Improved Large-Volume Sampler for the Collection of Bacterial Cells from Aerosol

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A modified large-volume sampler was demonstrated to be an efficient device for the collection of mono-disperse aerosols of rhodamine B and poly-disperse aerosols of bacterial cells. Absolute efficiency for collection of rhodamine B varied from 100% with 5-μm particles to about 70% with 0.5-μm particles. The sampler concentrated the particles from 950 liters of air into a flow of between 1 and 2 ml of collecting fluid per min. Spores of Bacillus subtilis var. niger were collected at an efficiency of about 82% compared to the collection in the standard AGI-30 sampler. In the most desirable collecting fluids tested, aerosolized cells of Serratia marcescens, Escherichia coli, and Aerobacter aerogenes were collected at comparative efficiencies of approximately 90, 80, and 90%, respectively. The modified sampler has practical application in the study of aerosol transmission of respiratory pathogens.

The operation and preliminary evaluation of a simple large-volume air sampler has been reported by Buchanan et al. (2). A copy of this sampler has been constructed and subsequently extensively modified, both physically and in its means of operation, in this laboratory. The present paper reports the evaluation of the efficiency of the modified sampler for the collection of mono-disperse particulate aerosols of rhodamine B and for the collection of experimental aerosols of vegetative cells of Aerobacter aerogenes, Escherichia coli, and Serratia marcescens as well as spores of Bacillus subtilis var. niger (BG). In the bacterial studies, the efficiency of the sampler was determined in relation to the all-glass impinger (AGI-30).

MATERIALS AND METHODS

The Defense Research Establishment Suffield (DRES)-modified sampler (cyclone scrubber) samples air at the rate of 950 liters per min (measured by the static pressure drop at the throat of the inlet horn which had been calibrated with a flowrator [Fischer and Porter flowrator meter, catalog no. 6565-521B, Fischer and Porter Co., Warminster, Pa.]) and concentrates the airborne particles into a liquid flow of between 1 and 2 ml/min (Fig. 1). It differs from the model of Buchanan et al. (2) in several respects. (i) That portion of the transition piece (Fig. 1, A) which is inserted into the collector (Fig. 1, B) was redesigned to ensure complete collection of fluid. (ii) Vacuum for the collection of the fluid is provided by using the pressure drop between the sample outlet tube (Fig. 1, C) and the motor, rather than by a pump. (iii) Collecting fluid is provided at a constant rate by means of a screw-driven syringe (Fig. 1, D). We have found this arrangement to be superior to a peristaltic pump in that: (1) at low flow rates (e.g., 1 ml/min), it is more accurate over an extended time period; (2) it eliminates the difficulties involved in adjusting flow when tubing is changed; and (3) sterility is much easier to maintain (on several occasions during extended operation of the sampler, we experienced widespread contamination by a Pseudomonas sp., which was apparently utilizing some component leached from the tubing employed [Tygon or Silastic]). (iv) The modified sampler contains a device (Fig. 1, E) for the metered addition of sterile, distilled water to replace that lost due to evaporation during sampling. Depending on the temperature and relative humidity (RH), evaporation can result in as much as a threefold concentration of the collecting fluid. This concentration effect has been shown to adversely affect the viability of aerosolized vegetative bacterial cells when certain high ionic strength collecting fluids were employed (L. A. White and D. E. Davids, unpublished data). (v) A removable preimpactor (Fig. 1, F), for the collection of particles in excess of 10-μm mass median diameter (MMD), has been designed and fitted to the sampler (R. Naylor and T. J. Malach, unpublished data). The preimpactor mode was not used in the experiments reported here. The absolute efficiency of the sampler was determined for particles in the range of 0.5 to 5.0 μm by the use of rhodamine B (Fisher). Mono-disperse aerosols were produced with a spinning disk generator (model 8330, Environmental Research Corp., St. Paul, Minn.) from ethanolic solutions of various concentrations of dye. Particle sizing was conducted with the aid of a light microscope, fitted with a graduated ocular. The particles were collected in the sampler employing a liquid flow of 1 ml/min of a 0.1% solution of Triton X-100. Rhodamine B concentration

335
was measured at 565 nm (Spectronic 20, Bausch and Lomb, Rochester, N.Y.). Modification of the spinning disk, by machining the edge to a 45° angle rather than the 90° angle as supplied, resulted in increased homogeneity of the aerosol, as well as reducing loss of dye due to excessive production of satellite droplets.

Vegetative cells of *A. aerogenes* (Macdonald College Culture Collection no. 112), *E. coli* (strain 162), and *S. marcescens* (local isolate) were aerosolized either from a laboratory scale Collison nebulizer (3), or a Venturi-type needle spray atomizer, developed in this laboratory. All cells were spray from Trypticase soy broth (BBL) cultures which had been grown at 37°C for 16 h and then aged at 4°C for at least 24 h. The Collison nebulizer produces an aerosol in which 90% of the particles are <3 μm (MMD), whereas the needle sprayer produces a slightly coarser aerosol, 70% of the particles having a MMD of 3 μm or less. Both values were determined by the use of the MRE impinger (7).

Cultures were sprayed in a wind tunnel (approximately 10 feet [3.048 m] wide by 10 feet high) into a moving stream of air drawn from the outside at a speed of 1 to 2 miles/h. The aerosol was, therefore, not exposed to direct sunlight. Spray time with the Collison nebulizer was 1 min, whereas it was only 15 s with the needle sprayer, since the latter device produces a higher density aerosol. Samplers were placed 35 feet from the point of aerosol generation. The cyclone scrubber and AG1-30 impingers (two were employed) were activated at the commencement of aerosolization and each was operated for 3 min.

All samples were assayed for viable bacterial cells by the standard drop plate technique (8) on plates of Trypticase soy agar. Plates were incubated at 37°C for 24 h.

The efficiency of several fluids for collection of the airborne bacteria was determined. The collecting media investigated were: (i) gelatin-glycerol milk phosphate (GGMP), a medium (gelatin-milk phosphate medium and 40% glycerol) which was devised in this laboratory for the collection of airborne *S. marcescens* under cold weather conditions (gelatin-milk phosphate medium in grams per liter: skim milk [Difco], 10.0; gelatin, 1.0; NaCl, 8.5; spermidine phosphate, 0.02; l-cysteine HCl, 1.0; KH2PO4·3H2O, 3.8, and KH2PO4, 1.2); (ii) gelatin milk (GM), a medium similar to gelatin-milk phosphate medium except for the omission of phosphate; GM was employed both at normal and one-half strengths; (iii) 0.06% Tween 80 in sterile glass distilled water (GDW), as employed by Buchanan et al. (2); (iv) a medium determined to be optimum for the uptake of [32P]orthophosphate by *A. aerogenes* (9); (v) a similar medium devised for *E. coli* (E. coli medium: tris(hydroxymethyl)aminomethane [Calbiochem], 2.5 × 10^-2 M; KCl, 10^-1 M; MgCl₂, 10^-4 M; glucose, 1 mg/ml; and N-2-hydroxyethyl-piperazine-N'-2'-ethanesulfonic acid [Calbiochem], 2.5 × 10^-2 M; adjusted to pH 7.2); and (vi) a medium devised for *S. marcescens* (S. marcescens medium: tris(hydroxymethyl)aminomethane, 5 × 10^-2 M; KCl, 10^-2 M; MgCl₂, 10^-4 M; NH₄Cl, 10^-3 M; and glucose, 1 mg/ml; adjusted to pH 7.5). The same collection fluid was used in all samplers in any one individual experiment.

Aerosol RH and temperature were recorded during each experiment. Trials were conducted using outside air over a wide range of temperatures and relative humidities.

**RESULTS**

Efficiency of the sampler for the collection of inert particles. The cyclone scrubber was shown to be an efficient device for the collection of mono-disperse aerosols of rhodamine B containing particles of 5, 2.3, 1.1, and 0.5 μm in
diameter. As is true with virtually all air sampling devices (1), the cyclone scrubber showed a greater collection efficiency for the larger particles. The efficiency was essentially 100% for particles down to 2.3 µm in diameter and fell off to approximately 70% with particles of 0.5 µm. The results were adjusted to allow for the loss of approximately 20% of the input dye due to the production of satellite particles, which were trapped in an internal filter in the aerosol generator. Studies with bacterial particles of between 0.5 and 1 µm in diameter demonstrated that slippage through the sampler was minimal.

**Efficiency for the collection of particles containing bacteria.** Table 1 presents the results of 92 separate tests by comparison of the recovery of BG spores in the cyclone scrubber with that in the AGI-30. The AGI-30 was selected as the reference sampler for the reasons stated by Buchanan et al. (2). The collection efficiency of the DRES-modified cyclone scrubber for BG spores was determined to be approximately 82%, which is similar to that previously reported (2). The apparent efficiency did not appear to be affected by the collection fluid employed. Since the BG spore is generally not damaged on collection in a liquid medium, no adverse effect due to the collection fluid was expected. No attempt was made to sample mono-disperse aerosols of BG spores produced as reported by Buchanan and his co-workers (2).

The cyclone scrubber has also been found to be an efficient means of collecting airborne vegetative microorganisms. The composition of the collecting fluid, however, is critical. The comparative collection efficiencies for *S. marcescens* are presented in Table 2. Collection efficiency was quite acceptable in all media except one. In the case of 0.06% Tween 80 solution, collection efficiencies could not be determined, since cells were killed in the AGI-30 when this medium was employed. The total number of cells collected in the cyclone scrubber compared favorably with the numbers collected in the other four media, however, indicating that, when employed in this sampler, a 0.06% Tween 80 solution is an adequate collecting medium for *S. marcescens*.

**Table 2. Efficiency of the cyclone scrubber for the collection of aerosolized cells* of S. marcescens***

| Medium           | No. of trials | % Efficiency* |       |       |
|------------------|---------------|---------------|-------|-------|
|                  | X  | SD* | Sample SE* |       |-------|
| 0.5 Gelatin-milk | 8  | 89  | 20.3       | 7.2   |
| Gelatin-milk     | 8  | 77  | 26.8       | 9.5   |
| Glycerol-gelatin milk phosphate | 8 | 70  | 14.8       | 5.2   |
| GDW/Tween 80     | 8  | —   | —          | —     |
| *S. marcescens* medium | 8 | 68  | 14.2       | 5.0   |

*Particles were either 90 or 70% <3-µm MMD, depending on the device used for generation of the aerosol.

* Temperature range, 6 to 20°C; RH range, 31 to 83%.

* As compared to the AGI-30.

* SD, Standard deviation.

* SE, Standard error.

* *S. marcescens* are killed in the AGI-30.

**Table 3. Efficiency of the cyclone scrubber for the collection of aerosolized cells* of *E. coli***

| Medium           | No. of trials | % Efficiency* |       |       |
|------------------|---------------|---------------|-------|-------|
|                  | X  | SD* | Sample SE* |       |-------|
| 0.5 Gelatin-milk | 5  | 79.0 | 5.2       | 2.3   |
| Gelatin-milk     | 5  | 70.8 | 3.7       | 1.7   |
| Glycerol-gelatin milk phosphate | 5 | 45.0 | 17.5     | 7.8   |
| GDW/Tween 80     | 5  | —   | —         | —     |
| *E. coli* medium | 4  | 67.0 | 2.8       | 1.4   |

*Particles were either 90 or 70% <3-µm MMD, depending on the device used for generation of the aerosol.

* Temperature range, 20 to 25°C; RH range, 31 to 48%.

* As compared to the AGI-30.

* SD, Standard deviation.

* SE, Standard error.

* *E. coli* are killed in the cyclone scrubber.

* *E. coli* are killed in the AGI-30.
The results obtained with *E. coli* are presented in Table 3. Collection efficiencies in the GM medium, full or half-strength, and *E. coli* medium were shown to be acceptable. GGMP is not suitable as a collecting fluid since cell death occurred on sampling in the cyclone scrubber. As was the case with *S. marcescens*, *E. coli* was killed in the AGI-30 when 0.06% Tween 80 was used as the collection fluid and, therefore, relative collection efficiency figures could not be calculated.

Table 4 presents the results obtained when aerosols of *A. aerogenes* were collected. This organism was demonstrated to be collected with greatest efficiency in full-strength GM and *A. aerogenes* media, although 0.06% Tween 80/ GDW and 0.5 GM were also quite effective. GGMP was unsuitable since *A. aerogenes* was killed by this fluid on collection in the cyclone scrubber.

**DISCUSSION**

The collection efficiencies in the better fluids were very high, indicating the cyclone scrubber to be an important and reliable tool for the collection of airborne bacterial spores and vegetative cells. Certain anomalies were observed during the study, however. *E. coli* and *A. aerogenes* were killed in the cyclone scrubber in GGMP, whereas *S. marcescens* was not. GGMP differs from GM media only in that the former contains 40% glycerol and 0.025 M phosphate buffer. Since this level of glycerol in either *A. aerogenes* or *E. coli* medium did not reduce collection efficiencies or numbers of viable cells collected from aerosols of *A. aerogenes* or *E. coli*, phosphate was suspected of being responsible for the observed death of the cells. The process of aerosolization, in addition to killing a large number of vegetative cells (4), undoubtedly causes damage, in particular to the cell envelope, which may only be lethal if the cells are placed in an adverse environment on collection. Phosphate, the entry of which would normally be regulated by the phosphate permease (9), may enter through ruptured portions of the membrane of these cells when collected in GGMP, resulting in lethal levels of this anion intracellularly.

In some cases, the standard deviations of collection efficiencies were quite large, but no more so than often observed in aerosol studies. Since it was our intention to evaluate versatility of the sampler for routine use, we deliberately made no attempt to select similar RH and temperature conditions under which to conduct our experiments. Viability of airborne bacteria is well known to be affected by variations in either or both of these parameters (4). We also made no attempt to maintain the bacterial cloud concentration constant between experiments and, in addition, we employed two devices with different spray characteristics. Any, or all, of these factors may have been responsible for the observed standard deviations. These variations might also be reflections of the different collection mechanisms of the two samplers. In the AGI-30, airborne particles are impacted into a constant volume of collecting fluid at a velocity very near that of sound. Particles are collected in the cyclone scrubber primarily by impaction on a moving film of liquid, but the impaction velocity is only approximately one-sixth of the speed of sound which should result in less mechanical damage to the cells. Since the stress effects on aerosolized bacterial cells and the sizes of bacteria-containing airborne particles vary with RH and temperature, it is not unreasonable to assume that the efficiencies of the two types of samplers could vary considerably. At a certain temperature and RH, one sampler might collect a higher percentage of the bacteria in a viable state than the other, or might have a higher collection efficiency for either the larger or smaller particles. In the relatively warm and dry conditions of summer daylight, May (7) has shown that the survival of *E. coli* is directly related to particle size.

With a concentration effect of 950,000 to 1 and the demonstrated high efficiency for collection of particles, both inert and biological, in the 1- to 5-μM MMD size range, the sampler described here and others similar to it (2)

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**Table 4. Efficiency of the cyclone scrubber for the collection of aerosolized cells* of *A. aerogenes**

| Medium              | No. of trials | % Efficiency | X   | SD* | Sample SE  |
|---------------------|---------------|--------------|-----|-----|------------|
| 0.5 Gelatin-milk    | 5             | 60.8         | 13.9| 6.2 |            |
| Gelatin-milk        | 5             | 79.0         | 10.2| 4.6 |            |
| Glycerol-gelatin    | 5             | 33.2  | 8.0 | 3.6 |            |
| phosphate           |               |              |     |     |            |
| GDW/Tween 80        | 3             | 69.7         | 13.3| 7.7 |            |
| *A. aerogenes* medium | 5          | 87.4         | 30.6| 13.7|            |

* Particles were either 90 or 70% <3-μM MMD, depending on the device used for generation of the aerosol.

* Temperature range, 24 to 33 C; RH range, 18 to 44%.

* As compared to the AGI-30.

* SD, Standard deviation.

* SE, Standard error.

* *A. aerogenes* are killed in the cyclone scrubber.
should have a wide practical application. For example, it should be useful in air pollution studies and in studies on factors affecting the aerosol transmission of respiratory disease. The importance of aerobiology to medical research on respiratory infections, disease pathogenesis, and host immune response has been recently discussed by Larson (6).

There is little doubt that data obtained using nonpathogenic vegetative cells bear more relation to the aerosol stability of respiratory pathogens than does data obtained with BG spores, which are highly resistant to death during aerosol generation, airborne travel, and collection. In contrast, aerosolized vegetative cells are much more susceptible to death due to drying, irradiation, or oxidation, as well as to stress induced by the collection method employed (4).

Our results clearly show that BG spores were collected with virtually the same efficiency in all seven collecting fluids examined, whereas with the vegetative cells considerable differences were noted between the collecting fluids. All fluids used, with the exception of the Tween 80 solution, were selected because they have proven to be effective for the collection of aerosolized vegetative cells in samplers such as the AGI-30. Thus, the successful collection of airborne vegetative cells in the cyclone scrubber is dependent on the correct collecting fluid. This becomes even more important when pathogenic bacteria are to be collected, since many of the same factors which are required for growth in artificial media, because of their fastidious nature, may also be required to prevent death on collection in the cyclone scrubber. For example, a complex chemically defined medium, NCDM (6), was found to be necessary for the optimum collection of \textit{Neisseria meningitidis} from a naturally occurring aerosol (J. F. Currie and D. C. O’Connell, Can. J. Public Health, 65:52, 1974).

The DRES-modified large-volume air sampler is simple in construction and employs readily available components. It can be easily operated by a semiskilled individual. Once the instrument is in operation, the only attention required is to collect samples, change syringes, and readjust the “make up” water supply (Fig. 1, E) as temperature and relative humidity change. This latter procedure need not be carried out more often than changing syringes.

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