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Biogeosciences Discussions

Interactive comment on "High-resolution ice nucleation spectra of sea-ice bacteria: implications for cloud formation and life in frozen environments" *by* K. Junge and B. D. Swanson

K. Junge and B. D. Swanson

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Interactive Discussion

We appreciate all the comments and suggestions by the referee and other contributors. We address the comments below in a point-by-point fashion and will prepare a revised manuscript.

C. Morris (Referee), cindy.morris@avignon.inra.fr, Received and published: 5 December 2007

1) Questions regarding the involvement of INA bacteria in polar cloud formation.

- In this study we examined the ice nucleation behavior of polar marine bacteria that

have the potential to initiate ice and enhance precipitation processes in polar clouds (as suggested by Bigg and Schnell, 2001). A discussion of the active ice formation process in tropospheric polar clouds themselves would be somewhat speculative and a review of what is known is beyond the scope of this paper. In the revised version we will add more specific terms such as ice nucleation (instead of nucleation only) and formation of 'ice' in polar clouds and change language that is misleading with regards to these terms. It is possible (given studies by others that found many of cloud bacteria examined to be CCN active, see Bauer et al., 2002 and Ahern et al., 2006) that polar marine bacteria are also CCN active but additional experiments are needed to examine this further (see hypothesis stated on pg. 4270, l.4). Our methodology and our experiments allow us to make inferences only about the potential of sea-ice bacteria to initiate ice formation in liquid droplets via the immersion freezing process. The questions posed by the reviewers are very good but could only be answered by a field study and not a laboratory study.

2) Questions regarding the choice of bacterial strains:

- We used these particular strains, since they were found to be the numerically dominant members of an Arctic sea-ice bacterial community (see Junge et al., 2001 for a detailed explanation on how this was determined). Furthermore, more recent studies have shown the predominance of the same groups and genera both in Arctic and Antarctic sea-ice samples (see Brinkmeyer et. al.,2003). Nevertheless, it is entirely possible that other strains (even of the same genera, such is the case with E. herbicola, where some strains show high INA others not, Steve Lindow, personal communication) could be ice nucleation active. Further tests of additional isolates are needed (as indicated on pg. 4269, l. 20) to examine this further. In case of the bacteriophage V9, there is no information available on the prevalence of this (or other cold-active) viruses in the Arctic (see Wells, 2007 for an in-depth review on cold-active viruses). V9 is of particular interest though since it represents one of the few cold-active viruses that have been characterized in detail. Furthermore, to our knowledge, the ice nucleation behavior of

BGD

4, S2618–S2624, 2008

viruses (temperate, warm or cold-active) has only rarely been tested in general. We will add a paragraph in the revised manuscript to point out that fact.

Brinkmeyer, R., K. Knittel, J. Jürgens, H. Weyland, R. Amann,and E. Helmke. Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. Appl Environ Microbiol. 2003 Nov ;69 (11):6610-9 14602620.

Wells, L. 2007. Cold-active viruses. In Rosa Margesin, Franz Schinner, Jean-Claude Marx and Charles Gerday (eds) 2007. Psychrophiles: From Biodiversity to Biotechnology, Springer-Verlag, Berlin. Chapter 10, pp. 157-173.

3) Questions regarding the ecological importance for INA in marine psychrophiles.

- An important question to assess the INA of bacteria is the magnitude of the change in nucleation temperature due to their presence in the droplet medium. The melting point of bulk seawater (with a salinity of 35ppt) or artificial seawater (ASW) is -1.8°C, small droplets though don?t freeze homogeneously until -42°C (see Figure 2 and 3, Table 1). When Pseudomonas syringae cells were suspended in artificial seawater, ice nucleation occurred at an Ft of about -12°C (thus about 30 degrees higher than the water would nucleate homogeneously [-42°C]), but also about 6 degrees lower than when suspended in distilled water (-6°C, see Figure 3). Thus there is a marked difference in nucleation behavior depending on the droplet medium. From our results for marine psychrophiles inferences can only be made from our data about such bacteria that - when suspended in marine waters and lofted into the air (to much colder temperatures) could be involved in the formation of ice within those lofted water droplets. From an ecological perspective we envisioned that this would make sense for psychrophilic sea-ice bacteria since this would ensure their enclosure into newly formed ice - the habitat in the Arctic and Antarctic where they are found predominantly (Helmke and Weyland, 2004). As our results show though, that is not the case for the predominant sea-ice bacteria studied here. In future experiments we plan to study the freezing behavior of sea-ice bacteria also in distilled water, since one can imagine an atmospheric

BGD

4, S2618–S2624, 2008

scenario where marine polar bacteria as part of the aerosol would act as CCN and form freshwater liquid water droplets that than could nucleate to ice. However, this was beyond the scope of the present study.

Helmke, E., and H. Weyland. 2004. Psychrophilic versus psychrotolerant bacteria–occurrence and significance in polar and temperate marine habitats. Cell Mol Biol (Noisy-le-grand). 2004 Jul ;50 (5):553-61 15559972.

4) Comment regarding the lack of ice nucleation induction in the strains.

- This is a very good point. We are not aware of studies of non ice-nucleation active bacteria under similar experimental conditions (i.e. ultra-pure and substrate free) and agree that it will be important to explore additional non-INA mesophilic bacteria to determine if this is indeed a form of special adaptation. We will add a sentence pertaining to this in the revised manuscript since it is not feasible for us to obtain and add another freezing profile at this time. - Furthermore some bacteria have the IN gene and others don't and we still don't know the ecological advantage of having this gene. Maybe freeze avoidance (avoiding the initiation of intracellular ice) can be done two ways; either initiating the formation of extracellular ice or attempting to avoid ice nucleation entirely. Secondly, most mesophilic bacteria will not be exposed to ice as much as psychrophilic bacteria that also might explain the difference. Furthermore, our SARS study showed the difference between the outer cell membrane of mesophilic versus psychrophilic bacteria (Laucks et al., 2005). Maybe membrane structure is another piece of the puzzle that warrants further exploration. Currently, we are working on improving the freeze tube technique by installing digital cameras that will shorten the data analysis time significantly and will allow for a more rapid screening of isolates.

5) Regarding specific comments.

p. 4265, l. 22: "The enclosure and freezing tube" WERE then purged..

- This will be corrected in the revised manuscript

BGD

4, S2618–S2624, 2008

p. 4266, l. 8: 2008 - This will be updated in the revised manuscript.

p. 4268, l. 7.

- This is a very good point. Jayaweera and Flanagan (1982) describe one of their ice-nucleating strains isolated from the Arctic atmosphere as 'Pseudomonas sp.' without further specifications. We will adjust our wording accordingly in the revised manuscript. We also have sent a copy of the paper to the referee.

p. 4268, l. 28. What does passivated mean? Is this a generally accepted term? (If so, sorry for my ignorance.)

- Passivation is a general term describing a process of making a material 'passive' in relation to another material. In the case we are describing here one is concerned with the potential for heterogeneous ice nucleation initiated by contact between the sample and the surrounding container or substrate. Typically a hydrophobic polymeric solution is used to coat the substrate thereby inactivating the substrate-induced heterogeneous IN process. The problem is that much testing is required to be assured that the role of the substrate is actually passive and that the observed freezing is initiated by the process one is interested in studying.

p. 4270, l. 20: The correct spelling of the bacterial species is Pseudomonas FLUO-RESCENS.

- This will be corrected in the revised manuscript.

p. 4271, l. 10: 240 what?? Are the units missing here?

- We originally used 240 ppt (units salinity), but the editor asked us to delete this, since there are no SI units for salinity

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Comment regarding the range of ice nuclation behavior of bacteria.

BGD

4, S2618–S2624, 2008

Interactive Comment

- As stated earlier in responding to the referees' comments, additional bacteria need to be tested to evaluate the significance of our results with respect to sea-ice bacterial adaptation and also atmospheric ice formation processes. As such, this study represents the starting point for further investigations of the range of polar bacterial ice nucleation temperatures under conditions resembling more closely those that are present in the atmosphere. For instance, initial single-run low resolution freezing spectrum of Pseudomonas antarctica showed freezing temperatures ranging from about -20 to -40°C in artificial seawater (unpublished results). These initial findings need to be verified, but if proven correct, it indicates that this bacterium contains compounds referring INA over a very broad range of temperatures previously not found for bacteria (and which were possibly undetectable with substrate-based methods, see Obata et al., 1999). Currently, we are exploring this further (in addition to testing other mesophilic isolates, such as INA-plus and INA-minus strains of Erwinia herbicola.

Obata, H., N. Muryoi, H. Kawahara, K. Yamade and J. Nishikawa. Identification of a novel ice-nucleating bacterium of Anarctic origin and its nucleation properties. Cryobiol. 38: 131-139. 1999.

H. Eicken, hajo.eicken@gi.alaska.edu, Received and published: 5 January 2008

1) Comment regarding the potential impact of ice-nucleation activity (INA) of bacteria on sea-ice crystal growth in brine inclusions.

- Our working hypothesis was that marine psychrophilic bacteria that are present in the water could, when lifted into the cold air in regions of active ocean-air transfer, cause ice formation in the air. Then, when these crystals fall back down into the water they would initiate the formation of sea ice rather than causing the growth of new ice crystals from within the water column. We will adjust the final text to make this point more clear.

2) Comment regarding the possibility of bacterial involvement in salt precipitation processes within brine inclusions.

BGD

4, S2618–S2624, 2008

- This is an interesting question and one that would be interesting to pursue further given that we did observe salt precipitates within natural sea-ice brine inclusions (using methods described in Junge et al., 2001). However, we didn?t observe bacteria being directly associated with the salt precipitates or that bacteria were present within the same brine pockets as the salt crystals. This might lead one to conclude that bacteria are not involved in the precipitation of salt within brine pockets but a more thorough investigation is warranted to prove or disprove this point.

3) Questions regarding the occurrence of organisms with INA at very small supercoolings ($<$ 1 K) and ice nucleation in waters underneath the Antarctic shelf.

- Observations of bacterially induced ice nucleation on plant surfaces at around -2 °C have been recorded (Lindow 1982). Warmer temperature observations (at -1 °C) have been made but only a small fraction of bacteria have this INA. We are not aware of claims of IN with supercoolings less than 1 °C and of bacterial community studies that show the presence of P. syringae - type bacteria in polar waters or of other bacteria that will nucleate ice at -2 °C or above. Thus, the jury is still out as to how such large ice crystals are being formed under the Antarctic shelfs. Perhaps a field study observing these processes in-situ could elucidate the processes involved.

Interactive comment on Biogeosciences Discuss., 4, 4261, 2007.