Elevated bacterial abundance in laboratory-grown and naturally occurring frost flowers under late winter conditions

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Abstract

Sea ice has been identified as an important microbial habitat, with bacteria and other microbes concentrated in the brine inclusions between ice crystals. Frost flowers, thought to draw brine from underlying sea ice, have not been characterized from a microbial standpoint. To test whether frost flowers serve as an upward vector of bacteria contained within sea ice brines we grew frost flowers in a freezer laboratory (air temperature of –21°C) from saline water spiked with the mesophilic (and thus passive under experimental conditions) bacterium Halomonas pacifica. Salinity of melted samples was measured and bacterial abundance determined by epifluorescent microscopy. Bacterial counts scaled to ice-melt volume averaged 2.82 x 106 ml–1 for frost flowers, compared to 9.47 x 105 ml–1 for underlying ice (3 x higher). Bacterial counts also correlated significantly with salinity (maximum value of 62.5 psu) for frost flowers, brine skim, and ice (df = 17, r = 0.59, p < 0.0001). Segregation coefficients were calculated to describe the efficiency of transport of both cells and salt from the starting solution into frost flowers. From these coefficients an enrichment index was calculated to test for bacterial concentration into frost flowers at a different rate than salt. Analysis with a Student's T-test (df = 24, t = 0.306, p = .76) indicated that cells and salt were not transported into frost flowers with a significantly different efficiency. To test these findings in the field we then collected frost flowers (and related samples) from new sea ice near Barrow, Alaska in April 2009. Bacterial counts were significantly elevated (again, a 3-fold increase) in natural frost flowers (mean = 2.73 x 105 ml–1) compared to underlying sea ice (mean = 8.46 x 104 cells ml–1). For all field samples collected (frost flowers, underlying brine skim and sea ice, as well as snow), bacterial abundance correlated significantly with salinity (maximum value 124 psu, df = 40, r = 0.60, p < 0.0001). The presence of elevated numbers of bacteria in frost flowers may have implications for the previously observed chemical reactions that take place in them, especially if microbial activity can be shown to occur in this unique low temperature, low water activity microbial habitat.

Introduction

Frost flowers (Fig. 1) are structures that form on the surface of new ice over both fresh and saline waters. Within the Arctic and Antarctic marine environments frost flowers can cover a substantial fraction of the surface of new sea ice.

In winter frost flowers are exposed to a steep temperature gradient. Where the coldest winter sea ice might reach −30˚C in the Arctic, the periphery of the frost flower reaches the corresponding temperature of the atmosphere.

As saline water freezes, the liquid fraction undergoes substantial changes to both ionic composition and water activity (Fig. 2). Sea ice retains a liquid fraction down to the eutectic point for a CaCl2/H2O brine at −54˚C. Although the brine chemistry of frost flowers is less well known, field analyses indicate higher bulk salinities than the underlying sea ice (Perovich and Richter-Menge, 1994). This raises the question of whether biological material might also be concentrated within frost flowers.

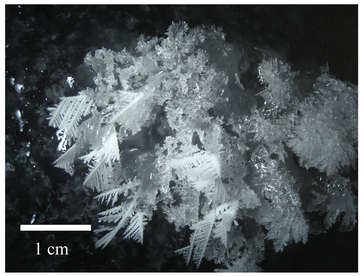

Fig. 1. Frost flower grown in the laboratory at −21˚C from a starting NaCl solution of 35 psu.

Any organism present within frost flowers will be exposed to the concurrent challenges of very low temperature, altered ion concentrations, and low water activity (Aw). Understanding the mechanisms by which Bacteria and Archaea may survive these conditions and possibly continue metabolic activity under them may provide unique insight into the habitability of many extraterrestrial environments that feature saline ice formations.

Concurrent environmental challenges to microbial life in water ice

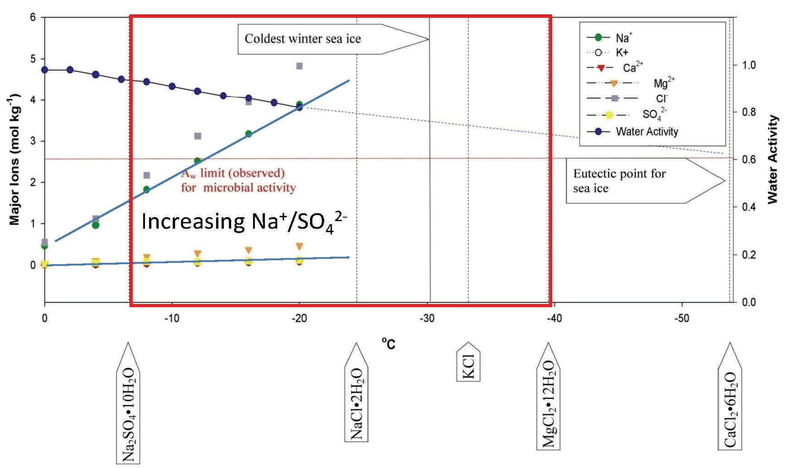

Fig. 2. Decreasing temperature is accompanied by decreasing water activity in sea ice brines. The precipitation of salts at predictable temperatures also dramatically changes the ionic composition of the brine (Deming and Eicken, 2007; Marion and Farren, 1999). The FREZCHEM (Marion et al.) model was used to calculate the concentration of individual ions and A_w to −20˚C. The dotted blue line below this temperature represents the general trend of A_w to the endpoint. The region of frost flower growth is given by the red box.

Methods

To test the hypothesis that microbial cells are concentrated in frost flowers, we grew frost flowers in a freezer room using the device pictured in Fig. 3, modeled after the method of Style (2007). After establishing a mechanisms for reliably growing frost flowers, the 35 psu NaCl source solution was spiked with cells of the mesophilic bacteria *Halomonas pacifica*. A mesophile was selected to ensure passive transport. Frost flowers, the surface "skim", and ice underlying ice were collected, melted, and fixed with 2 % paraformaldehyde. Prior to fixation salinity was determined with a handheld salinometer. Bacteria were enumerated by epifluorescent microscopy using the DNA-specific stain DAPI.

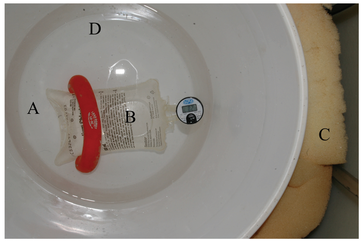

Fig. 3. A) NaCl/H20 solution B) Ethanol filled bag for overflow C) Insulation to prevent side freezing D) Bottom heat source (not visible).

Frost flowers were collected in the field at Barrow, Alaska on April 6 and 7, 2009 at a site where tidal action had forced sea ice brines to the ice surface. Frost flowers grew readily each night over the newly exposed brine. Salinity and cell counts were determined in the same manner as for laboratory experiments.

Additional frost flowers were collected on September 2, 2009 from the central Arctic Ocean during LOMROGII onboard the Swedish icebreaker *Oden*. Salinity and cell counts were determined in the same manner as for Barrow and laboratory grown frost flowers.

Results

Microbial cells were found to correlate significantly with salinity in both laboratory and environmental frost flowers (Figs. 4 and 5). This suggests that microbial cells and ions are sharing the same transport mechanism from sea ice to frost flower.

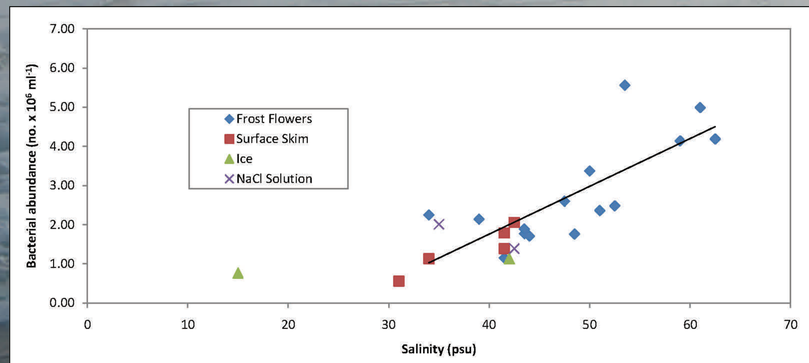

Fig. 4. Correlation between bacterial abundance and salinity (df = 13, r = 0.75, P = 0.001) for laboratory grown frost flowers. The regression line has been fit only to the frost flowers, though the brine skim values can be seen to closely follow this trend.

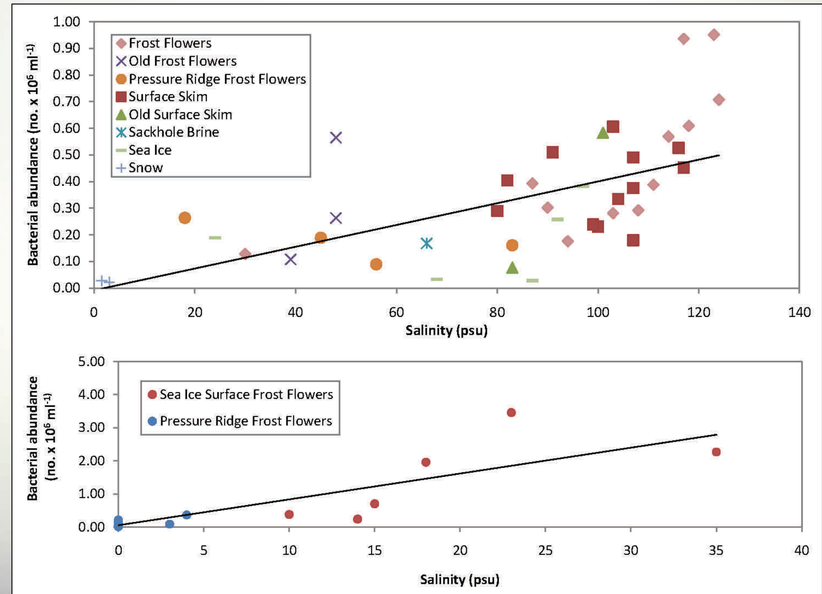

Fig. 5. Correlation between cells and salinity for Barrow (top, df = 42, r = 0.60 and p < 0.0001) and central Arctic Ocean (bottom, df = 22, r = 0.83 and p = 0.0024) samples. For Barrow. A strong correlation is observed between bacterial abundance and salinity across the sea ice surface environment. The regression line is fit to all data. For the central Arctic Ocean. Frost flowers from atop a pressure ridge as well as from atop newly formed lead ice were sampled.

Discussion

The correlation between bacterial abundance and salinity over a wide range of values measured in frost flowers suggests that both parameters are controlled by the same physical transport and concentration mechanisms.

The presence of bacteria in elevated numbers within the frost flowers has possible implications for cold adapted physiologies, to brine and atmospheric chemistry, and the dispersal of bacteria. Future work will focus on quantifying microbial activity in frost flowers *in situ*, and in the laboratory under simulated environmental conditions. We are undertaking assessing microbial diversity in frost flowers, skim, underlying sea ice, and hoar frost derived from atmospheric moisture. These analyses will provide information on the possible biogeochemical roles of frost flower microbial communities. Results will also suggest whether or not taxon-specific bacteria are preferentially transported into frost flowers or retained within the underlying sea ice matrix.

Acknowledgements

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