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Characterization of Acremonium and Isaria ice nuclei

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Until recently, the only known fungal ice nuclei (IN) were a few exponents of lichen mycobionts and *Fusarium* spp. [Kieft and Ruscetti 1990, Pouleur et al. 1992, Hasegawa et al. 1994, Tsumuki et al. 1995], as well as two strains of mold [Jayaweera and Flanagan 1982]. Other investigated species did not show any IN activity [Pouleur et al. 1992, Iannone et al. 2011, Pummer et al. 2013]. In the last few years, IN-activity has been discovered in some rust and smut fungi [Morris et al. 2013, Haga et al. 2013], *Acremonium implicatum* (*Acr.*) and *Isaria farinosa* (*Isa.*) [Huffman et al. 2013] and a handful of other airborne and soil fungi [unpublished data].

We started characterizing the IN of *Acr.* and *Isa.*: Like other non-bacterial biological IN, they can be easily separated from the cells in aqueous suspension, and keep their activity. The IN-active aqueous suspensions were processed by filtration (5 μm, 0.1 μm, 300 kDa, 100 kDa) and exposure to heat (60˚C) or guanidinium chloride (6 M). The IN activity of the processed samples was measured by a freezing assay of droplets, as described by Pummer et al. [2013]. Via the Vali formula, we calculated the amount of IN per gram of mycelium, which is higher than 10^5 g^{-1}.

The initial freezing temperature was -4˚C for *Isaria* and -8˚C for *Acremonium* IN. Both were completely knocked out by 60˚C or guanidinium chloride. The *Acremonium* IN are in a mass range between 100 and 300 kDa. The *Isaria* IN seem to be either a bit larger, or more attached to larger particles, since not all of them pass through the 300-kDa-filter. It is likely that both of these new IN are proteinaceous like the IN of *Fusarium* spp. and lichen mycobionts, which belong to the *Ascomycota* phylum. Since the *Isaria* IN show a high onset freezing temperature and are rather large for single molecules, they might be agglomerates.

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