

Biogeosciences Discuss., referee comment RC1  
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## Comment on bg-2022-113

Anonymous Referee #1

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Referee comment on "Ice nucleating properties of the sea ice diatom *Fragilariopsis cylindrus* and its exudates" by Lukas Eickhoff et al., Biogeosciences Discuss.,  
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### **Ice nucleating properties of the sea ice diatom *Fragilariopsis cylindrus* and its exudates by Eickhoff et al.**

This study presents measurements of the ice nucleating activity of the sea ice diatom *Fragilariopsis cylindrus* isolated and cultured from Antarctic sea ice. The authors analyzed whole cells, as well as fragments and exudates which pass a 0.22  $\mu\text{m}$  syringe filter. Eventually they compare the results with experimental results from other marine/sea-ice related diatoms and present a mass-based parametrization of the ice nucleation activity for the use in models.

Eickhoff et al. present valuable results with a potential significant contribution to a better understanding of the microphysical processes in the ocean and the (polar) atmosphere. However, in my opinion, some chapters could benefit from some shortening/rewriting, while the major findings should be rather put in a nutshell. Furthermore, I think, this manuscript is still lacking a look on the bigger picture and a critical discussion of its (atmospheric) significance and implications. See below for more specific comments.

#### **1. Introduction**

L34-39 and L61-71.: Could you be more specific, which of the findings hold true for the Arctic, the Antarctic or both polar regions? This appears very important to me to not mix them up, since certain features of the Arctic and Antarctic are quite different.

L34-35 Please add a reference.

L40-42 Please recheck, if Wilson et al. (2015) or Aslam et al. (2018) are suitable references for supporting the statement of "the production of so-called ice-binding proteins (IBPs)"

L52-54 Which reference states that EPS do have good ice-binding properties? Is there any knowledge under which conditions (chemical composition) EPS do have these properties?

L46-60 It might be important to clarify the relationship between the terms "ice-binding protein", "antifreeze protein" and "ice-nucleating protein" here. Do I get it right that less efficient ice-nucleating substances (in the sense that they are active at lower temperatures) can be at the same time antifreeze-substances at higher temperatures?

L59-60 "Here we explore whether a similar ice-nucleating effect does occur also for IBPs from *F. cylindrus*" -> What is the outcome? This might be an important result that deserves to be mentioned in chapter "5. Summary and Conclusions"

## **2. Material and methods**

L76 Please add the months, when ANT XVI/3 took place.

L76-81 When did the laboratory steps happen after the isolation in 1999? Back in 1999 or just recently, just before the ice nucleation experiments started? I just wonder how many cells (of those  $10^8$  cells) were still alive after a storage of approximately 20 years.

L84-94 This section could be shortened, since it contains much redundant information. It might be enough to refer to Tabel S1 in regard of the exact composition of the artificial sea salt.

L 94, 100, 104, 110, 118, ... If you only used one type of filter throughout this study, it might be enough to mention the filter type just once in the beginning.

L103 I guess replacing "*F. cylindrus* cells" with "*F. cylindrus* samples" might make it more accurate.

L106 Any reference that states that fcIBP11 is a soluble macromolecule detached from the cell? Or could it be connected to the cell surface of the diatoms as well?

L109-110 Is it possible to give an approximate estimate of the extend of cell loss?

L136 "...belongs to the DUF3494 IBP family,....".. was already mentioned in the introduction (L 43). Why is it relevant to mention it here again?

L145-184 Is it possible to shorten these sections onto the relevant information since all these methods have been published before? Of course, the main principle and deviations from the original protocol should be mentioned. If you want to keep all details, maybe it is possible to shift part of these descriptions to the SI? Instead I would appreciate a short explanation, why you chose two different setups (2.3.1, 2.3.2) for the ice nucleation experiments.

L185. You should check the appropriate use of "INP" throughout the manuscript. Maybe "ice nucleating molecules" or "ice nucleating entities" could be more correct here? I recommend to check the terminology proposed by (Vali et al., 2015).

L186-188 Is this true? I think the methodology in the literature is just different to yours, where bigger droplets with higher volume per droplet were used. E.g. (Budke and Koop, 2015) used in the BINARY 1  $\mu$ L droplets. Why did you choose for these small volumes of 380 pL (l 190) then?

L189-192: I find this statement quite surprising, since you started your experiments with a solution of a highly concentrated culture (see 2.1). Then you performed several dilutions. And now you are stating that the number of INP was not enough for your experimental setup. Why did you perform dilutions then?

L199-308 Is it possible to shift this section (or parts of it) into the SI?

### **3. Results and Discussion**

General: Is it possible to bring this part more on a point? It feels like reading and re-reading the same or similar aspects.

L 326 How can freezing at  $-44^{\circ}\text{C}$  be still relevant, when water droplets usually freeze at  $-38^{\circ}\text{C}$  homogeneously? Is it because of the small volume of the droplets in your setup?

Figure 5: Just as another example, where sentences can be shortened. Instead of: "b): Freezing temperatures of the same samples shown in panel (a), but after filtration with a pore size of  $0.22\ \mu\text{m}$ ." you could write: "b): Freezing temperatures of the filtered ( $0.22\ \mu\text{m}$ ) samples."

Line 369 "all diatoms" (?) or all cells?

L378 Within the whole manuscript, proteins are proposed as likely ice nucleating molecules. What about polysaccharides? Any tests performed into this direction?

L379 "macromolecules" or maybe "polysaccharides", to be more specific. Why is the comparison of diatoms with birch pollen relevant here?

#### **4. Discussion and Implications**

General:

What is the difference between the chapters "3. Results and Discussion" and "4. Discussion and Implications"? It appears to me that the text (or at least parts) of chapter 4 in the current version of the manuscript might still represent a subchapter of "3. Results and Discussion".

This study was mainly motivated with the atmospheric relevance of INPs (e.g lines 64-72, lines 534-536). However, a critical discussion of these (new) findings for atmospheric implications are still missing and could fit here. Some of the following aspects should be discussed in this section:

- Which atmospheric residence time would you expect for diatoms, their fragments and exudates? Whole marine diatoms are rather big and might precipitate within few

seconds or minutes. Can it be expected that complete cells/fragments will make it into the atmospheric layers relevant for mixed-phase clouds or even cirrus clouds? (as implied in lines 66-70)

- Which are the atmospheric concentrations of diatoms/*Fragilariopsis cyclindus* or fragments in the ambient air?
- In Figure 10, you nicely compare the ice nucleating activity of *Fragilariopsis cyclindus* with several diatoms from other studies. However, a rating of the importance of diatoms as INP in the polar regions/Southern Ocean in comparison to other types of INP (such as marine bacteria, mineral dust, ...) in regard of abundance and/or ice nucleating efficiency is missing.
- The Antarctic is known for a low number of efficient atmospheric INPs in comparison to the Arctic (e.g. (McCluskey et al., 2018; Wex et al., 2019; Hartmann et al., 2021; Zeppenfeld et al., 2021)). Considering this fact, how would you evaluate the importance of your findings for a better understanding of the Antarctic environment/atmosphere?

Specific:

L494 You performed own elementary analysis for obtaining the carbon content in your samples? Could you please add the method to chapter "2. Materials and Methods"? Few lines might be sufficient.

Figure 10: Is it anyhow possible to still extend this figure with the experimental freezing results on the diatom *Thalassiosira pseudonana* by (Wilson et al., 2015) or (Knopf et al., 2011)?

Figure 10: Now you show values which are normalized on mass (total mass?) At which part did you include the carbon content (L 494-495) then?

L505-507: "All temperatures were corrected for the freezing point depressions of different buffers..." How did you do it? Did you follow the approach by Koop and Zobrist (2009)?

L508-510 Is it necessary to mention this in the main text? It could be sufficient to add this information as a footnote in Figure 10.

L528 Is there any reason, why you convert °C to K at this late part of the manuscript? You have not done it before, so why here?

## 5. Conclusions

General:

The current version of the text rather represents a summary of the manuscript. However, conclusions are still sparse in this section. I'd recommend adding some real conclusions and a renaming of this chapter "Summary" or "Summary and Conclusions".

**Minor comments:**

L67 "can be transported"

L538: Remove "s" from "seas-ice"

L538 Check for a consistent writing of "sea-ice diatoms" versus "sea ice diatoms" throughout the manuscript

**References:**

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