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Evidence for More Than One Division of Bacteria Within Airborne Particles

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When the protocol that we had used to demonstrate a single division of bacterial cells in airborne particles was changed to one that increased the glycerol content of the atomizer fluid from 1 to 5% (vol/vol), thus producing larger particles, more than two (and nearly three) divisions of bacteria occurred within 6 h of aerosol time.

In a series of papers, we presented evidence that a bacterium (*Serratia marcescens*) can undergo at least one division within small (2-μm diameter, more or less), airborne, aqueous particles containing nutrients (2, 3, 6).

This paper presents evidence that when the droplet size was increased to 4 to 6 μm in diameter, more than one division occurred.

MATERIALS AND METHODS

The techniques, instrumentation, and species (*S. marcescens* 8UK) described in the previous papers were used in this study with the following exceptions: (i) the glycerol content of the atomizer fluid was increased from 1% (vol/vol) to 5% (vol/vol) for the purpose of creating larger particles at equilibrium, with the moisture content of air holding the aerosol (4); and (ii) Coulter Counter studies were not done, since we had satisfied ourselves that the increase in viable bacterial numbers above that of the initial numbers was not artifactual (not caused by wall effects within the aerosol chambers) and that the settling rate of the contained particles was measured adequately by continuous light-scatter (nephelometric) measurements (1).

RESULTS

Data from two replicate runs wherein maximal growth occurred are shown graphically in Fig. 1A and B. Logistical problems prevented our taking a 12-h sample at night; hence, hypothetical data points at 12 h of aerosol time (indicated with asterisks) were derived by interpolation of the observed decay of viable cell numbers. We include them for clarity and for estimating a potentially maximal number of new cells produced.

On this basis, the increase in numbers of viable airborne cells, corrected for fallout, was sixfold in one test (Fig. 1A) and slightly more than sevenfold in another (Fig. 1B). On the basis of the 24-h samples, the increases were fivefold and sixfold, respectively. Since samples were taken in impingers, wherein the bacteria are distributed as individual cells within sampler fluid before the dilution and plating step, the corrected numbers (triangles in Fig. 1) do not represent an increase in airborne particles, but rather an increase in the number of cells within particles remaining in the volume of air sampled.

The increased physical decay of the aerosol during the first 6 h, measured as light scattered from particles, showed that the median particle size (1) was larger than those of aerosols previously tested (3) and was approximately 4 μm in diameter, with some particles as large as 6 μm and some as small as 1 μm. The heterogeneity of the particle sizes and the probability of the distribution of bacteria within the initially formed particles prevented a valid quantitative analysis of the data, but taken on face value, they point to an initial doubling time of about 1 h. After that time, the doubling time increased to about 5 h. Under ideal conditions, when media in flasks held in an incubator that provides aeration by agitation are employed, the doubling time of this species is about 45 min. Either some newly formed cells began to die after about 2 h of aerosol time, or the environment within the particles became inhibitive; the latter is a more reasonable explanation since the decay rate of viable cell numbers from 12 to 24 h of aerosol time is the same as the physical decay for that time, indicating no loss of viable cell numbers.

The results show unequivocally that it is possible for bacteria held in moist, airborne particles to double at least twice and very likely three times when the particle size is 4 to 6 μm in diameter (i.e., the volume is 50 to 200 times that of the cell volume).

DISCUSSION

Aside from a desire to obtain new fundamental knowledge of the extent of bacterial capabilities for growth in unusual environments, we are

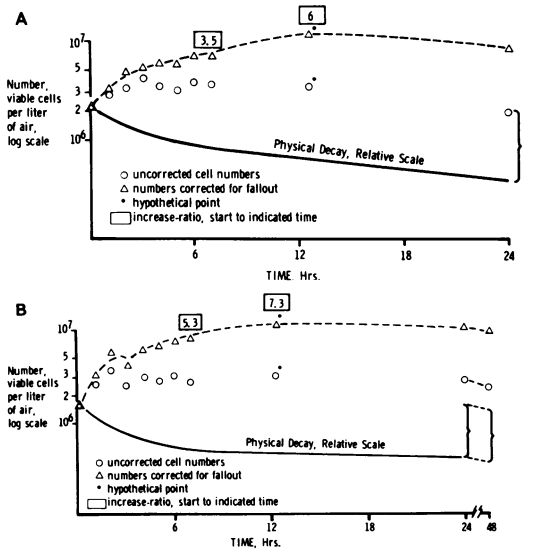

FIG. 1. *Concentration of cells of S. marcescens, as a function of time, in a rotating-drum, aerosol container at 31°C and saturated humidity. "Physical decay" curve depicts the change, with time, of all aerosol particles as a result of gravitational removal. "Uncorrected cell numbers" means concentration of cells, as measured. "Corrected cell number" means concentration of cells which would have been present had no particles that contained cells undergone "physical decay." "Increase-ratio" is corrected number of cells, at time t,/number of cells, at time zero.*

making these studies to determine whether microbial forms might survive and increase in number in a gaseous menstruum such as the Jovian atmosphere. These atmospheres appear to have easily detectable levels of organic compounds, but little, if any, oxidizing capability (5). Any failure to demonstrate survival and growth of bacteria in particles suspended in a "simulated" Jovian atmosphere, however, could be attributed either to the possible toxic or inhibitory qualities of reducing atmospheres or to the inherent inability of bacteria to grow while suspended in a gas. We have shown that growth beyond a single doubling is possible if the particles are moist and contain nutrients, although propagation beyond three generations may be limited because of accumulation of waste products within the particle or lack of nutrients.

At least one more study is needed before simulated atmospheres should be tested. For the continued aerial propagation of a species, additional nutrients would have to be obtained by the cell from the atmosphere. Material in the vapor phase contacts airborne particles more readily than particulate substances can coalesce. Although we have preliminary evidence that ammonium acetate vapor is readily adsorbed by airborne particulates and does not injure airborne cells that were grown in ammonium acetate medium, we have yet to obtain evidence that these cells obtained nutritional value from the vapor.

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