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## New species of ice nucleating fungi in soil and air

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Primary biological aerosol particles (PBAP) are ubiquitous in the atmosphere (1,2). Several types of PBAP have been identified as ice nuclei (IN) that can initiate the formation of ice at relatively high temperatures (3, 4). The best-known biological IN are common plant-associated bacteria. The IN activity of these bacteria is due to a surface protein on the outer cell membrane that catalyses ice formation, for which the corresponding gene has been identified and detected by DNA analysis (3).

Fungal spores or hyphae can also act as IN, but the biological structures responsible for their IN activity have not yet been elucidated. Furthermore, the abundance, diversity, sources, seasonality, properties, and effects of fungal IN in the atmosphere have neither been characterized nor quantified.

Recent studies have shown that airborne fungi are highly diverse (1), and that atmospheric transport leads to efficient exchange of species among different ecosystems (5, 6). The results presented in Fröhlich-Nowoisky et al. 2012 (7) clearly demonstrate the presence of geographic boundaries in the global distribution of microbial taxa in air, and indicate that regional differences may be important for the effects of microorganisms on climate and public health.

DNA analyses of aerosol samples collected during rain events showed higher diversity and frequency of occurrence for fungi belonging to the Sordariomycetes, than samples that were collected under dry conditions (8). Sordariomycetes is the class that comprises known ice nucleation active species (Fusarium spp.). By determination of freezing ability of fungal colonies isolated from air samples two species of ice nucleation active fungi that were not previously known as biological ice nucleators were found. By DNA-analysis they were identified as Isaria farinosa and Acremonium implicatum. Both fungi belong to the phylum Ascomycota, produce fluorescent spores in the range of 1-4  $\mu$ m in diameter, and induced freezing at -4 and -8°C. The IN seem not be bound to cells because they can be easily washed off the mycelium. They pass through a 0.1  $\mu$ m filter and can be inactivated by 60°C treatment.

Ongoing investigations of various soil and air samples indicate that diverse ice nucleation active fungi from more than one phylum are not only present in air and soil but can also be abundant components of the cultivable community. A recently discovered group of IN fungi in soil was also found to possess easily suspendable IN smaller than 300 kDa. Ice nucleating fungal mycelium may ramify topsoils and release cell-free IN into it. If some of these IN survive decomposition or are adsorbed onto mineral surfaces this contribution will accumulate over time, perhaps to be transported with soil dust and influencing its ice nucleating properties.

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