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DYNAMIC SIZE SPECTROMETRY OF AIRBORNE MICROORGANISMS: LABORATORY EVALUATION AND CALIBRATION

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Abstract—Bioaerosol samplers need to be calibrated for the microorganisms of interest. The Aerosizer, a relatively new aerodynamic size spectrometer, is shown to be a suitable dynamic instrument for the evaluation and calibration of such samplers in the laboratory, prior to their use in the field. It provides the necessary reference count against which the microbiological response of the sampler can be compared. It measures the health-significant aerodynamic diameters of microorganisms down to 0.5 μm, thus including most of the bacteria, fungi and pollen found in outdoor and indoor air environments. Comparison tests with a laser size spectrometer indicate that the suspension of microorganisms needs to be washed several times before aerosolization to avoid coating of the airborne microorganisms with nutrients and microbial slime from the suspension, and to reduce the residue particles to sizes below the lowest size of the aerosolized microorganisms.

Key word index: Bioaerosol, bacteria, microorganism, aerodynamic diameter, sampler calibration.

INTRODUCTION

In recent years, the measurement of airborne microorganisms has gained increasing importance because of the many bioaerosols in outdoor and indoor environments that have been found to cause adverse health effects. In ambient environments, airborne microorganisms such as ragweed pollen may cause allergy in humans (Solomon *et al.*, 1983). In agricultural environments, airborne microorganisms from both plants and animals may cause health impairment. For example, airborne spores of thermophilic actinomycete may cause farmer's lung disease (Davies, 1968). In the construction field, demolition of old buildings may release into the surrounding air high and therefore potentially hazardous concentrations of molds and fungi. In indoor environments, such as homes and air-conditioned offices, some airborne microorganisms have had significant health impacts on the occupants (Morey *et al.*, 1986; Kodama and McGee, 1986; Morey *et al.*, 1990). The occurrence of pathogenic airborne bacteria in hospitals, schools and hotels has caused diseases, such as tuberculosis, measles and legionellosis (Riley *et al.*, 1962, 1978; Fraser, 1980; Snider and Roper, 1992).

Common methods for sampling airborne microorganisms employ either collection onto a filter, impaction onto an agar surface, or impingement into a liquid. Filter collectors differ by the size of the filter, the filter material used and the sampling flow rate through the filter (Lippmann, 1989; Lee and Ramamurthi, 1993). An impactor collects the airborne microorganisms on one or more nutrient surfaces (Andersen, 1958; Nevalainen *et al.*, 1992, 1993). Examples of commercially available bioaerosol impactors are the Andersen six-stage viable particle sizing sampler (Graseby-Andersen Samplers Inc., Atlanta, GA), and the Mattson–Garvin slit to-agar air sampler (Barramundi Corp., Homosassa Springs, FL). An impinger impacts the airborne microorganisms into a liquid (May and Harper, 1957; Nevalainen *et al.*, 1992; Juozaitis *et al.*, 1994). Examples of widely used impingers are the AGI-4 and AGI-30 (Ace Glass Inc., Vineland, NJ).

In reporting the data obtained with any of these instruments, the sampling device needs to be specified, as each device covers a different particle size range and has different physical and biological collection efficiencies (Delmore and Thompson, 1981; Kang and Frank, 1989a–c; Jensen *et al.*, 1992). As each bioaerosol sampler inlet is different in size and shape, the inlet efficiencies of these samplers have different wind and particle size sensitivities (Brockmann, 1993; Grinshpun *et al.*, 1994). Similarly, impactors and impingers

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differ by the geometry and flow characteristics in the impaction region, so that each device has its own unique cut-size below which less than 50% of the microorganisms are collected (Nevalainen *et al.*, 1992, 1993; Willeke *et al.*, 1993; Juozaitis *et al.*, 1994). In impactors, the microorganisms may be injured during the sampling process, e.g. by desiccation (Thompson *et al.*, 1994). Damaged or injured microorganisms may not grow on the selected collection medium (Burge *et al.*, 1977; Burge and Solomon, 1987). During incubation, the colonies formed from the microorganisms collected on the agar surface may overlap each other, lending to an underestimation of the bioaerosol concentration (Chang *et al.*, 1994).

As all of these parameters may affect the precision and accuracy of the reported bioaerosol concentration, each device needs to be calibrated for the airborne microorganisms it collects. This study was undertaken to find a suitable method for dynamically calibrating bioaerosol samplers. A new aerodynamic size spectrometer was found to be particularly suitable for dynamically measuring the concentration of airborne microorganisms over a wide particle size range. By generating test microorganisms into a particle-free air flow, the biologically detected aerosol concentration of the bioaerosol sampler being tested is compared to the size-spectrometer-measured concentration of airborne microorganisms upstream of the test sampler.

EXPERIMENTAL MATERIALS AND METHODS

In this section, the new laboratory setup for evaluating and calibrating bioaerosol samplers will be described in detail. Additional methods used to confirm and validate the new technique will also be highlighted. Finally, we will explain the microbiological procedures used in the evaluations.

Experimental setup

Figure 1 shows the experimental setup. A Collison nebulizer with three nozzles (BGI, Inc., Waltham, MA) was used to nebulize a suspension of cultured and washed microorganisms or of standard calibration particles. Since the aerosol concentration from this nebulizer is well above the coincidence limit of the particle size spectrometers used, about $2\,\ell\,min^{-1}$ of the effluent flow from the nebulizer, $Q_{neb} = 5\,\ell\,min^{-1}$, were discharged, and the remaining $3\,\ell\,min^{-1}$ were diluted by dry dilution air, Q_{dil}, of about $45\,\ell\,min^{-1}$, resulting in a test flow rate, Q_{test}, of about $48\,\ell\,min^{-1}$. Dilution of the aerosol from the nebulizer with dry air also avoids agglomeration of the droplets and drives off the water content. Thus, milliseconds before the aerosol enters the test samplers the aerosol contains a mixture of dry microorganisms or particles and of much smaller residue particles, resulting from droplets not containing either a microorganism or a test particle. The test aerosol passes through an electrical charge neutralizer with a 10 mCi Kr-85 radiative source (TSI, Inc., St. Paul, MN) to reduce the electrical charge on the nebulized aerosol to Boltzmann charge equilibrium (Wen *et al.*, 1984).

The test aerosol enters an open cup from which aerosol is withdrawn at a sample flow rate, Q_{sample}, of $5\,\ell\,min^{-1}$. To avoid release of microorganisms to the laboratory, all components are contained in a biological safety cabinet that is vented to the outside after treatment (Model 6TX, Baker Co., Inc., Sanford, ME). The temperature and relative humidity at the sample location were kept constant at 20°C and 30%, respectively.

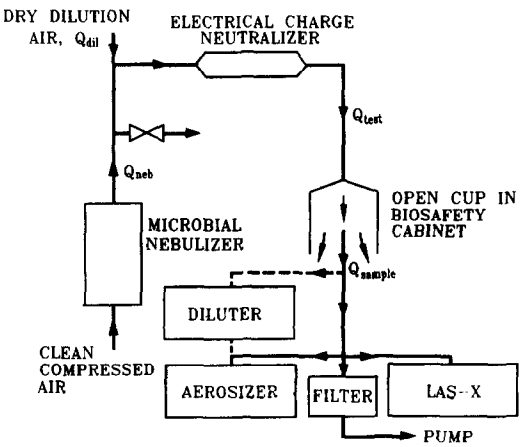

Fig. 1. Schematic diagram of experimental test setup.

The Aerosizer, a relatively new aerodynamic particle size spectrometer (Amherst Process Instruments Inc., Hadley, MA), was found to be most suitable for evaluating bioaerosol samplers with airborne microorganisms larger than 0.5 μm. The principle of the Aerosizer is based on the acceleration of particles in a sonic expansion flow: small particles are aerodynamically accelerated to greater velocities than large particles. The particle velocity, measured by two laser beams, is thus an indicator of the particle's aerodynamic size. Although this device sizes spherical particles down to 0.3 μm, it should be used for reliable size and concentration information—in its present configuration—only for particles 0.5 μm in diameter or larger (Cheng *et al.*, 1993a). As the instrument's upper size limit is about 200 μm, it covers the size range of single-cell bacteria (0.5–30 μm), fungi (about 0.5–30 μm) and pollen (about 10–100 μm) (Nevalainen *et al.*, 1993). In our experiments only bacteria of sizes near the Aerosizer's lower limit of detection were tested. Other instruments, such as the Aerodynamic Particle Sizer (APS, TSI Inc., St. Paul, MN), the Laser Aerosol Spectrometer (LAS-X, Particle Measuring Systems Inc., Boulder, CO) and inertial impactors were found to have less desirable characteristics than the Aerosizer. Since the APS reliably measures particles only above 0.8 μm, and the mean size of many bacteria is between 0.8 and 1.0 μm, the APS can only be used for larger airborne microorganisms. The LAS-X size-discriminates down to about 0.1 μm, but it records the data in terms of optical equivalent diameters, which—as shown later—need to be calibrated for each microorganism to relate them to their health-significant aerodynamic diameters. Inertial cascade impactors measure the concentrations within specific ranges of aerodynamic diameter; but they are not dynamic and have low particle-size discrimination, since each stage covers a relatively wide range of particle sizes (Marple *et al.*, 1993).

Prior to using the Aerosizer with microorganisms, the instrument's factory calibration was checked with monodisperse polystyrene latex (PSL) test spheres ranging from about 0.3 to 3.0 μm in size (Bangs Laboratories, Inc., Carmel, IN). The suspension of PSL particles was deagglomerated for 5 min in an ultrasonic bath (Model 220, Branson Cleaning Equipment Co., Shelton, CT).

In order to test the Aerosizer's linearity in response to different concentration levels of airborne microorganisms,

a diluter (Model 3302, TSI, Inc., St. Paul, MN) was inserted ahead of the Aerosizer, see Fig. 1. The dilution ratio, R, of the aerosol flow, $Q_{aerosol}$, is the ratio of the flow through the diluter's filter, $Q_{dil.filter}$, to the sum of $Q_{aerosol}$ and $Q_{dil.filter}$.

Sampling with the polycarbonate filter required a minimum of 5 min for a sufficiently high bacteria count. After sampling, the bacteria on the polycarbonate filter were washed into a test tube containing 5 ml of deionized water. The tube with the filter was vortexed for 30 s to ensure maximum detachment of the microorganisms from the filter. The suspension was then transferred from the tube to a brightline hemocytometer with a 0.1 mm deep chamber (Hausser Scientific Partnership, Horsham, PA). The bacterial number in the chamber was counted using a phase contrast microscope (Labophot-2, Nikon Co., Tokyo, Japan). In order to get good statistical results, about 400 bacteria were counted in each sample (Jones and Simon, 1975). Since some microorganisms did not detach from the filter during vortexing, the filter was removed from the test tube, placed onto a tryptic soy agar plate and incubated for 18 h to allow the remaining bacteria to grow. The bacterial colony count on the filter was added to the bacterial count from the suspension. It was generally less than about 5% of the total bacterial count.

An LAS-X laser aerosol spectrometer, measuring over an optical particle diameter range 0.1–3.0 μm, was used in parallel with the Aerosizer to give information on the residue particles resulting from the residual nutrients, salt and bacterial slime in dried droplets not containing bacteria. As seen in Fig. 1, some of the sampled airborne microorganisms were also collected onto a filter for comparison of the dynamic size-spectrometric measurements with conventional enumerations from the filter. A standard 25 mm in-line filter holder (Gelman Sciences, Inc., Ann Arbor, MI) with a 0.1 μm pore size polycarbonate filter (Nuclepore Co., Pleasanton, CA) was connected to a common sampling port for all three samplers. Q_{sample} was always kept constant to avoid potential biases in the sampling line.

Most bacteria are rod shaped or spheroids in chains. As they are accelerated in the near sonic expansion flow in the Aerosizer (Cheng *et al.*, 1993a), the particle shape and density may affect the accuracy of its aerodynamic diameter measurement, as has also been noted for the APS aerodynamic size spectrometer (Baron, 1986; Brockmann and Rader, 1990; Cheng *et al.*, 1993b). In order to investigate the Aerosizer's response to non-spherical bacteria, the sixth stage of a six-stage Marple Cascade Impactor (Model 266, Sierra Instruments Inc., Carmel Valley, CA) with a 1.0 mm nozzle diameter was used to inertially calibrate the LAS-X to compare the bacterial aerodynamic sizes measured by both instruments. The surface of the impaction substrate was coated with a thin layer of petroleum jelly to avoid particle bounce, and the impaction stage was operated at flow rates ranging from 1 to 10 $\ell\,min^{-1}$, corresponding to aerodynamic cut sizes of 2.1–0.64 μm. The LAS-X measured optical size, for which the aerosol concentration is reduced to 50% of the concentration without the impaction stage, corresponds to the cut-size of the impaction stage (Marple *et al.*, 1993). *Pseudomonas fluorescens* bacteria were used for this test. As shown later, these bacteria can be clearly differentiated from the residue particles if the bacterial suspension is washed six times.

To obtain statistically meaningful results, the experiments were repeated three times, and the data have been presented as averages of these. The standard deviations are indicated in the figures wherever they are significant.

Microbial preparation

To evaluate our new method, *Pseudomonas fluorescens* ATCC 13525 (American Type Culture Collection Inc., Rockville, MD) was selected as our model bacterium because it is non-pathogenic and is a typical rod-shaped airborne bacterium (Nevalainen, 1989). It appears as single-cells, i.e. it does not clump (Breed *et al.*, 1957; Luria, 1960; Palleroni, 1984).

This bacterium was prepared as follows: *Pseudomonas fluorescens* was streaked onto tryptic soy agar plates (Difco Laboratories, Detroit, MI), which were then incubated for 18 h at 25°C. The cells were then removed from the plates and washed in a centrifuge at 2860 g (Marathon 6K, Fisher Scientific, Pittsburgh, PA). As many as six washings were performed to reduce the amount of residue in the liquid suspension. The number of washings and the time of washing are expected to affect the bacterial survival. This effect, however, has not been tested because the total (but not viable) bacterial concentration was of interest in this study. The number of washings was found to affect the bacterial size (the results are discussed below). To see the effect of residue removal on the response of the size spectrometers tested, each bacterial suspension was brought to the same level of absorbance (about 1.21 at a wavelength of 600 nm), as measured by a spectrophotometer (Spectronic 21D, Milton Roy Co., Rochester, NY).

The reported size of *Pseudomonas fluorescens* varies among authors: Breed *et al.* (1957) report the cell to be 0.3–0.5 μm in diameter and 1.0–1.5 μm in length, while Palleroni (1984) reports the cell to be 0.7–0.8 μm in diameter and 1.5–3.0 μm in length. The small, but significant differences in reported size for a particular bacterium may be due to differences in the measurement methods used, e.g. light-microscopy versus electron-microscopy. In addition, the microbial size may depend on the microbial growth stage during which the measurement is performed (Rogers, 1983). We used Scanning Electron Microscopy (SEM) (Hitachi S-570, Hitachi Co., Tokyo, Japan) to check the shape and size of the *Pseudomonas fluorescens* used in our experiments. Two types of bacterial collection were analyzed: One from the liquid suspension placed onto a glass slide, and the other from the polycarbonate filter that sampled from the aerosolized bacteria. Each sample was dried and coated with gold/palladium in a coater sputter (Desk II, Denton Vacuum Co., Cherry Hill, NJ), and was observed and photographed by SEM. The SEM micrographs showed that the *Pseudomonas fluorescens* used was rod-shaped, 0.4–0.7 μm in width and 1.5–2.0 μm in length, i.e. of width and length between those of the quoted references.

In addition to our model bacterium, several other bacteria of relatively small size, such as 0.7–0.9 μm wide and 3–4 μm long *Bacillus alcalophilus* ATCC 21522, and 1.2–1.5 μm wide and 2–5 μm long *Bacillus megatherium* ATCC 14581, and a coccus, *Streptococcus salivarius* ATCC 13419, with a diameter of 0.8–1.0 μm (exists in irregular chains) were aerosolized from suspensions that had been washed three times.

RESULTS AND DISCUSSION

Figure 2 shows the particle size distribution measured by the LAS-X laser aerosol spectrometer for several aerosolized suspensions of *Pseudomonas fluorescens* bacteria. The number concentration, N, recorded for each of the instrument's channels, is divided by the logarithmic interval of the corresponding size range, and is plotted as a function of the optical equivalent diameter, d_{opt}. Each data point on the logarithmic size scale represents the geometric mean of the corresponding size range.

When the suspension of *Pseudomonas fluorescens* bacteria is aerosolized without any removal of the nutrients or slime and capsular material from the bacteria, i.e. the number of washings is zero, the data

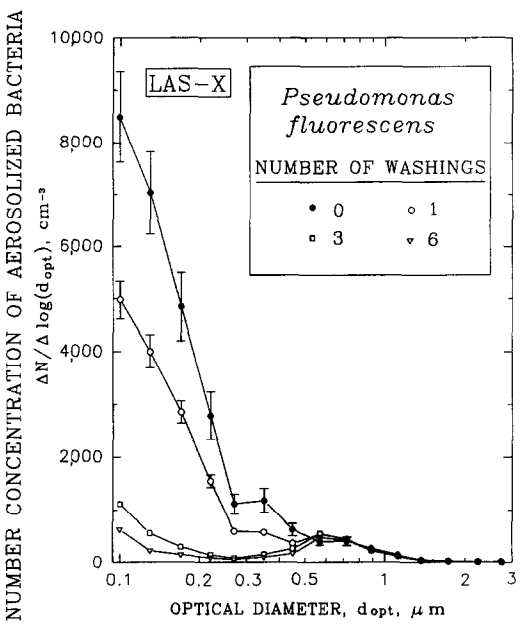

Fig. 2. Effect of repeated washings of the bacterial suspension on the concentration of residues measured by the LAS-X optical size spectrometer.

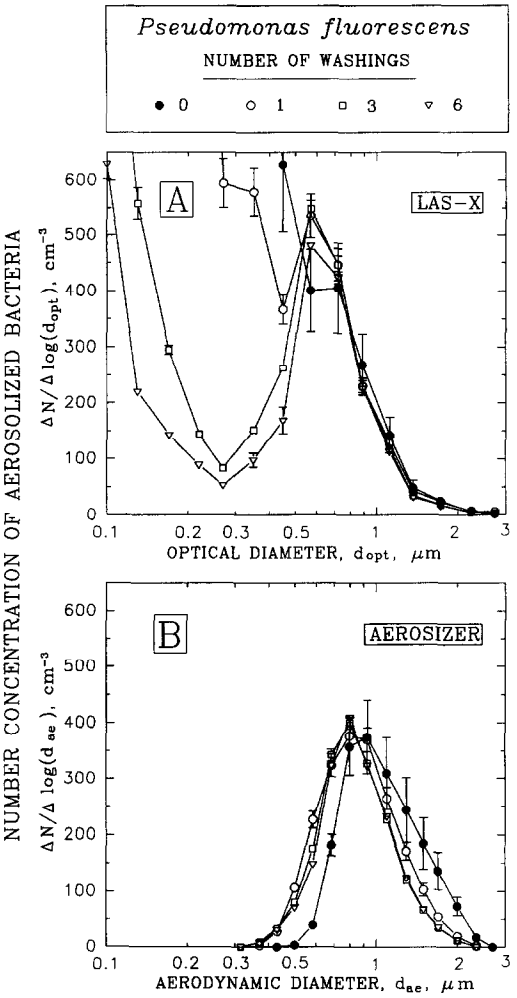

Fig. 3. Size distributions of aerosolized bacterial suspensions of *Pseudomonas fluorescens*, as measured by the (A) LAS-X optical size spectrometer, (B) Aerosizer aerodynamic size spectrometer.

of Fig. 2 show a very high concentration of residue particles, and no bacterial peak. As the number of washings increases, the concentration of residue particles decreases and a bacterial peak becomes clearly recognizable at the optical equivalent diameter of about 0.6 μm. After six washings, the bacteria are clearly distinguishable from the residues. Figure 2 also shows that the standard deviation is high for the residue particles before any washing is performed, and that it decreases with the number of washings performed. The high standard deviation of the residue concentration, aerosolized from the original suspension, appears to be due to the ever-changing content of bacterial slime in the suspension, which depends on factors such as the microbial species, its growth stage and its nutrition supply. SEM micrographs of the filter collection from an aerosolized bacterial suspension that had been washed three times showed that the residue particles were 0.25 μm or smaller in size and that they were clearly differentiated in size from the 0.4–0.7 μm wide, 1.5–2.0 μm long *Pseudomonas fluorescens* bacteria.

The bacterial peak, measured by the LAS-X optical size spectrometer, is enlarged over ten times in Fig. 3A. For a *Pseudomonas fluorescens* suspension that had been washed six times, it shows a peak concentration of about 480 bacteria per m^3 at an optical diameter, d_{opt}, of about 0.6 μm with a geometric standard deviation of about 1.40.

Figure 3B shows the same aerosolized suspensions measured by the Aerosizer as a function of aerodynamic diameter, d_{ae}. Prior to measurements with microorganisms, the Aerosizer was evaluated with monodisperse PSL particles which the instrument was found to resolve well, as also found by other investigators (Cheng *et al.*, 1993a). Since the bacterial peak size measured by the Aerosizer is larger than 0.5 μm and the Aerosizer measures concentrations properly for particles larger than about 0.5 μm (Cheng *et al.*, 1993a), we believe the Aerosizer-measured peaks in Fig. 3B to be true peaks, not the thresholds for the lower limit of detection. As seen in Fig. 3B, the Aerosizer-measured peak concentration for *Pseudomonas fluorescens* after six washings is 430 bacteria per m^3 at an aerodynamic diameter of about 0.78 μm with a geometric standard deviation about 1.35. Since the measured geometric standard deviation of the Aerosizer data is somewhat smaller than that of the LAS-X optical size spectrometer, the peak concentration measured by the Aerosizer should have been somewhat higher, not lower than that of the LAS-X. We have not yet been able to resolve this discrepancy. Some of the smaller bacteria at or below 0.5 μm may

have been counted with decreased efficiency leading to a narrowing of the distribution by the Aerosizer, and some residue particles may have been counted as bacteria by the LAS-X due to the partial overlap of the optical size distribution for the bacteria with the one for the residue particles. Since the two instruments operate on different principles, we accept the difference, at this time, as being within the limits of experimental and instrumental variability.

Figure 3B also shows that the physical size of the aerosolized bacteria decreases after the first washing and a little more after additional washings. We conclude that a significant amount of nutrients and bacterial slime coat the bacteria if not washed. The procedure and degree of bacterial washing should, therefore, be specified for calibration purposes.

As residue material is removed from the bacteria, their shape may change and, thus, the width of the measured size distribution. At this time, it is not known whether elongated particles are randomly distributed in the sensing zone of the Aerosizer, as was observed with the functionally similar APS aerodynamic size spectrometer (Brockmann and Rader, 1990; Cheng *et al.*, 1993b). As seen in Fig. 3B, the size distribution appears to narrow somewhat, as its peak shifts to a smaller size with increased washings. Such a narrowing may also occur upon approaching the lower limit of detection.

Measurements with the Marple-cascade-impactor-calibrated LAS-X of the aerosolized *Pseudomonas fluorescens* suspension after six washings indicated an average aerodynamic size of about 0.7 ± 0.1 μm. Starting with the measured average width of 0.55 μm and length of 1.75 μm for the *Pseudomonas fluorescens* bacteria, we have calculated the aerodynamic diameter (Baron and Willeke, 1993) for a density of $1.16\,g\,cm^{-3}$ (Nevalainen *et al.*, 1993) and different shape factors depending on the bacteria's orientation (Fuchs, 1989). The calculated aerodynamic diameter is thus 0.76 μm for bacteria aligned perpendicular to the flow, 0.84 μm for bacteria in parallel alignment and 0.78 μm for bacteria in an orientation that is the statistical average of the two. These calculations are approximately within the range of the inertially calibrated LAS-X size of 0.7 ± 0.1 μm. The small differences between the actual bacterial sizes and the calculated ones may be attributed to the assumption in the theoretical calculations that the average bacterial size represents the entire bacterial size range 0.4–0.7 μm in width and 1.5–2.0 μm in length.

The Aerosizer-measured mean size of 0.78 μm is somewhat larger than the LAS-X measured average aerodynamic size, but it is within the LAS-X measured size range of 0.6–0.8 μm. The larger size measured by the Aerosizer may, in part, be due to the following three factors: the reduced pressure in the Aerosizer may lead to an overestimation of submicrometer particle sizes; the non-spherical shape of the bacteria may lead to different orientations and thus drag forces in the sensing zone; and the difference in density between the *Pseudomonas fluorescens* bacteria and the unit-density PSL particles used for calibration may affect the measurement of aerodynamic equivalence (Cheng *et al.*, 1993a). Also Fig. 3A shows that some residue particles overlap with the bacteria even after six washings. While this does not show up on the Aerosizer data, Fig. 3B, because the residue particles are of a size smaller than or close to the Aerosizer's limit of detection, they may nevertheless affect the Aerosizer and impactor calibrated LAS-X measurements by a small amount.

The total bacterial concentrations measured by the Aerosizer were about 13% less than those measured by the LAS-X in the optical size range of 0.3–3 μm for bacterial suspensions that had been washed six times. This small difference may be due to remaining residue particles that still existed in this optical size range after six washings and were detected by the LAS-X but not the Aerosizer. The bacterial concentrations, measured by the Aerosizer, were also compared to the bacterial concentrations enumerated from simultaneous collections on the polycarbonate filters. The filter count was slightly less than that of the Aerosizer count, but never by more than 10%.

Figure 4 shows how the Aerosizer measured bacterial concentration decreases with increasing dilution of the aerosol flow, when a diluter with a dilution filter is inserted upstream of the Aerosizer. As seen, the concentration decreases linearly with dilution ratio, R, as expected, even for *Pseudomonas fluorescens* bacteria below 0.5 μm. While the Aerosizer's absolute counting efficiency decreases below 0.5 μm, this result shows that it can be used for relative concentration measurements down to 0.3 μm.

The aerodynamic particle size distributions of *Bacillus alcalophilus* and *Bacillus megatherium* are similar in shape to that of *Pseudomonas fluorescens*. The peak size of *Bacillus alcalophilus* was measured to be

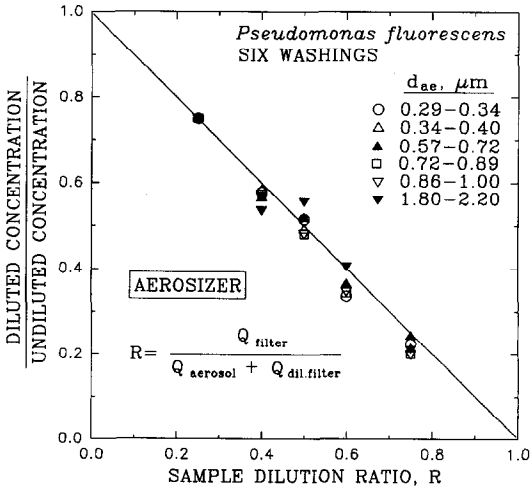

Fig. 4. The Aerosizer's linearity of concentration measurement with increasing clean air dilution.

0.70 μm with a geometric standard deviation of about 1.56. The peak size of *Bacillus megatherium* was 1.2 μm with a geometric standard deviation of about 1.38. Nebulization of *Streptococcus salivarius* was found to not always disagglomerate the chain into single cells which complicates the interpretation of results from such experiments.

CONCLUSION

This study has shown that the Aerosizer aerodynamic size spectrometer can be used to dynamically size microorganisms exceeding 0.5 μm in size. The instrument is ideally suited for calibrating or evaluating bioaerosol samplers in the laboratory, in preparation for their use in the field.

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REFERENCES

Andersen A. A. (1958) New sampler for the collection, sizing and enumeration of viable airborne particles. *J. Bacteriol.* **76,** 471–484.

Baron P. A. (1986) Calibration and use of the Aerodynamic Particle Sizer (APS 3300). *Aerosol Sci. Technol.* **5,** 55–67.

Baron P. A. and Willeke K. (1993) Gas and particle motion. In *Aerosol Measurement: Principles, Techniques, and Applications* (edited by Willeke K. and Baron P. A.), pp. 23–40. Van Nostrand Reinhold, New York.

Breed R. S., Murray E. G. D. and Smith N. R. (eds.) (1957) *Bergey's Manual of Determinative Bacteriology*, 7th ed., pp. 89–152. The Williams and Wilkins Co., Baltimore, MD.

Brockmann J. E. (1993) Sampling and transport of aerosols. In *Aerosol Measurement: Principles, Techniques and Applications* (edited by Willeke K. and Baron P. A.), pp. 77–111. Van Nostrand Reinhold, New York.

Brockmann J. E. and Rader D. J. (1990) APS response to nonspherical particles and experimental determination of dynamic shape factor. *Aerosol Sci. Technol.* **13,** 162–172.

Burge H. A., Boise J. R., Rutherford J. A. and Solomon W. R. (1977) Comparative recoveries of airborne fungus spores by viable and non-viable modes of volumetric collection. *Mycopathologia.* **61,** 27–33.

Burge H. A. and Solomon W. R. (1987) Sampling and analysis of biological aerosols. *Atmospheric Environment* **21,** 451–456.

Chang C. W., Hwang Y. H., Grinshpun S. A., Macher J. A. and Willeke K. (1994) Evaluation of counting error due to colony masking in bioaerosol sampling. *Appl. Envir. Microbiol.* **60,** 3732–3738.

Cheng Y. S., Barr E. B., Marshall I. A. and Mitchell J. P. (1993a) Calibration and performance of an API Aerosizer. *J. Aerosol Sci.* **24,** 501–514.

Cheng Y. S., Chen B. T. and Yeh H. C. (1993b) Behavior of compact nonspherical particles in the TSI Aerodynamic Particle Sizer model APS33B: ultra-Stokesian drag forces. *Aerosol Sci. Technol.* **19,** 255–267.

Davies C. N. (1968) Farmer's lung. *Ann. Occup. Hyg.* **11,** 377–378.

Delmore R. P. and Thompson W. N. (1981) A comparison of air sampler efficiencies. *Med. Device Diag. Ind.* **3,** 45–48.

Fraser D. W. (1980) Legionellosis: evidence of airborne transmission. *Ann. New York Acad. Sci.* **353,** 61–66.

Fuchs N. A. (1989) *The Mechanics of Aerosols*, pp. 37–46. Dover, New York

Grinshpun S. A., Chang C. W., Nevalainen A. and Willeke K. (1994) Inlet characteristics of bioaerosol samplers. *J. Aerosol Sci.* **25,** 1503–1522.

Jensen P. A., Todd W. F., Davis G. N. and Scarpino P. V. (1992) Evaluation of eight bioaerosol samplers challanged with aerosols of free bacteria. *Am. Ind. Hyd. Assoc. J.* **53,** 660–667.

Jones J. G. and Simon B. M. (1975) An investigation of errors in direct counts of aquatic bacteria by epifluorescence microscopy, with reference to a new method for using membrane filters. *J. Appl. Bacteriol.* **39,** 317–329.

Juozaitis A., Willeke K., Grinshpun S. A. and Donnelly J. (1994) Impaction onto a glass slide or agar versus impingement into a liquid for the collection and recovery of airborne microorganisms. *Appl. Envir. Microbiology* **60,** 861–870.

Kang Y. J. and Frank J. F. (1989a) Evaluation of air samplers for recovery of artificially generated aerosols of pure cultures in a controlled environment. *J. Food Protection* **52,** 560–563.

Kang Y. J. and Frank J. F. (1989b) Evaluation of samplers for recovery of biological aerosols in diary processing plants. *J. Food Protection* **52,** 655–659.

Kang Y. J. and Frank J. F. (1989c) Comparison of airborne microflora collected by the Andersen sieve sampler and RCS sampler in a dairy processing plant. *J. Food Protection* **52,** 877–880.

Kodama A. M. and McGee R. I. (1986) Airborne microbial contaminants in indoor environments. Naturally ventilated and air-conditioned homes. *Archives Envir. Health* **41,** 306–311.

Lee K. W. and Ramamurthi M. (1993) Filter collection. In *Aerosol Measurement: Principles, Techniques and Applications* (edited by Willeke K. and Baron P. A.), pp. 179–205. Van Nostrand Reinhold, New York.

Lippmann M. (1989) Sampling aerosols by filtration. In *Air Sampling Instruments for Evaluation of Atmospheric Contaminants*, 7th ed. (edited by Hering S. V.), pp. 305–336. American Conference of Governmental Industrial Hygienists, Inc. Cincinnati, OH.

Luria S. E. (1960) The bacterial protoplasm: composition and organization. In *The Bacteria, a Treatise on Structure and Function* (edited by Gunsalus I. C. and Stainer R. Y.), pp. 1–34. Academic Press, New York.

Marple V. A., Rubow K. L. and Olson B. A. (1993) Inertial, gravitational, centrifugal, and thermal collection techniques. In *Aerosol Measurement: Principles, Techniques and Applications* (edited by Willeke K. and Baron P. A.), pp. 206–232. Van Nostrand Reinhold, New York.

May K. R. and Harper G. J. (1957) The efficiency of various liquid impinger samplers in bacterial aerosols. *Brit. J. Ind. Med.* **14,** 287–297.

Morey O., Chatigny M., Otten J., Feeley J., Burge H., LaForce F. M. and Peterson K. (1986) Bioaerosol. Airborne viable microorganisms in office environments: sampling protocols and analytical procedures. *Ann. Ind. Hyg.* **1,** R19–R23.

Morey P. R., Feeley J. C. and Otten J. A. (eds.) (1990) *Biological Contaminants in Indoor Environments*, pp. 136–162. American Society for Testing and Materials, Philadelphia, PA.

Nevalainen A. (1989) Bacterial aerosols in indoor air. Ph.D. dissertation, pp. 61–62, University of Kuopio, Finland.

Nevalainen A., Pastuszka J., Leibhader F. and Willeke K. (1992) Performance of bioaerosol samplers: collection characteristics and sampler design considerations. *Atmospheric Environment* **26A**, 531–540.

Nevalainen A., Willeke K., Leibhaber F., Pastuszuka J., Burge H. and Henningson E. (1993) Bioaerosol sampling. In *Aerosol Measurement: Principles, Techniques, and Applications* (edited by Willeke K. and Baron P. A.), pp. 471–492. Van Nostrand Reinhold, New York.

Palleroni N. J. (1984) Family I. *Pseudomonadaceae.* In *Bergey's Manual of Systematic Bacteriology* (edited by Kreig N. R. and Holt J. G.), Vol. 1, p. 165. The Williams and Wilkins Co., Baltimore, MD.

Riley R. L., Mills C. C., O'Drady F. O., Sultan L. U., Wittestadt F. and Shivpuri D. N. (1962) Infectiousness of air from a tuberculosis ward. Ultraviolet irradiation of infected air: comparative infectiousness of different patients. *Am. Rev. Respir. Dis.* **84**, 511–525.

Riley E. C., Murphy G. and Riley R. L. (1978) Airborne spread of measles in a suburban elementary school. *Am. J. Epidemiol.* **107**, 421–432.

Rogers H. J. (1983) Bacterial morphology. In *Topley and Wilson's Principles of Bacteriology, Virology and Immunity* (edited by Wilson G., Miles A. and Parker M. T.), Vol. 1, p. 16. The Williams and Wilkins Co., Baltimore, MD.

Snider D. E. and Roper W. L. (1992) The new tuberculosis. *N. Engl. J. Med.* **326**, 703–705.

Solomon W. R., Burge H. A. and Muilenberg M. L. (1983) Allergen carriage by atmospheric aerosol—I. Ragweed pollen determinants in smaller micronic fractions. *J. Allergy Clin. Immunol.* **72**, 443–447.

Thompson M. W., Donnelly J., Grinshpun S. A., Juozaitis A. and Willeke K. (1994) Method and test system for evaluation of bioaerosol samplers. *J. Aerosol Sci.* **25**, 1579–1593.

Wen H. Y., Reischl G. P. and Kasper G. (1984) Bipolar diffusion charging of fibrous aerosol particles—I. Charging theory. *J. Aerosol Sci.* **15**, 89–101.

Willeke K., Grinshpun S. A., Donnelly J., Juozaitis A., Thompson M., Chang C. W., Liebhaber F. and Nevalainen A. (1993) Physical and biological sampling efficiencies of bioaerosol samplers. In *Indoor Air '93—Indoor Air Quality and Climate* (edited by Kalliokoski, P., Jantunen M. and Seppänen O.), Vol. 4, pp. 131–136, Gummerus Oy, Jyväskylä, Finland.