Effect of Relative Humidity on the Survival of Airborne Unicellular Algae

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A method is described which is suitable for assessing the effects of relative humidity (RH) on the viability of two unicellular algae in experimental aerosols. Viable cells of Nannochloris atomus collected from the airborne state were detected by plating onto agar surfaces of an appropriate growth medium, whereas viable airborne cells of Synechococcus sp., because of unreliable growth on solid media, were determined by a liquid assay system. The assays were performed at intervals during short-term and prolonged storage of algal aerosols in chambers preconditioned to a selected RH and temperature. Both species showed the greatest loss in viability during the first minute after atomization, and the extent of this inactivation, as a function of RH, reflected the subsequent long-term survival. The airborne eukaryotic alga was unable to survive at an RH below 91%, whereas the airborne prokaryotic alga was comparatively stable over a wide humidity range. Initial inactivation was least and long-term survival best, for both species, at 94% RH.

Direct sampling of the atmosphere over terrestrial, marine, and fresh water environments has established algae as part of the naturally occurring aerial biota (15). The atmosphere over three regions of the United States, for example, has been shown to contain a total of 54 taxa of algae, representing four phyla (15). Natural sources of airborne algae include: (i) formation of oceanographic aerosols by wave action and the bursting of bubbles at the water-air interface (18), (ii) aquatic foams which become airborne (14), (iii) aerosols created by turbulent streams and rivers, (iv) air movement over dry and dusty soil (15), and (v) aeration of sewage (15).

The effects of atmospheric conditions on survival and dispersal of airborne algae are poorly understood. Some species may be better adapted to aerial dispersal and this may partly account for their geographical distribution. According to Schlichting (15), the nature of the aerial biota may act as an indicator of climatic conditions, and thus qualitative sampling could lead to a better understanding of the movement of air masses. Airborne algae may even influence the physical and chemical nature of a given environment, since the invasion and subsequent succession of organisms in a new habitat affects its abiotic structure. In addition, adaptation to airborne stresses may represent a force of natural selection which could have played an important role in algal phylogeny.

Since experimental studies of airborne algae have not been reported, the present investigation was undertaken to establish a protocol for such studies under controlled environmental conditions. We were particularly interested in determining under what atmospheric conditions algae survive best. In this initial paper we will show that two species of unicellular algae, selected to represent both prokaryotic and eukaryotic forms, are differentially susceptible to such atmospheric stresses as dehydration and rehydration. In this respect, they behave in the aerosol state much like other microorganisms (11).

MATERIALS AND METHODS

Algal species. Two isolates of this laboratory, Nannochloris atomus (DWE-4) and Synechococcus sp. (R-3), were used in this study. N. atomus was isolated from a water sample collected at Bodega Bay, Calif., whereas Synechococcus sp. was found in phytoplankton collected from a rice field at Knight's Landing, Calif. Both species were selected on the basis of their unicellularity and small cellular size, because colonial and multicellular algae might be disrupted by the stresses of atomization. Moreover, unicellular forms >15 nm in diameter would exhibit fast settling rates, thus preventing long-term survival studies in aerosols.

Growth conditions and spray suspensions. The algae for aerosol experiments were grown in flasks
containing 100 ml of growth medium on a Metabolyte water bath shaker (model G77, New Brunswick Scientific Co., N.J.) with continuous shaking (100 rpm) and illumination. N. atomus was grown in half-strength medium "I" (9) at 25 C. Synechococcus sp. was grown at 25 C in the medium of Hughes et al. (12), as modified by Allen (2).

The algal cells were allowed to reach a concentration of approximately 2 x 10^7/ml. The culture was used directly as the spray suspension or the algae were harvested by low-speed centrifugation and then suspended in 10 ml of fresh growth medium before atomization.

**Aerosol methodology.** Algal aerosols were generated by a Wells reflux atomizer, modified by installing a stainless-steel peripheral air jet into a 500-ml glass bulb (5). The atomizer was operated at 15 lb/in^2. The primary aerosols were mixed with measured volumes of filtered wet and dry air to achieve the desired relative humidity (RH), which was determined by the wet-dry bulb method. In all experiments, aerosols were maintained at 21 ± 2 C. Aerosols were transported into either a 500-liter rotating drum or down a dynamic aerosol transport apparatus (DATA). Details of the construction and operation of the rotating drum have been reported by Goldberg et al. (8), whereas those for the DATA were previously described by Hatch and Dimmick (10). Briefly, the DATA is a 45-foot (13.7-m) duct, 6 inches (15.2 cm) in diameter, being inserted 2 feet (61 cm) into another 45-foot duct, 8 inches (20.3 cm) in diameter. Each duct was equipped with numerous sampling ports.

**Aerosol sampling and assay.** Algal aerosol samples were collected from the drums or DATA at periods selected to represent specified aerosol ages. The airborne algae were collected either by impaction on agar surfaces of the appropriate growth medium in a slit sampler (3) operated at 5.9 liters/min, or by impaction across AGI-30 sampler (4) operated at 12.5 liters/min and containing 20 ml of the appropriate growth medium.

Cells of N. atomus collected on agar surfaces were incubated at 22 C under continuous illumination, whereas those collected in liquid were serially diluted and the appropriate decimal dilution was plated on half-strength medium "I" containing 1.0% ion agar (Oxoid Ltd., London). The plates were incubated as described above for 20 days, and individual colonies were enumerated.

Since Synechococcus sp. did not always exhibit reliable growth on agar surfaces, a liquid assay system was devised. Disposable clear plastic trays (12) containing 25 individual wells (Fig. 1A) measuring 2.8 by 4.1 by 1.6 cm were filled with 9 ml of liquid growth medium per well (2). An inverted empty tray was then placed over the filled wells to minimize evaporation and prevent contamination; it was removed only once to inoculate the wells with appropriate dilutions of algal suspensions, at which time each well in a given row (starting with the row on the left, Fig. 1B) received a 1-ml inoculum of the lowest dilution of spray or impinger fluids to be tested. Subsequent rows received 10-fold fewer algal cells in the inoculum dose. Thus, a total of five different decimal dilutions were routinely assayed, in quintuple, in each of the five rows of wells. The trays were subsequently incubated at 22 C under continuous illumination for 10 days, and rows showing from 10 to 100 individual colonies in the liquid were counted (Fig. 1B and C). To evaluate the efficiency of the liquid assay system for detection of individual viable alga, the total number of algal cells in cultures was determined using the well-known method for counting cells in the Petroff-Hauser counting chamber. Estimates of total cells per milliliter of undiluted suspensions were then compared with the viable counts per milliliter of the same suspension, as ascertained by the liquid assay system.

**Physical and biological decay of aerosols.** The overall total decay rates (K_t) of the aerosols (defined as the combined total of the physical and biological losses as estimated by the viable counts at various aerosol ages) were determined by the recovery of viable algae on agar or in the liquid assay system. Physical fallout or decay rates (K_p) were monitored by the method of Dimmick et al. (7) and Dimmick (6) with a forward-angle light scatter monitor and a Universal photomultiplier photometer (model PH-200, Eldorado Electronics Co., Concord, Calif.). The light scatter monitor output was recorded (model G-14, Varian Associates, Sunnyvale, Calif.). The biological loss of colony-forming ability or decay rates (K_b) was determined by the formula: K_b = K_t - K_p. The curves shown in Fig. 2 through 4 have been corrected for physical losses and represent rates of biological decay, as determined by data points of three mean samples per each aerosol age from three or more aerosols at the relative humidities tested.

**RESULTS**

**Enumeration of viable algae by the liquid assay system.** The liquid assay system is depicted in Fig. 1. Rows of filled wells inoculated with the lowest serial dilution of Synechococcus sp. suspension displayed heavy growth (Fig. 1B, left row), but as the inoculum dose was reduced sufficiently individual algal colonies were observable (Fig. 1B and C). This was possible because as individual cells replicated in the liquid growth medium, it was apparent that mother and daughter cells remained attached. Cells in subsequent generations also remained attached together to the ever increasing algal colony, and eventually the algal colony was macroscopically visible (Fig. 1C) and could be counted as a colony-forming unit. The colony-forming units per milliliter, representing the sample mean of five wells, were compared with counts obtained by the Petroff-Hauser method. A good correlation was found (Table 1) between the total number of algal cells per milliliter of plating suspension and the viable cell count obtained for that suspension. Thus, most of the Synechococcus sp. in suspensions used in this study were considered viable.
FIG. 1. Liquid assay system used to determine viable cells of *Synechococcus* sp. in pre- and postaerosol samples. A 1-ml inoculum of serial 10-fold dilutions of a given sample was added to each well containing 9 ml of liquid growth medium. Progeny from a single viable alga adhered together and produced a macroscopically visible colony. (A) View of trays under incubation conditions (1/3x). (B) View of trays showing algal growth in rows of wells inoculated with five different dilutions of the alga (2/3x). (C) Enlargement of a single well showing individual algal colonies in the liquid growth medium (2.5x).

**Stability of algae to reflux atomization.**
Since it is known that atomization stress can be detrimental to some microorganisms (11), stability of both species of algae to reflux atomization was determined by comparing the number of viable cells in the spray suspension before and after each experiment. In all cases these counts remained relatively constant, indicating no significant loss of viability due to the process of aerosolization.

**Physical decay of algal-laden particles in aerosols.** Table 2 shows the physical decay rates of algal-laden particles in aerosols held at various humidity levels in the DATA and rotating drum. Mean decay rates of 1.15, 1.35, 1.5, 2.1, and 4.1%/min were observed in the DATA at 19, 40, 60, 80, and 92% RH, respectively. These results indicate that the primary cause of particle fallout in these experimental aerosols was the total particle size, as influenced by RH, and not the microbial cells within the particles.

Similar results were found with aerosols held in a rotating drum, where the rate increased from 0.2 to 0.4%/min concomitantly, with a rise in RH from 19 to 94%.

**The effect of relative humidity on airborne survival.** The survival of airborne *Synechococcus* sp. in the DATA was determined at 19, 40, 60, 80, and 92% RH. The results show (Fig. 2) that, although the alga remained viable throughout the range, survival was measurably different at each RH. At 1- and 6-min
TABLE 1. Comparison of the viable counts of Synechococcus sp. obtained with the liquid assay system and total counts obtained with a Petroff-Hauser counting chamber

| Algal suspension | Total count (cells/ml) | Viable count (cells/ml) |
|------------------|-----------------------|-------------------------|
| 1                | 7.5 × 10^7           | 6.5 × 10^7              |
| 2                | 5.0 × 10^7           | 4.2 × 10^7              |
| 3                | 1.0 × 10^8           | 1.2 × 10^8              |
| 4                | 9.5 × 10^7           | 1.0 × 10^8              |
| 5                | 1.5 × 10^8           | 9.3 × 10^7              |

TABLE 2. Physical loss of airborne Synechococcus sp. and N. atomus in dynamic and aged aerosols at various RHs

| Organism          | Aerosol apparatus | RH (%) | Physical loss a (%)/min |
|-------------------|-------------------|--------|-------------------------|
| Synechococcus sp. | DATA              | 19     | 1.15                    |
| Synechococcus sp. | DATA              | 40     | 1.35                    |
| Synechococcus sp. | DATA              | 60     | 1.5                     |
| Synechococcus sp. | DATA              | 80     | 2.1                     |
| Synechococcus sp. | DATA              | 92     | 4.1                     |
| Synechococcus sp. | Rotating drum     | 19     | 0.2                     |
| Synechococcus sp. | Rotating drum     | 94     | 0.4                     |
| N. atomus         | DATA              | 19     | 1.2                     |
| N. atomus         | DATA              | 40     | 1.4                     |
| N. atomus         | DATA              | 60     | 1.6                     |
| N. atomus         | DATA              | 80     | 2.1                     |
| N. atomus         | Rotating drum     | 92     | 4.2                     |
| N. atomus         | Rotating drum     | 94     | 0.4                     |

a The particle decay in aged aerosols was measured in rotating drums over 4 h, whereas the decay in the DATA was measured for 12 min.

b Physical decay rates of particles in aerosols were determined by light-scatter measurements. The values presented represent geometric means of three or more aerosols at each RH. The rates varied no more than 5% for any given humidity range.

sampling times, there occurred a progressive decrease in the number of viable cells collected, with an increase in RH from 19 to 80% followed by a rapid increase in stability at 92%. To determine the survival of airborne Synechococcus sp. over an extended time period at 19 and 94% RH, the algae dispersed as an aerosol was maintained in a rotating drum for 4 h. The results of this long-term experiment (Fig. 3) indicated a rapid loss in colony-forming ability at 19% RH and a comparatively less, but progressive, loss at 94% RH.

In contrast, the aerosol behavior of the eukaryotic alga was markedly different. At the selected relative humidities in the DATA, viable cells of airborne N. atomus could not be demonstrated below 92% RH (Fig. 2). However, over the interval from 1 to 6 min at 92% RH in the DATA (Fig. 2) and over a period of 4 h at 94% RH in the rotating drum (Fig. 4), N. atomus proved to be comparatively more stable than Synechococcus sp.

To illustrate the dramatic effect of RH on the initial survival of the airborne algae, the number of viable algal cells of both species recovered at 1-min aerosol time was compared with the number which could be theoretically recovered if no loss in viability had occurred. This was based on atomized fluid output and air volumes involved, according to the method of Warren and Hatch (17). The results shown in Table 3 indicate that by the time the first sample was taken significant loss in viability, of both species at all relative humidities, had occurred and the extent of this loss reflected the subsequent long-term survival of the algae. Thus, the initial viability loss of Synechococcus sp. decreased with an increase in RH from 19 to 80% with a rapid recovery at 91%, whereas both algae exhibited the minimal initial loss at 94% RH.

Fig. 2. Effect of RH on survival of unicellular algae in dynamic aerosols at 21 C for 6 min. Data shown are corrected for physical loss and represent biological decay. Curves are delineated from mean values of three or more replicate experiments at each humidity level. No single value deviated more than 15% from those shown. Symbols: ○, Synechococcus sp.; Δ, N. atomus.
that the greatest loss in viability occurred directly after atomization and the RH of the experimental chamber significantly influences the survival of the fraction of algae which was not inactivated by the immediate stresses of aerosolization. The fact that only a small percentage of these algae, at RHs at which survival was recorded, were viable immediately after atomization would imply that most of the cells of these species, which become airborne due to natural processes, are initially inactivated. This implication would be valid only if the stresses which occur immediately after experimental atomization are similar to those occurring under

FIG. 3. Survival of Synechococcus sp. in aged aerosols at 21 °C and various RHs. Data shown are corrected for physical loss and represent biological decay. Figure was obtained by plotting the mean values of at least three replicate experiments at each RH. No single value deviated more than 15% from those shown. Symbols: ◆, 94% relative humidity; x, 19% relative humidity.

DISCUSSION

The results of this investigation show that accurate estimates of viable unicellular algae in experimental aerosols can be successfully achieved and, thus, the behavior of these microorganisms under varied atmospheric conditions may be examined in the airborne state. It is known that airborne microorganisms are influenced most dramatically by the water content of the air environment in which they are suspended (1, 11). The data presented here are then consistent with the survival patterns reported for both airborne bacteria and viruses, in

FIG. 4. Survival of N. atomus in aged aerosols at 21 °C and a RH of 94%. Data shown are corrected for physical loss and represent biological decay. Figure was obtained by plotting the mean values of at least three replicate experiments. No single value deviated more than 15% from those shown.

TABLE 3. Loss of viability at 1 min after aerosolization of Synechococcus sp. and N. atomus at various RHs

| Organism          | RH | Aerosol chamber | Spray suspension (cells/ml) | Calculated theoretical aerosol concn at time 0 (cells/liter) | Viable cells recovered at 1 min* (cells/liter) | Surviving fraction at 1 min (%) |
|-------------------|----|-----------------|----------------------------|-------------------------------------------------------------|---------------------------------------------|---------------------------------|
| Synechococcus sp. | 19 | DATA*           | 1.8 × 10⁴                  | 5.0 × 10³                                                   | 5.0 × 10³                                   | 1.00                            |
| Synechococcus sp. | 40 | DATA            | 1.8 × 10⁴                  | 5.0 × 10³                                                   | 3.4 × 10³                                   | 0.70                            |
| Synechococcus sp. | 60 | DATA            | 1.8 × 10⁴                  | 5.0 × 10³                                                   | 2.5 × 10³                                   | 0.50                            |
| Synechococcus sp. | 80 | DATA            | 1.8 × 10⁴                  | 5.0 × 10³                                                   | 2.5 × 10³                                   | 0.50                            |
| Synechococcus sp. | 92 | DATA            | 1.8 × 10⁴                  | 5.0 × 10³                                                   | 3.0 × 10³                                   | 0.60                            |
| Synechococcus sp. | 94 | Rotating drum   | 4.4 × 10⁴                  | 4.4 × 10³                                                   | 3.0 × 10³                                   | 0.70                            |
| N. atomus         | 92 | DATA            | 9.1 × 10⁷                  | 2.6 × 10⁵                                                   | 1.9 × 10⁴                                   | 7.10                            |
| N. atomus         | 94 | Rotating drum   | 5.3 × 10⁷                  | 5.0 × 10⁴                                                   | 4.8 × 10⁴                                   | 9.60                            |

* Mean values of three replicate samples per aerosol and at least three aerosols at each RH.
* See reference 10.
natural conditions. Of the natural processes known to cause algae to become airborne, only those which aerosolize these microorganisms from an aqueous environment (e.g., wave action, bursting of bubbles, aquatic foams, turbulent streams, and aeration of sewage) could be considered similar to the process of reflux atomization employed in this study. Under these conditions, the algae are subjected to rapid dehydration, regardless of the RH of the air in which they become suspended. It seems likely then that algae adapted to terrestrial habitats would be more resistant to the stress of dehydration and, thus, better adapted to aerial dispersal. This suggestion is supported by the field studies of Schlichting (15), who has found greater abundance of aerial algae in air over dry, dusty soil than in sea air.

Detailed studies on the effect of RH on the survival of airborne bacteria (11) have shown, in many cases, the most lethal humidities to be in the midrange. Although both algae exhibited initial airborne inactivation similar to other microorganisms (1, 11), only the prokaryotic alga, *Synechococcus* sp., showed a pattern of survival resembling those reported for bacteria in that it remained viable throughout the RH spectrum and exhibited the greatest loss in viability at an RH (80%) between the extremes. This is viewed as supporting the contention that blue-green algae are closely related to bacteria (16) in that they show a definite prokaryotic behavior. In marked contrast was the survival of *N. atomus*, a eukaryote, which remained viable at only near saturated humidities. Until additional data on the survival of prokaryotic versus eukaryotic algae in the airborne state are obtained, it is premature to make generalizations. However, the differential survival patterns of these two species are seen to indicate that some algae are better adapted to aerial dispersal than others.

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