J. Rech. Atmos., 19 (1985), n° 2-3, pp. 139-145.

BACTERIA AS CONDENSATION NUCLEI

by

J.R. SNIDER[1], R.G. LAYTON[2], G. CAPLE[3], D. CHAPMAN[3].

RÉSUMÉ

On a constaté que deux souches de bactéries aptes à la nucléation de la glace (INA), Psuedomonas syringae *et* Erwinia herbicola*, produisaient la condensation à 100 % d'humidité sur des substrats de filtres. Un mutant de* P. syringae*, non-INA, n'a pas produit de gouttes dans des conditions semblables. A 0,5 % de sursaturation, les aérosols de bactéries INA et non-INA sont des noyaux de condensation actifs. Ces découvertes préliminaires : 1) impliquent que l'activité de congélation après immersion à température positive et l'aptitude à la condensation peuvent être interdépendantes, 2) rendent compte de l'aptitude possible des bactéries à la nucléation après un transport dans l'air, et 3) améliorent notre compréhension du mode de nucléation de la glace par les bactéries dans l'atmosphère et à la surface des plantes. Les changements de l'activité nucléante après immersion de* P. syringae *dus à la dilution, au pH ambiant et à l'alimentation ont également fait l'objet de recherches. Les concentrations atmosphériques en bactéries ont été suivies en deux endroits ; les concentrations observées en organismes viables varient en fonction du lieu de collecte, de l'humidité relative, et des dimensions des particules associées aux bactéries recueillies.*

ABSTRACT

Two strains of ice nucleation active (INA) bacteria, Psuedomonas syringae *and Erwinia herbicola, were observed to produce condensation at 100 % humidity on filter substrates. A* P. syringae *mutant, which is non-INA, produced no drops under comparable conditions. Both INA and non-INA bacterial aerosols were observed to be active condensation nuclei at 0.5 % supersaturation. These preliminary findings : 1) imply that warm immersion freezing activity and condensation ability may be interdependent, 2) are significant in terms of the ability of the bacteria to remain viable during airborne transport, and 3) increase understanding of the mode of bacterial ice nucleation in the atmosphere and on plant surfaces. Changes in* P. syringae *immersion freezing activity due to dilution, media pH and nutrient supply were also investigated. Ambient atmospheric concentrations of bacteria were monitored at two sites ; the observed concentrations of viable organisms varied depending on the location of collection, relative humidity, and particle size associated with the collected bacteria.*

(1) *Department of Atmospheric Science, University of Wyoming, Laramie, WY 82071, U.S.A.*
(2) *Department of Physics, Northern Arizona University, Flagstaff, AZ 86011, U.S.A.*
(3) *Department of Chemistry, Northern Arizona University, Flagstaff, AZ 86011, U.S.A.*

condensation nuclei at supersaturations less than 1 %. Aerosols were generated with a Devilbiss Glass Nebulizer (The Devilbiss Co., Somerset Pa.) whose output was calibrated. The generated aerosol was mixed with dried air and stored in a 0.1 m^3 mylar bag. Droplets which activated in a thermal diffusion chamber (TDC) at 0.5 % supersaturation were counted photographically.

An Andersen multistage impactor was employed to segregate by size viable airborne bacteria at a rural lakeside site 20 miles east of Flagstaff, and from the roof of the NAU chemistry building in Flagstaff, Arizona. Sampling was conducted between August and November 1983. Petri plates containing trypticase soy agar supplemented with 0.1 % cycloheximide were employed for the collections.

RESULTS.

a) Condensation nucleation.

Drops large enough to count were apparent on filter surfaces 10 hours after placing dried filters in the humidifying chamber. On the filters covered with bacteria the drops appeared uniform in size and were evenly dispersed over the filter surface. The range in drop sizes and the coverage on the blank filters were less uniform. The collected data are presented in Figure 1. Although the background is high and variable, a significant difference is noted between the number of drops appearing on the blank filters compared with those filters containing INA bacteria. The experiment indicates that the two organisms which were observed to also be INA possess wettable cell surfaces. The lack of proportionality between filter droplets and bacteria is thought to be due to interactions between the growing droplets (e.g. competition for vapor and coalescence). Although the colony morphology and the responses to a battery of biochemical tests were identical to the INA *P. syringae*, the non-INA organism was not observed to produce any significant condensate under comparable conditions.

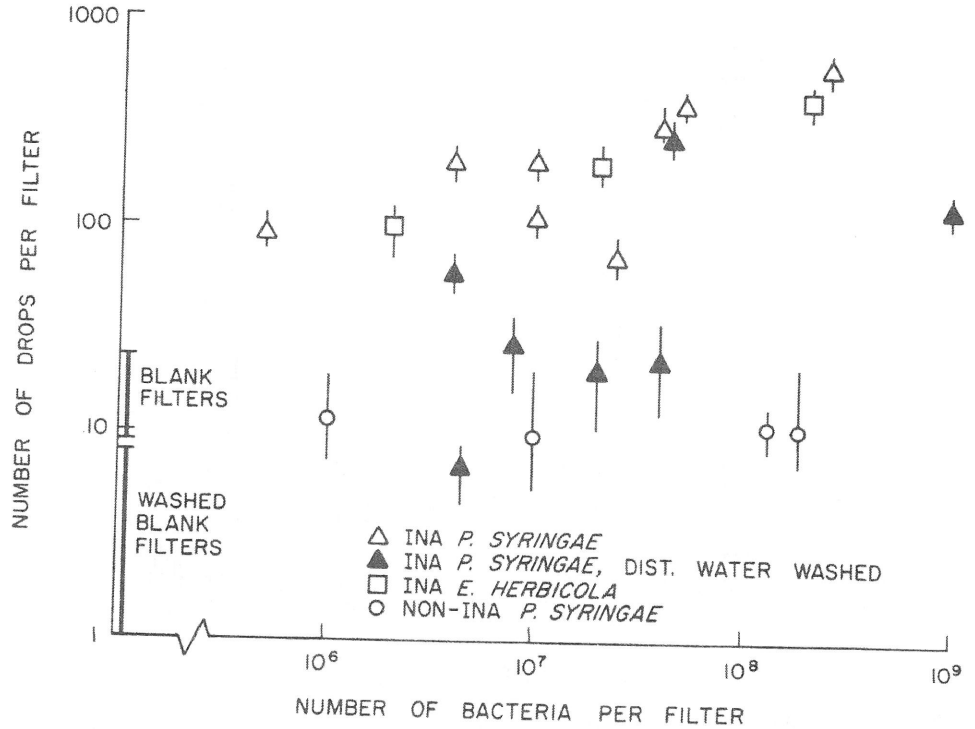

FIG. 1. — Number of counted drops vs. the number of live bacteria deposited on the filter surface. Each data point is an average of 2-4 replicate filters. Vertical lines indicate the range of observed values.

fog indicate that live cell concentrations were higher in foggy air. Cell death due to desiccation and other factors such as increased vertical mixing and solar insolation are thought to be important factors contributing to this difference.

c) Immersion freezing studies.

We have observed significant pH changes during the exponential growth phase of *P. syringae* cultures. The freezing spectra of a stationary culture (pH = 9.0) diluted either in KCB (pH = 6.8) or in culture filtrate (pH = 9.0) are compared in Figure 3. When filtrate is used as a diluent, freezing activity between −6 and −8 °C is enhanced. The KCB diluent was found to promote nucleation at temperatures warmer than −2 °C. The difference in freezing behavior at warm temperatures is thought to reflect the dependence of bacterial nucleation on oxidizable organic molecules (i.e. citrate). Differences at temperatures colder than −6 °C may be the result of changes in cell surface characteristics (e.g. surface charge) induced by the pH alteration.

An additional feature of the differential spectra shown in Figure 3 is the enhanced activity apparent in all INA samples between −5 and −3 °C. Dilution in either KCB or

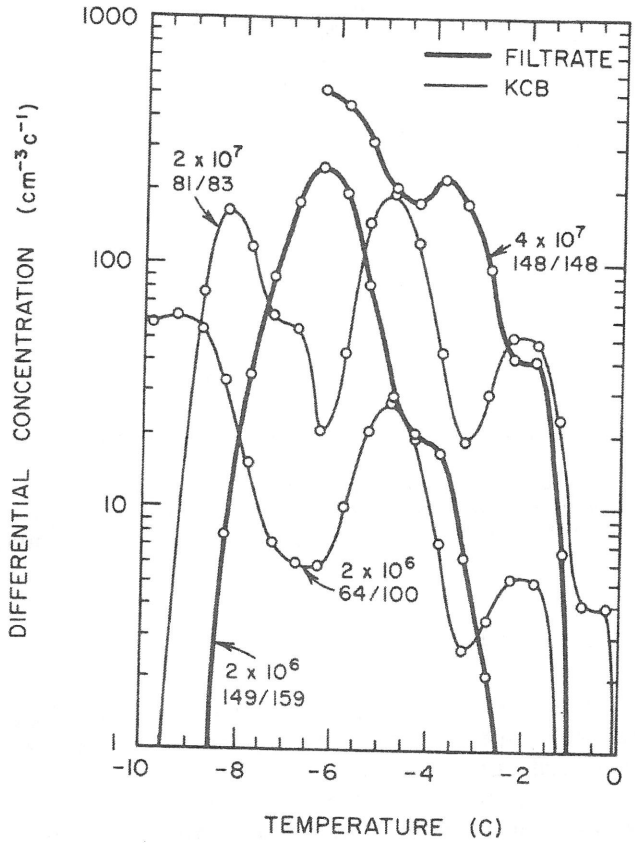

FIG. 3. — Differential freezing spectra obtained via dilution of stationary phase *P. syringae* cultures. Labels indicate the live cell concentration (cm^{-3}) and the fraction of tested drops frozen by −10 °C.

culture filtrate produces a monotonic decrease in the height of this peak. Nucleus concentrations in this temperature interval are plotted versus live cell concentration in Figure 4.

The growth and freezing histories of *P. syringae* in a broth which contains ethanol as a substitute for citrate are also shown in Figure 4. This comparison indicates that ethanol initially inhibits both freezing and reproductive activity, however by 30 hours the

surface. Perhaps as intriguing is the possibility that microbial nuclei represent a means of interaction and feedback between surface biological processes, the physics of precipitation development and the efficiency of its delivery.

ACKNOWLEDGEMENTS.

The authors wish to thank the Herman Frasch Foundation and The Organized Research Committee of Northern Arizona University and The Montana State Agricultural Station for support of this work.

REFERENCES

BOVALLIUS, A., B. BUCHT, R. ROFFEY and P. ANAS, 1978 : Three-year investigation of the natural airborne bacterial flora at four localities in Sweden. *Appl. Microbiol.*, 35, pp. 847-852.

DINGLE, A.N., 1966 : Pollens as condensation nuclei. *J. Rech. Atmos.*, 2, pp. 231-237.

GREGORY, P.H., 1973 : The microbiology of the atmosphere, 2nd ed., Leonard Hill Books, Alysbury, G.B., 377 p.

KEENE, W.C., J.N. GALLOWAY and J.D. HOLDEN, Jr., 1983 : Measurement of weak organic acidity in precipitation from remote areas of the world. *J. Geophys. Res.*, 88, pp. 5122-5130.

LEVIN, Z., 1984 : Bacterial condensation and freezing. Private communication.

LEVIN, Z., and S.A. YANKOFSKY, 1983 : Contact versus immersion freezing of freely suspended droplets by bacterial ice nuclei. *J. Climate Appl. Meteor.*, 22, pp. 1964-1966.

MAKI, L.R., E.L. GALYAN, M.C. CHIEN and D.R. CALDWELL, 1974 : Ice nucleation induced by *Psuedomonas syringae. Appl. Microbiol.*, 28, pp. 456-459.

PRUPPACHER, H.L., and J.D. KLETT, 1978 : *Microphysics of Clouds and Precipitation.* Reidel, Dordrecht, Holland.

SCHNELL, R.C., 1977 : Ice nuclei in seawater, fog water and marine air off the coast of Nova Scotia : Summer 1975. *J. Atmos. Sci.*, 34, pp. 1299-1305.

SNIDER, J.R., and G.A. DAWSON, 1985 : Tropospheric light alcohols, carbonyls, and acetonitrile concentrations in the Southwestern United States and Henry's Law data. *J. Geophys. Res.*, 90, pp. 3797-3805.