Possible Application of Bacterial Condensation Freezing to Artificial Rainfall Enhancement

Z. LEVIN,* S. A. YANKOFSKY,† D. PARDES* AND N. MAGAL†

Tel Aviv University, Ramat Aviv 69978 Israel

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ABSTRACT

Both gram-positive and gram-negative bacteria become excellent condensation nuclei when lyophilized to dryness. The same freeze-dry procedure does not inactivate the highly effective freezing nuclei produced by ice nucleation active bacteria. Therefore, irrespective of their contact nucleation potential, ice nucleation-active bacteria ought to effect condensation freezing at −10°C or warmer in cloud systems. Output from a numerical cloud model suggests that the condensation freezing capability of ice nucleation-active bacteria at warmer temperatures could be exploited to produce rainfall from clouds too warm to respond positively to inorganic nucleants like silver iodide.

1. Introduction

It is the presence or absence of ice particles in sufficiently large amounts which normally determines whether or not a continental cloud will give rain. But, except for low concentrations of highly effective biogenic ice nuclei (Schnell and Vali, 1976, Vali et al., 1976), the atmosphere is generally a poor source of ice nuclei active at temperatures warmer than −15°C. Indeed, even nuclei effective at −20°C average only about 1 l^{-1} (Pruppacher and Klett, 1980). The consequence of this deficiency in effective ice nuclei is that continental convective clouds with ceiling temperatures higher than about −12°C give little rain (Pruppacher and Klett, 1980).

Artificial seeding of relatively warm clouds with high concentrations of effective ice nuclei has been used as a means of enhancing natural rainfall. Prominent among the inorganic nucleants now used for this purpose are aerosols of silver iodide (AgI). The principal advantage of such aerosols as cloud seeding agents is that relatively uniform small nuclei can be readily produced in very large numbers. In addition to operational convenience, small particle size ensures efficient contact nucleation. On the other hand, in terms of the maximum temperatures in which they act, AgI aerosols are only a few degrees more effective than natural ice nuclei already present in clouds. The most abundant natural ice nuclei in clouds act at −15°C and below, as compared to about −10°C and below for AgI (Yankofsky et al., 1981). If so, artificial seeding with ice nuclei more effective than AgI aerosols might further increase precipitation yields from clouds cold enough to respond to AgI. More significantly, introduction of even better seeding agents might make it possible to obtain worthwhile amounts of water from some of the warmer clouds not influenced by AgI.

A few of the *Pseudomonas* and *Erwinia* species normally found on plant surfaces possess freezing nuclei as effective as any yet discovered in natural environments. Associated with intact cells of all such ice nucleation-active (INA) bacteria are a range of freezing nuclei active at temperatures between −2° and −10°C (Maki and Willoughby, 1978; Lindow et al., 1978; Yankofsky et al., 1981b, Lindow et al., 1982). Since the comparative freezing spectrum of AgI is about −8° to −16°C (Yankofsky et al., 1981b), there can be little doubt that the bacterial particles will, on average, incite ice formation at significantly smaller supercoolings than the inorganic nucleants now in use as cloud-seeding agents. However, it remains to be established whether the freezing mode activity of INA bacteria suspended in large drops of water in the laboratory reflects a corresponding ability to form ice crystals in the very different environment of a relatively warm cloud.

Existing methods for large-scale bacterial culture could easily furnish enough material for cloud-seeding operations at reasonable cost. Moreover, at least in the case of Bacterium M1, an Erwinia-like INA isolate from citrus (Yankofsky et al., 1981a), it has been shown that lyophilized (i.e., freeze-dried) cell preparations contain highly effective contact as well as freezing nuclei (Levin and Yankofsky, 1982). It is doubtful, however, whether the established contact nucleation ability

* Department of Geophysics and Planetary Sciences, Faculty of Exact Sciences.

† Department of Microbiology, George S. Wise Faculty of Life Sciences.

of INA bacteria would actually find practical expression in clouds. Because of their large size (relative to aerosols of AgI), collisions between bacterial cells and water droplets would be simply too infrequent. Fortunately, the potential effectiveness of INA bacteria as cloud-seeding agents does not depend on their contact nucleation potential. As will be demonstrated, anhydrous bacteria are excellent condensation nuclei. Hence, INA bacteria should be able to initiate raindrop growth via condensation-freezing.

2. Bacterial strains employed

The INA and non-INA bacteria employed in these studies are listed in Table 1. Bacterium M1 and Bacterium L3 are gram-negative, rod-shaped, facultatively anaerobic and asporogenic isolates from the same frost-damaged citrus leaf. Both exhibit the general characteristics of *Erwinia herbicola* strains (Yankofsky et al., 1981). However, Bacterium L3 does not produce freezing nuclei active at −10°C or warmer. Although not found on plants, members of the genus *Escherichia* are distantly related to the *Erwinia* on morphological, physiological and phylogenetic grounds. The particular strain of *E. coli* dealt with here, DG-76, is a leucine-requiring and thymine-requiring derivative of *E. coli* K12. It stops multiplying when deprived of leucine (an essential protein amino acid) and dies when starved for thymine (an essential DNA base). Like the vast majority of bacteria, *E. coli* DG-76 is not nucleation active. Bacterium M5, a gram-negative and strictly aerobic rod with the characteristics of *Pseudomonas syringae,* produces cell-associated nuclei comparable to those of Bacterium M1 in all respects (Yankofsky et al., 1981b). Finally, unlike all of the other bacteria described above, *Bacillus cereus* is gram-positive and forms heat-resistant endospores. It has no INA potential and is unrelated to the other species listed.

It might also be worth noting that exponential-phase cultures of both Bacterium M1 and Bacterium M5 generally contain only about one nucleation-positive cell in 1000 (Yankofsky et al., 1981b, Yankofsky et al., 1983). Also, the freezing nuclei of active cells are not uniformly effective. Thus, of the individuals active at −8° to −10°C (type III activity) in populations of Bacterium M1, only 10%, at most, will also nucleate at −2° to −4°C (type I activity), and even the fraction exhibiting activity at −5° to −7°C (type II) varies (Yankofsky et al., 1981a, Yankofsky et al., 1983).

TABLE 1. Bacterial strains.

Bacterium	INA Phenotype
Bacterium M1 (*Erwinia herbicola*)	+
Bacterium L3 (*Erwinia herbicola*)	−
Escherichia coli DG-76	−
Bacterium M5 (*Pseudomonas syringae*)	+
Bacillus cereus	−

3. Experimental methods

a. Construction of INA⁺ strains of E. coli

The genetic vector used to introduce the *ice* genes of Bacterium M1 into *E. coli* was pBR322. This multicopy plasmid of *E. coli* carries genes which render its bacterial host resistant to ampicillin and tetracycline. Digestion with the restriction endonuclease *Bam*HI converted circular molecules of pBR322 DNA into linear ones by introducing a single double-strand break within the *tet* (i.e., tetracycline resistant) gene. The same restriction endonuclease converted chromosomal DNA of Bacterium M1 to many relatively small fragments of varying length. Thus, when a mixture containing approximately equal amounts of restricted plasmid and bacterial DNA was incubated with T4 DNA ligase, three kinds of circular plasmid structures were formed: 1) normal plasmids, products of simple rejoining of the two ends of linear pBR322 molecules; 2) multimeric plasmids, due to intermolecular resealing of two or more linear pBR322 DNA molecules by the ligase; and 3) recombinant plasmids, circular structures with one or more fragments of restricted M1 DNA covalently inserted into the *tet* gene of pBR322. In other words, the ligated DNA mixture contained a range of plasmids carrying different segments of the Bacterium M1 genome. Suitable DNA transformation techniques (Lederberg and Cohen, 1974) were used to introduce these recombinant plasmids into an appropriate strain of *E. coli* (Hb 101). Cells which received a plasmid (recombinant or not) during the transformation process were easy to select for, since they alone were capable of forming colonies on agar containing ampicillin. It was also easy to establish which of the transformed (i.e., ampicillin-resistant) cells had received recombinant plasmids. The latter cell lines were recognized by their inability to develop on media supplemented with tetracycline. Colonies able to develop in the presence of ampicillin, but not on tetracycline, were screened, one by one, for ability to nucleate drops of water at −10°C. Colony number 151 proved to be nucleation active. This clone, designated Rec 151, was the source of the INA^+ plasmid (hereafter referred to as pBM1NB). Purified pB1NB DNA effectively transformed all strains of *E. coli,* including DG-76, from INA^- to INA^+. A detailed exposition of the standard recombinant DNA techniques (Cohen et al., 1978, Maniatis et al., 1982) used to construct pMBM1NB and clone it into *E. coli* DG-76 will be presented elsewhere.

b. Preparation of dry bacterial powders

All of the bacteria listed in Table 1 were aerobically cultivated on nutrient broth (Difco) supplemented with 5 g l^{-1} casein hydrolysate (Difco) at 30°C as described in Yankofsky et al. (1981a). In addition, *E. coli* DG-76/pBM1NB, the INA^+ transformant obtained by introducing the recombinant plasmid from Rec 151 into

competent DG-76 cells, was cultivated in the presence of added L-leucine and thymine at 30°C on aerated mineral salts medium (Davis and Mingioli, 1950) containing 2 g l^{-1} glucose as the general source of energy and carbon. Inocula of *E. coli* DG-76/pBM1NB were allowed to develop in the presence of enough thymine (100 μg ml^{-1}) to completely satisfy the pyrimidine requirement, but were not given enough L-leucine for unlimited growth. After overnight incubation, such amino acid-limited cultures were then diluted fivefold in medium containing 100 μg ml^{-1} each of thymine and L-leucine, allowed to grow out to late exponential phase with shaking at 30°C, and then placed in a refrigerator at 4°C (without agitation) for 2–12 h. Allowing cells which had recovered from amino acid starvation to incubate at low temperatures produced cell populations in which the number of cell-associated freezing nuclei active at −10°C or above was approximately equal to the number of cells. Regardless of identity, or how cultivated, cells were recovered from cultures by centrifuging at 5000 × *g* at 4°C, resuspended in a volume of saline solution (9 g NaCl l^{-1}) equal to the volume of the original growth medium, again pelleted as previously mentioned, rapidly frozen in an acetone-dry ice mixture, and then sublimated to dryness in a refrigerated Leybold lyophilizer at reduced pressure. The dry powder yield per liter of bacterial culture varied from 1.2 to 1.5 g.

c. *Measurement of bacterial condensation*

The granular bacterial mass obtained by lyophilization (previously presented) was reduced to a finer powder by dry grinding in a mortar and then transferred to a large flask. The flask was next brought into contact with a vortex-type stirrer and connected to a compressed air source (see Fig. 1). This combination of vigorous shaking with a strong jet of air into the dry powder was designed to generate and maintain a uniformly dispersed cloud of dry bacteria in which the degree of cell-to-cell aggregation would be as small as possible. Maintaining positive pressure in the cloud-generating flask throughout each experiment also served to induce continuous flow of dispersed bacteria into the haze chamber to which the flask was attached.

The continuous-flow haze chamber into which the dry bacterial cloud was allowed to flow consisted of two parallel glass plates (30 × 160 cm), separated by an air space of 1.3 cm and sealed all around except for air and water ports at the top and bottom (Fig. 1). The interior wall of each plate was covered with a thin sheet of absorbent paper and kept wet by continuous downflow of water. This served to maintain chamber humidity at saturation. The chamber was kept at room temperature (usually around 23°–25°C) and its relative humidity was monitored at discrete intervals by a dewpoint hygrometer sited about two-thirds of the way down the chamber.

Dispersed bacteria entered the haze chamber from the cloud-generating flask through the upper air port and were forced through it at a flow rate of 6 cm^3 min^{-1} (determined by the pumping rate of the optical counter). The excess volume of bacterial cloud, created by differences in flow rate between the generating flask and optical counter pump, was allowed to escape through an opening above the haze chamber (see Fig. 1). Data on particle number and diameter measured by the counter (Particle Measuring Systems-CSSP100) was directly outputted to a Tektronix 4051 microcomputer for on-line processing and storage. Individual particles averaged 200 s of growth by condensation before exiting the haze chamber at the applied rate of air flow.

The initial particle-size distribution of dry bacteria in the cloud-generating flask was determined by connecting it directly to the aerosol optical counter. Particles ranging in diameter from 0.3 to 20 μm were analyzed by the counter over a period of 16 min in each wet or dry run.

d. *Measurement of drop-freezing spectra*

Procedures and instrumentation used to determine drop-freezing spectra of bacterial suspensions have been detailed elsewhere (Yankofsky et al., 1981b). In the present experiments, dry bacterial powders were suspended in distilled water at 5 mg cm^{-3} and sets of 5-μl drops from each suspension were distributed, 30 at a time, over the stage of a freezing nucleus spectrometer. Stage temperature was then slowly lowered from room temperature to −12°C and the precise temperature at which each drop in the set froze was recorded. Precisely the same operations were also carried out on serial tenfold dilutions of each primary suspension. The cumulative distribution of frozen drops in each set was then plotted as a function of stage temperature in each case. In some cases, concentrations of type I and type III freezing nuclei in bacterial suspensions were determined at −4°C and −11°C as detailed elsewhere (Yankofsky et al., 1983).

4. Experimental results

a. *Condensation growth of dry bacteria*

Figure 2 compares the apparent size distribution of dry bacteria in the cloud-generating flask to that of wet bacteria after passage through the haze chamber. The natural INA bacteria respectively represented in Figs. 2a and 2b are Bacterium M1 (*E. herbicola*) and Bacterium M5 (*P. syringae*). In both cases, a distinct shift to particles of larger diameter occurred together with the expected decrease in the concentration of smaller particles when dry bacteria were exposed to water vapor in the saturated haze chamber. Calculated mean diameters of dry and wet particles are presented in Table 2. As can be seen, 200 seconds in the chamber sufficed

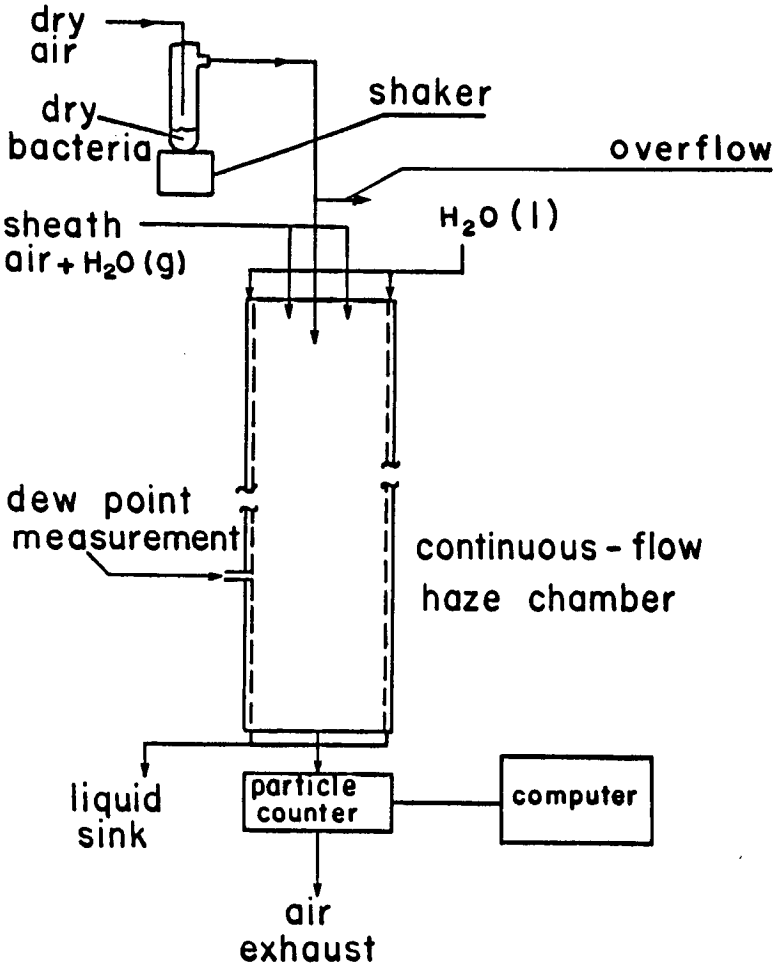

FIG. 1. Schematic diagram of apparatus for measuring condensation growth of dry bacteria.

to respectively increase the average diameter of particles in the Bacterium M1 preparation by a factor of 4, and by a factor of 2 in the case of Bacterium M5. Therefore, condensation growth was responsible for 64-fold and 8-fold increases in mean particle volume in these two cases. It should, however, be noted that the unit dry particle from which condensation growth was initiated was larger than a single bacterium in most cases. Measured mean particle diameters (assumed to represent spherical particles) were 1.0 μm or larger, whereas individual bacteria of the type dealt with here are typically rods of about 0.5-μm diameter and 1-μm length (Yankofsky et al., 1981a).

Condensation growth similar to that seen with natural INA bacteria was also observed when the genetically engineered INA strain of *E. coli* was tested in the haze chamber (Fig. 3a). Mean diameter and volume changes of about 3- and 27-fold were respectively obtained (Table 2). However, almost precisely the same result was also observed when the original *E. coli* strain lacking *ice* genes was examined (Fig. 3b, Table 2). Therefore, there is not necessarily any connection between condensation nucleation and ice nucleation. Further confirmation of this latter idea was obtained when other non-INA bacteria were tested. Thus, both Bacterium L3 and *B. cereus* proved to be effective condensation nuclei (Table 2).

b. Freezing nucleus spectra of dried bacteria

The experiments described before clearly show that freeze-dried bacteria will quickly surround themselves with a layer of liquid water when introduced into a saturated water vapor environment. Therefore, to the extent that they retain their original freezing nucleus capability, lyophilized INA bacteria ought to act as highly effective centers of condensation freezing. Shown in Fig. 4 is the freezing nucleus distribution in a freeze-dried preparation of Bacterium M1. Drops averaging 2.5×10^{-10} g of dried M1 cells still froze at -9°C. Since the mean dry weight of one bacterium is 2×10^{-13} g, the above result evaluates to at least one

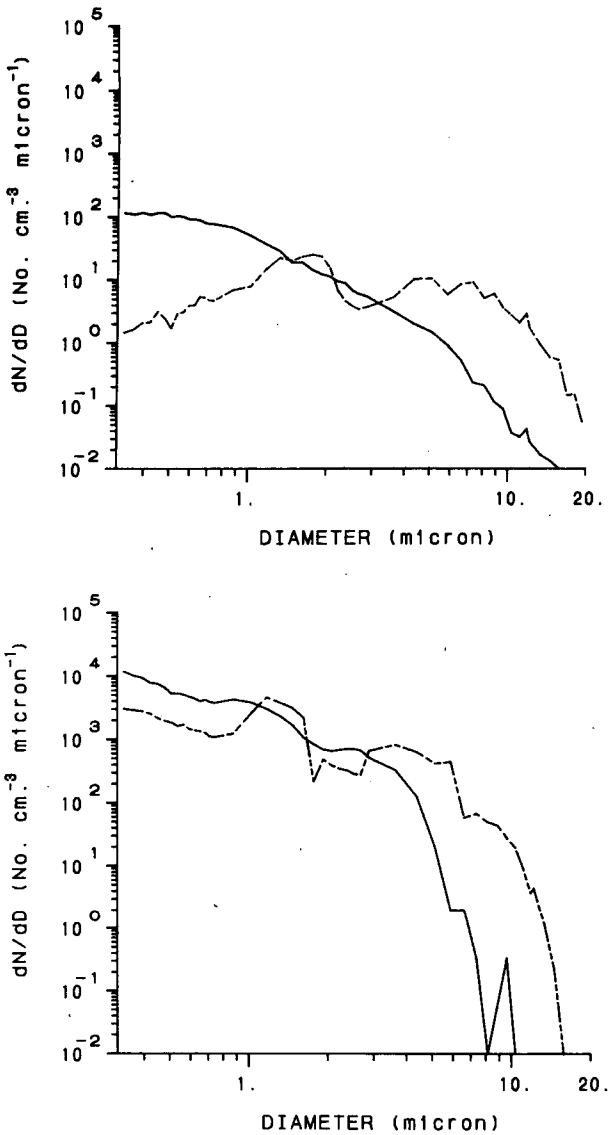

FIG. 2. Size distribution of dry (solid curve) and wet (dashed curve), particles of (a) Bacterium M1 (*Erwinia*) and (b) Bacterium M5 (*Pseudomonas*). Total particle concentrations measured were 98 and 91 cm^{-3} for dry and wet cells of Bacterium M1, respectively; the corresponding concentrations for dry and wet Bacterium M5 were 6593 and 6207 cm^{-3}, respectively.

active bacterial ice nucleus per 1200 cells. Taking into account the fact that the average dry particle is probably an aggregate of two or more individuals (Table 2), the actual ratio of active to inactive cells in the dry population is probably no different than the 1 in 1000 ratio previously reported for actively dividing cell populations of bacterium M1 (Yankofsky et al., 1983). Also similar to the situation in normal cell populations was the −3°C activity maximum of the most effective, and rarest, nuclei present. This latter finding suggests that dessicated INA bacteria will contain the same range of freezing nuclei as the viable cell population from which they were derived. Indeed, there is reason to believe that even the number distribution of each freezing nucleus type is not drastically altered during the drying process.

A major drawback to the use of lyophilized INA bacteria as cloud seeding agents is the fact that only a minority of cells are nucleation active at any given moment. There are normally 100 to 1000-fold fewer nuclei than cells in cultures of natural INA bacteria (Maki & Willoughby, 1978; Yankofsky et al., 1983). Likewise, the percentage of nucleation active cells in strains of *E. coli* engineered to carry and express *ice* genes is normally low. For example, despite the fact that many copies of *ice* genes were present per cell, *E. coli* DG-76 transformed with the multicopy pBM1NB plasmid produced only about one nucleation active cell in 100 during normal culture on medium supplemented with excess leucine and thymine. What is more, the engineered strain expressed far fewer type I nuclei (i.e., particles active at −2 to −4°C) than the strain of Bacterium M1 from which its *ice* genes were derived. Thus, while the ratio of type I to type III nuclei in Bacterium M1 was normally about 0.1, ratios of 10^{-5}–10^{-6} were the rule in all INA^+ strains of *E. coli* containing the recombinant pBM1NB plasmid. Fortunately, it was possible to almost completely overcome both of these limitations.

As will be recalled, strain DG-76 of *E. coli* cannot develop on media lacking preformed molecules of leucine. Lack of this amino acid prevents the synthesis of new proteins. In the case of INA derivatives of strain DG-76, the inability to synthesize new proteins extends to those specified by *ice* genes. As it turns out, leucine deprivation does more than merely block the synthesis of new freezing nuclei. Not only did leucine-starved cells fail to produce new nuclei, they rapidly lost whatever activity had been present before the amino acid was removed. It did not prove difficult to restore nucleation activity, however. Simply returning leucine to the medium and aerating for 2 or more hours at 30°C restored freezing nucleus levels to at least their starting values. In fact, in many trials, nucleation activity transiently increased to the point where the number of freezing nuclei equaled the number of cells. A way to

TABLE 2. Mean diameters and volumes of dry and wet bacteria from condensation growth experiments.

Bacterium	Mean particle diameter (μm)		Mean volume diameter (μm)	
	Dry	Wet	Dry	Wet
Bacterium M1	1.3	5.4	7.5	11.0
Bacterium M5	1.2	2.5	3.3	6.7
E. coli DG-76/pBM1NB	1.2	3.4	2.9	8.7
E. coli DG-76	1.0	3.3	2.3	8.9
Bacterium L3	1.0	4.2	2.2	8.7
B. cereus	1.0	3.1	3.3	8.0

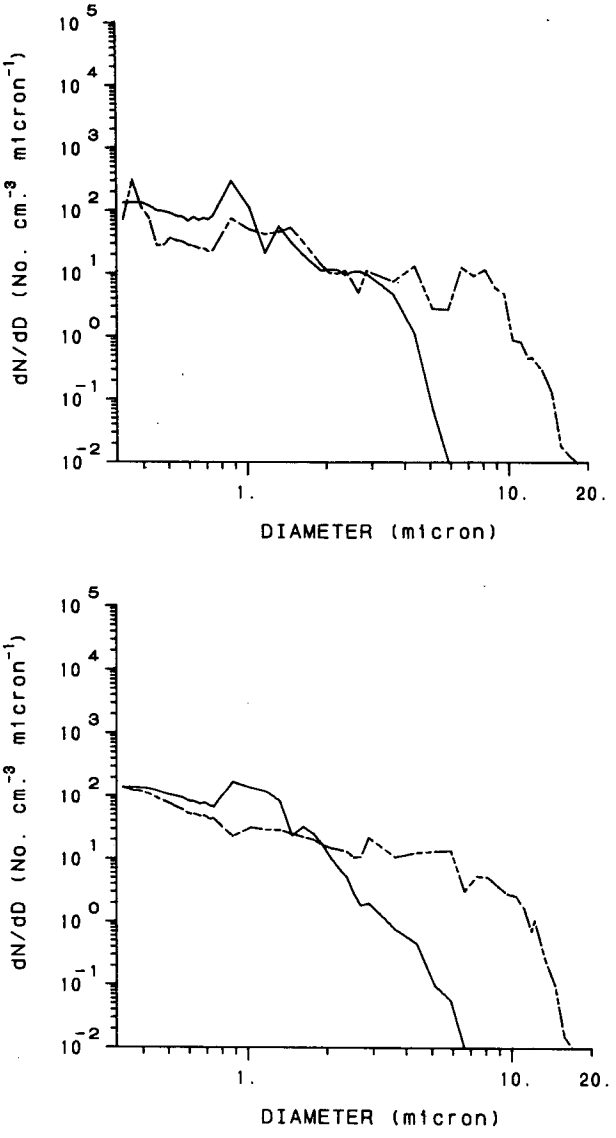

FIG. 3. Size distribution of dry (solid curve) and wet (dashed curve) particles of (a) *E. coli* DG-76/pBM1NB and (b) *E. coli* DG-76. Total particle concentrations measured were 144 and 144 cm^{-3} for dry and wet cells of *E. coli* DG-76/pBM1NB, respectively; the corresponding concentrations for dry and wet *E. coli* DG-76 were 142 and 145 cm^{-3}, respectively.

stabilize this "induced" activity was found. Transfer of the cell suspension from 30° to 4°C (without aeration) anywhere from 5 min to 4 h after adding back leucine induced the appearance of freezing nuclei in all cells even more reliably than did continued aeration at elevated temperature. Elevation of bacterial freezing nucleus concentrations at low temperatures has also been observed in natural INA bacteria (Maki & Willoughby, 1978). Equally interesting, nuclei induced by combining recovery from leucine starvation with temperature downshift contained a much higher proportion of type I particles and remained stable as long as the temperature was not raised above 4°C.

The mechanism of the above induction process will be dealt with elsewhere. Here, suffice it to say that induction of freezing nuclei in INA^+ cells of *E. coli* is independent of cell density. It is just as easy to induce 10 billion cells to become nucleation active in 1 ml as in 1 l. By contrast, while also possible, induction of nucleation activity in populations of natural INA bacteria is not nearly so simple, convenient and reliable a process (Yankofsky et al., 1983). Additional reasons for preferring engineered INA strains of *E. coli* to natural INA bacteria as potential cloud seeding agents will be presented in the Discussion.

Preservation of induced freezing nuclei in lyophilized preparations of *E. coli* DG-76/pBM1NB (INA^+) is demonstrated in Fig. 5. About 55% of the drops containing 2.5×10^{-12} g of dry material (curve dash-dot-dot) froze at temperatures of −10°C or warmer. Assuming unicellular particle size and a Poisson distribution of particles among drops would yield values of 0.8 nuclei and 10 cells per drop (1 cell averages 2.5×10^{-13} g dry weight). Correcting for the fact that there are probably several bacteria in an average dry particle brings the above ratio closer to unity. Thus, every particle in the dry preparation probably contained at least one active freezing nucleus. Moreover, the data also suggest that at least 2% of the particles will nucleate at temperatures warmer than −6°C (curve dash-dot-dash). Accordingly, bacterial ice nucleus technology has now reached the stage where practical experiments in actual clouds can be contemplated. What is more, further efforts should yield even better (and cheaper) material for cloud seeding, perhaps including cell-free preparations.

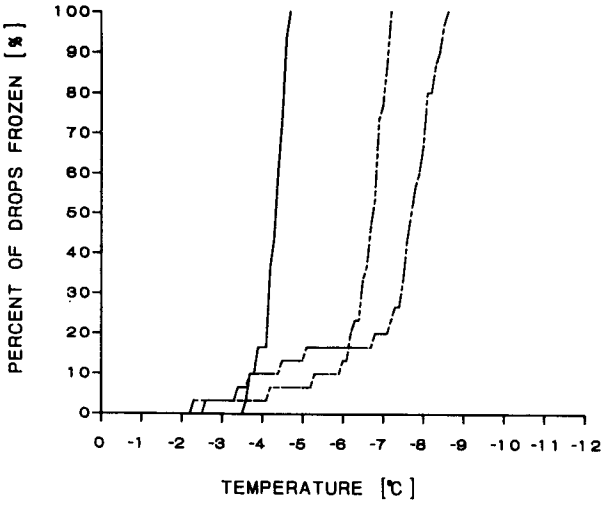

FIG. 4. Drop-freezing spectrum of dry Bacterium M1 cells. The plotted curves derive from a single preparation diluted to contain 2.5×10^{-5} g $drop^{-1}$ (solid line), 2.5×10^{-8} g $drop^{-1}$ (long-short-short-dashes) and 2.5×10^{-10} d $drop^{-1}$ (long-short dashes).

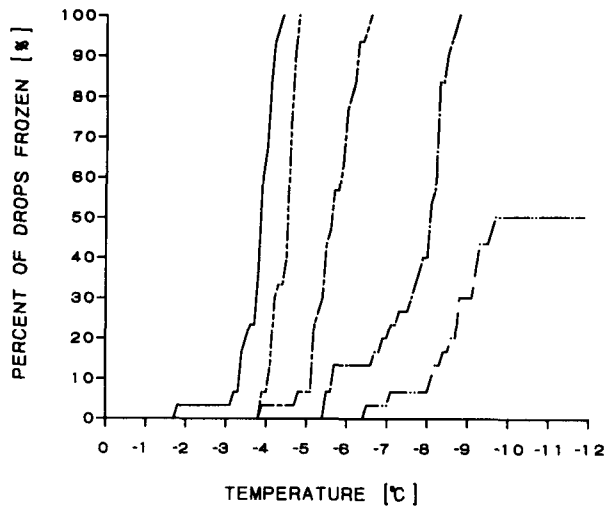

FIG. 5. Drop-freezing spectrum of dry *E. coli* DG-76/pBM1 cells. The plotted curves derive from the same preparation diluted to contain 2.5×10^{-6} g drop^{-1} (——), 2.5×10^{-8} g drop^{-1} (— — — —), 2.5×10^{-10} d drop^{-1} (— — —), 2.5×10^{-11} g drop^{-1} (—·—) and 2.5×10^{-12} g drop^{-1} (—· ·—).

5. Model simulation of cloud seeding with dry INA bacteria

Dry bacteria absorbed enough water to form droplets of 15–20-μm diameter during an average 200 s passage through the haze chamber. Even bigger droplets would be expected to form if the same kinds of particles were allowed to ascend from the base of a real cloud to higher regions. After all, the supersaturated conditions in a cloud would ensure a faster rate of condensation growth and the relatively large distances traveled would provide considerably more than 200 s of growth time. Nor is there any a priori reason why the relatively large droplets expected to condense around dry INA bacteria in a cloud should not freeze when suitably low (−5° to −10°C) temperatures are reached, and then continue to grow larger as a result of vapor deposition and subsequent riming. In spite of this, it is not obvious that seeding relatively warm clouds with dry INA bacteria will enhance rainfall, particularly from clouds too warm to be affected by AgI.

A useful method for testing the effectiveness of dry INA bacteria as seeding agents is by simulation in a numerical cloud model. Here we briefly present data from a preliminary study carried out with a time dependent, one-and-a-half dimensional model which treats the microphysics in detail but assumes a constant cloud radius and allows for entrainment. It is identical to the electrical cloud model of Tzur and Levin (1981) except that electrical processes were omitted. The model simulates cloud development from the initial nucleation of droplets through their growth by condensation, stochastic collection, ice nucleation and ice-crystal growth by deposition, riming, and, finally, rain. Ice is assumed to evolve as in a continental cloud without ice multiplication. In other words, the concentration of naturally produced ice crystals at any given temperature during the developing stages of the cloud cannot exceed the concentration of ice nuclei active at that temperature. The actual ice nucleus distribution with temperature used in the model was taken from Gagin (1975).

Seeding was simulated by introducing small ice crystals (1.2 μm radius) at a single level of the model cloud, or at several levels corresponding to a range of temperatures similar to that of the experimental drop freezing spectrum presented in Fig. 5. The former case was equivalent to seeding with a population of dry INA bacteria of uniform efficiency, whereas the latter approach allowed for the fact that not all individual particles in an INA population will be associated with freezing nuclei of the same efficiency. Additional tests to simulate seeding with AgI aerosols were also performed. In these latter cases, the ice crystals were introduced at colder temperatures corresponding to the AgI freezing spectrum reported by Yankofsky et al. (1981b). Nevertheless, initial conditions for all tests were the same. The temperature profile in the atmosphere was taken as 6.4°C km^{-1} up to a temperature of −13°C (about 5000 m) capped with a deep isothermal layer. This profile limited the growth of the model cloud to temperatures of about −14°C.

Simulated seeding with AgI was performed by introducing 0.5 ice crystals per liter at −10°C, 1.5 ice crystals per liter at −12°C, 2.5 ice crystals per liter at −12.5°C and 5.5 ice crystals per liter at −14°C. Seeding was done 2000 s into the run while the cloud was still in its developing stage. In accordance with their greater nucleation efficiencies, simulated seedings with ice crystals representing dry INA bacteria were initiated at warmer temperatures. The timing of ice crystal introduction was, however, the same. Also common was the unseeded run included for comparison. A summary of results of all of the above trials is presented in Fig. 6. Plotting accumulated rain on the ground as a function of time from the initiation of each computer run revealed that the unseeded cloud yielded a total rain accumulation of 4.5 mm during its lifetime. By contrast, 5.1 mm of rain fell from the model cloud after multilayer seeding with AgI. It may also be worth noting that the rainy period after AgI-like seeding was 200 s shorter than in the case of the unseeded cloud. This latter result implies that rainfall intensity is also enhanced by seeding. The 5.1 mm value obtained by a simulated seeding of the model cloud with AgI was surpassed by both single-level and multilevel seeding with bacteria-like nuclei. Thus, a single-level seeding at −5°C with 10 ice crystals per liter produced 6.5 mm of rain on the ground, as compared to 7 mm of rain after introducing 0.8 ice crystals per liter at −5°C, 4.2 ice crystals per liter at −8°C and 5.0 ice crystals per liter at −9°C in a multilevel test. The reason for the

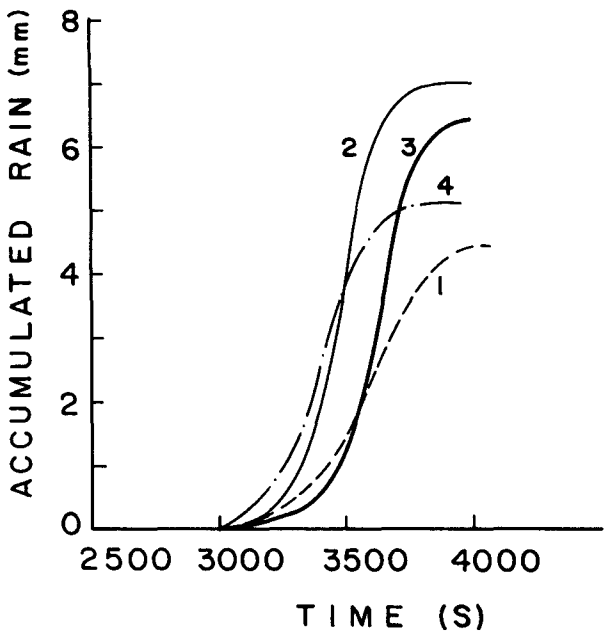

FIG. 6. Computed rainfall versus time from a numerical cloud model. Curves 1 through 4 represent unseeded, multilevel seeding with dry bacteria, single-level seeding with dry bacteria and multilevel seeding with AgI, respectively. Details are given in the text.

observed difference in predicted precipitation yields is not clear.

In this model, the increase in rain stems from the growth of seeded ice crystals and the increase in buoyancy. Initiation of freezing at temperatures as warm as −5°C allows for growth from the vapor to occur over a relatively long period during the ascent. Apparently, this longer period of growth is more than enough to compensate for the slower rate of growth of ice crystals at −5° to −10°C (Pruppacher and Klett, 1980), resulting in more rain than when small clouds are seeded with AgI at −10°C or colder.

Represented in Fig. 7 are the respective water and ice distributions in an unseeded model cloud as a function of height and particle size at 2000 s (Fig. 7a) and 3000 s (Fig. 7b) after the initiation of the run. The contours are of constant liquid (solid lines) and ice (dashed lines) contents (g m^{-3}). Vertical development of the cloud is limited to about 5500 m (ca. −14°C) by the superimposed isothermal layer. The corresponding case at 3000 s for an identical cloud seeded at different levels with dry INA bacteria is presented in Fig. 8. Comparison with Fig. 7b reveals that seeding produced an "explosion" of the cloud and an extension of its top to about 6600 m (ca. −18°C). In other words, the release of latent heat was large enough to push the cloud top up against the inhibiting effect of the isothermal layer.

The results of the numerical simulations presented above are only preliminary. Considerably more work is needed to refine the model and quantify the comparative effectiveness of dry INA bacteria and AgI. Nevertheless, it is already evident that bacterial ice nuclei show more promise as cloud-seeding agents than AgI. Actual trials with AgI in Israel show that winter continental clouds whose tops fail to reach at least −11°C are not significantly affected by seeding (Gagin & Neumann, 1981). The studies described herein suggest that clouds whose tops do not even reach −10°C could be made to produce considerable rainfall by seeding with dried INA^+ *E. coli* cells previously induced to high specific nucleation activity as described in this article.

6. Discussion

Data presented herein suggest that freeze-dried INA bacteria might be considerably more effective as cloud seeding agents than AgI. Moreover, knowledge of how to prepare bacterial ice nuclei suitable for delivery into warm cloud systems on a large scale at reasonable cost already exists. Still, the number of intact bacterial cells we are proposing to release into the atmosphere is enormous. At 10 nuclei l^{-1}, it would require approximately 2.5×10^{14} active bacterial particles to seed a 2 km thick layer in just one cloud with a diameter of 4 km. Consequently, the question of environmental impact must also be considered.

It is, first of all, highly unlikely that even the most frequent and widespread release of INA bacteria into clouds would significantly add to the numbers of such bacteria already present on plant surfaces and in the atmosphere. However, let us, for the sake of argument, suppose that local plants and animals at ground level might be at least transiently exposed to larger numbers of INA bacteria than usual. What ecologically undesirable consequences might be even remotely anticipated from such enhanced contact?

All natural INA bacteria are known plant epiphytes and pathogens (Lindow, 1983). It is therefore conceivable, even if not very likely, that certain natural and cultivated plants may be harmed by contact with raindrops containing INA bacteria. Seeding with an engineered INA strain of *E. coli* in place of natural INA bacteria avoids this potential problem altogether. The natural habitat of enteric bacteria like the *Escherichia* is the vertebrate gut. There is more than a century of bacteriological experience to confirm that *E. coli* is not a phytopathogen, and cannot even colonize plants. Equally well established is the benign nature of the interaction between this enteric bacterium and its animal hosts. In particular, laboratory strains of *E. coli* are entirely harmless to humans and animals, even when frequent contact with huge numbers of viable bacteria is involved. At most, handlers of concentrated bacterial powders who fail to take precautions against inhaling the dry material might develop a mild lung irritation (Rylander, 1982).

One could still argue that large enough additions of

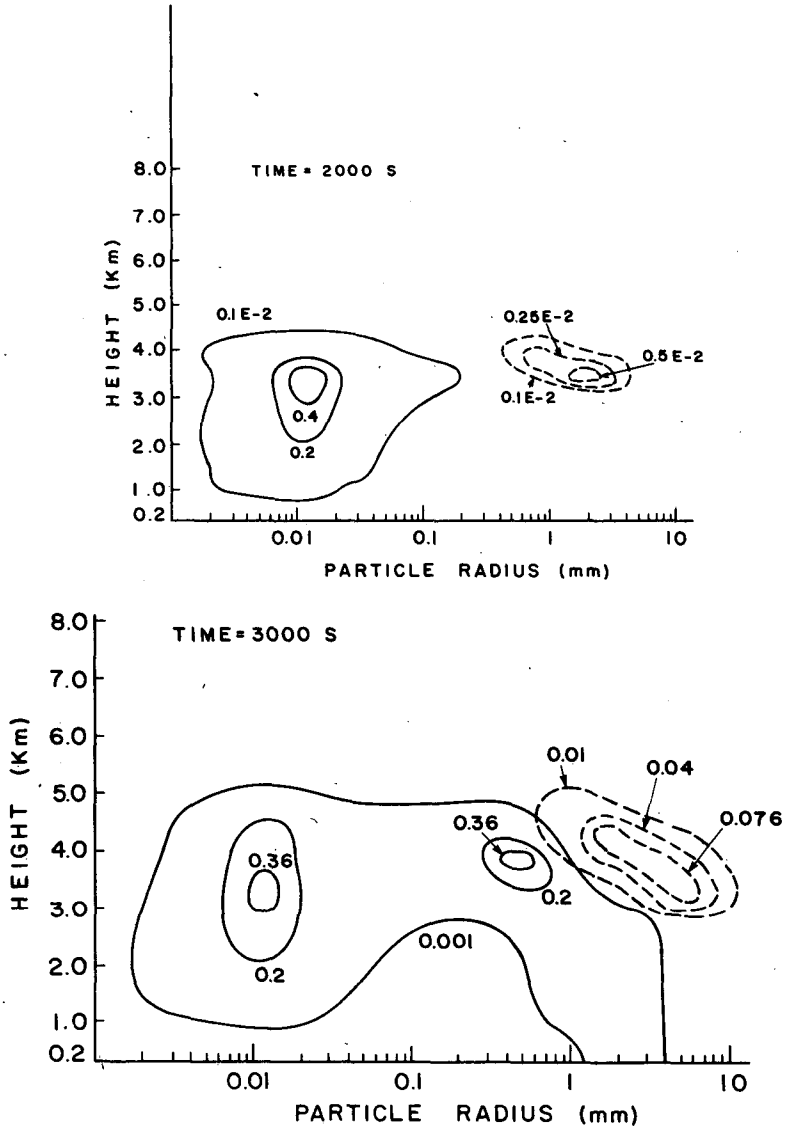

FIG. 7. Water (——) and ice (- - -) contents (g m^{-3}) versus altitude and particle radius (a) 2000 s and (b) 3000 s after initiation of the computer run of an unseeded cloud.

E. coli cells to the environment could somehow upset natural balances in much the same way that all environmental pollution does. Precautions against even this rather nebulous possibility can also be taken. Nutritionally defective mutants of *E. coli* are not found in nature. More to the point, the survival probability of strains with amino acid and, worse yet, pyrimidine requirements is vanishingly small. An exponential decline in the viable cell concentration was observed when populations of *E. coli* DG-76 were deprived of thymine for longer than 1–2 h. In short, as far as long-term reproductive potential is concerned, strains like DG-76 are effectively dead from the moment they leave the controlled environment of the growth flask. One can, in fact, biologically prepare inert bacterial nucleants with no particular difficulty.

But what of the possibility that, viable or not, INA bacteria precipitating onto plant surfaces after cloud seeding might subsequently incite warm-temperature frost damage? After all, natural INA bactera in situ do cause this kind of injury to plants (Hirano & Upper, 1983). Against this eventuality is the fact that local atmospheric conditions conducive to rain in regions prone to frost damage would not be accompanied by freezing weather at ground level. In addition, as already noted, lack of essential amino acid would lead to effective loss of freezing nuclei in a mutant strain like *E. coli* DG-76 within several hours. All told, and pro-

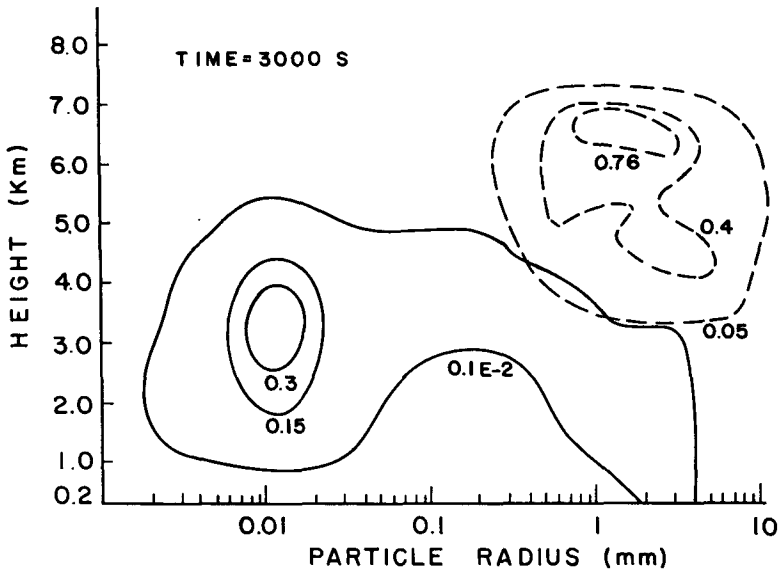

FIG. 8. Water (—) and ice (- - -) content (g m^{-3}) versus altitude and particle radius 3000 s after initiation of the computer run of a seeded cloud. The simulation in this case was multilevel seeding with ice crystals representing dry INA bacteria.

viding that the right strains are used, there is no obvious ecological reason for disqualifying intact dry INA bacteria as potential cloud-seeding agents. That is not to say, however, that cell-free bacterial ice nuclei of equivalent efficiency would not be preferable to intact cells on economic and meteorological grounds.

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