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ASM News
Cloudy with a Chance of Microbes

Terrestrial microbes swept into clouds can catalyze the freezing of water and may influence precipitation on a global scale

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In trying to disprove spontaneous generation, Louis Pasteur helped to launch experimental aeromicrobiology. Recognizing that virtually any microbe could be transported and disseminated in air, he developed sampling procedures to study airborne cells and soon showed that microbial abundances decreased at higher altitudes; precipitation scrubbed dust and cells from the atmosphere; and dust particles helped to disperse microbes in the atmosphere. Since then, aeromicrobiology has contributed to our understanding of airborne pathogens and the life cycles of other microbes.

Recent developments challenge the belief that microorganisms are passive travelers in the atmosphere. Indeed, evidence that microorganisms affect meteorological processes is invigorating research to define the role of biology in atmospheric processes and to understand how those processes might affect the abundance, dispersion, and viability of aerosolized microbes. In particular, some airborne microbes, called ice nucleators (IN), efficiently catalyze ice formation. This ice-nucleating phenotype (Ice\(^+\)), first described nearly 40 years ago, appears important for dispersing microorganisms in precipitation and might play a role in the formation of precipitation in clouds.

Independent Research Efforts Led to the Discovery of Bacterial Ice Nucleators

The formation of ice in clouds is important for the processes that lead to snow and most rainfall. At temperatures warmer than about −36°C, aerosol particles are required either to nucleate ice from water vapor or to freeze water droplets directly. Abundant mineral dusts and soot are presumed to serve as the major sources of IN in clouds (Fig. 1). IN are substrates on which supercooled water molecules aggregate into a metastable form, called an embryo. As water molecules are added to bring an embryo to a critical size, the probability for a phase change in which ice crystals form steadily increases. The maximum temperature at which IN can initiate the ice phase is specific to the nucleating material. Knowledge about the nature of IN in the atmosphere thus is fundamental to understanding ice phase precipitation and the radiative balance on Earth.

In the late 1950s, atmospheric scientists be-

Summary

- Knowledge about microbial and other ice nucleators (IN) in the atmosphere is fundamental to understanding ice phase precipitation and the radiative balance on Earth.
- Independent research groups, one consisting of atmospheric physicists and the other of microbiologists, independently discovered ice nucleating bacteria during the 1970s.
- A number of bacteria is known to produce similar ice-nucleating proteins that bind water efficiently and may explain their superiority as IN over dust particles.
- While borne in clouds and dispersed widely by atmospheric currents, a large abundance of Ice\(^+\) microorganisms would be expected to influence freezing and the processes which lead to precipitation.
- Despite growing knowledge and interest, the microbiota of the atmosphere remains a relatively unexplored frontier that appears ripe for further discovery.
gan to explore the sources of warm-temperature IN in samples of snow, rain, and hailstones. Laboratory simulations of clouds indicate that supercooling below \(-15^\circ C\) is required for ice nucleation in common mineral dust aerosols. Paradoxically, ice phase precipitation occurs in clouds at temperatures warmer than \(-15^\circ C\).

Organic-rich soils are enriched in IN, according to Gabor Vali of the University of Wyoming. Heating the soils to 200°C abolishes this activity, according to his collaborator Russell Schnell. In delving further during the summer of 1970, Schnell left leaf and grass samples soaking in distilled water and later noticed that these infusions were turbid. Testing indicated that the water froze at \(-1.3^\circ C\). “At that point, I suspected that the most active ice nuclei might be related to living microorganisms,” he later commented. Shortly thereafter, University of Wyoming microbiologist Leroy Maki screened bacteria and fungi from leaf surfaces for IN activity and found a pair of isolates with ice nucleation temperatures identical to that of the decaying leaf samples. By 1972, Maki identified the isolate as a strain of *Pseudomonas syringae* and demonstrated that its ice-nucleating efficiency strongly depended on the culture incubation temperature and media nutrients.

Independently, Paul Hoppe at the University of Wisconsin, who was breeding corn plants resistant to northern corn leaf blight, noticed in 1964 that frost damage occurred only on plants inoculated with dried preparations of *Exserohilum turcicum*-infected leaves. His observation remained unexplained until Steve Lindow joined Christen Upper at the University of Wisconsin in 1973 and tested leaf extracts that became contaminated by microbial growth. Plating the extract led Lindow to identify *P. syringae* as a strain that actively incited freezing on plants. The specific mechanism by which the bacterium incited frost damage remained elusive to Upper and Lindow until 1975, when the results from the group of atmospheric scientists and microbiologists at the University of Wyoming were brought to their attention. Thus two research groups with completely different scientific backgrounds and objectives independently discovered ice nucleating bacteria.

**Advantages of Being an Efficient Freeze Catalyst**

In addition to *P. syringae*, proteinaceous IN are also found in a variety of other bacteria such as *P. viridiflava, P. fluorescens, Pantoea agglomerans,* and *Xanthomonas campestris* and fungi such as *Fusarium avenaceum*, as well as algae, lichens, pollens, and freeze-tolerant animals. The ice nucleation protein of *P. syringae* (InaZ) is a 120- to 180-kDa protein containing a repet-
itive central domain flanked by nonrepetitive N- and C-terminal domains.

The ice-nucleating proteins from *P. syringae* as well as other Ice+/H11001 bacteria require a lipid environment for optimal activity. These proteins can form large homoaggregates, each containing as many as 50 protein molecules, on the surface of the bacterial outer membrane. The tandem octapeptide repeats (AGYGSTET) of the central domain of these proteins likely form a secondary structure of antiparallel β-strands, creating a hydrogen bonding environment that favors the binding of water molecules in a configuration similar to the ice crystal structure, according to modeling (Fig. 2).

The ability to bind water molecules in an ordered fashion that mimics the ice lattice is a characteristic that could explain the superiority of biological IN over mineral dust and other particulates as warm-temperature IN. Nucleic acid sequence comparisons of *ina* genes from various ice-nucleating bacteria indicate that coding portions of the N- and C-terminal gene product ends are homologous. Moreover, the repetitive sequences in the central domain vary from 832 to 1,280 amino acid residues. However, the number of repeats apparently is not a crucial determinant of ice-nucleating efficiency.

A considerable portion of microbes with the Ice+ phenotype associate with plants or are frank phytopathogens. Their ability to catalyze freezing at elevated temperatures may benefit these microorganisms in one or more ways. For example, inducing plant tissues to freeze would free nutrients from damaged cells and provide access to promote disease. The Ice+ phenotype might also help microbes to survive freeze-thaw cycling and could also protect host tissues against damage from supercooling and low-temperature ice formation. Because water vapor tends to accumulate on a frozen versus an unfrozen surface at subzero temperatures, Ice+ species may survive longer on plants by preventing their desiccation during extended periods of low-temperature quiescence.

Moreover, the capacity of *P. syringae* and other Ice+ microorganisms to catalyze the formation of ice could play a role in the dissemination of these microbes through the atmosphere. Due to their aerodynamic properties, particles the size of bacterial cells can remain suspended as aerosols for weeks. However, the modeled atmospheric residence time for an Ice+ microbe is about 20-fold shorter than that for cells incapable of ice nucleation. Thus, a mechanism that takes advantage of ice-phase scavenging might facilitate the return of airborne microbes to surface habitats that support their metabolism and growth.

**Microbial Influences on Meteorology and Precipitation**

Microbes can be aerosolized from virtually any surface and transported both horizontally and vertically in the atmosphere. They are ubiquitous in the near-surface and free troposphere, and are found in clouds at concentrations of about 10^6 cells m^{-3}. There are even reports of viable bacteria and fungi being collected from 10- to 50-km altitudes in the stratosphere and 50 to 100 km above the Earth in the mesosphere. Nevertheless, very little is known about...
the flux, abundance, and diversity of microorganisms in the Earth-atmosphere system.

Remarkably, certain atmospheric conditions may support microbial growth. Rates of heterotrophic production in supercooled cloud droplets suggest that cloud-borne bacterial biomass has the potential to increase by as much as 20% per day. Hence, microbes and their metabolic activities could affect meteorological processes in the atmosphere both by changing cloud chemistry and serving as nuclei for precipitation.

In 1978, David Sands at Montana State University collected Ice\(^{-}\) P. syringae on petri plates containing selective media that he thrust through the window of a small airplane flying at altitudes up to 2,500 m. From such sampling experiments, he concluded that P. syringae is capable of disseminating in the atmosphere via precipitation, perhaps over great distances. He speculated that airborne Ice\(^{-}\) P. syringae and other bacterial IN trigger precipitation as a means of moving to new plant hosts via the atmosphere, a process he called bioprecipitation (Fig. 3). In support of this general concept, P. syringae detaches from plant surfaces under dry conditions, becomes aerosolized, and can be transported in the air both horizontally and vertically, with net upward fluxes on the order of 50 to 500 CFU m\(^{-2}\) sec\(^{-1}\), according to Upper at the University of Wisconsin and his collaborators.

Soon after I met David Sands and his longtime collaborator Cindy Morris in 2005, I began collaborating with them in an effort to detect and quantify biological IN in fresh snowfalls collected in the vicinity of Bozeman, Mont. We soon expanded the range of our sampling to include sites in Louisiana, the French Alps and Pyrenees, Antarctica, and the Yukon. Although we did not expect biological IN in every precipitation sample analyzed, that is exactly what we found. For instance, melted snow and rain contain concentrations of 5 to 500 IN per liter that are active at temperatures warmer than \(-10^\circ\)C. Based on these analyses, about 95% of the IN are biological particles and at least 40% originate from bacteria. Further IN active above \(-10^\circ\)C are up

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**FIGURE 3**

Proposed life cycle of P. syringae between the phyllosphere and the atmosphere. Cells scrubbed in precipitation may encounter new plants directly or in rain or snow melt used for irrigation.
to 8 times higher in the near-surface atmosphere of agricultural compared with forested areas, according to Noah Fierer and his colleagues at the University of Colorado. *P. syringae* is detected in freshly fallen precipitation, atmospheric aerosols, and cloud water. To date, every strain of *P. syringae* that has been isolated from snow and rain is Ice\(^+\), whereas those isolated from other ecosystems it inhabits such as lakes, rivers, plants, and rock surfaces are less frequently ice nucleation active, according to Morris. This striking observation argues strongly that *P. syringae* has an active role as ice nuclei in the atmosphere. However, *P. syringae* typically constitutes only a small fraction of the total microflora deposited in the precipitation. In many precipitation samples that we have analyzed, we find significant levels of bacterial IN but no culturable populations of *P. syringae*, suggesting that *P. syringae* and comparable bacteria maintain their ice nucleation activities while losing culturability. Alternatively, such samples may contain novel microbial IN.

**Effect of Biological IN on Climate: a Game of Numbers**

Biological IN particles might play a role in the hydrological cycle and radiative balance of Earth. Enormous numbers—10\(^{24}\) to 10\(^{26}\) cells—of microorganisms inhabit leaf surfaces globally. About one-third of the ice crystal residues in clouds sampled over Wyoming are biological particles, providing direct evidence for the involvement of bacteria, fungi, and/or plant material in ice-cloud processes, according to Kimberly Prather of the University of California San Diego and Paul DeMott at Colorado State University. In addition, IN active above −25°C that originate from the Amazon rainforest are dominated by biological particles, and the cloud condensation nuclei are primarily composed of secondary organic aerosols from volatiles produced by terrestrial biota, according to Ulrich Pöschl of the Max Planck Institute for Chemistry and his colleagues. Taken with our findings and those of Fierer and his colleagues, these observations support the hypothesis that plant canopies are significant terrestrial sources of biological IN. Further, land use practices appear to influence the flux of biological IN to the atmosphere, and some microbial species disseminate in the atmosphere via precipitation.

Despite the fact that biologically derived materials are the most active naturally occurring IN, there are few measurements of their concentrations in the atmosphere. Moreover, their diversity and sources have not been systematically investigated, and their role in generating precipitation and influence over global climate remains speculative.

The challenges and complexities in measuring the Earth-atmosphere system demand interdisciplinary approaches to investigate the full meteorological role of biological IN. This interdisciplinary strategy is a fitting tribute to the atmospheric scientists, cloud physicists, microbiologists, and plant pathologists who crossed traditional boundaries to discover microbial IN several decades ago. Thanks to them and subsequent research efforts, we now recognize that the atmosphere plays a fundamental role in dispersing microorganisms, that clouds may support cellular reproduction, and that airborne microbes and their metabolic activities could affect the global climate. Even so, the microbiota of the atmosphere remains a relatively unexplored frontier that appears ripe for further discovery.

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**SUGGESTED READING**


