Looking Back: An Account of How Ice Nucleation by Bacteria Was Discovered (1963 to about Mid-1980s). Part II: Broadening the Scope
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ABSTRACT: In Part I, we described the discoveries we and our associates made in the 1960s and 1970s about biological ice nucleating particles (bio-INPs). The bio-INPs are far more effective than mineral INPs at temperatures above −10°C. The bio-INPs were found in decayed vegetation and in ocean water, and then, bacteria were identified as being the most active source for this remarkable activity. In this Part II, we recount how, within a few years, the worldwide distribution of bio-INP sources was shown to correlate with climate zones, as was the abundance of INPs in precipitation. Oceanic sources were further studied, and the presence of bio-INPs in fog diagnosed. The potential for release of bio-INPs from the ground to the atmosphere was demonstrated. Bacterial INPs were found to play a crucial role in a plant’s frost resistance. These and other early developments of biological INPs are described. A bibliography of related recent literature is presented in the online supplemental material (https://doi.org/10.1175/BAMS-D-23-0114.s1).

SIGNIFICANCE STATEMENT: In the decade following the discovery of biological ice nucleating particles (bio-INPs) in the 1960s, evidence was found for the abundance of bio-INPs in plant litters and in rain that correlated with the climate the vegetation grew in, and for the factors governing their presence in the oceans where they were being produced by marine bacteria. These findings laid the groundwork for the current recognition of the atmospheric role of bio-INPs.

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1. Introduction

This is the second of two articles giving a historical account of the discovery of biological ice nucleating particles (bio-INPs). In Vali and Schnell (2024, hereafter Part I), a description was given of the discovery of three major types of bio-INPs: the leaf-derived nuclei (LDN), the ocean-derived nuclei (ODN), and the bacteria-derived nuclei (BDN) (using the designations given at the time). Those results were at hand by the early 1970s (Schnell 1971, 1974a; Schnell and Vali 1976; Vali et al. 1976).

Findings described in this Part II deal with the worldwide distribution of biological INPs and their relationship to climate zones, and the similar variation with climate of INPs in precipitation. It was shown that natural clay particles acquire high activity by attachment of bio-INPs. The release of bio-INPs from the ground over various vegetation surfaces was demonstrated. A case was found showing the critical role of bacterial ice nucleators in the frost survival of a tropical plant. A few related and previously unpublished findings are also included. At the same time as these findings about the natural sources and distribution of bio-INPs were reached, studies of the nature and role of the bacterial ice nucleators in the biosphere also led to important discoveries. A summary of what was known by the mid-1990s is given in the book by Lee et al. (1995), and a more recent historical account is given by Lindow (2023). An annotated bibliography of recent results directly relevant to the material in this article is given in the online supplemental material (https://doi.org/10.1175/BAMS-D-23-0114.s1).

2. Worldwide and regional sources of LDN

a. Climate determines LDN abundance at the surface and in precipitation. Following the identification of highly active INPs as products of the decay of tree leaves (LDN), a wider range of samples were collected in order to examine how widespread the phenomenon is. After completing his Master of Science degree (Schnell 1971), RCS\(^1\) gathered samples as he traveled east across Canada, flew to Europe, then traveled by train from the Netherlands through Germany (West and East), Poland, and Russia, and traveled east across Russia to Vladivostok port on the Pacific Ocean. He collected litter samples in the top 2 cm at regular intervals. After traveling through Japan, he turned west traveling through Hong Kong, Thailand, Burma, East Pakistan, India, Nepal, Iran, and Great Britain. Returning to Alberta, he collected surface litter samples there and then drove south to Wyoming. The leaf litter samples collected from the top 2 cm of the leaf litter deposits were transported in a backpack in small plastic bags at ambient temperatures. In total, he collected hundreds of decayed leaf litter samples that were tested for INP with the drop freezing technique within weeks after RCS returned to the United States, as discussed in Schnell and Vali (1976).

The leaf litter samples were tested for INP by adding 1 g of leaf litter mixed into 100 mL of highly distilled water and tested with the drop freezing technique. The same procedures, weight of the material tested and the amount of water into which the samples were added, remained constant for the drop freezing measurements as discussed in Part I and in this article (Part II). It was noted that the INP activity of the leaf litter samples fell into three distinct
groupings correlated with Earth’s climate zones as defined by the Köppen climate classification system (Peel et al. 2007). As shown in Fig. 1a from Schnell and Vali (1973), the greatest number and most active freezing nuclei were from leaf litters collected in “microthermal” D climates in the latitudes of Canada and Russia, and the lowest concentration of active INP was from “tropical” A type climates such as those in southern Burma, southern Thailand, and India. Plant litters from “humid mesothermal” C climates such as the eastern United States and portions of the cooler subtropics contained freezing nucleus concentrations between the D and A climates. Differences are seen in the temperatures at which the spectra start, but the most significant difference is in terms of concentration: at −10°C, there are $10^7$ more INPs per gram of leaf litter in the samples from the D climate zone than from the A climate zones. This difference probably arises from the fact that organic material accumulates readily in soils under D climates. In contrast, there is more complete decay in the A climate zones in the tropics. These findings lend support to the notion that decay of leaf tissue is the source of LDN, not cellulose, or other inert material.

1) INPs in rain. The availability of INPs in surface soils (plant litters) is a plausible first link to INP abundance in the atmosphere and in precipitation. The large numbers of rain analyses accumulated over the years in our laboratory opened the opportunity to examine the link directly. Results for a total of 440 rain samples were available. Samples came from southern Florida, various locations throughout the United States, and also from west-central Canada. Results presented in Fig. 1b reveal that groupings of INP spectra by climate region mirror, although with a smaller magnitude, the differences by the climate zone in leaf litter seen in Fig. 1a of the same figure. This is no proof of the link between INPs in surface sources and those in precipitation, but the fact that most of the rain samples originated from convective storms where local aerosol sources dominate lends more support for the possible link (Schnell and Vali 1976).

2) LDN as atmospheric INPs. Bigg and Stevenson (1970) presented INP measurements from filter samples of ambient air collected at 44 sites scattered across the globe under a routine sampling strategy. A reanalysis of these results in Schnell (1974a; Fig. 7.7) revealed that the measured INP concentrations varied with the climate zone in the same direction as in soil and precipitation data. These measurements were made at −15°C in an ice thermal diffusion chamber with only 3 mm between the ice-covered plate and the filters to obtain as large a supply of water vapor as possible. The data show factors of 2–3 increases in INPs between climate zones A and D as seen in Fig. 1 in the global distribution of LDN. This smaller magnitude of the variation here, compared to Fig. 1, is due in part to the very different INP test method used: drop freezing technique for the LDN samples and an ice diffusion chamber for the air filter samples. It is reassuring that the relative trends are consistent between the datasets obtained by two very different measurement systems.

b. A possible link between LDN and atmospheric INP. The potential for LDN to be INPs in the atmosphere was demonstrated by showing that LDN can modify the ice nucleating ability of kaolinite, one of the minerals often found in atmospheric aerosols. In a relatively simple experiment, Schnell (1977a) packed reagent grade kaolinite that contained few INPs at $T > −15°C$ as tested by the drop freezing technique, into 25-cm-long, 5-cm-diameter glass tubes and added layers of 70-S-14 decayed leaf litter on top of the kaolinite. To simulate rainfall, distilled water was periodically dripped onto the top of the LDN/kaolinite columns keeping them wet until liquid reached the bottom of the column months later. The columns were left to dry after which 1 g from the bottom of the kaolinite columns was mixed into 100 mL of distilled water and tested for INP content by the drop freezing technique.
Before the tests, the kaolinite sample had $10^3$ g$^{-1}$ INPs at $-15^\circ$C and the LDN sample 70-S-14 had orders of magnitude more per gram of litter, as shown in Fig. 2. After the rain simulation, INP activity of the kaolinite increased by three orders of magnitude at $-15^\circ$C. When this activated kaolinite was baked at 500$^\circ$C, INP activity returned to that of the original reagent grade sample. These tests show quite clearly that mineral particles can acquire greater INP activity by adhered trace materials from more active LDN sources (Schnell and Vali 1976). This possibility is in concert with the general finding that biogenic particles bind well with clay particles (Delany and Zenchelsky 1976). Recent investigations of the composition of aerosol particles further confirm the near-ubiquitous presence of organic trace components in atmospheric aerosol.

**c. Release of INPs from soils.** A first step to assess how readily INPs get lofted from the surface was taken with measurements conducted using a portable wind tunnel over three vegetated surfaces at the Big Spring Field Station, Amarillo, Texas (Schnell et al. 1977). The surfaces were “green vegetation” consisting of pasture grass, postharvest hay crop “plant litter,” and...
“highly eroding” Amarillo loamy fine sand. Airflow through the wind tunnel was 11–12 m s\(^{-1}\). Aerosol samples were collected on 0.45-μm pore diameter Millipore filters 15 cm above ground level at the tunnel exhaust. The filters were processed in a static diffusion chamber at temperatures between −10° and −20°C at or exceeding saturation with respect to water.

The wind tunnel tests showed that the hay field plant litter released more INPs than eroding sandy soil under the same wind conditions. Air samples collected over undisturbed hay field plant litter (no wind tunnel air) contained elevated levels of active INP. Green vegetation did not release appreciably greater numbers of INP than the eroding sand soil. Taken together, these results show that there were more active INPs in the hay field plant litter that were aerosolized than from the aerosolized sandy soil or green grass.

d. Equatorial hail and LDN. The highest recorded occurrence of hail on Earth is near tea plantations near Kericho, Kenya, about 40 km south of the equator. There have been up to 132 annual hail days over the tea plantations (Linacre and Geerts 1998).

Samples of surface plant litters (top 2 cm) were collected in the Kericho tea estates, from the undisturbed tropical vegetation leaf litters in the Mau Forest, and in commercial eucalyptus plantations west of Kericho. The samples were stored at ambient temperature and flown to the University of Wyoming, Laramie, for measurements of INP content.

Data presented in Fig. 3, from the drop freezing tests performed weeks after collection, show that the tea leaf litters have consistently greater numbers of active freezing nuclei than the Mau Forest and eucalyptus plantation litters. Also, samples from the top of the leaf litters contained far more INPs, uniformly 3°–5°C warmer than samples collected 5 cm lower in the deep leaf litter.

It should be noted that these results reflect a sort of steady state in these samples (after dry storage over several weeks), not what may be the case if freshly decaying litter had been tested with, possibly, active bacteria INP present. It is also of interest that the INP spectra follow relatively close to INP spectra from Köppen’s A type (tropical) climate zones presented in Fig. 1.

Thus, as an early step leading to the formation of hail in the Kericho cumulus clouds, it was suggested in Schnell and Tan-Schnell (1982) that INPs from the tea litters could be released to the atmosphere either naturally or assisted by the feet of hundreds of tea pickers going...
about their daily tea leaf harvest. Once airborne, these small, light particles could be drawn aloft into the growing cumulus clouds to initiate hail formation.

e. Was the Sahelian drought linked to the removal of LDN sources by overgrazing? In the late 1960s, intense overgrazing by livestock occurred in the Sahel regions of sub-Saharan Africa as a result of improved veterinary practices, animal inoculations, and water wells dug by aid programs. This overgrazing was followed by a massive drought in the region that decimated livestock numbers. After 1974, the absence of livestock allowed the grasslands to recover somewhat. Rainfall returned in the succeeding years. Based on the correlation between soils (leaf litters) and rain on the scale of climate regions (section 2a), RCS hypothesized that the removal of vegetation and associated INPs may have led to the reduced chance of rainfall due to fewer INPs available to initiate rain in the local cumulus clouds.

To test the hypothesis, RCS spent a month in the Sahel, collecting soil and plant litter samples on west-to-east and north-to-south transects covering hundreds of miles. Most of the Sahel was practically devoid of grass though scattered areas had some grass cover as the livestock that would have eaten the grass had died a year before.

In total, 76 samples were obtained. The samples were stored at ambient temperatures and flown to the University of Wyoming where INP measurements were made within weeks of collection by the drop freezing technique, as reported by Schnell (1974b). INP spectra fell into three groups (Fig. 4) with fresh organic litter exhibiting greater concentrations of INPs active at temperatures warmer than −10°C than inorganic soils. Soils with a moderate content of organic material are also intermediate in INP content. These results lent some support to the hypothesis that reduced rainfall may be linked to the removal of vegetation by overgrazing.

3. ODN as atmospheric INPs

The initial finding of high activity is near-surface samples of ocean water (Part I; Schnell and Vali 1975) were further confirmed by correlation between airborne INPs and oceanic sources by Schnell (1975, 1977b). Bigg (1973) published the results of shipboard samples of INPs over the Southern Ocean. RCS’s analysis showed that the highest concentrations of INPs coincided...
with high marine phytoplankton production zones. It was argued that INPs in the air near the ocean surface originate from sea spray and bubble bursting providing a link of ODN to the presence of phytoplankton.

More direct observations of ODN were obtained by Schnell (1977b) during 2 weeks onboard the U.S. Naval Ship (USNS) Hayes Fog Research Cruise (28 July–11 August 1975). Seawater and fog water samples were tested upon collection for INP content with a portable drop freezer. It was found important to perform the tests within minutes to an hour of collection as ODN activity was observed to decrease significantly within a few hours.

The main findings in Schnell (1977b) were that the ODN contents of ocean surface water and of associated fog-derived nuclei (FDN) were almost identical in some cases but quite different in others. Sample results shown in Fig. 5 illustrate cases when both high and low activity samples of ODN and FDN were similar (upper panel) and others when there are large differences (lower panel). In the latter cases, the fog samples have higher INP content than the local seawater. This is not surprising as fog is advected over distances without dissipating.

The research crew on the Hayes also included two marine microbiologists who were successful in isolating bacteria from the seawater and fog water samples. Some bacteria cultures washed off the agar plates were found to be highly active, with −2.0°C initial freezing temperatures. The bacteria were not identified. Infrared reflectance analyses of sweater and fog water were conducted throughout the cruise (Baier 1976) to look for glycoproteins. It was observed that the samples of seawater and fog water with highly active INPs also contained the highest glycoprotein levels measured on the cruise. These data suggest an organic or bacterial origin for ODN.

4. Other sources of atmospheric INPs compared with LDN and ODN

a. Volcanic ash. Volcanic aerosols have been long suggested as being atmospheric INP sources. Schnell and Delany (1976) collected aerosol samples on filters upwind and downwind of eruption plumes from Mount Saint Augustine, Alaska, using the National Center for Atmospheric Research (NCAR) Electra aircraft. Testing the filters for INPs in an ice thermal diffusion chamber showed that the ash made no contribution to ice nucleating activity above that measured upwind of the volcano. Similar results were shown by Radke et al. (1976) in the Mount Baker (Washington) 1975 eruption plume.

During the eruption of Mount Saint Helens in 1980, ash was collected on air sampling filters with a light aircraft upwind and within the plume. From these measurements and for ash samples aerosolized into a Mylar lined tent, the ash was found to have few INP events at temperatures warmer than −10°C and had INP concentrations of only ∼10 g−1 active at −18°C as shown in Fig. 6 (Schnell et al. 1982). The volcanic ash from Mount Saint Helens had INP content similar to that of reagent grade kaolinite (section 2b).
**b. Coal-fired power plants.** Aerosols were collected on filters with a light aircraft upwind and within the plumes of three different coal-fired power plants in the western and one in the eastern United States. The filters were stored at ambient temperature and processed in an ice thermal diffusion chamber. It was found that none of the power plants had any effect (increase or decrease) on the natural INP concentrations measured upwind of the plants (Schnell et al. 1976; Pueschel et al. 1979).

**5. Lobelia telekii survives frosts due to ice nucleation by bacteria in the interior of the plant**

RCS worked in Kenya, Africa, for 2 years in the 1970s on a high-elevation meteorological research project. He noticed that *L. telekii* plants (Fig. 7) survived nightly temperatures down to −10°C. The plant accomplished this by having the liquid in their interior freeze when the air temperature cooled below about −2°C. This initiation of freezing protected the plant from greater tissue damage that would have been caused by freezing occurring at colder temperatures. Latent heat released by the freezing of the liquid also helped *L. telekii* to survive.

RCS hypothesized that freezing of the liquid may be bacteria mitigated. To test this hypothesis, he collected liquid samples from the interior of *L. telekii* and tested them for INP activity by the drop freezing technique within hours of collection. Nearly every liquid sample had INP activity warmer than −4°C and some froze at −1.5°C. Liquid samples as well as portions of intact *Lobelia* plants were frozen on dry ice immediately after collection and flown to the United States. Professor Ray Fall, University of Colorado, tested the samples with the drop freezing technique and found two species of bacteria that initiated ice when added to droplets of distilled water, at temperatures of −2°C, as warm as the most active *P. syringae* INP strains. After extensive purification and testing, the INP bacteria were identified as *Pseudomonas viridiflava* and *Pseudomonas chlororaphis*.

![Fig. 5. Relationship between ODN in seawater and FDN in fog water. (top) Cases where the ODN and FDN spectra are similar for both high- and low-activity situations. (bottom) Cases where the ODN and FDN spectra are diametrically opposite even though the respective samples were collected at the same time. The 2°C melting point depression is applied to ODN samples (figure reproduced from Fig. 3 in Schnell 1977b).](image-url)
6. Where did this work lead?
Insights and new research directions that flowed from the work presented in Part I and Part II of this article are sketched here briefly, without the addition of literature citations. An annotated bibliography in the online supplemental material (https://doi.org/10.1175/BAMS-D-23-0114.s1) provides a guide to recent results that better indicate the full extent of what is now known about bio-INPs and their impacts.

A handful of bacterial species were identified early on as active ice nucleators. By now, the number and variety of known bio-INP must be in the hundreds. The catalog of bio-INPs is amazingly extensive: many species of living and dead bacteria, fungi, and macromolecules. They exist in a huge range of environmental conditions and in many geographical areas. Isolating and identifying these bio-INPs is an ever-growing process with the participation of many researchers around the world. New tools have been developed for diagnosing and analyzing bio-INPs via increased knowledge in molecular biology and with the help of miniaturization and huge computational power. All this is done within the framework of aerosol chemistry and physics, including long-range transport and transformation processes.

The association of activity with proteins on the outer surface of bacteria and fungi is being elaborated by direct analyses and by molecular simulations. These studies are closely linked to the general question of what surface characteristics lead to ice nucleation on different materials.

The important roles of bio-INPs in the atmosphere have been quite successfully demonstrated. Samples of rain and snow from many parts of the world show INP spectra similar to those discussed in early sections of this document. The strong contribution by organic components to INP activity is well documented. The correlation of INP abundance with climate zone has more and more evidence. Both as a consequence and as a cause of climate change, elements of the link between vegetation, bacteria, and other bio-INPs are known and will surely be subjects of much future research.

New instruments capable of detecting atmospheric INPs active at temperatures above −15°C accelerated knowledge about bio-INPs. Through chemical analyses of these bio-INPs, more is learned about their sources. The impact of these bio-INPs on tropospheric clouds is an important element of the progress that weather and climate models seek.

Many other scientific and practical issues are connected to bio-INPs. Bacterial INPs, specifically SNOMAX, are widely utilized for artificial snowmaking. SNOMAX has become almost a reference standard in testing ice nucleation instruments and to study basic ice nucleation physics.
Ice nucleation as the key process, and the identification of biological agents promoting ice nucleation, has led to advances in understanding, and perhaps managing, frost resistance in flora and fauna. Much has been learned about the natural freeze resistance that organisms create. Modification of frost resistance via changes in ice nucleating properties has been proven possible.

Important recent work is moving closer to identifying how ice nucleation is taking place on the molecular scale. Both by direct observation and by simulations, specific sites and their characteristics are identified as most favorable for ice germ formation and nucleation. Some of this work focuses on bacterial cell surfaces which provide a different challenge.

Much more could be said regarding the state of knowledge about bio-INPs and related matters. The number of leads to follow in future research is huge. Practical applications exist and will grow. Observations of the atmospheric INPs and of cloud processes are steadily improving via in situ and remote sensors. Model simulations are sharpening knowledge and gauging the weather and climate impacts of bio-INPs. In the biological realm, the marvelous developments of tools for molecular and genetic analyses and manipulation have made progress truly exciting. Progress over the past half a century clearly shows that more is to follow.

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Data availability statement. No new data or new analyses were used in this article. Data depositories were not in use at the time work here described was published. Some of the data used in the original papers may be available by request to the authors.
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