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Water-to-Air Fractionation of Bacteria

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Differences in the ability of bacterial species to be transported from water to air by bursting bubbles were investigated. Bubbles were generated in suspensions of mixed bacterial cultures, and the concentration was measured for each species in the top jet drop ejected. This concentration divided by the concentration in the bulk menstruum is the concentration factor (CF). Bubbles were generated 2 cm below the liquid surface, and jet drops with diameters from 34 to 136 μm were studied. *Serratia marinorubra* and *Micrococcus euryhalis* had CFs which were generally 10 to 100 times greater than those of *Escherichia coli*, *Pseudomonas bathycetes*, and spores of *Bacillus subtilis*. *P. bathycetes* never had a CF significantly greater than 1 at any drop size, and spores of *B. subtilis* had a maximum CF of 4. *E. coli* had a maximum CF of 6 for 1- or 2-day old cultures, but this increased to 80 when a 5-day-old culture was used. This change in the CF with age of the cells indicates that composition of the cell may be a factor influencing its ability to concentrate in jet drops.

Bacteria can be transported into the atmosphere by bubbles bursting at an air-water interface. The jet drops produced by these bubbles can contain bacteria in concentrations up to 1,000 times greater than the concentrations in the bulk liquid (6, 11). Recently, similar findings have been reported for viruses (2, 3). This implies that bubbles bursting in waters which contain large numbers of bacteria or other microorganisms could contribute to the microbiological pollution of the atmosphere.

Studies around sewage treatment plants have shown that there are higher atmospheric concentrations of indicator organisms, such as *Escherichia coli* and *Enterobacter aerogenes* downwind than there are upwind from the plant (1, 12-14). Katzenelson et al. (15) were able to connect the use of wastewater for spray irrigation with the incidence of disease in Israeli communities.

If various bacterial species differ in their ability to be transported from water to air, then this difference must be taken into account when assessing the health hazards of pathogens aerosolized from polluted waters. F. B. Higgins (M.S. thesis, Georgia Institute of Technology, Atlanta, 1964) conducted wind tunnel experiments with mixed bacterial aerosols and estimated that *Serratia marcescens* is 20 times more effectively aerosolized than spores of *Bacillus subtilis*. Using natural water samples, Bezdek and Carlucci (4) found that various types of bacteria differ in their concentrations in drops produced by bursting bubbles.

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The purpose of the present work was to determine the amount of the differences among several bacterial species in their ability to be ejected into the atmosphere by bubbles rising through water and bursting at the air-water interface.

MATERIALS AND METHODS

Organisms and culture conditions. Three species of marine bacteria, *S. marinorubra*, *Pseudomonas bathycetes*, and *Micrococcus euryhalis*, were tested for their ability to be aerosolized. These cultures were maintained on 2216 marine agar slants (Difco). Cultures of *E. coli* and *B. subtilis* were maintained in nutrient agar. A mixed suspension of two or three species was used in each experiment to eliminate between-experiment variance. Before each experiment, cultures were grown at 23 to 25°C in petri dishes on either marine or nutrient agar. After 2 days of growth the cells were harvested by washing the agar surface with 5 ml of the suspending liquid to be used in the subsequent bubbling experiments. This was sterile 3% NaCl in experiments with marine bacteria or 0.85% NaCl in experiments with *E. coli* or spores of *B. subtilis*.

After harvesting, each bacterial suspension was adjusted to specific optical densities, agitated on a Vortex mixer for 30 s, and examined microscopically to assure that the cells were not aggregated. A volume of each suspension sufficient to give a final concentration of 10^4 cells per ml for each species was then added to the bubbling dish.

Aerosol formation and quantification. The bubbling apparatus used was similar to that described by Blanchard and Syzdek (7) and is shown schematically in Fig. 1. The glass bubbling dish rotated on a turntable at 16 rpm. Bubbles were produced in a single stream by air released 2 cm below the water surface by a nozzle made from a Pasteur pipette drawn out to form bubbles of a given diameter. The rate at which the

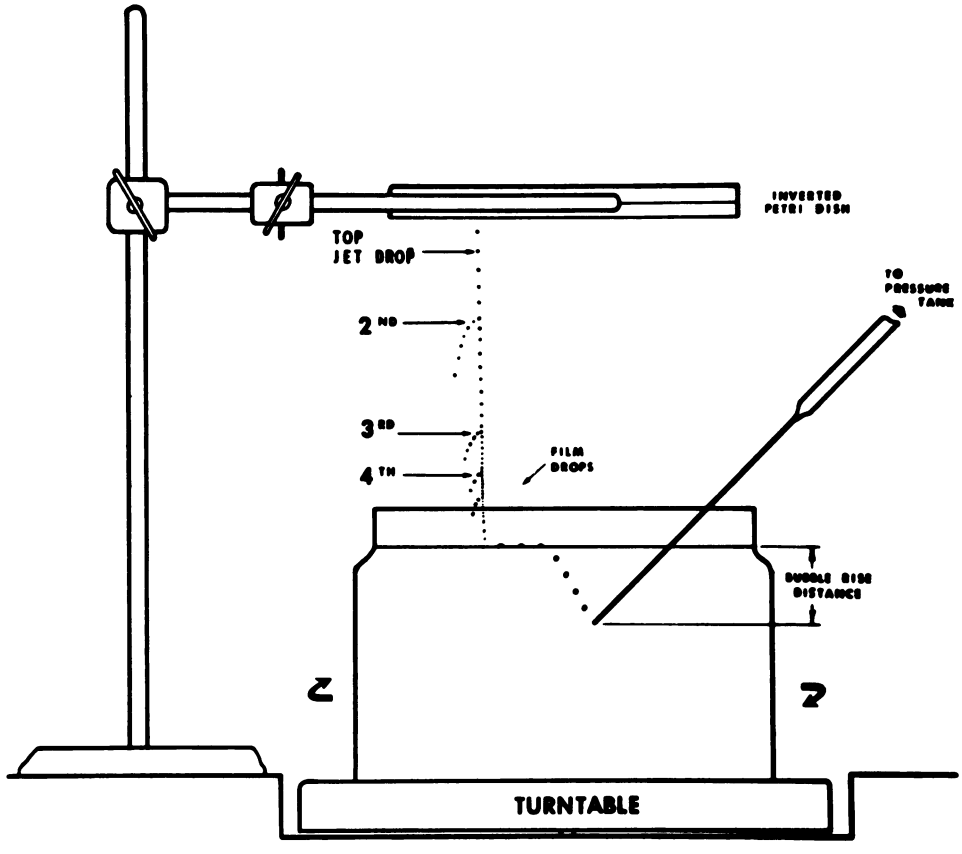

FIG. 1. *Schematic diagram of bubbling apparatus. Agar surface is about 0.5 cm below the ejection height of the top jet drop. The bubble rise distance used in these experiments was 2 cm.*

bubbles were produced was measured with a stroboscopic light.

The average size of the top jet drop was measured by impacting them on MgO-coated glass slides (9) and using the mean of 10 impaction points to obtain the mean drop diameter (16) from which the drop volume was calculated. Bacterial concentrations in the jet drops were measured by collecting the top jet drop from each bursting bubble on an agar plate held inverted above the bubble chamber. The agar surface was positioned 0.5 cm below the ejection height of the top jet drop. After exposure, a drop of sterile saline solution was added to each plate and spread over the agar surface. Quintuplicate plates were inoculated for each drop size tested. The concentration of bacteria in the bulk suspension was determined from five separate samples taken from a depth of 2 cm at the start and finish of an experiment.

Definition of terms. The concentration factor (CF) is defined as the concentration of a given species in the top jet drop (Fig. 1) divided by its concentration in the bulk suspension (C_b). The enrichment of the species in the jet drop relative to the bulk suspension is its CF minus one. The fractionation of species A relative to species B is the CF of A divided by the CF of B. These relationships may be summarized as follows. The CF for species A is: $CF_A = (A)_{drop}/(A)_{bulk\ suspension}$. The enrichment of species A is: $E_A = CF_A - 1$. The fractionation of species A relative to B is: $F_{A/B} = CF_A/CF_B$.

RESULTS

The CFs found over a range of jet drop diameters for *S. marinorubra* and *M. euryhalis* are shown in Fig. 2. This experiment was conducted in a 3% NaCl solution at 23°C. The error bars represent 95% confidence intervals for the mean at each point. The maximum CF for *S. marinorubra* was 140 at a drop diameter of 45 μm. *M. euryhalis* had a maximum CF of 40 at 35 μm diameter, with the CF for both organisms decreasing at a diameter of 90 μm. In this experiment, *S. marinorubra* was enriched in the jet drops 3 to 11 times more than *M. euryhalis*, depending on the drop diameter.

When *S. marinorubra*, *E. coli*, and *P. bathycetes* were used together, *S. marinorubra* had CFs up to 30 times greater than that of *E. coli* for 60-μm drops and 50 times higher than that of *P. bathycetes* for 42-μm drops (Fig. 3). The maximum CF for *S. marinorubra* in this experiment was 92; *E. coli* had a maximum of 6; and *P. bathycetes* showed no significant enrichment

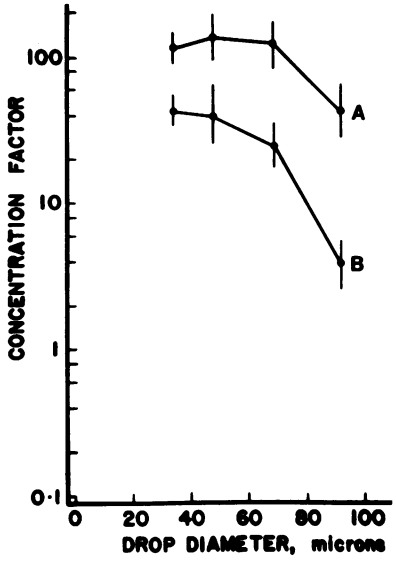

FIG. 2. *Comparison of CFs for (A) S. marinorubra (C_b = 4.9 × 10^4 cells per ml) and (B) M. euryhalis (C_b = 1.8 × 10^4 cells per ml). The suspending menstruum was 3% NaCl at 23°C.*

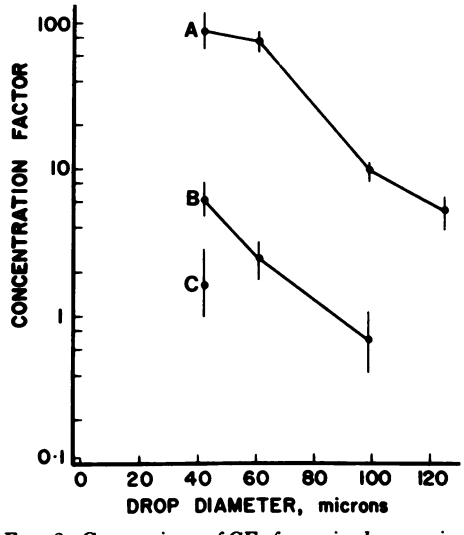

FIG. 3. *Comparison of CFs for a single experiment using (A) S. marinorubra (C_b = 0.89 × 10^4 cells per ml), (B) E. coli (C_b = 1.1 × 10^4 cells per ml) and (C) P. bathycetes (C_b = 2.9 × 10^4 cells per ml). The suspending menstruum was 0.85% NaCl at 23°C. Missing points for E. coli and P. bathycetes indicate too few colonies to determine drop concentrations accurately for these points.*

in the top jet drops. CFs considerably less than 1 were indicated for *P. bathycetes* at drop diameters of 61, 99, and 125 μm, although too few colonies were present to determine the drop concentrations accurately for larger drop diameters. CFs below 1 may be due to migration of *P. bathycetes* away from the surface of the liquid, as was indicated by a crude sampling of the surface layer with a capillary pipette.

Several variables were tested to determine their effects on the fractionation process. Changing the temperature, salinity, bubble rise distance, and bacterial concentration in the bulk menstruum did not affect the fractionation between species significantly. The bubble rise distance and the bulk concentration did, however, affect the individual CFs as others (4, 7, 8) have reported.

A variability in the fractionation of *S. marinorubra* and *M. euryhalis* relative to *E. coli* was noted in one set of experiments which seemed to be related to the incubation time of the cultures before each experiment. The effect of culture age on the CFs of *S. marinorubra* and *E. coli* is shown in Fig. 4. One trial was done after 1.5 days of culture growth and another was done after 5 days. The 1.5-day culture of *E. coli* showed the usual low CFs relative to *S. marinorubra*. The CFs of a 5-day-old culture of *E. coli* showed a 15-fold increase over those of the younger culture and were comparable to those of *S. marinorubra*, which remained unchanged.

DISCUSSION

Concern has been expressed over the possibility of aerosolizing potentially pathogenic organisms from sewage treatment plants (12–14). Typically the organisms looked for are *E. coli* or *E. aerogenes*, with the assumption that if one

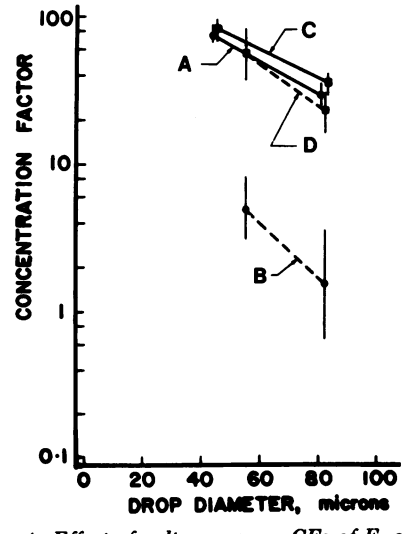

FIG. 4. *Effect of culture age on CFs of E. coli (A, 5 days, C_b = 0.82 × 10^4 cells per ml; B, 1.5 days, C_b = 1.4 × 10^4 cells per ml) and S. marinorubra (C, 5 days, C_b = 0.73 × 10^4 cells per ml; D, 1.5 days, C_b = 1.4 × 10^4 cells per ml). Suspending menstruum was 0.85% NaCl at 23°C.*

finds these indicators then pathogens may also be present. Under such an assumption, one presumes that all species of bacteria are aerosolized with equal efficiency. Our findings however, indicate that this is not the case. When grown and aerosolized under similar conditions, various bacterial cultures of identical ages have CFs that differ by as much as two orders of magnitude (Fig. 2 and 3).

In developing a model to explain the shape of the concentration curves such as those in Fig. 2 and 3 of this paper, Bezdek and Carlucci (4) reasoned that the bacteria ejected within the jet drops (into the atmosphere) are derived from the bacteria suspended in the bulk solution and the bacteria concentrated in the surface microlayer at the air-water boundary. Their model predicted that as the drop size decreased, the proportion of material derived from the surface layer would increase until a maximum CF was reached. As the drop diameters decrease further, the density of the cells per unit area of the surface film relative to the drop diameter becomes so small that the chance of entraining a bacterium decreases with decreasing drop diameter. Subsequent experimentation with radioactive stearate microlayers (5) confirmed the hypothesis that smaller drops contain a greater proportion of material from the surface layer. The bacteria in the jet drops appear to be concentrated in the surface microlayer and are drawn with it in drop formation. The reasons for the existence of a bacteria-rich surface layer have not been established but, our results comparing the jet drop concentration of 1.5- and 5-day-old cultures of *E. coli* suggest some component of the cell itself is involved. At this point we can only speculate on the nature of the cellular material. For example, an increased lipid content, particularly in the cell envelope, could mediate concentration of cells in the surface microlayer. A *Brevibacterium* species has been reported to partition itself in the oil phase of an oil-water mixture (17), and this organism was found to increase its lipid content with age. Age effects in other bacterial species could possibly affect their ability to partition between the surface layer and the bulk menstruum.

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LITERATURE CITED

1. **Adams, A. P., and J. C. Spendlove.** 1970. Coliform aerosols emitted by sewage treatment plants. Science **169**:1218–1220.
2. **Baylor, E. R., M. B. Baylor, D. C. Blanchard, L. D. Syzdek, and C. Appel.** 1977. Virus transfer from surf to wind. Science **198**:575–580.
3. **Baylor, E. R., V. Peters, and M. B. Baylor.** 1977. Water-to-air transfer of virus. Science **197**:763–764.
4. **Bezdek, H. F., and A. F. Carlucci.** 1972. Surface concentration of marine bacteria. Limnol. Oceanogr. **17**: 566–569.
5. **Bezdek, H. F., and A. F. Carlucci.** 1974. Concentration and removal of liquid microlayers from a seawater surface by bursting bubbles. Limnol. Oceanogr. **19**:126–132.
6. **Blanchard, D. C., and L. D. Syzdek.** 1970. Mechanism for the water-to-air transfer and concentration of bacteria. Science **170**:626–628.
7. **Blanchard, D. C., and L. D. Syzdek.** 1972. Concentration of bacteria in jet drops from bursting bubbles. J. Geophys. Res. **77**:5087–5099.
8. **Blanchard, D. C., and L. D. Syzdek.** 1974. Importance of bubble scavenging in the water-to-air transfer of organic material and bacteria. J. Rech. Atmos. **8**:529–540.
9. **Blanchard, D. C., and L. D. Syzdek.** 1975. Electrostatic collection of jet and film drops. Limnol. Oceanogr. **20**: 762–774.
10. **Carlucci, A. F., and H. F. Bezdek.** 1972. On the effectiveness of a bubble for scavenging bacteria from sea water. J. Geophys. Res. **77**:6608–6610.
11. **Carlucci, A. F., and P. M. Williams.** 1965. Concentration of bacteria from seawater by bubble scavenging. J. Cons. Cons. Perma. Int. Explor. Mer **30**:28–33.
12. **Fannin, K. F., J. J. Gannon, K. W. Cochran, and J. C. Spendlove.** 1977. Field studies on coliphages and coliforms as indicators of airborne animal virus contamination from wastewater treatment facilities. Water Res. **11**:181–188.
13. **Goff, G. D., J. C. Spendlove, A. P. Adams, and P. S. Nicholes.** 1973. Emission of microbial aerosols from sewage treatment plants that use trickling filters. Health Ser. Rep. **88**:640–652.
14. **Hickey, J. L. S., and P. C. Reist.** 1975. Health significance of airborne microorganisms from wastewater treatment processes. Part I: summary of investigations. J. Water Pollut. Control Fed. **47**:2741–2757.
15. **Katzenelson, E., I. Brium, and H. Shuval.** 1976. Risk of communicable disease infection associated with wastewater irrigation in agricultural settlements. Science **194**:944–946.
16. **May, K. R.** 1950. The measurement of airborne droplets by the magnesium oxide method. J. Sci. Instr. **27**:128–130.
17. **Severance, M. M., and P. A. LaRock.** 1973. Thermal death of a hydrocarbon bacterium in a nonaqueous fluid. J. Bacteriol. **116**:1287–1292.