polymerase mix (TaqKaRa Ex Taq from Takara Shuzo). PCR was performed in a total volume of 25 μl containing 1 μl (~1 to 5 ng/μl) DNA, 200 μM deoxynucleoside triphosphates (dNTPs), 1.5 mM MgCl₂, 0.2 μM primers, and 2.5 units of TaqKaRa Ex Taq polymerase. Amplification conditions were 95°C for 3 min and 40 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s, with a final extension step at 72°C for 10 min. PCR bands between 300 and 500 bp were excised, purified, and cloned with a Qiagen PCR cloning kit (Qiagen, Hilden, Germany) or a pCR2.1 TA cloning kit (Invitrogen, Carlsbad, CA) cells were transformed with the ligation products and screened using blue/white coloration. Thirty colonies were picked, and the plasmid was extracted by using an alkaline lysis method described elsewhere previously (12).

Unique EcoRI restriction fragment length polymorphism groups from the positive clones were sequenced (Macrogen Inc., South Korea), and the sequences were trimmed of the vector sequence and aligned by using the CLUSTAL program in MEGA, version 4 (57). Reference sequences were recruited from the NCBI (2) and CAMERA (51) databases and added to the alignment. The phylogeny of the translated sequences was constructed with MEGA4.0 based on the neighbor-joining algorithms with 500 bootstrap replicates and Bayesian analysis computed using MrBayes (45), providing confidence estimates for the inferred topologies.

**Nucleotide sequence accession numbers.** The sequences reported in this study have been deposited in the GenBank database under the following accession numbers: GU206107 and GU338798 to GU338849.

### RESULTS

A total of 52 positive and distinct PR sequences were obtained from about a thousand analyzed clone samples, with 17 positive cDNA sequences and 35 genomic DNA sequences. Clones were reported for all five sampling sites and were found to be distributed in the top, middle, and bottom sections of the sea ice (see Table 2).

Here, we report the first findings of PR-bearing bacteria within Antarctic sea ice by PCR-based gene surveys of PR genes and PR mRNA. We amplified both genomic DNA extracts and cDNA using the six PR primer combinations recommended previously by Atamna-Ismaael et al. (5). All primer combinations (Table 1) gave PCR bands, but only one set (RYIDW and GWA1YP) was positive for PR. The spatial distribution of our sequences within the sea ice core is illustrated in Table 2. We found more PR sequences in the bottom of the sea ice than in the middle, while the top of the sea ice had the lowest number of PR sequences. For both the genomic DNA and cDNA samples, more PR-bearing bacteria were found in the bottom-ice sections at CR, GH, and MCM, whereas a higher proportion of clones from the middle-ice section were seen in samples from TNB and CE. Overall, more PR clones were reported for the northern sampling sites than at the higher-latitude sites (Table 2).

**Phylogenetic analysis of PR genes.** Phylogenetic analysis showed that the PR sequences clustered within two known bacterial phyla, the Bacteroidetes (Flavobacteria) and the Proteobacteria, with two unique genomic DNA clades (clades I and II) (Fig. 3) formed within the Bacteroidetes cluster. As shown in Fig. 3, our clones showed close relationships to cultivated bacteria and also aligned to previously reported yet uncultured PR bacteria from different aquatic environments. PR clones from the bottom-ice, middle-ice, and brine samples were found widely distributed in sea ice from the Ross Sea at sites more than 300 km apart (Fig. 1). There was a segregation of clones by geographical region, whereby samples collected from the same location were grouped together most of the time. All derived clones, regardless of genomic DNA or cDNA origin, clustered into clades based on their translated nucleotide sequences and were distinct from the outgroup Salinibacter ruber (Fig. 3).

Overall, clones with PR genes amplified from genomic DNA found within the Proteobacteria group in this study showed 98% bootstrap support and 100% posterior probability values (Fig. 3B). Both values indicate that our clones within this group have well-supported alignment to previously reported sequences within this group. Six of our sequences in this clade were of genomic DNA origin, and they aligned mainly with samples reported from the GOS project (8) and the SAR92 clade (55). Clone TNB-C-3M1 clustered within the Alphaproteobacteria group and is grouped with the SAR11 representative Pelagibacter ubique. Each clone in the figure is genetically distinct from each other. For example, whether clones originated from the same geographic location and sea ice section (e.g., TNB-B-65-27 and TNB-C-3M20) or from different loca-

### Table 1. Primers used in this study

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<th>Primer</th>
<th>Direction</th>
<th>Sequence (5’–3’)</th>
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* Reported in reference 5.

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<th>Genomic DNA</th>
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* GPS, global positioning system.
FIG. 3. Phylogenetic tree of genetic and complementary PR genes in Antarctic sea ice and brine based on neighbor-joining analysis of translated nucleotide sequences. (A) The Bacteriodetes group of sequences. (B) The Proteobacteria group of sequences. Numbers on branches represent bootstrap values (distance/posterior probability) of 50% or greater indicated on the branches. Sequences obtained from this study are labeled by location, type, and clone number (i.e., TNB-C-3M20 indicates a sample collected at TNB, collected as sea ice core [C], and clone number 20 from the Middle section of the 3rd core).
tions and sea ice sections (e.g., GH-B-120A3 and TNB-C-3M42), their translated nucleotide sequences were quite distinct (Fig. 3B).

Within the Bacteroidetes group, our samples aligned to both cultured and uncultured environmental PR Flavobacteria at a bootstrap value of 72%. PR gene sequences that were isolated from TNB and CR core samples were all genetically similar to Polaribacter  riggedii, although bootstrap support was low (Fig. 3A). Our cDNA clones from CE and GH glacier were also found to be related to another cultivated PR-containing species, Psychroflexus torquis, although with lower confidence levels. One of our clones obtained from the bottom ice of GH was aligned with a 50% probability value to a coastal sample (GOS10) from the GOS project (8).

Two distinct groups of clones, unique genomic DNA clades I and II, from the GH sea ice were placed within the phylum Bacteroidetes but did not cluster with any known PR clones (Fig. 3A). Clone GH-B-80C9, isolated from brine collected at GH at an 80-cm depth, was adjacent to a sequence from a German freshwater lake (5), although the sequences are genetically distinct, while clone GH-B-120A7, also from a brine sample at GH, aligned between environmental clones from the Indian Ocean and GOS samples collected from the open ocean and coast (8); again, the sequences are genetically quite distinct, as shown in Fig. 3A.

Active proteorhodopsin. To identify bacteria actively expressing PR genes in natural populations, we used reverse transcription-PCR to identify PR gene mRNA from the environment. Sequences of PR genes obtained from RNA samples stored in RNAlater and converted to cDNA were grouped into the same two phyla as those from genomic DNA (Fig. 3). Eight of the cDNA sequences were found in the Proteobacteria clade, showing at least a 50% posterior probability value compared to the genomic DNA sequences. The majority of the cDNA transcripts within the phylum Bacteroidetes were found clustered in the Flavobacteria group (Fig. 3A). Even within this cluster, intersite and intracore genetic differences are obvious. The cDNA transcripts derived from the MCM and CR sites, which are ~300 km apart, exhibited distinct genetic variability, and even the three cDNA transcript-translated nucleotide sequences from the CR bottom ice showed genetic differences (Fig. 3B).

Within the Bacteroidetes PR cluster, one cDNA clone (MCM-B-1C9) from the MCM bottom ice is distinctly related to a South China Sea clone (96% bootstrap support), and the other cDNA clones clustered closely to the cultured PR representatives Polaribacter sp. strain MED152 and Dokdonia donghaensis MED134 (Fig. 3A).

From Fig. 3, several differences from the genomic DNA results were evident. A large number of genomic DNA clones formed two separate unknown clusters (unique clades I and II) with no corresponding cDNA sequences. We also noted that a larger percentage of cDNA clones was found from the middle and top regions of the ice (52%) than genomic DNA (23%) (Table 2). However, the cDNA sequences within the tree were generally distinct from the genomic DNA sequences. The majority of the cDNA sequences have low bootstrap and ≤50% probability values compared to the genomic DNA sequences.

Spectral tuning of PRs. Ten and thirty-eight of the predicted PR proteins retrieved in this study contained leucine or methionine at position 105, respectively, suggesting that they are green-absorbing PRs. Only four of the clones (Fig. 3) had glutamine in this position, which suggests that they are blue-absorbing PRs. We found green-absorbing PRs throughout the ice, but three of the blue-absorbing species were found in the midregion of the ice at TNB and GH, and only one of the blue-absorbing PRs were found in the MCM bottom ice. The two blue-absorbing PR clones from midcore samples at TNB are genetically quite distinct. Clone TNB-C-3M1 is closely related to blue-absorbing GOS samples (99% bootstrap support), while clone TNB-C-3M42 is most closely matched to clones from MCM and GH despite the sites being more than 300 km apart. All blue-absorbing PRs were found among members of the Proteobacteria, with three out of four in the Gamma-proteobacteria class (Fig. 3B).

DISCUSSION

PR-bearing bacteria account for 13 to 70% of the overall microorganisms in the euphotic zone (49, 60) and may thus play an important role in all marine ecosystems. The findings presented here confirm the presence of PRs within the Antarctic sea ice matrix. This work is a part of New Zealand’s Latitudinal Gradient Project (LGP), which is a multidisciplinary program studying marine and terrestrial ecosystems over 6° of latitude in the Ross Sea region (29). We identified PR-bearing bacteria at all depths in sea ice (Table 2) from LGP-designated sites—TNB and GH—located ~300 km apart within the Ross Sea region (Fig. 1). PR clones from each site were clustered together and were generally genetically distinct from clones identified at the other sites (Fig. 3).

Fourteen positive PR sequences from the Proteobacteria and 38 from the Bacteroidetes were identified in sea ice, with many aligning alongside environmental metagenome sequences from the GOS project, including those from estuarine, open-ocean, and coastal environments (46). This illustrates that PR bacteria are widely distributed in marine waters. However, the majority of our clones are generally distinct from their GOS relatives (Fig. 3).

Our genomic DNA data revealed that a number of sea ice PR-bearing bacteria were distributed into two unique clades. Given that both clades were well separated from the rest of the reported Flavobacteria group of clones, we suspect that the sequences in both clades indicate a wider diversity of rhodopisin genes within the phylum Bacteroidetes than previously thought. The closest known match to those clusters is Spirosoma linguale DSM74. Hence, there might be more PR-similar genes in other taxonomic subgroups beyond what has been reported to date (25, 26, 62). Our sequences are the first set to be reported for a new group of PR analogues in the Bacteroidetes.

The SAR11 clade is one of the most numerically abundant microorganisms in the ocean, at ~2.4 × 10^28 cells (42). However, in the Southern Ocean, limited diversity (61) and seasonally variable concentrations (23) of SAR11 were noted. Only one clone in this study, TNB-C-3M1, was closely aligned with SAR11 representatives. Also, none of our clones aligned with the SAR86 clade of PR genes, which was reported to comprise the majority of Antarctic pelagic PR sequences in a previous study (10). The choice of PCR primers (5), molecular tech-
nique biases (50, 58), and the physiochemical conditions within the sea ice matrix (17, 22, 44) could explain the low number of SAR11 clade-affiliated PR clones, reflecting some degree of environmental selection.

Several clones were closely matched genetically to an Antarctic bacterium, *P. igensii* (27) (Fig. 3). Sequences from *P. igensii* are well represented in the Antarctic region (13) and were one of the most common Antarctic OTUs detected in a latitudinal study by Abell and Bowman (1). Cottrell and Kirchman commented previously that PR genes from the Northern and Southern Hemispheres are significantly different (19). In support of this, we noted that none of the clones obtained in the current study matched closely with Arctic Ocean sequences. Even within the 300-km radius encompassed in our study, there were distinct differences in PR community structures encountered at different sites. Some of our samples aligned with GOS samples collected from temperate and tropical regions (46), although they were always genetically distinct. It is apparent that PalE6, which was previously reported near Palmer Station, Antarctica (10), is quite distinct from any of the clones detected within this study (Fig. 3). Our results therefore suggest that, in general, PR-bearing bacteria might be geographically isolated in the Ross Sea from other regions in the Southern Ocean. However, the limited sample size precludes major conclusions at this point.

Over half of the sequences identified in the genomic DNA analysis were placed into an unknown group related to the *Bacteroidetes* (unique genomic DNA clades I and II). However, no sequences in the cDNA set were found from this group, which indicates that microorganisms identified here may be dormant in sea ice (32). Eight *Gammaproteobacteria* clones from the midsection of the core and four from the bottom section were found in the cDNA analysis. None of these clones were closely related to the known sequences included in the analysis, and they also appear to be different from the three *Gammaproteobacteria* sequences identified in the genomic DNA sequence analysis. This suggests that, while they appear to be physiologically active, these 12 clones may be in relatively low abundance and were therefore not found in our initial screening of genomic DNA. Further studies using high-throughput 454 pyrosequencing (52) or quantitative PCR may be required to properly evaluate this question.

For the first time, we show by the use of cDNA methods that sea ice bacteria are indeed actively expressing PR mRNA, meaning that microorganisms within this ecosystem may use PR as a proton pump to harvest light energy for cellular metabolism. Our results suggest that bacteria exploiting PR phototrophy might be physiologically active in situ, which may be of ecological importance to the Antarctic sea ice ecosystem, as chlorophyll-based phototrophy by microalgae may not be the sole source of energy within the ice matrix (4, 34). Recent studies by Martin et al. (39) have shown that sea ice bacteria exhibit high single-cell metabolic activity and are likely to be actively involved in the microbial loop. Other studies have also reported a high proportion of viable bacteria in the Southern Ocean (4, 43, 44). However, the exact role and contribution of PR-bearing bacteria in the sea ice ecosystem have yet to be clearly defined.

Lami et al. (33a) recently suggested that light influences the growth of PR-containing bacteria and the regulation of PR mRNA synthesis in natural communities. In addition, cultivated *Dokdonia donghaensis* MED134 cells grown in dissolved organic matter (DOM)-enriched media under light conditions *in vitro* have been shown to exhibit higher growth rates than when grown under reduced-light and/or DOM conditions (25). Furthermore, Walter et al. (60a) showed that PR allows transformed *E. coli* cells to resist environmental respiration challenges by harvesting light energy. Clones in our study were collected during the summer period, where light is present for 24 h at intensities that vary more than 1,000-fold from the top to the bottom of the ice (16, 17). The highest concentrations of genomic DNA and cDNA clones detected were at the bottom of the sea ice, where light intensity is relatively low (Table 2). Given that the bottom of the sea ice has the highest biomass concentration (Fig. 2) compared to that of the upper portion, with lower concentrations of bacteria (22, 56), it is perhaps not surprising that the amount of PR sequences obtained from this section was larger.

Solar radiation, which regulates the production and growth of SIMCO (22), is highly scattered in sea ice, and the more-energetic blue light predominates (16). However, given the abundance of microalgae in the bottom 5 to 10 cm of annual fast ice, the only available light for prokaryotic phototrophs at the bottom of the ice will be in the green waveband (31). The disjunct distribution of green- and blue-absorbing PRs over a distance of more than 300 km uncovered in our study suggests a response to the light environment and further indicates that these organisms may be functional in the ice and not simply trapped there during ice formation. If this is true, they may play an important role in the microbial sea ice ecosystem either as a means of energy harvesting or via a sensory role (38, 53). PR might thus confer a selective advantage and/or enhancement to bacteria for other cellular functions, and phototrophy is likely to be beneficial to the organisms considering the ecological costs of maintaining essential genes and synthesizing proteins (21). Some organisms might use PR only as a mechanism of survival under harsh conditions, which would not be readily demonstrated *in vitro*.

Marine PRs may harbor a diverse array of physiological, biogeochemical, and ecological functions that warrant further investigation. The information gathered from the thousands of PR sequences acquired using cultivation and metagenomic techniques from different geographical and ecological environments suggests that environmental control and natural selection significantly affect the distribution and evolution of PR (11). A better understanding of the roles of PR might help us predict some potentially important and imminent impacts of global change on marine communities (21).

Here, we present the first report of PR-bearing bacteria from Antarctic sea ice. Although we illustrate similarities to previously reported PR-containing bacteria, our sea ice-derived bacterial sequences are generally distinct. We also suggest that these ice-associated bacteria are physiologically adapted to a unique environment (22). These results also suggest that the SAR11 clade PR-containing bacteria may not be as abundant in Antarctic sea ice as in the North Atlantic (18) or the Southern Ocean (23) and that previously undetected and unreported PR-containing bacteria might be present in this extreme environment.

Ultimately, field experiments will need to be conducted to
establish the ecological roles of PR in the energetics of micro-

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