ICE NUCLEATION STUDIES ON BACTERIA AEROSOLS

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1. INTRODUCTION

One species of epiphytic bacteria, Pseudomonas syringae, has been identified as a principal natural ice nucleant (IN) initiating frost damage in leaves of frost-prone North American plants (at temperatures warmer than -5°C (Lindow et al. 1978)). In the absence of these specific bacteria, living vegetation has been observed to supercool to -6°C and to recover without loss of vigor. This IN active bacteria has been collected in the atmosphere in a viable state above and downwind of field crops (Lindemann, et al. 1979) and in clouds over the Arctic Ocean (Planagen and Jayaweera, 1980). In both studies, the bacterium was found to have maintained its ability to initiate ice at supercoolings of -5°C or warmer.

In cloud chamber tests, P. syringae, has been observed to be a good IN at temperatures colder than -8°C (Schnell, 1976, Makl and Willoughby, 1978) which is somewhat colder than the -1.5°C to -2.5°C threshold nucleation temperatures observed in bulk suspensions of the bacteria (Vali, et al. 1976). In this report we present data on a series of experiments in which the IN properties of P. syringae aerosols were determined under controlled laboratory conditions.

2. METHODS

Water suspensions of actively growing P. syringae cultures were nebulized into a 2.75 m^2 aerosol tent constructed of Mylar. A typical aerosolization consisted of approximately 1.5 x 10^7 bacteria suspended in 10 cm^2 of water sprayed into the tent over a 1 minute period. The relative humidity in the tent never exceeded 50% during the tests reported in this paper.

Aerosol size and number distributions in the tent were monitored with two overlapping laser aerosol spectrometers (active scattering aerosol spectrometer (ASASP-X) and a forward scattering aerosol spectrometer (FSASP)) measuring particle radii from 0.045 µm to 23.5 µm in eight partially overlapping ranges of 15 bins each (Knollenberg, 1976). Total suspended aerosol mass per unit volume of air sampled in the tent (averaged over a 5-min period) was determined by integrating under the DV/D log R curves in a manner outlined by Barrett et al. (1979), assuming a density of 1 for the suspended bacteria.

At intervals while the aerosols were aging in the tent, 25-2 samples of the tent contents were filter sampled using 0.45-µm pore diameter Sartorius hydrophobic membrane filters, and the IN content of the captured particulates determined within 10 minutes of collection using a combination membrane filter-drop freezing technique (Schnell, 1979). In this IN measurement technique, a filter is bonded to a clean aluminum-foil-covered copper plate which can be thermoelectrically cooled at a slow and steady rate such that the freezing temperatures of an array of distilled water drops placed on the filter can be monitored. The freezing temperatures of the drops are then used to produce a freezing-nucleus spectrum for the aerosol as illustrated in Figures 2 and 3 of this report.

3. RESULTS

A DV/D log R plot of a P. syringae aerosol at 1 hour after aerosolization is shown in Figure 1. From this Figure, it may be observed that the aerosol distribution peaked in the range of 1.0 µm to 1.5 µm radius (2.0 to 3.0 µm diameter) and had a mass of 225.6 µg/m^3. Since individual P. syringae cells are about 1.0 to 1.5 µm in length, these data suggest that some clumping of cells occurred in the aerosol. This suggestion is supported by observations that when exceptionally high concentrations of bacteria were aerosolized into the tent, (thereby increasing the probability for clumping), the peak of the distribution shifted to larger diameters, in one case, up to a diameter of 8 µm.

The IN spectra from a typical P. syringae aerosol aging experiment are shown in Figure 2. From this figure it may be observed that immediately following aerosolization (time, 0) the aerosol contained IN active at -1.6°C in concentrations of 10 ± 1 active at -2.0°C. One hour later, the concentration of active IN had
decreased appreciably (time, 1 hr) and by 3 hours after aerosolization (time, 3 hr) threshold nucleation had decreased to −7.2°C with a concentration of 10 F−1 active at −12.5°C. The aerosol distribution shown in Figure 1 corresponds to the time of the nucleus spectra 1 hour after aerosolization in Figure 2.

As mentioned earlier, the IN activity of the bacteria on the filters was determined within 10 minutes of filter exposure. This was done as earlier research (Scnell et al. 1980) had shown that natural IN, once deposited upon filters, tended to lose activity over relatively short periods of time. To monitor the potential change of IN activity on the filters exposed in this study, triplicate filters were exposed simultaneously, then developed later. The results of one such experiment are shown in Figure 3 where it may be observed that appreciable IN activity was lost within the first 2 hours of storage with a proportionately smaller loss over the following 24 hours. In other tests on these aerosols, it was observed that beyond 24 to 36 hours further loss of ice nucleus activity was not appreciable (up to one month).

Figure 1. Volume-size plots of a P. syringae aerosol at 1 hour after aerosolization. The aerosol at this time possessed the ice nucleation characteristics depicted in Figure 2, time 1 hr.

Figure 2. Ice nucleus spectra of P. syringae aerosol in a 2.75 m² tent as a function of time after aerosolization.

Figure 3. Ice nucleus activity losses of P. syringae aerosols stored at room temperature on membrane filters.
The above data show that it is possible to aerosolize P. syringae into an enclosed volume and recover associated IN activity in appreciable concentrations over periods up to 3 hours. In other tests conducted on the same bacteria, IN were recovered up to 8 hours after aerosolization although the IN were present only in small concentrations and possessed activity only marginally better than background IN. The average concentration of ice forming bacteria (as measured by active IN on the filters) in the tent aerosol was in the region of 1 \( \times 10^{-1} \) active at \(-10^\circ\text{C}\) which is less than the average concentration of 1.7 \( \times 10^{-1} \) viable bacteria (maximum 6.5 \( \times 10^{-1} \)) observed in natural air over midwest agricultural crops (Lindemann et al., 1979). But, the concentrations of bacteria in the tent were considerably greater than the average of 0.04 \( \times 10^{-1} \) living bacteria in the atmosphere capable of producing IN reported in this same study. In the only other study of atmospheric bacterial IN known to the authors, Flanagan and Jayaweera (1980) report concentrations of up to 150 viable bacteria per liter of air sampled in stratus clouds over the Arctic Ocean in June 1980, with the total count of bacteria, living plus dead, occasionally observed to be in excess of 2000 \( \times 10^{-1} \). A proportion of the bacteria they collected were of the genus Pseudomonas and were active IN at temperatures as warm as \(-5^\circ\text{C}\). It is possible that some of the dead (dormant) bacteria could have been IN as Maki et al. (1974) have shown that dead P. syringae may retain IN active at \(-5^\circ\text{C}\).

In summary, we suggest that at some point in the tent studies, the concentration of IN active bacteria may have been similar to those observed in the natural atmosphere. If this was so, our data show that aerosolized P. syringae may remain airborne for appreciable periods of time and maintain threshold IN activity at temperatures in the region of \(-5^\circ\text{C}\) and warmer. Thus, our observations support the results of other scientists which suggest that IN active bacteria in the atmosphere may be playing important roles in transmitting frost sensitivity between agricultural crops, and that the bacteria have a potential to act as atmospheric IN.

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