

Contact Versus Immersion Freezing of Freely Suspended Droplets by Bacterial Ice Nuclei

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ABSTRACT

Droplets freely suspended in the air stream of a wind tunnel were nucleated with desiccated bacterial cells in either the contact or immersion mode. Immersion freezing seemed to give a noncontinuous frequency distribution of freezing with temperature, whereas the corresponding curve for contact was monotonic. Although the latter nucleation mode was more efficient by $\sim 2^\circ\text{C}$, the temperature ranges over which droplets froze by either mode of nucleation were closer to 0°C than those so far published for nonbiogenic ice nuclei of natural origin.

1. Introduction

The existence of bacterial ice nuclei active as efficient immersion freezers has already been established (Schnell, 1976; Vali *et al.*, 1976; Maki *et al.*, 1974; Levin *et al.*, 1980; Yankofsky *et al.*, 1981a), and their role in the formation of ice on plants has also been demonstrated (Lindow, 1977; Lindow *et al.*, 1978; Yankofsky *et al.*, 1981b).

It has been suggested (Maki and Willoughby, 1978 and Levin *et al.*, 1980) that such ability to freeze water at temperatures as warm as -2°C could possibly be used in cloud seeding operations to produce ice crystals at warmer temperatures than presently obtainable with silver iodide as the artificial nucleus. However, considerable additional research will be necessary before the use of bacteria as cloud seeding agents can even be contemplated. For example, although ice nucleation-active (INA) bacteria are exceptionally efficient immersion freezers, their ability to act as contact, deposition or condensation freezing nuclei, remains to be determined. This communication deals with the question of contact nucleation by freeze-dried cells of Bacterium M1. As already demonstrated (Yankofsky *et al.*, 1981a), the above microorganism has been shown to be as efficient in the immersion mode as any other yet described (Maki *et al.*, 1974; Schnell, 1976; Lindow *et al.*, 1978).

Pitter and Pruppacher (1973) have shown that supercooled droplets in free fall freeze at warmer temperatures when impacted by dry inorganic nuclei than when the same nuclei are pre-immersed in the droplets. This implies that ice nucleation by contact may be

intrinsically more efficient than immersion freezing. Cooper (1974) has attempted to explain the apparently higher relative efficiency of contact nucleation on theoretical grounds. Nevertheless, the underlying mechanism of the process is by no means clear. Nor, for that matter, is it certain that all nuclei will show the same behavior. Here we demonstrate that organic freezing nuclei of bacterial origin are also somewhat more efficient in the contact mode.

2. Experimental method

a. Wind tunnel operations

The vertical wind tunnel facility at the University of California, Los Angeles, was used. This tunnel can hold droplets of $15\ \mu\text{m}$ to several mm radius in free suspension (Pruppacher and Neiburger, 1968; Beard and Pruppacher, 1969). It has an inner tunnel (located in the test section) where position with respect to air flow can be adjusted during operation for the purpose of preventing of turbulence due to heat transfer from the outside during operation at temperatures below ambient (Pitter and Pruppacher, 1973). This latter addition provides greater flexibility in controlling the position of suspended drops or ice crystals.

In the present experiment, drops of radii between 220 and $360\ \mu\text{m}$ were individually suspended in the tunnel. Drop sizes were determined by the air speed needed to keep them in stationary suspension. The dry and dew point temperatures of the tunnel were continuously monitored, the first by a protected thermometer and the latter by a Cambridge System dew point hygrometer.

Two modes of ice formation were tested. In the contact mode, single drops composed only of double-distilled water were introduced into the wind tunnel while temperature was being lowered and allowed to come to equilibrium with the air. Since the air was normally somewhat subsaturated, slow evaporation occurred. Since it was impossible to maintain the air in the tunnel at constant temperature, all experiments were carried out while air was either cooling or warming. However, the rate of change of temperature never exceeded 1° per 3 min and no drop was ever suspended for longer than about 4 min. For contact freezing, once the desired temperature was reached, a small cloud of desiccated bacteria was manually generated upwind of the drop by a pulse of compressed air blown through a tube containing the dry material. The duration of the pulse was only a fraction of a second. The injected nuclei flowed past the suspended drop, where, because of the large number of nuclei used, some invariably made contact with it. Three criteria were used to determine whether a given drop froze. These were: 1) reduced fall velocity after freezing—caused by the increase in both volume and drag; 2) color changes after freezing—detected with crossed polaroid filters; and 3) bouncing when the frozen drop finally impacted on the tunnel wall. Immersion freezing experiments with dry bacteria pre-suspended in double-distilled water were carried out essentially as described above for contact nucleation, except that the pulse of dry nuclei at a predetermined temperature was omitted. Trials in which immersion freezing did not occur after the temperature in the tunnel had dropped by more than 3°C were discontinued because of excess evaporation and temperature control difficulties. Such drops were obviously not included in the cumulative plot of frozen drops (Fig. 1b). They were, on the other hand, counted as non-freezers over the temperature interval of the test run (Fig. 1a). Temperature and dew point values were continuously recorded in all trials.

Since the temperature of the wind tunnel changed relatively slowly, it was assumed that the drops rapidly cooled down to temperatures close to that of the air (Pruppacher and Klett, 1978). However, since the dew point temperature was invariably lower than the dry air temperature, evaporation of drops always occurred. This was in agreement with the fact that air speed in the tunnel had to be continuously adjusted downward during any given trial. However, the net loss of mass was never found to exceed 18% of initial drop mass (6% change in radius as determined by wind tunnel speed adjustment). The surface temperature of any given drop was thus always lower than that of air because of evaporative cooling. Surface temperature is, in fact, close to the wet bulb temperature (T_w) of the air ($T_d < T_w < T_{\text{air}}$). The value of T_w for each experiment was, therefore, evaluated from T_d and T_{air} using standard meteorological charts.

b. Preparation of dry nuclei

Culture of Bacterium M1 on standard bacteriological medium has already been described (Yankofsky *et al.*, 1981a). Cells in cultures were harvested by centrifugation ($8000 \times g$, 20 min, 4°C) rapidly frozen by immersion in a methanol-dry ice bath, and lyophilized to dryness under vacuum. The resulting dry powder was finally pulverized in a mortar to give a fine suspension.

3. Results and discussion

The absence of efficient freezing nuclei in distilled water batches was demonstrated by the fact that sets of 20 drops of 1 mm radius remained unfrozen at $-5 \pm 1^\circ\text{C}$ for several hours. By contrast, drops of the same size containing about 5×10^6 dried bacterial cells each, rapidly froze under the same conditions.

Control experiments in the wind tunnel were also conducted on a regular basis. In these, drops of distilled water were suspended in the tunnel and the temperature lowered. No freezing of these drops was observed down to -14°C , showing that freezing over the temperature range examined required the presence of an outside source of efficient ice nuclei.

Figure 1a represents the percentage of frozen drops in each temperature interval relative to the total number of drops tested in that interval. Fig. 1b, on the other hand, is the same data plotted as a cumulative distribution of frozen drops versus temperature. Comparative plots of contact versus immersion freezing are presented in both figures. Regardless of how plotted, contact freezing increases monotonically with decreasing temperature and reaches 100% activity at about -7°C . The immersion freezing curves may suggest non-monotonic increase of activity at around -5 to -8°C . While this observation would be in keeping with our earlier finding (Yankofsky *et al.*, 1981a) that cell-associated nuclei active in the freezing mode at temperatures warmer than -8°C appear less frequently in populations of the INA bacterium in question than those active below -8°C , the number of drops tested was too small to be statistically certain that we were, in fact, observing 2 distinct types of freezing nuclei. There can, however, be no doubt that 100% freezing by immersion occurred about 2°C lower than was the case for contact.

The monotonic increase in freezing frequency with decreasing temperature obtained in the contact nucleation mode is difficult to explain. For one thing, the total number of bacteria pulsed into the air stream of the tunnel was not accurately determined, and, for another, there was no way to tell how many cells were present in even the finest particles of the dry powder. Nor could the number of collisions between bacterial particles and drops be precisely assessed. However, even without knowing the type of nucleus involved in

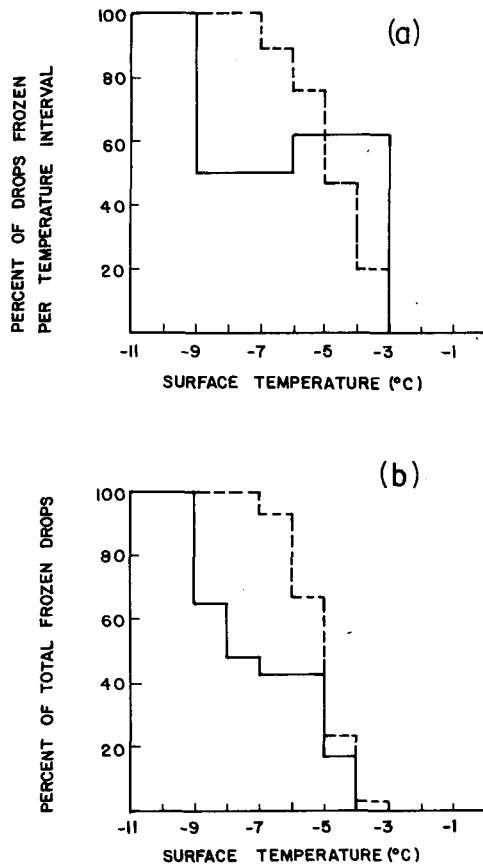


FIG. 1. Comparison of contact (dashed line) and immersion (solid line) nucleation temperature distributions for drops in free fall. (a) Percent of drops tested in a given temperature interval which froze. The indicated intervals were chosen so that the number of drops tested per interval was comparable. Total number of drops tested was 48 in the contact mode and 32 in the immersion mode. (b) Cumulative plot of frozen drops with temperature. The same data as in (a) are presented except that only the 30 and 23 drops which respectively froze during contact and immersion trials are considered.

contact nucleations, it is obvious that freezing by contact is more effective. Whatever the reason for this apparent difference in contact versus immersion nucleation efficiencies, it is not as large as previously reported by Pitter and Pruppacher (1973) for inorganic nuclei.

Efficient contact nucleation by INA bacteria at relatively warm temperatures does not necessarily imply superior cloud seeding potential. Given their large size relative to agents in current use, intact bacteria would probably collide with supercooled water droplets in

clouds far less frequently than, for example, AgI particles. However, the possibility of obtaining small active fragments from disrupted bacteria cannot be ruled out. Thus, although the active bacterial fragments now available do not operate as freezing nuclei above -8°C (Maki and Willoughby, 1978; Yankofsky *et al.*, 1981a), future efforts may well furnish smaller particles with the requisite efficiency. Also there is the possibility that dried bacteria will also be efficient condensation-freezing nuclei. This latter possibility is under current investigation.

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REFERENCES

- Beard, K. V., and H. R. Pruppacher, 1969: A determination of the terminal velocity and drag of small water drops by means of a wind tunnel. *J. Atmos. Sci.*, **26**, 1066–1072.
- Cooper, W. A., 1974: A possible mechanism for contact nucleation. *J. Atmos. Sci.*, **31**, 1832–1837.
- Levin, Z., N. Sandlerman, A. Moshe, T. Bertold and S. A. Yankofsky, 1980: Citrus derived bacteria as freezing nuclei at -2.5°C . *Proc. Int. Conf. Cloud Physics*, Clermont-Ferrand, Int. Assoc. Meteor. Atmos. Phys. 45–47.
- Lindow, S. E., 1977: Leaf surface bacterial ice nuclei as incitants of frost damage to corn and other plants. Ph.D. dissertation, University of Wisconsin, Madison, 361 pp.
- , D. C. Arny and C. D. Upper, 1978: *Erwinia herbicola*: A bacterial ice nucleus active in increasing frost injury to corn. *Phytopathology*, **68**, 523–527.
- Maki, L. R., and K. J. Willoughby, 1978: Bacteria as biogenic sources of freezing nuclei. *J. Appl. Meteor.*, **17**, 1049–1053.
- , E. L. Galyan, Chien Mei-Mong Chang and D. R. Caldwell, 1974: Ice nucleation induced by *Pseudomonas syringae*. *Appl. Microbiol.*, **28**, 456–459.
- Pitter, R. L., and H. R. Pruppacher, 1973: A wind tunnel investigation of freezing of small water drops falling at terminal velocity in air. *Quart. J. Roy. Meteor. Soc.*, **99**, 540–550.
- Pruppacher, H. R., and M. Neuberger, 1968: Design and performance of the UCLA cloud tunnel. *Proc. Int. Conf. on Cloud Physics*, Toronto, Amer. Meteor. Soc., pp. 389–392.
- , and J. D. Klett, 1978: *Microphysics of Clouds and Precipitation*. Reidel, 714 pp.
- Schnell, R. C., 1976: Bacteria acting as natural ice nucleants at temperatures approaching -1°C . *Bull. Amer. Meteor. Soc.*, **57**, 1356–1357.
- Vali, G., M. Christensen, R. W. Fresh, E. L. Galyan, L. R. Maki and R. C. Schnell, 1976: Biogenic ice nuclei. Part II: Bacterial sources. *J. Atmos. Sci.*, **33**, 1565–1570.
- Yankofsky, S. A., Z. Levin, T. Bertold and N. Sandlerman, 1981a: Some basic characteristics of bacterial freezing nuclei. *J. Appl. Meteor.*, **20**, 1013–1019.
- , —, and A. Moshe, 1981b: Association of ice nucleating bacteria with citrus plants in Israel and their possible role as causative agents of frost damage. *Curr. Microbiol.*, **5**, 213–217.