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Collection of Airborne Microorganisms by Electrostatic Precipitation

G. Mainelis, S. A. Grinshpun[*]*, K. Willeke, T. Reponen, V. Ulevicius*[†]*, and P. J. Hintz*

AEROSOL RESEARCH AND EXPOSURE ASSESSMENT LABORATORY, DEPARTMENT OF ENVIRONMENTAL HEALTH, UNIVERSITY OF CINCINNATI, P.O. BOX 670056, CINCINNATI, OH 45267-0056, USA (G.M., S.A.G., K.W., T.R., AND V.U.), CENTER FOR DISEASE CONTROL-NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH,ENVIRONMENTAL INVESTIGATIONS BRANCH, DIVISION OF RESPIRATORY DISEASE STUDIES, 1095 WILLOWDALE RD., MORGANTOWN, WV 26505, USA (P.J.H.)

ABSTRACT. The applicability of electrostatic precipitation as a method for bioaerosol collection was investigated by using a modified Electrostatic Aerosol Sampler (EAS) (Model 3100, TSI Inc., St. Paul, MN). The physical and biological efficiencies of this method were determined. The tests were performed using three bacterial species which were collected onto agar, into water, and onto filters. The physical collection efficiency was higher than 80% when using a sampling flow rate of 1 L/min. When the *Bacillus subtilis* var *niger* (BG) spores were collected on agar, about 50–60% of the collected culturable organisms formed colonies. The bioefficiency exceeded 90% when the BG spores were collected on a filter, but was only 15–22% when collected into water. The *Mycobacterium bovis* BCG bacteria recovered at the 0–8% level on all three collection media. The least number of colonies were formed when *Pseudomonas fluorescens* bacteria were collected on any of the collection media. The data show that the process of electrostatic collection is very complex for sensitive airborne bacteria and thus several effects should be considered when assessing its bioefficiency. In separate tests conducted without aerosol flow through the sampler, bacteria placed onto the collection media did not show any significant reduction in bacterial recovery while exposed to a strong electric field. It was found that evaporation from the collection media, such as agar or water, increases the humidity inside the EAS and may affect the size distribution of the particles being collected, resulting in decreased physical and biological efficiencies of the electrostatic precipitation method. For hardy microorganisms such as BG spores, the bioefficiency for electrostatic collection is high, thus encouraging further explorations of the electrostatic method for sampling bioaerosols.

INTRODUCTION

Exposure to airborne microorganisms is usually assessed by using air samplers designed for monitoring viable microbial aerosols. Among the available sampling methods, the ones most commonly used collect the microorganisms onto an agar plate by impaction, into a liquid by impingement, or onto a dry, nonnutrient surface by filtration. When the resulting data are quoted,

[*] Corresponding author.
[†] On leave from the Institute of Physics, Vilnius, Lithuania.

the instrument used always needs to be identified, as differences in the physical and biological efficiencies in the collection method may result in significantly different enumerations. Available bioaerosol samplers differ by their inlet efficiency (Grinshpun et al. 1994), their physical collection characteristics, such as the particle cut-off size (Nevalainen et al. 1993), and their ability to maintain microbial viability during and after collection (Juozaitis et al. 1994). It has been shown that with certain collection mechanisms, such as impaction, there is a trade-off between the physical collection efficiency and the viability of collected microorganisms: high collection efficiency is often achieved through high impaction velocity but may cause increased damage to the microorganisms (Stewart et al. 1995). Particle velocities in impactors and impingers are equal to or less than air velocities through the samplers' nozzle(s). For instance, some particles travel at near sonic velocities in an AGI-30 all-glass impinger.

Electrostatic precipitation has the potential for "gentle" microbial collection, and its use as a bioaerosol sampler was explored in this study. With electrostatic collection, the airborne particles are electrically charged and then subjected to a strong electrical field, which causes their drift to and deposition on the collection substrate. When aerosol particles are collected electrostatically, their velocity component perpendicular to the collection medium is about two to four orders of magnitude lower than that in bioaerosol impactors or impingers at comparable sampling flowrates; Table 1. Thus, the gentle motion of airborne microorganisms towards the collection medium is expected to be an advantage of the electrostatic collection method over the impaction and impingement methods.

The electrostatic method of aerosol collection is in common use for the control of airborne dust in residential and industrial settings (Boelter and Davidson 1997). Attempts to utilize this method for bioaerosol sampling were already made in the first half of this century. In the electrostatic apparatus constructed by Berry (1941), *Serratia marcescens* bacteria acquired electrical charge in an ionizer and were collected on an agar surface. However, the bacterial recovery rates were not reported. Berry stated that impaction played a significant role in the collection process. In the 1960's, a large-volume electrostatic air sampler (LVS) was developed to recover airborne viruses (Gerone et al. 1966). The LVS sampler was operated at a flow rate of 1000 L/min. A modified version of this sampler had a ten times higher flow rate (Decker et al. 1969). In all of these devices, charging of the microorganisms was achieved by corona discharge, which is known to produce ozone and nitrogen oxides. The latter may adversely affect the viability of some airborne microorganisms (Cox 1987).

In this study, the feasibility of using the electrostatic technique for bioaerosol collection was investigated by performing experiments with a modified Electrostatic Aerosol Sampler (EAS)

TABLE 1. Particle Velocities towards Collection Medium in Bioaerosol Samplers.

Sampler	Sampling Flowrate, L/min	Velocity Perpendicular to Collection Medium, m/s
6th Stage of Andersen Six-Stage Viable Particle Sizing Sampler	28.3	24
Burkard Sampler	10.0	11.9
Surface Air System Sampler	180	17.3
Casella Airborne Bacteria Sampler MK-II	30.0	51.4
All Glass Impinger AGI-30	12.5	265.2
Electrostatic Sampler[a]	1–10	0.01–1

[a] 1–10 kV/cm precipitating field, 10–1000 electric charges per 1 μm particle.

(Model 3100, TSI Inc., St. Paul, MN). The EAS was originally designed as a nonbiological particle sampler (Liu et al. 1967) and thus has certain limitations as a bioaerosol sampler; e.g., the corona discharge mechanism used for particle charging may reduce the viability of microorganisms collected by this sampler. However, the EAS was found suitable as a tool for the exploration of biological particle collection utilizing electrical forces. In order to collect the microorganisms for subsequent microbiological analysis, the EAS was modified by cutting a well into the collection surface and inserting a trough with the tested collection medium into the well.

COLLECTION OF MICROORGANISMS BY ELECTROSTATIC PRECIPITATION

The collection of microorganisms in an electrostatic precipitator is schematically presented in Figure 1. The schematic represents the three essential sections of the modified EAS used. Typical ambient aerosols consist of a mixture of microorganisms and various biologically inert particles. The latter are not shown in Figure 1. The schematic illustrates the potential paths and fates of microorganisms that are culturable before entering the electrostatic precipitator. Microorganism "**a**" acquires insufficient electrical charge in the charging section or may be too far from the collection surface to be collected in any of the three indicated sections. Microorganism "**b**" gets close to the collection surface, but is removed from the air stream in the outlet section. The lost particles, N_{LOST}, contribute toward reducing C_{DOWN}, the airborne concentration of microorganisms at the device's exit, which may be considerably lower than C_{UP}, the concentration entering the device. Microorganism "**c**" is collected on the collection substrate in the precipitation section; its culturability is unaffected by the sampling process. The intent of airborne microorganism collection by electrostatic precipitation is to maximize N_{CFU} (CFU = colony forming unit), the number of collected microorganisms that will form colonies choosing subsequent incubation. Microorganism "**d**" is also collected on the collection substrate, but is rendered unculturable by continuing exposure to the electric field after collection. The experiments discussed further below address the likelihood of this occurrence. Microorganism "**e**" is rendered unculturable by the electric field while it is still airborne. Microorganism "**f**" is rendered unculturable already in the charging section, while microorganism "**g**" is physically lost in that section. Thus, the number of collected microorganisms, $N_{COLLECTED}$, consists not only of the microorganisms that will form colonies, N_{CFU}, but potentially also of microorganisms that were initially culturable, but became nonculturable in the electrostatic precipitator. The experiments shown below address these potential losses.

MATERIALS AND METHODS

Modified Electrostatic Aerosol Sampler

In its original design, the EAS consists of an inlet, flow straighteners (honeycombs), an ion source, charging and precipitating voltage plates, a collection surface (glass slides), and an outlet. The airflow carrying aerosol particles enters the EAS through the inlet and is distributed uniformly across the channel through two perforated metal plates (honeycombs), as shown in Figure 2. The space above the ion source (corona wire) is the charging region in which the aerosol particles are exposed to positive ions and become charged. The positive ions are produced by a 0.05 mm tungsten wire maintained at a voltage of 3.5 kV. The charging voltage applied to the charging plate is in the form of clipped sine waves of 60 cps with peak amplitudes of 800 V. The charged particles then enter the precipitating region. Due to their exposure to the electric field created by 4.2 kV of precipitating voltage, the particles (or a fraction of them) are collected onto a collection substrate. The EAS was originally designed to be used with microscope grids and slides as collection substrates. In this study, other collection media were used, including

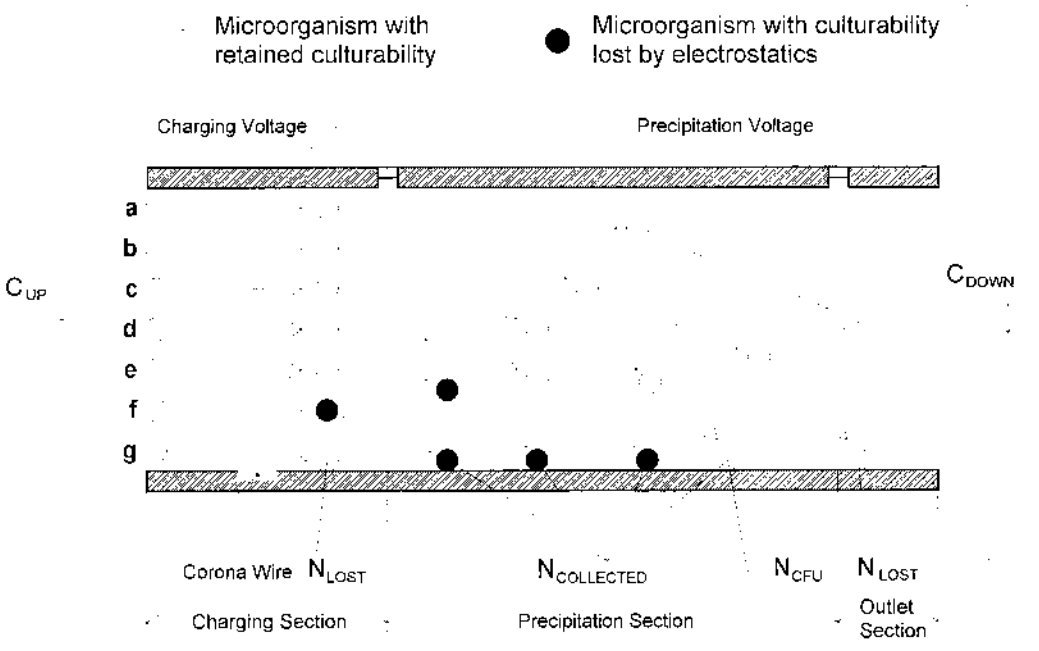

FIGURE 1. Schematic illustration of potential microorganism loss to enumeration by colony forming when the microorganisms are collected in a standard electrostatic precipitator.

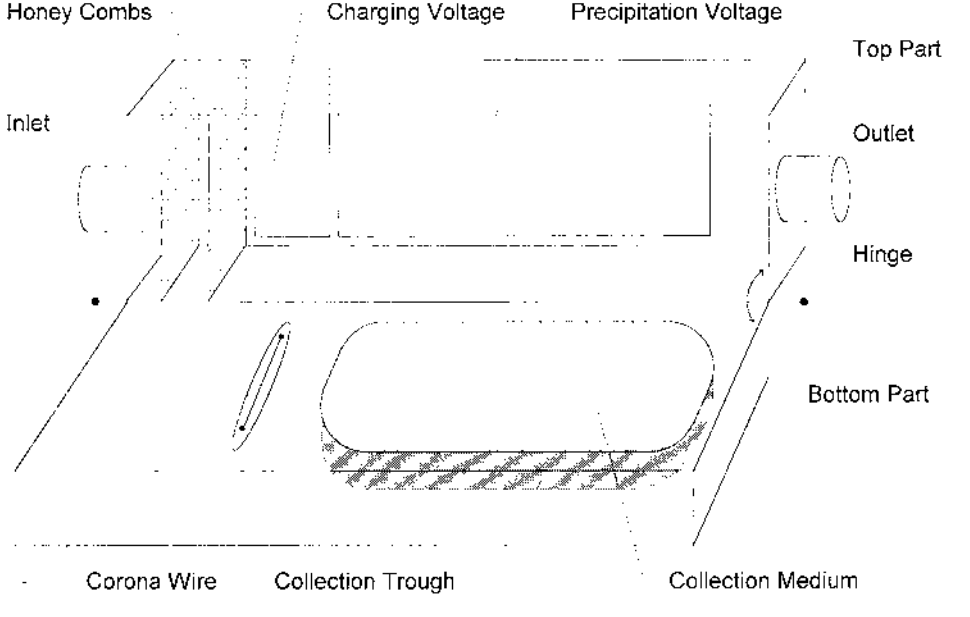

FIGURE 2. Schematic representation of the Electrostatic Aerosol Sampler (Modified Model 3100, TSI Inc.). Shown open.

liquid. To accommodate these, the EAS was modified by milling a 0.8 cm deep, 4.8 cm wide, and 18 cm long well into the collection area so that a rectangular trough containing the collection medium could be placed inside, as shown in Figure 2. The uncollected aerosol particles exit the sampler via its outlet.

Experimental Setup

The test system that we developed earlier for the evaluation of bioaerosol samplers (Juozaitis et al. 1994; Thompson et al. 1994) was modified for this study as shown in Figure 3. A Collison nebulizer (BGI Inc., Waltham, MA) was used as the bioaerosol generator. It aerosolized the microbial particles from a liquid suspension at a flow rate (Q_{NEB}) of 6 L/min. The air going into the nebulizer was dry, filtered, and compressed. The resulting aerosol was diluted with dry, filtered, and compressed laboratory air at a dilution flow rate (Q_{DIL1}) ranging from 35 to 40 L/min. A total flow of 41–46 L/min $(Q_{NEB} + Q_{DIL1} = Q_{TOTAL})$ entered an open sampling chamber from which the aerosol was sampled into the electrostatic precipitator at Q_{SAMPL} = 1 L/min for 3, 10, and 30 min. The upstream and downstream aerosol concentrations, C_{UP} and C_{DOWN}, were determined by an Aerosizer (Amherst Process Instruments, Inc., Hadley, MA). This multichannel aerodynamic particle sizing instrument has previously been proven to measure particle sizes starting from about 0.5 μm, thus covering most of the bacterial size range (Qian et al. 1995).

In most of our experiments the EAS sampling flow rate was equal to or less than the Aerosizer sampling flow rate of 5 L/min (Q_{AER}). The dif-

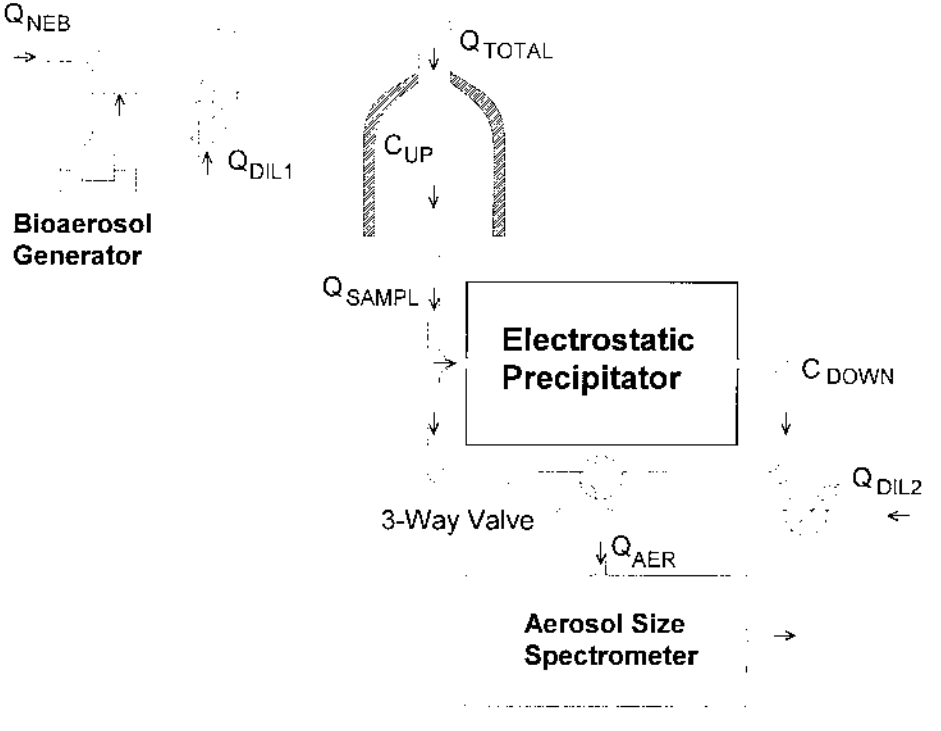

FIGURE 3. Experimental setup.

ference in air flow was added through the dilution air $Q_{DIL2} = Q_{AER} - Q_{SAMPL}$. The number of particles retained by the EAS was determined from the measured difference between C_{UP} and C_{DOWN}, the sampling flow rate, and the sampling time.

The entire test system was placed in a Class II, Type B2, biological safety cabinet (SterilchemGARD; Baker Company, Sanford, ME) so that any aerosol particles not collected by the sampler were properly eliminated. The temperature was kept at 22–26°C and the relative humidity at 30–50% during all experiments.

Test Microorganisms and Preparation of the Microbial Suspension

The following bacterial species were used in this study: *Bacillus subtilis* var *niger* spores (BG), vegetative cells of an avirulent strain of *Mycobacterium bovis* (*M. bovis*) BCG (Bacillus of Calmette-Guerin), and vegetative cells of *Pseudomonas fluorescens* (*P. fluorescens*). The rod-shaped Gram-negative *P. fluorescens* bacteria are commonly found in ambient air (Nevalainen 1989) and represent sensitive bacteria (Neidhardt et al. 1990), whereas the rod-shaped Gram-positive BG spores are known to be very resistant to many adverse conditions (Sneath 1986). Avirulent *M. bovis* BCG was chosen because of its biochemical similarity with pathogenic *Mycobacterium tuberculosis* (TB) bacteria, which have a relatively high resistance to drying, chemical disinfectants and other environmental factors (Freeman 1985). As 5.3 million workers are exposed to TB at work in the US (ACGIH 1997), it is especially important to explore new efficient methods for TB detection and enumeration.

Dry spores of BG were obtained from the US Army Edgewood Laboratories (Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, MD). Stock cultures of *M. bovis* BCG (ATCC 35737) and *P. fluorescens* (ATCC 13525) were obtained from the American Type Culture Collection (Rockville, MD). BG spores are rod-shaped, approximately 0.7–0.8 μm in width and 1.5–1.8 μm in length (Johnson et al. 1994). Rod-shaped *M. bovis* BCG cells have a large length-to-width ratio and range from 3 to 14 μm in length (Schafer et al. 1998). *P. fluorescens* vegetative cells range from 0.7 to 0.8 μm in diameter and from 1.5 to 3 μm in length (Qian et al. 1995).

The BG spores were received in dry form and did not need additional cultivation. Prior to their aerosolization, a small amount of these spores was dissolved in deionized and sterilized water. This suspension was then kept at 55°C for 25 min to activate the spores. The *M. bovis* BCG cultures were grown at 35–37°C in Bacto Dubos liquid medium supplemented with Bacto Dubos Medium Albumin (Difco Laboratories, Detroit, MI) and 1.0% Tween 80. The *M. bovis* BCG cells were kept in the early log phase by recultivating the cultures every 4–5 days in fresh broth. To prevent clumping of the cells, the culture was mixed 1–2 times a day using a Vortex Touch Mixer (Fisher Scientific, Pittsburgh, PA). After about two weeks of growth, the amount of *M. bovis* BCG cells was sufficient to generate high enough concentrations of airborne bacteria. The *P. fluorescens* culture was grown in Trypticase Soy Broth (Becton Dickinson Microbiology Systems, Cockeysville, MD) at 30°C while kept for 18 h in a Gyrotory Water Bath Shaker (Model G76, New Brunswick Scientific, Edison, NJ). The *M. bovis* BCG and *P. fluorescens* cells were harvested from their suspensions by centrifugation at 4800 g for 10 min (Marathon 6K Centrifuge, Fisher Scientific, Pittsburgh, PA). The resulting pellets were washed three times with deionized and sterilized water (5 Stage Milli-Q Plus System, Millipore Corp., Bedford, MA). To obtain suspensions of desired bacterial density, the initial suspensions of all three microorganisms were diluted with deionized and sterilized water. The concentrations of bacteria in the air ranged from 1 to 80 spores/cm^3 for BG, from 0.3 to 10 cells/cm^3 for *M. bovis* BCG, and from 5 to 80 cells/cm^3 for *P. fluorescens*.

Prior to every experiment, the fraction of culturable bacteria ($F_{CULTURABLE}$) in each suspension was determined as a ratio of the number of culturable bacteria to the total number of bacteria in the suspension. The culturable bacteria in a unit volume of suspension were enumerated by taking an aliquot, diluting it, and cultivating dilutions on agar media. The media and incubation conditions were the same as those used for aerosol samples and are described in the next section. The total count of bacteria per unit liquid volume was obtained by placing the suspension in a hemacytometer (Petroff-Hausser Counter, Hausser Scientific Partnership, Horsman, PA) and counting the number of cells under a phase-contrast microscope (Labophot-2, Nicon Corp., Tokyo, Japan) at a magnification of 400X.

Collection Media and Colony Enumeration

Three different collection media were used in this study: agar, deionized and sterilized water, and a filter material (Whatman 42 Ashless, W & R Balston, Ltd., England). Trypticase Soy Agar (Becton Dickinson Microbiology Systems, Cockeysville, MD) was used for *P. fluorescens* and BG, while Middlebrook 7H10 Agar (Difco Laboratories, Detroit, MI) was used for *M. bovis* BCG.

The incubation and enumeration procedures were dependent on the type of collection medium and the microorganism species used. When collecting microorganisms on agar or into water, 30 ml of the respective substrate was used. This amount was sufficient for sample analysis and fully filled the collection trough.

In the tests using agar as the collection medium, melted agar was poured into the sampling trough and allowed to solidify for approximately 30 min. The sampling trough was then inserted into the EAS prior to sampling. After sampling, the agar in the trough was divided into three equal portions—one near the charging section, one near the outlet section, and one in between the two. Each portion was then placed into an empty Petri dish for incubation. By placing the agar in covered Petri dishes the agar media were prevented from desiccation and contamination after sampling creating optimal conditions for culturing bacteria.

When sampling with water, 30 ml of deionized and sterilized water was poured into the sampling trough prior to sampling. After sampling, water from the trough was removed with a BenchMate pipette (Oxford Labware Group, Saint Louis, MO). Aliquots of 0.2–0.3 ml were then cultivated on appropriate agar media and incubated as described below. When sampling for 30 min, the suspension extracted from the trough was diluted to increase the accuracy of colony enumeration.

When sampling with filter as the collection medium, a conducting support was placed into the trough underneath the filter to ensure that the collection surface was at the same height as when sampling with agar and water. The strength of the applied electric field was the same as for the other collection media. When microbial collection on a filter was completed, the filter was placed into a vial containing 25 ml of deionized and sterilized water. The microorganisms were eluted from the filter by shaking the vial for 1 min with a Vortex Touch Mixer (Fisher Scientific, Pittsburgh, PA). After the elution procedure, 0.2–0.3 ml aliquots of suspension were transferred onto agar and incubated.

The *P. fluorescens* vegetative cells and BG spores collected on agar (or transferred onto agar from water or filter samples) were incubated for 24 h at 28°C and 37°C, respectively. After incubation, the colonies formed by these microorganisms were enumerated. The *M. bovis* BCG vegetative cells collected on or transferred onto agar were incubated at 37°C in the presence of 7.5% CO_2. *M. bovis* BCG is a slow growing microorganism; it takes 25–90 days to form colonies (Murray et al. 1995). To prevent agar desiccation during this long incubation time, the Petri plates were wrapped with CO_2-permeable plastic wrap (All-Purpose Laboratory Wrap, Fisher Scientific, PA). The agar

plates containing *M. bovis* BCG bacteria were examined weekly for the presence of colonies which were counted 4, 6, and 8 weeks after incubation.

The sampling times were 3, 10, or 30 min in the tests with all three collection media, and the sampling flow rate was between 1 and 5 L/min. To obtain statistically meaningful results, the experiments were repeated at least three times; the data are presented as averages of these repeated tests. The standard deviations are indicated in the figures wherever they are significant.

Coupling between Bacteria and Agar

Once the bacteria gently land on the agar surface, they may be insufficiently coupled with the nutrients, preventing sensitive bacteria, such as *P. fluorescens*, from forming colonies. The effect of embedding on the bacterial recovery was examined by treating the bacteria collected onto agar in three different ways. First, the bacteria were incubated using the standard sampling procedure; i.e., no additional embedding was attempted after collection. Second, the coupling between the bacteria and the agar was improved by smoothing the agar surface after collection with a spreader in the same manner as when the bacteria are cultivated on agar in a Petri dish. Third, 15 ml of melted agar (conditioned at 55°C) was poured into the sampling trough on top of the agar immediately after collection to ensure that the bacteria were completely engulfed by nutrients. The sampling times for these experiments was limited to 30 s so that possible negative effects caused by desiccation and/or ozone production were minimized. *M. bovis* BCG cells were not used in these experiments because of their slow growth rate.

Retention Efficiency and Particle Losses inside the Sampler

The aerosol penetration through the sampler is defined as the ratio of the measured aerosol concentration downstream to the concentrations upstream of the sampler:

$$\text{Penetration Efficiency} = C_{\text{DOWN}} / C_{\text{UP}}. \quad (1)$$

The aerosol particle retention efficiency of the EAS is the numerical difference between unity and the penetration efficiency:

$$\text{Retention Efficiency} = 1 - \text{Penetration Efficiency}. \quad (2)$$

The retention efficiency accounts for the particle deposition in all segments of the EAS, including the inlet, the charging chamber, the sampling trough, and the outlet. In the ideal situation, when losses do not occur inside the sampler (i.e., all the particles removed from the air by the EAS are deposited in the precipitation section, Figure 1), the retention efficiency is equal to the physical collection efficiency of the sampler. Otherwise,

$$\text{Collection Efficiency} = \text{Retention Efficiency} - \text{Loss Fraction}. \quad (3)$$

Because of the rather complicated geometry inside the EAS, the particle losses were hard to quantify. A semiquantitative loss assessment was performed by observing the deposition pattern of fluorescein dye particles (Eastman Kodak Co., Rochester, NY) inside the sampler under operational conditions. Fluorescein dye particles were dissolved in water, aerosolized in a Collison nebulizer, and then sampled with the EAS on agar at the flow rate of 1 L/min for 4 h. After sampling, the entire inner surface area of the EAS was divided into 15 segments and the deposition of fluorescein dye particles was estimated by wipe samples from each of those segments. Kimwipes EX-L (Kimberly-Clark, Ontario, Canada) moistened with ethanol were used for wiping. The wipes were analyzed under long-wave UV light (model MR-4, George Gates & Co., Inc., New York, NY), and the amount of fluorescein dye on every wipe was estimated visually on a scale from 1 to 10 for each segment inside the ESP.

Biological Efficiency

The biological efficiency for the tests in the EAS was assessed using measurements of the bacterial recovery rate, which we define as the ratio of the number of colonies formed by the collected microorganisms to the number of initially culturable microorganisms retained by the EAS:

$$\text{Bacterial recovery rate} = \frac{N_{\text{CFU}}}{(N_{\text{COLLECTED}} + N_{\text{LOST}})F_{\text{CULTURABLE}}}, \quad (4)$$

where N_{CFU} is the number of colonies formed on the collection medium (precipitation section); $N_{\text{COLLECTED}} + N_{\text{LOST}}$ is the number of microorganisms retained by the EAS, including those collected in the precipitation section $(N_{\text{COLLECTED}})$ and those retained in other sections (N_{LOST}); and $F_{\text{CULTURABLE}}$ represents the fraction of culturable microorganisms in the initial nebulizer suspension ($F_{\text{CULTURABLE}}$ was determined for every experiment as indicated above). The value of $(N_{\text{COLLECTED}} + N_{\text{LOST}})$ was found by measuring the aerosol concentrations C_{UP} and C_{DOWN}. The value of $(N_{\text{COLLECTED}} + N_{\text{LOST}})F_{\text{CULTURABLE}}$ represents the total number of the initially culturable microorganisms retained by the sampler. It is equal to N_{CFU} if the bacterial recovery rate is 100% and there are no losses. When microbial collection was performed on agar, the value of N_{CFU} was determined through colony enumeration on the agar. However, when the microorganisms were collected on a filter or in a liquid medium, the colony enumeration was performed in an aliquot as n_{CFU}, from which the number of culturable microorganisms in the entire sample was calculated:

$$N_{\text{CFU}} = n_{\text{CFU}} \frac{V_{\text{SAMPLE}}}{V_{\text{ALIQUOT}}}, \quad (5)$$

where V_{SAMPLE} is volume of the sample (30 ml for water samples, 25 ml for filter samples) and V_{ALIQUOT} is volume of the aliquot (0.2–0.3 ml).

RESULTS AND DISCUSSION

Effect of Collection Medium and Bacterial Species on Bacterial Recovery

The physical retention efficiency of all three microorganisms measured with the Aerosizer was found to be sufficiently high (80–90%) when the EAS was operated at a flow rate of 1 L/min, the flow rate used for most of the tests in this study.

Figure 4 shows the bacterial recovery rate for microorganism collection on agar. It was discovered that the recovery rate greatly depended on the bacterial species collected. The highest recovery rate (50–60%) was observed when collecting BG spores $(F_{\text{CULTURABLE}} = 10\%)$. The recovery rate of *M. bovis* BCG cells $(F_{\text{CULTURABLE}} = 25\%)$ ranged from 3 to 7%. When collecting *P. fluorescens* bacteria $(F_{\text{CULTURABLE}} = 90\%)$, very few colonies formed on agar resulting in a recovery rate for this bacterium close to zero. For each microorganism tested, the bacterial recovery rate did not show any dependence on collection time within the experimental error.

When the microorganisms are collected electrostatically onto the agar surface, desiccation stress may result from their continued exposure to the air flow, especially if the microorganisms are not efficiently coupled with the nutrients. The stress induced due to the insufficient coupling between bacteria and the nutrients occurs not only during the relatively short sampling period but also during 24 h of subsequent incubation. For sensitive microorganisms, such as *P. fluorescens*, the lack of nutrients and possibly the desiccation stress may have been so significant that very few colonies were formed. The BG spores are known to be hardy and resilient microorganisms. Even a minimal amount of nutrient may be sufficient for them to form colonies (Sneath 1986), which is seen in Figure 4. Another potential cause of the low recovery rate of *P. fluorescens*, and also *M. bovis* BCG, may have been a stress induced upon bacteria during their aerosolization (Griffiths et al. 1996).

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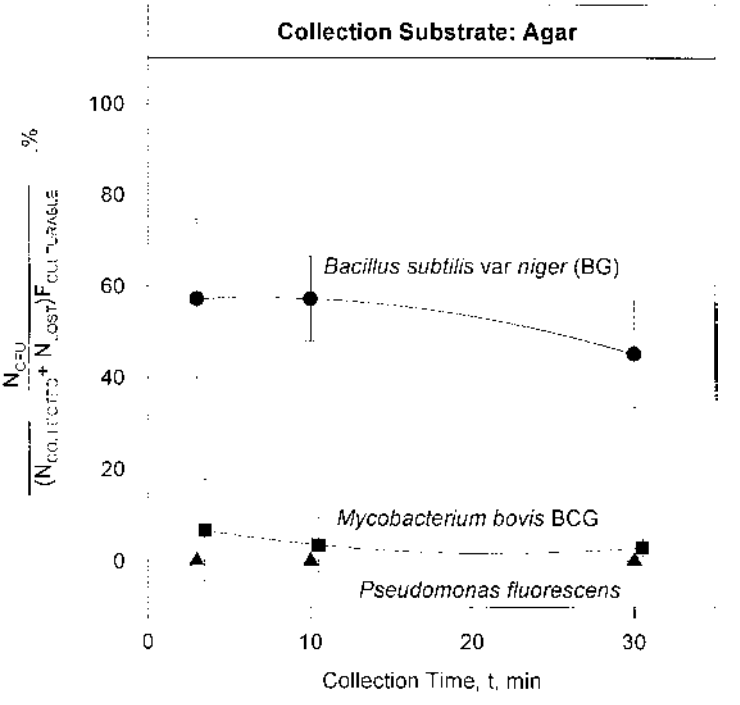

FIGURE 4. Effect of collection time and bacterial species on the bacterial recovery in the EAS when collecting on agar. Q_{SAMPL} = 1 L/min.

However, a study by Reponen et al. (1997) has shown that the relative recovery of sensitive *P. fluorescens* bacteria when aerosolized with Collison nebulizer was 83% ± 19% and did not decrease over 60 min. Since *M. bovis* BCG are known to be resistant to many adverse conditions, we expect it to endure the aerosolization stress at least as well as *P. fluorescens* does. The recovery rate of *M. bovis* BCG was higher than that of *P. fluorescens* but lower than that of BG spores.

The relatively high recovery rate of BG spores on agar shows that the electrostatic method is feasible for agar collection of hardy bacteria. To avoid insufficient coupling between the microorganisms and the media, separate experiments were performed with all three microorganisms collected into water at the same flow rate. When the microorganisms land on the water surface, they presumably sink and are engulfed by the water. Thus, they are protected from the desiccation stress. Upon subsequent embedding in agar they are expected to have a high recovery rate. However, the recovery rate of BG spores $\left(F_{\text{CULTURABLE}} = 10\%\right)$ was only 15–22%, and the recovery rates of *M. bovis* BCG cells $\left(F_{\text{CULTURABLE}} = 25\%\right)$ and *P. fluorescens* $\left(F_{\text{CULTURABLE}} = 90\%\right)$ cells were close to 0%, which indicates lower recovery of bacteria for collection in water than for collection on agar, as shown in Figure 5A.

It was hypothesized that once the charged microorganisms have submerged in the water, they may still be influenced by the electric field and, consequently, migrate towards the bottom of the sampling trough and attach to it. So, after a period of sampling, a portion of the collected microorganisms would no longer be suspended in the collection fluid, but rather be deposited on the bottom of the aluminum trough. This

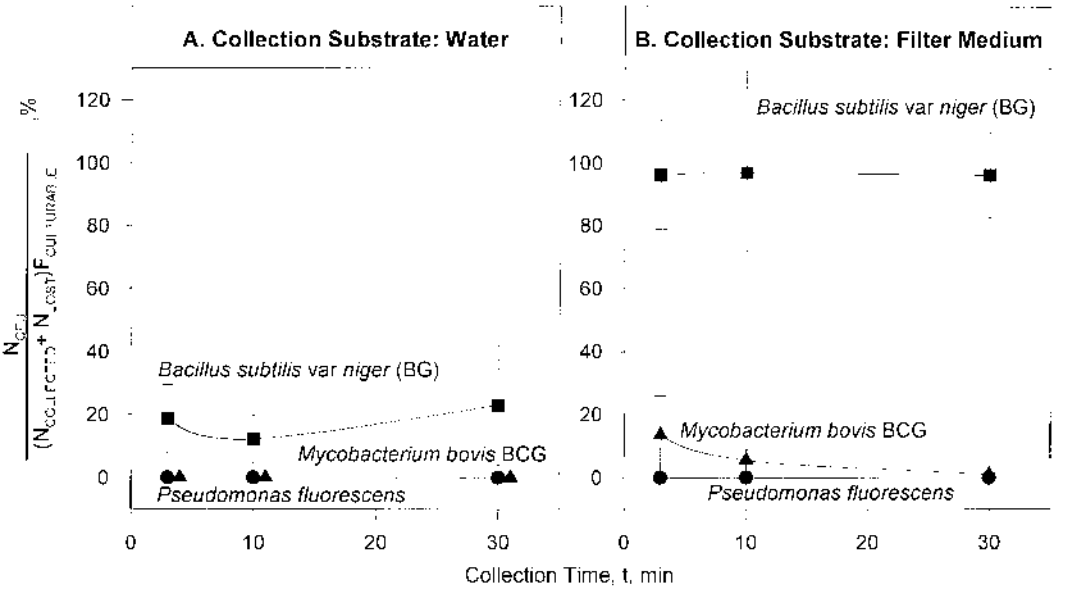

FIGURE 5. Effect of collection time and bacterial species on the bacterial recovery in the EAS when collecting in water (A) and on filter (B). Q_{SAMPL} = 1 L/min.

may reduce the recovery rate due to the microbial stress caused by the charged bacteria-metal surface interaction. To check this hypothesis, the trough was first covered with 15 ml of agar and was then filled with 15 ml of water, and BG spores were then collected for 3 and 10 min. The number of colonies formed by the spores extracted from the water layer was 10–20% of the total number of culturable spores retained, which is comparable to the percentage of spores recovered from the trough when it was filled with water only. The percentage of colonies formed on the agar covering the trough's metal surface was approximately 15%, i.e., about the same as formed in the water layer. Thus, electrical migration of microorganisms collected into water in the EAS is significant and should be taken into account when refining this collection method. In this experiment, the agar in the trough covered not only the bottom but also the sides of the trough, and it was observed that colonies were also formed on the sides of the trough. This suggests that the bacteria are charged and that their movement follows the electric field lines.

The results of tests with the filter as a collection medium are shown in Figure 5B. More than 90% of the collected culturable BG spores $(F_{CULTURABLE} = 10\%)$ were able to form colonies. This number was only 0–8% for *M. bovis* BCG cells. In these tests, $F_{CULTURABLE}$ of *M. bovis* BCG was about 2%, much lower than that in previous experiments. We found that initial viability of *M. bovis* BCG varied significantly if cells were taken from different batches. For BG spores, the recovery rates on the filter were higher than those on agar; for *M. bovis* BCG and *P. fluorescens*, these recovery rates were comparable to each other. *P. fluorescens* cells $(F_{CULTURABLE} = 90\%)$ formed very few colonies after collection on the filter—similar to the tests conducted with this bacterium on the other collection media. The filter tests confirm that the BG spores are the least sensitive among the three tested species, while the *P. fluorescens* cells are the most sensitive.

Particle Retention and Deposition inside the EAS

Microbial species differ in size and shape, which may affect their electrical migration towards the collection substrate when sampled by an EAS. One may also hypothesize that the electrical migration of biologically active particles may differ from that of nonbiological particles because biological particles are more complex in their composition and naturally contain electrical charges. Performance of the EAS for nonbiological particles was examined by testing with monodisperse spherical Polysterene Latex (PSL) particles. The results are shown in Figure 6. For the lowest flow rate of 1 L/min, more than 95% of particles of all three sizes entering the EAS were retained inside. The aerodynamic diameter (d_a) of the PSL particles was calculated from their physical diameter and their natural density of 1.05 g/cm^3 (Hinds 1982; Baron and Willeke 1993). When collecting 0.5 μm PSL at flow rates of 3 and 5 L/min, the retention efficiency decreased to 75% and 57%, respectively. This decrease in collection efficiency is due to the decreased distance of migration toward the collection surface when the particles' residence time in the sampler is decreased with increasing air flow rate through device. For particles of 1.0 μm in diameter the retention efficiency was at levels similar to those for particles of 0.5 μm in diameter. However, when 1.6 μm diameter particles were sampled at 3 and 5 L/min, the retention efficiency increased to about 85%. This increase for the larger particles was attributed to an increase in particle losses inside the sampler, as the airflow inside the EAS follows a curved path.

When the three rod-shaped test microorganisms were sampled at 1 L/min, 80–90% of them were retained inside the EAS—about the same fraction as found for biologically inert PSL par-

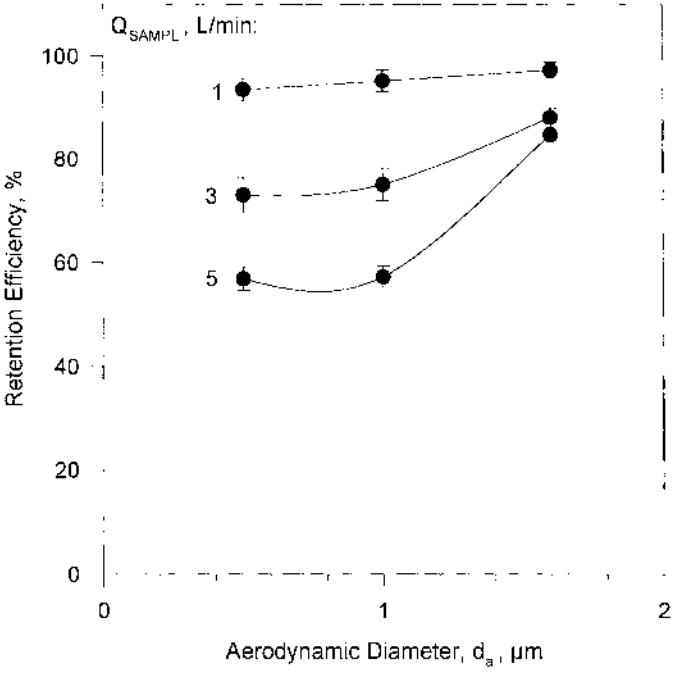

FIGURE 6. Dependence of the EAS retention efficiency on particle size for PSL test particles passing through the sampler at different flow rates. Particle collection is on agar for these tests.

ticles of the same size range. It follows that neither the biological nature of the tested microorganisms nor their shape significantly affects their retention by electrostatic forces inside the tested EAS.

Tests performed with fluorescein dye particles, covering an aerodynamic diameter range of 0.5–3 μm, found fluorescein dye particles in all segments of the EAS. Their deposits around the ion source were even visible with the naked eye. This finding is consistent with the findings of Liu et al. (1966), who concluded that the major part of the losses occur in the charging region of the EAS, apparently resulting from the space charge of the ions in the charging zone. Only about 24% of the fluorescein dye particles were found on the collection substrate. Although the fluorescein dye particles and the bacteria used in this study differ somewhat in size and charging characteristics, the results of the semiquantitative analysis with fluorescein dye particles can be used to interpret the data obtained with bacterial collection in the EAS. It is known that many bacterial species have their own natural electrical charge (Gittens and James 1963; Harden and Harris 1953; Sleytr 1978; Sutherland 1977). The charge can be as high as 10^5 elementary charges per cell (Sherbet and Lakshmi 1973). Because of their differences in shape and in cell wall structure, some species may accept external charges more readily than others and the highly charged cells may deposit inside the EAS well before they get to the collection trough. A separate study should be performed to determine the bacterial behavior in electrical fields by applying a well-controlled external charge to different Gram-positive and Gram-negative species and studying their behavior in electrical fields.

Effect of Embedding on Bacterial Recovery

The effect of the embedding conditions on microbial recovery for *P. fluorescens* cells and BG spores collected on agar is presented in Table 2. *M. bovis* BCG cells were not used in these experiments because of their slow growth rate. As seen from the table, the recovery rate of *P. fluorescens* bacteria was approximately zero for all three embedding conditions. The percentage of retained culturable BG spores that formed colonies was comparable with that observed in earlier experiments (Figure 4) and did not significantly depend on the embedding conditions. It can be concluded that although bacterial embedding in the collection medium may increase the survival of the organisms and their ability to form colonies (study with impactors by Stewart et al. 1995), insufficient embedding of bacteria collected on agar in the EAS does not explain the low recovery rate of *P. fluorescens* cells in the EAS.

Exposure of the Microorganisms to the Electric Field

When bacteria are sampled by an electrostatic precipitator, the air passes through the device with transport velocity $V_{\text{TRANSPORT}}$. Neglecting

TABLE 2. Bacterial Recovery Rate Determined when Additional Coupling between the Collected Bacteria and Agar is Applied after Sampling with the EAS (Sampling Time = 30 s).

Collection conditions	$\frac{N_{\text{CFC}}}{(N_{\text{COLLECTED}} + N_{\text{LOST}})F_{\text{CULTURABLE}}}$, %	
	P. fluorescens	*B. subtilis* (BG)
Usual collection (no additional imbedding)	(0)	71.0 ± 11.4
Additional imbedding after sampling	(0)	54.2 ± 10.3
Liquid agar poured on top of sample	(0)	72.3 ± 31.9

gravitational settling, the charged particles are attracted to the collection surface with electrical drift velocity V_E. Thus, the particle path follows the resultant velocity vector V_R, as shown in Figure 7. The transport of bacteria and their collection into one of the three media is schematically shown for three moments in time, t. Period t_1 represents the time during which a microorganism inside the sampler is being carried by the airflow and the electrical field to the collection medium. Period t_2 represents a time interval between the moment of particle-medium contact until the moment when the particle velocity is decreased to 0. For collection on a solid agar or a filter, $t_2 = 0$. If the charged microorganism lands on the top of a liquid, such as water, it is likely to submerge, as shown in Figure 7b, $t_2 > 0$. Under the influence of the electric field and gravity it will migrate towards the bottom of the sampling trough. Once the bottom is reached, the microorganism remains there (time period t_3). If sampling for long periods of time, the extended exposure of the microorganisms to the electric field may inactivate them (Figure 1, cases "**c**" and "**d**"). When a solid or semisolid collection medium is used, such as an agar or a filter, the exposure conditions do not change from t_2 to t_3.

Exposure of microorganisms to an external electrical field can cause conformational changes in the protein helix-coil or metastable conformational transitions in the polynucleotide mode (Varekhov and Smirnov 1993). An applied electrical field may hyperpolarize the membrane facing the anode and depolarize the side facing the cathode (Harold and Caldwell 1990). Jaffe (1977) suggested that an applied electrical field might redistribute charged, mo-

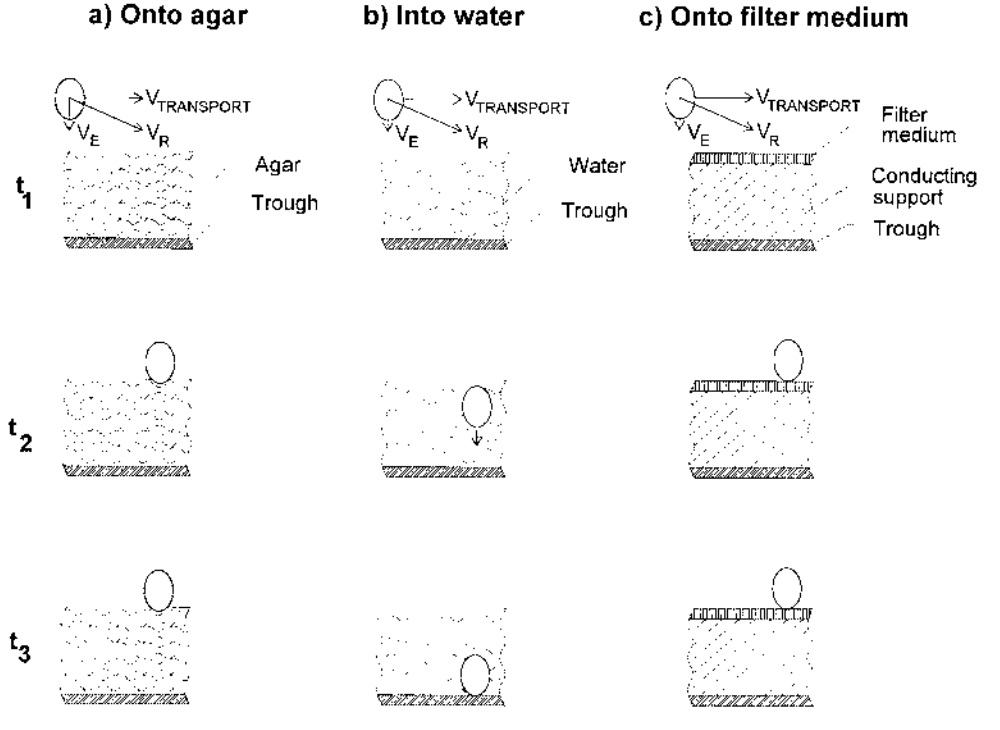

FIGURE 7. Transport of bacteria toward and onto/into different collection media.

bile components in the plane of the plasma membrane.

Changes in microorganism viability due to their exposure to an electric field were investigated by inoculating all three species of bacteria on agar and in water and exposing them to the electric field of 4.2 kV in the EAS. In this experiment, no sampling was involved: air suction and electrical charging were turned off to avoid the potentially negative effects of desiccation and ozone production. The filter medium was not used in these experiments because the process of bacterial landing on the filter surface and the conditions of microbial exposure to the electric field were believed to be similar to those for collection on agar. Figure 8 compares the number of colonies formed by bacteria exposed to the electric field with those formed by unexposed bacteria. It was found that exposures as long as 2 h did not reduce the culturability of three bacteria. The data show that even sensitive cells, such as *P. fluorescens*, were not significantly inactivated by the electric field after their collection on agar and water.

Effect of Humidity on the Bacterial Size Distribution in the EAS

When *P. fluorescens* cells were collected with the EAS, two types of bacterial size distributions were measured by the Aerosizer downstream of the sampler: one had a mean aerodynamic diameter of 0.8 μm (the same as upstream), but the other showed a shift to larger particles with the mean aerodynamic diameter of about 1 μm. This bacterial size shift occurred only when an evaporative sampling medium was used, such as water or agar. The evaporation of these materials inside the EAS apparently created an environment that was humid enough to change the mean aerodynamic diameter of *P. fluorescens* cells by approximately 25%. However, similar effects were not observed when collecting BG spores or *M. bovis* BCG cells. The observed change in

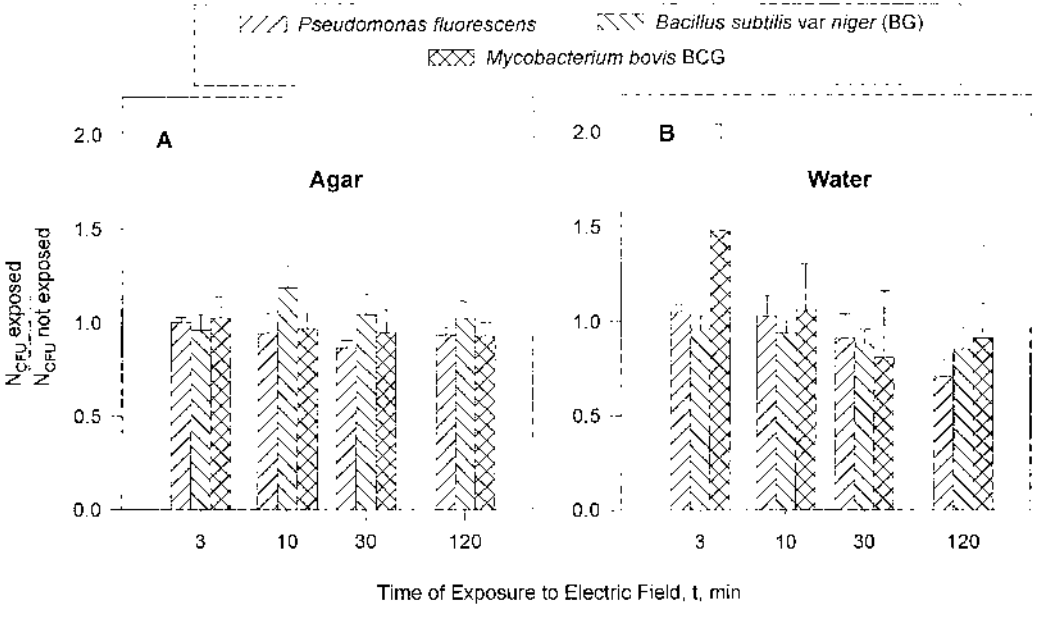

FIGURE 8. Effect of exposure of three bacterial species to an electric field of 4.2 kV/cm. (A) bacteria placed on agar and (B) bacteria suspended in water.

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bacterial size distribution during sampling complicates the estimation of collection efficiency for the electrostatic collection method.

When determining the collection efficiency of *P. fluorescens* cells, the upstream and downstream aerosol concentrations were determined from the data in the particle size range of 0.74–1.6 μm, covering all sizes of the tested airborne bacteria. This procedure may not be sufficiently accurate when some residue particles are present in the bacterial aerosol and the aerodynamic size of those particles is increased into the size range of single cells due to the condensation. Although some aerosol particles (especially salts) may grow in size considerably in the presence of high humidity, very little is currently known about the hygroscopic growth properties of biological aerosols (Reponen et al. 1996). In order to estimate the effect of humidity on the physical collection efficiency of the EAS, airborne *P. fluorescens* cells were collected into water for up to 3.5 h. During this time, the retention efficiency of the EAS decreased by a factor of 3 from 92% to 31%.

In humid environments, the strength of an electric field decreases (Abdel-Salam 1992). This may have contributed to the decreased retention efficiency of *P. fluorescens* cells in the EAS, but cannot explain such a significant drop in the particle retention efficiency. A major factor contributing to this finding may be the way the *P. fluorescens* cells were aerosolized in the experiment. When the bacterial suspension is aerosolized, each droplet ideally contains one bacterium. However, in order to avoid the presence of more than one bacterium per droplet, the bacterial concentration in the liquid is kept low enough so that each droplet contains only one bacterium or, in most cases, zero bacteria. It also may contain biological or nonbiological residue. When the liquid is evaporated, the empty droplets or those containing residue become small particles that are generally below the bacterial size range. In the presence of high humidity they may grow, returning to their original size range, and be counted by the Aerosizer as bacteria in the downstream effluent. To prove that the shift in the size distribution of *P. fluorescens* bacteria was caused by the humidity inside the EAS and not by any other effects, airborne *P. fluorescens* cells were collected onto agar for about 13 h until the agar medium had completely desiccated. As a result, humidity was no longer elevated. During the first 3–4 h of sampling, the bacterial size shift was clearly seen and the retention decreased from an initial 90% to values ranging from 16% to 31%. The retention efficiency again increased, reaching 90% after 10 h. At that time, the bacterial size shift was no longer seen. This suggests that the initial humidity inside the EAS was restored in the absence of evaporation from agar medium. When PSL particles of 1.024 μm diameter were collected into water for more than 3 h, no particle size shift was observed and the retention efficiency of the EAS decreased only from 98% to 85%. Similar results were obtained with BG spores.

These results show that the hygroscopic properties of *P. fluorescens* cells differ from those of BG, *M. bovis* BCG, and PSL particles. This may be one of the reasons for the low recovery rate of *P. fluorescens* bacteria. When a cell becomes coated by a water layer, it increases in size and mass while carrying the same number of elementary charges. As its charge-to-mass ratio decreases, the movement of the bacterium is less influenced by the electric force. Thus, the bacterium may not be collected in the EAS precipitation section by this force. However, since the bacterial mass has increased, the same bacterium may be more readily deposited in other sections of the EAS due to impaction or sedimentation.

CONCLUSIONS

The tests conducted with three bacterial species and with agar, water, and filter as collection media show that the electrostatic precipitation method has the potential for collecting viable airborne microorganisms. When collecting BG spores on agar, about 50–60% of the collected culturable microorganisms formed colonies in the EAS. However, a much lower recovery rate

was obtained for *P. fluorescens* and *M. bovis* BCG cells, which suggests that these sensitive bacteria may have sustained injuries during their sampling in the tested EAS. Additional embedding of the collected bacteria into agar did not result in an increase of the bacterial recovery rate. Exposure of all three microorganism species to the electric field after their collection did not change the bacterial recovery rate. The processes that may adversely affect the microorganisms include corona discharge, ozone production, and a strong electrical field affecting the microorganisms while they are still airborne. It was found that evaporation of the collection medium, such as water or the liquid component of agar, increases the humidity inside the EAS. This may affect the size distribution of the particles being collected and thus result in decreasing physical and biological efficiencies of the electrostatic precipitation method. This effect may become pronounced when the bacteria are collected for about 1 h or longer. To utilize the electrostatic precipitation method successfully for the collection of a wide range of bioaerosol particles, including those sensitive to stress, a new electrostatic bioaerosol sampler needs to be designed with the electrostatic and other sampling parameters optimized.

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