Effect of Plant Species and Environmental Conditions on Ice Nucleation Activity of Pseudomonas syringae on Leaves

R. DOUGLAS O'BRIEN† AND STEVEN E. LINDOW*

Department of Plant Pathology, 147 Hilgard Hall, University of California, Berkeley, California 94720

Received 19 January 1988/Accepted 17 June 1988

Selected plant species and environmental conditions were investigated for their influences on expression of ice nucleation activity by 15 Pseudomonas syringae strains grown on plants in constant-temperature growth chamber studies. Ice nucleation frequencies (INFs), the fraction of cells that expressed ice nucleation at −5 or −9°C, of individual strains varied greatly, both on plants and in culture. This suggests that the probability of frost injury, which is proportional to the number of ice nuclei on leaf surfaces, is strongly determined by the particular bacterial strains that are present on a leaf surface. The INFs of strains were generally higher when they were grown on plants than when they were grown in culture. In addition, INFs in culture did not correlate closely with INFs on plants, suggesting that frost injury prediction should be based on INF measurements of cells grown on plants rather than in culture. The relative INFs of individual strains varied with plant host and environment. However, none of seven plant species tested optimized the INFs of all 15 strains. Similarly, incubation for 48 h at near 100% relative humidity with short photoperiods did not always decrease the INF when compared with a 72 h, 40% relative humidity, long-photoperiod incubation. Pathogenic strains on susceptible hosts were not associated with higher or lower INFs relative to their INFs on nonsusceptible plant species. The ice nucleation activity of individual bacterial strains on plants therefore appears to be controlled by complex and interacting factors such as strain genotype, environment, and host plant species.

Bacteria are a major environmental source of ice nuclei that are active at temperatures greater than −10°C (10, 18, 23). Ice nucleation-active (INA) bacteria are present in water (18), on plants (1, 9–11, 13, 16), and in the atmosphere (8, 18). Bacterial ice nuclei have a proposed role in precipitation processes (18, 23) and plant frost injury (15). Many frost-sensitive plants supercool and avoid damage from ice formation at temperatures above −5°C if INA bacteria are absent (9, 15). When present, these bacteria limit the supercooling of water in plant tissue and incite frost injury. Of the several INA bacterial species, Pseudomonas syringae is the most common and most active (1, 9–11, 13). Different strains and pathovars of this species exist as epiphytes or pathogens on most plant species (1, 3, 13). Treatments that reduce the population size or ice nucleation activity of epiphytic INA bacteria have resulted in less plant frost injury (9, 11, 12, 16).

The ice nucleation frequency (INF) of INA bacteria, expressed as the logarithm of the number of cells per ice nucleus in a population of cells at a given assay temperature, varies widely in response to physical and biological factors and among strains (2, 17). Not every cell expresses the ice nucleation phenotype at a given time (17). Most measurements of INF have been performed in culture. Consequently, little is known about the natural habitat of INA strains, the variability in INF of different strains on plants, or the influence of different plant species and climatic conditions on this phenotype. One report indicated that cells of a single strain of P. syringae were more likely to produce ice nuclei active at −5 or −9°C when grown in culture than when grown on corn (9); however, other variables were not examined. The average INF of INA bacteria on different field-grown plants varies widely (9), presumably because of uncontrolled factors such as physical environmental conditions, chemical or nutritional variables, and the different compositions of INA bacterial strains. The entry of pathogens into susceptible hosts has been associated with frost injury to plants (6, 20). However, the role of ice nucleation in disease reactions between compatible host plants and pathogenic P. syringae strains has not been established. Pathogenic strains should express ice nucleation efficiently on susceptible plants, if this is a factor that determines virulence to such plants. An analysis of the specificity of expression of ice nucleation activity on leaf surfaces would therefore assist in predicting the frost sensitivity of the crops on which they reside, the quantity of nuclei available for release into the atmosphere, and the role of ice nucleation in plant disease.

The purpose of this study was to examine selected environmental factors for their influence on bacterial INFs in a random sampling of diverse P. syringae strains on various plant hosts. (A preliminary report [abstract] of this work has appeared previously [R. D. O'Brien and S. E. Lindow, Phytopathology 76:1068, 1986].)

MATERIALS AND METHODS

Bacterial strains. Fifteen P. syringae strains were collected from a range of plants and geographical areas and tested in three groups: strains isolated from asymptomatic plants as epiphytes (experiment 1); strains pathogenic to tomato (Lycopersicon esculentum L.), cucumber (Cucumis sativus L.), and bean (Phaseolus vulgaris L.) plants (experiment 2); and strains pathogenic to pea (Pisum sativum L.), corn (Zea mays L.), and oat (Avena sativa L.) plants (experiment 3) (Table 1). Spontaneous P. syringae mutants resistant to 100 ppm (μg/ml) of rifampin were selected after 10⁶ cells of each strain were spread onto King B medium (KB) (4) containing 100 μg of rifampin per ml (KBR). All strains were stored in 15% glycerol at −80°C. P. syringae strains and appropriate control strains were tested for
growth on minimal media, ice nucleation activity by a droplet freezing assay (14), pathogenicity on several plant hosts, levan production (7), oxidase reaction (19), tobacco hypersensitivity (5), and arginine dihydrolase reaction (21). For pathogenicity tests, all strains were spray inoculated and infiltrated into bean, pea, tomato, cucumber, oat, corn, and potato (Solanum tuberosum L.) leaves and stems at a concentration of 10^8 cells per ml. All strains were also infiltrated into these plant species at a concentration of 10^9 cells per ml. Pear fruit and citrus seedlings were wounded by stabbing them with a sterile toothpick, and the wounds were swabbed with cell suspensions of 10^6 cells per ml. Water soaking or lesion development in leaves after 2 to 10 days was considered evidence of pathogenicity. Desiccation and necrosis of leaf tissue at the site of inoculation within 24 h was considered a hypersensitive reaction.

**Inoculation and incubation of plants.** All bacterial strains were grown for 48 h at 24°C on KBR to obtain sufficient cells for inoculation. Cells were harvested with a loop and suspended in sterile water. Cell concentrations were determined spectrophotometrically and adjusted to 5 × 10^9 cells per ml by diluting them with sterile distilled water. Cell concentrations were confirmed by plating samples of appropriate dilutions of each inoculum suspension onto KBR.

The bacterial suspensions were sprayed with an atomizer until there was a runoff on 4- to 6-week-old greenhouse-grown plants in 20-cm-diameter pots. Plants were not watered from above prior to inoculation to keep leaf surfaces relatively free of bacteria. The strains isolated as epiphytes were sprayed onto bean (Bountiful), tomato (Bonny Best), oat (Cal Red), and potato (White Rose) plants. Both groups of pathogenic strains were sprayed onto bean (Eagle), tomato (Peto 95), oat (Cayuse), corn (PX20), cucumber (National Pickling), and pea (Spring) plants. Each strain was sprayed onto one pot of each plant species. Each experiment was repeated 4 times to constitute four replicates of each treatment in a randomized complete block design. For each experiment, all inoculated plants were sequentially subjected to two different physical environmental conditions: humid with low light levels for 48 h followed by dry, high-light incubation for 72 h. The moist environment was achieved by enclosing the plants, which were moistened by spray inoculation, into clear plastic bags in a large growth chamber (model PGW36; Conviron), at 24°C with a 10-h light period (240 microeinsteins/m²). Plants were then unbagged and left in the growth chamber and incubated under dry conditions (40% relative humidity and 24°C with a 14-h daily light exposure of 960 microeinsteins/m²). The temperature was kept constant at 24°C, which is within the optimum range of expression of bacterial ice nucleation (17). The light intensity was kept low in the bagged incubations to avoid a greenhouse effect within the bags. A higher light intensity during the unbagged incubations was used to simulate that of natural sunlight.

Because contaminating rifampin-resistant or INA bacteria on leaves would affect estimates of INFs, leaf samples were taken prior to inoculation to determine the background contamination by such bacteria. Cells washed from uninoculated plants as described below were dilution plated onto KBR, onto KB containing 50 μg of benomyl per ml and 100 μg of cycloheximide per ml (KBCB), and onto SSM media (12.0 g of sorbitol, 0.8 g of K2HPO4, 3H2O, 0.8 g of K2PO4-3H2O, 0.13 g of MgSO4·7H2O, 0.2 g of L-histidine, 128 mg of mixed alkytrimethylammonium bromide (Cetrimide), and 15.0 g of agar in 1 liter of H2O). KBCB and SSM media enabled the determination of rifampin-resistant bac-

teria and of total and pseudomonad background contamination, respectively. The plates were incubated at 24°C, and bacterial colonies were counted after up to 6 days. Representative colonies were transferred with velvet to paraaffin-coated aluminum foil and sprayed with sterile distilled water, and the sheets were floated on a refrigerated ethanol bath at −9°C and observed for ice nucleation activity to estimate the population size of INA bacteria (13). No INA or rifampin-resistant bacteria were detected on noninoculated leaves. The average of the log of bacterial population sizes prior to inoculation was 3.4 and 1.8 cells per g (fresh weight) on KBCB and SSM media, respectively, for all experiments and all plant species. Population sizes of contaminating bacteria did not differ on the various plant species (data not shown).

**Ice nucleation activity measurements.** The INFs of *P. syringae* strains were measured at −5 and −9°C, both in vivo and in vitro, by using a droplet freezing assay similar to that reported previously (14, 16). Cells for in vitro INF testing were grown for 48 h at 24°C on KBCB medium. A total of 15 to 40 individual leaves (15 to 20 g) were collected from each pot of plants, after incubation under both wet and dry conditions, to determine bacterial INF. The leaves were submerged in 100 or 200 ml of sterile washing buffer (0.1 M potassium phosphate buffer containing 0.1% Bacto-Peptone [Difco Laboratories Detroit, Mich.] [pH 7.0]) and sonicated for 7 min in an ultrasonic cleaner (Branson Sonic Power Co., Danbury, Conn.) to remove cells from the leaves. A collection of 40 10-μl droplets of appropriate dilutions of leaf washings or suspensions of cells harvested from KBR plates after 48 h of growth at 24°C were placed onto the surface of a paraffin-coated aluminum foil sheet floating on the surface of a refrigerated circulating ethanol bath that was maintained at −5 or −9°C (14). The cumulative number of ice nuclei per milliliter active at temperatures of −5 or −9°C or warmer, n(T), was calculated from the fraction of frozen droplets (f) at each of these two temperatures and the volume of each drop (V) in milliliters by the method of Vali (22) as n(T) = ln [1/(1−f)]/V.

In samples in which ice nucleation activity was low, 30-ml samples of leaf sonicates were centrifuged for 20 min at 28,000 × g, the supernatant was discarded, the cells were suspended in 1 ml of sterile distilled water, and ice nuclei were quantified as described above. Appropriate 10-fold serial dilutions of the cells that were removed from the leaves were plated onto KBCB containing 100 μg of rifampin per ml to obtain the cell density. The INF of a sample was calculated from the number of ice nuclei per gram of leaf tissue (n) and the number of cells per gram of leaf tissue (C) as log (C/n).

**Statistical methods.** Statistical computations were made by using software provided by Statistical Analysis Systems (release 5.16; SAS Institute, Inc., Cary, N.C.). The Statistical Analysis System general linear models procedure was used to perform analysis of variance on log-transformed data to determine the significant biological and environmental effects that influence the INF. Mean comparisons were made with the Ryan-Einot-Gabriel-Welsh multiple range test. This test controls the type I experimentwise error rate and has a lower type II experimentwise error rate than many other tests (Statistical Analysis System, SAS Institute, Inc.). Linear regression analysis was used to test for a direct relationship between INF in culture and on plants.

**RESULTS**

Results of biochemical and physiological tests confirmed that all bacterial strains used were prototrophic and indistin-

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guishable from \textit{P. syringae} (data not shown). Pathogenic strains infected only one host species, with only two examples of cross infectivity (Table 1). Limited water soaking and small lesions were observed when 10^6 cells of strains 407R and 655R per ml were infiltrated into pea and cucumber plants, respectively. All strains, including those with unknown pathovars, induced necrosis on most nonhost plants following infiltration at 10^6 cells per ml. Occasional hypersensitive reactions were also observed at 10^6 cells per ml. Although strain 563R was isolated from healthy citrus leaves as an epiphyte, it was also pathogenic to citrus. Disease symptoms were observed after dry incubation in all experiments for all pathogen and susceptible host combinations except strain 821R on tomato plants.

Analysis of variance of INFs on plants. Plant species and incubation environment significantly affected the INFs of the strains in all three experiments (Table 2). Interactions among strains, environmental conditions, and plant species sometimes occurred. Similar factors influenced INFs at both -5 and -9°C (data not shown).

Effects of strain, cultural condition, and plant species on ice nucleation activity. The INF means from all significant main effects were compared to show the range of INFs resulting from the effects of single factors. Individual strains differed significantly when INFs of each strain at -5°C, averaged over all plant species and both in planta environments, were compared (Table 3). Strains also differed significantly in INFs when grown in culture (Table 3).

Cultural conditions (culture on KBR or wet or dry incubation on plants), when averaged over all strains, affected bacterial INFs at -5°C significantly in all experiments (Table 2). Generally, the ice nucleation activities of strains in culture were lower than those after growth on plants (Table 3). However, the light intensity and humidity to which plants were subjected affected INFs differently in the different experiments. Bacteria had higher ice nucleation activities after wet incubation in the experiment with epiphytically isolated strains, while the INFs were favored after dry incubation in the experiments when strains isolated as pathogens were used. Regression analysis failed to show a significant prediction of INFs of cells on plants at -5°C by measurements of INFs of cells grown in culture (r^2 = 0.10).

The effect of different plants on bacterial INFs was estimated by comparing bacterial INFs at -5°C on each plant averaged over both environments and all strains (Table 4). For each experiment, plant species which significantly increased or decreased bacterial INFs were identified. Bacteria were applied to tomato, bean, and oat plants in all three experiments; bacterial INFs could thus be ranked on these host plants by using the Spearman coefficient of rank correlation (24). Estimates of bacterial INFs from strains inoculated onto tomato, bean, and oat plants, when blocked over the three experiments, did not differ at P = 0.05 by this test (data not shown).

Interactions among strains, cultural conditions, and plants in determining INFs. Analysis of variance indicated the likelihood that the light intensity and humidity to which

### TABLE 1. Characteristics of \textit{Pseudomonas} strains used in ice nucleation activity experiments

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Bacterial strain</th>
<th>Plant source*</th>
<th>Geographical origin of strain</th>
<th>Supplierb</th>
<th>Host infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{P. syringae}</td>
<td>4R</td>
<td>Pear</td>
<td>Lafayette, Calif.</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>\textit{P. syringae}</td>
<td>6R</td>
<td>Pear</td>
<td>Lafayette, Calif.</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>\textit{P. syringae}</td>
<td>9R</td>
<td>Strawberry</td>
<td>Salinas, Calif.</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>\textit{P. syringae}</td>
<td>468R</td>
<td>Almond</td>
<td>Modesto, Calif.</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>\textit{P. syringae}</td>
<td>563R</td>
<td>Citrus</td>
<td>Visalia, Calif.</td>
<td>1</td>
<td>Citrus</td>
</tr>
<tr>
<td>\textit{P. syringae}</td>
<td>584R</td>
<td>Citrus</td>
<td>Fresno, Calif.</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>\textit{P. syringae}</td>
<td>714R</td>
<td>Almond</td>
<td>Tulelake, Calif.</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>\textit{P. syringae}</td>
<td>1109R</td>
<td>Potato</td>
<td>Hillsboro, Oreg.</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>\textit{P. syringae}</td>
<td>1199R</td>
<td>Potato</td>
<td>Hillsboro, Oreg.</td>
<td>1</td>
<td>None</td>
</tr>
</tbody>
</table>

* Epiphytes were recovered from washings of healthy leaves. Pathogens were recovered from lesions.

b Suppliers of strains were as follows: 1. S. E. Lindow, University of California, Berkeley; 2. J. Lindemann, Advanced Genetic Sciences, Oakland, Calif.; 3. J. B. Jones, University of Florida, Gainesville; 4. M. L. Powell, Oregon State University, Corvallis; 5. S. Hiran, University of Wisconsin, Madison; 6. C. Leben, Ohio State University, Wooster.

c Strain 821R was pathogenic after stab inoculation but not after spray inoculation.

### TABLE 2. Summary analysis of variance of biological and environmental effects influencing the INFs of \textit{P. syringae} strains on plants at -5°C

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>Expt 1</th>
<th>Expt 2</th>
<th>Expt 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>1</td>
<td>0.43***</td>
<td>1.505***</td>
<td>1.3068***</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>8</td>
<td>10.92***</td>
<td>2.2969***</td>
<td>7.316***</td>
<td></td>
</tr>
<tr>
<td>E \times S</td>
<td>8</td>
<td>1.79**</td>
<td>0.12**</td>
<td>2.482***</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>3</td>
<td>9.06***</td>
<td>5.194***</td>
<td>5.75**</td>
<td></td>
</tr>
<tr>
<td>E \times P</td>
<td>3</td>
<td>0.31</td>
<td>0.57</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>S \times P</td>
<td>24</td>
<td>0.72</td>
<td>2.26***</td>
<td>10.116*</td>
<td></td>
</tr>
<tr>
<td>Block</td>
<td>3</td>
<td>12.92***</td>
<td>3.24*</td>
<td>3.52</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>159</td>
<td>0.78</td>
<td>0.44</td>
<td>113.39</td>
<td></td>
</tr>
</tbody>
</table>

* Abbreviations and symbols: df, Degrees of freedom; MS, mean square; **, significant at P = 0.001; ***, significant at P = 0.01; *, significant at P = 0.05. Means squares with no asterisks are nonsignificant. Experiments were as follows: expt 1, strains were isolated as epiphytes; expt 2, strains were pathogenic to tomato, cucumber, or bean plants; expt 3, strains were pathogenic to pea, oat, or corn plants.

* Data were analyzed in a randomized complete block design. E, Environment; S, strain; P, plant.
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TABLE 3. INFs of P. syringae strains on plants and in culture at -5°C  

| Expt and strains tested | INF (log cells/ice nucleus)  
|-------------------------|-----------------------------  
|                         | In vitro | In plants in the following environments:  
|                         | Wet | Dry | Mean  
| Expt 1b                |     |     |     2.6 ab  
| 9R                      | 2.9 a AB | 2.1 a A | 3.1 ab B |  
| 563R                    | 3.3 ab B | 1.5 a A | 2.7 a A | 2.0 ab  
| 6R                      | 3.3 abc C | 2.0 a A | 2.7 a b | 2.3 ab  
| 584R                    | 3.4 abc C | 1.5 a A | 2.3 a B | 2.0 a  
| 1190R                   | 4.3 bcd B | 2.4 a A | 2.5 a b | 2.4 ab  
| 1190R                   | 4.4 bcd C | 2.0 a A | 3.3 a B | 2.7 ab  
| 4R                      | 4.5 d A | 3.4 b A | 4.3 a A | 4.1 c  
| 468R                    | 4.7 d B | 2.4 a A | 3.1 a B | 2.8 b  
| 714R                    | 4.9 d B | 2.2 a A | 2.4 a b | 2.3 ab  
| Mean                    | 4.0 | 2.2 | 2.9 | 2.6  
| Expt 2c                 |     |     |     2.6 ab  
| 821R                    | 3.2 a B | 2.4 b A | 1.9 b A | 2.1 b  
| 407R                    | 5.9 b B | 1.7 a A | 1.2 a A | 1.4 a  
| 765R                    | 6.8 c B | 3.9 c A | 3.3 c A | 3.5 c  
| Mean                    | 5.3 | 2.7 | 2.1 | 2.3  
| Expt 3d                 |     |     |     3.5 b  
| 230R                    | 5.1 a b | 4.4 b B | 2.8 b A | 3.5 b  
| 655R                    | 7.2 b B | 1.9 a A | 1.4 a A | 1.6 a  
| 22R                     | 8.7 c B | 4.2 b A | 3.5 c A | 3.9 c  
| Mean                    | 7.0 | 3.5 | 2.6 | 3.0  

For each column, means followed by the same lowercase letter do not differ according to the Ryan-Einot-Gabriel-Welsch multiple range test (P = 0.05). For each row, means followed by the same capital letter do not differ by this test. Analysis of variance of these data is given in Table 2.  

a The strains were pathogenic to tomato, bean, and cucumber plants, respectively. Means reported are the average of four replicates of each strain.  
b The strains were pathogenic to oat, corn, and pea plants, respectively. Means reported are the averages of four replicates of six different plants for each strain.

DISCUSSION  

INFs varied among P. syringae strains by up to 1,000-fold in culture and 100-fold on plants (Table 3) (2). The growth temperature, which greatly influences INF in culture (17), was held constant in this study. The variability of INFs in culture was solely due to differences in strain identity. Variability of the expression of this phenotype on plants was attributed to changes in several individual factors such as strain identity, plant species, and light intensity and humidity. Strong interactions among these factors complicated the attribution of the INF to each, however. Although different strains, plants, and environments could be separated by the INFs that were observed, each single factor was modified by other treatments, to the extent that general predictions of environmental and plant species effects on the group of P. syringae strains taken as a whole were not possible. Nevertheless, the effects of single factors should be considered in future studies. The strain composition on leaves and the environmental conditions under which strains grow all contribute to the number of active ice nuclei. Of these factors, strain composition is of paramount importance because of the great range in expression, both in culture and on plants. Bacterial INFs on plants colonized by mixtures of naturally occurring strains have been reported previously. Bacteria growing on potato and navel orange plants produced from 6.5 to 40 times more ice nuclei per cell that were active at -5°C than those that grew on tomato, pear, and almond plants (9). The composition of P. syringae strains on plants can be complex (1). While these results are consistent with the effects of plant species on the INFs of P. syringae observed here, results from this study also indicate that
several other factors, including strain identity, humidity, and light intensity, must be considered before the influence of a particular plant species on bacterial ice nucleation activity can be predicted in the field.

Pathogens of a given plant species apparently do not exhibit significantly different INFs on susceptible hosts compared with those on nonsusceptible plants. No difference in INFs, even 5 days after inoculation, when lesions and water soaks were observed on susceptible host plants, could be attributed to such a compatible reaction. Frost damage as a method of bacterial entry into plants has been proposed (6, 20); however, there was no evidence for increased expression of ice nucleation activity by pathogens on susceptible hosts in the study presented here. Even the low level of expression of the ice nucleation phenotype observed in culture may be sufficiently high that induction by host plants may not be necessary to incite plant frost damage.

Growth phase differences at the time of INF measurement may have contributed to INF differences among strains since the INF (from in vitro studies) is known to be higher for cells in the stationary growth phase than for cells in the log phase or those entering the death phase (17). Bacterial populations varied significantly among strains, plants, and environments (data not shown); however, there was no clear relationship between the estimated growth phase (based on population size) and INFs. For dry incubation conditions, strains on their susceptible hosts were probably in the log phase (low INFs), while those on resistant plants were probably in the death phase (also low INFs). For wet incubation conditions, there was no correlation between high bacterial population (probably the stationary phase) and high INFs (data not shown). This is not conclusive, however, since growth phase data were not obtained and could only be estimated. Experiments designed with the growth phase in mind would clarify this issue.

The contribution of dead yet still INA cells to INF estimates must also be addressed, particularly after dry incubation. In experiments 2 and 3 this would explain the higher INFs after the dry incubation (massive cell death) than the wet incubation but would not explain the opposite result, which was obtained in experiment 1. The contribution of these dead cells to INFs in this study thus appears to be minimal.

The INF of cells in culture poorly predicts the potential of a particular bacterial strain to express ice nucleation on plants. This indicates that the genetic control of ice nucleation among strains, which is strongly controlled by culture age, substrate composition, liquid versus solid medium, aeration, and growth temperature in vitro (17), is also modified by the conditions on plants. Since the INFs of P. syringae strains grown on leaves were generally much higher than those of strains grown under optimum conditions in culture, leaves may harbor significantly more ice nuclei than expected, based only on estimates of the bacterial population size and estimates of ice nucleation activity made from cells in culture. Higher environmental concentrations of ice nuclei, with implications for frost risk and release into the atmosphere, are indicated.

INFs of P. syringae strains grown on plants are determined by interactions of biological and environmental factors. By testing 15 P. syringae strains, seven plant species, and two contrasting environments, a range of responses was obtained which indicates the great variability of the Ice phenotype under natural conditions. Frost risk assessment of particular crops, based on assessment of the INA bacterial strains present and previous environmental conditions, may be possible. More work, however, is needed before the magnitude of contributions of bacterial ice nuclei from plants to the atmosphere can be assessed.

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LITERATURE CITED


