

COMPATIBLE SOLUTES: EXPLAINING HOW ARCTIC MICROBES SURVIVE EXTREME SALINITY FLUCTUATIONS, WITH RELEVANCE TO THE HABITATION OF ICY MOONS E. A. Firth¹, S. D. Carpenter¹, and J. W. Deming¹ ¹University of Washington School of Oceanography, Box 357940, Seattle, WA 98195-7940, firth.evan@gmail.com

Introduction: In terms of microbial habitats, Arctic sea ice provides a useful terrestrial analogue for ice-covered oceans on Europa and Enceladus. The low temperature record of -20°C for bacterial activity came from measurements made under *in situ* conditions on Arctic winter sea ice [1], where bacteria concentrate within the liquid brine fraction of the ice matrix (up to $2 \times 10^8 \text{ mL}^{-1}$ brine) [1,2,3]. Sea ice is necessarily a salty environment, consistent with the salty liquid water and ice on both Europa [4] and Enceladus, and likely represented in the mission-accessible plumes of both moons [5,6]. In addition to the stresses posed by *static* low temperatures and high salinities, bacteria in Earth's sea ice environments experience *dynamic* temperature and salinity stresses on timescales of hours to days and longer [7]. One mechanism for withstanding variable salinities is through the use of *compatible solutes* – well characterized in certain halotolerant bacteria, but untested in marine psychrophiles. This research resolves that knowledge gap with observations of compatible solute usage by *in situ* Arctic sea ice microbial communities, as well as by two cold-adapted bacteria available in pure culture: the model marine psychrophile *Colwellia psychrerythraea* strain 34H; and a psychrotolerant *Psychrobacter* species strain 7E, recently isolated from Arctic winter sea ice brine, that is also capable of growing over a wide range of salinities (euryhaline).

Methods: Using the model marine psychrophile *Colwellia psychrerythraea* strain 34H, the euryhaline marine psychrotolerant *Psychrobacter sp.* strain 7E, and natural microbial communities from brines within the sea ice cover of Kobbefjord, Greenland, we measured the extent to which ^{14}C -radiolabeled choline (precursor to the known compatible solute glycine betaine) was utilized upon salinity shifts of 50–200% *in situ* salinity (final salinities of 25.5–146 ppt) at -1°C . The fate of the ^{14}C -choline was calculated in terms of total cellular uptake, incorporation into macromolecules, respiration to CO_2 , retention in intracellular pools, and extracellular release.

Results: We found that choline was used in a manner consistent with that of a compatible solute. When external salinity was increased, both of our test organisms and the natural brine communities preferentially imported and retained the choline in large quantities, respiring very little of it. When salinity was decreased,

the choline was released on a sub-second time scale (*C. psychrerythraea* 34H and *P. sp.* 7E) and the remainder preferentially respired on a longer (hour-day) time scale (*C. psychrerythraea* 34H and natural communities). The short-term releases were presumably from mechanosensitive ion channels, as the genomes of *C. psychrerythraea* 34H and *Psychrobacter arcticus* (97% similarity to *P. sp.* 7E) contain genes for this mechanism [8].

Discussion: Bacterial tolerance of *dynamic* salinity is often overlooked in favor of bacterial tolerance of extremely low temperatures or extremely high salinities. However, the ability to tolerate both *static* extremes and *dynamic* fluctuations in temperature and salinity plays an important role in microbial survival and activity in sea ice environments on Earth. Understanding the mechanisms of bacterial survival in these environments can contribute to understanding and predicting the types of life that could potentially be present on Europa, Enceladus, or other icy bodies. As ice appears to be the most prevalent form of water in our solar system, and sea ice appears to be the most habitable form of ice on Earth [3], research into the adaptations utilized by natural Arctic sea ice communities will continue to provide valuable biological perspective to the astrobiological scientific community.

References: [1] Junge, K. et al. (2004) *Appl. Environ. Microbiol.*, 70(1), 550-557. [2] Collins, R.E. et al. (2008) *J. Mar. Systems*, 74, 902-917. [3] Deming, J.W. and Eicken, H. (2007) *Planets and Life: The Emerging Science of Astrobiology*, W.T. Sullivan and J.A. Baross, eds. 292-312. [4] Khurana, K. K. et al. (1998) *Nature*, 395. [5] Roth, L. et al. (2014) *Science* 343, 171-174. [6] Postberg, F. et al. (2011) *Nature* 474, 620-622. [7] Ewert, M. and Deming, J. W. (2014) *FEMS Microbiol. Ecol.* 89(2), 476-489. [8] Ewert, M. (2014) *U. Washington thesis*, 204-226.

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