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Measurement of electrical charges carried by airborne bacteria laboratory-generated using a single-pass bubbling aerosolizer

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ABSTRACT

Widely used bioaerosol generators like Collison nebulizer probably produce electrostatically charged particles, but the electrical charges carried by laboratory-generated airborne microorganisms using bubbling aerosolizers are poorly understood. In this study, we measured the fraction of neutral particles and number of elementary charges per particle as a function of the aerodynamic diameter of airborne bacteria (*Escherichia coli* and *Enterococcus hirae*). Bioaerosols were produced by a liquid sparging aerosolizer-type bubbling generator, with particle sizes ranging from roughly 0.6 to 2 μm. The experimental setup included an electrostatic precipitator and real-time devices including an electrometer, aerodynamic particle sizer, and electrical low-pressure impactor. Experimental results obtained for various operating conditions showed that aerosols produced with a higher bubbling airflow contained a larger proportion of neutral particles (from around 30% to 50%) and that bacteria carried a greater average absolute number of elementary charges (from around −10 to −60 elementary units) than those under lower airflow. Under the investigated conditions, a neutralization step is unnecessary because it may have a negative effect on the viability of sensitive microorganisms. Our results suggest that the neutral fraction can be used downstream of an electrostatic precipitator, and that this setup may have advantages over bipolar neutralizers.

Introduction

Most aerosolization processes are likely to provide electrostatically charged particles, especially during the first few minutes after their dispersal. The number of electrical charges carried by airborne particles, including microorganisms and other airborne biological entities, can considerably modify their deposition in the lung, their collection efficiency on filters and other separation devices, their transport (e.g., deposition in sampling lines) and their behavior during sampling processes (Azhdarzadeh, Olfert, Vehring, & Finlay, 2014; Brockmann, 2011; Liu, Pui, Rubow, & Szymanski, 1985; Raynor, Leith, Lee, & Mukund, 2011; Yao & Mainelis, 2006).

There are limited data available describing the electrical charges carried by particles in workplace aerosols, and even less for bioaerosols. Lee et al. (2004) indicated that airborne microorganisms in a range of field environments were negatively charged. Consistent with this, Yao and Mainelis (2006) reported that airborne microorganisms encountered in indoor and outdoor environments carried either net negative or net positive charges. Results obtained by Shen, Wei, and Yao (2013) also showed that the positively and negatively charged culturable bacterial aerosol concentration and diversity varied with sampling environment. After dispersal in a field environment, the microorganisms gradually return to their natural charge state, which depends on their cell structure (Lee et al., 2004). Wei, Zou, and Yao (2014) estimated bioaerosol charge distributions for both indoor and outdoor environments. Their results revealed that, regardless of charge polarity, outdoor culturable bacterial aerosol charge levels became normally distributed with a peak around 21–29 elementary charge units, while the indoor ones were skewed toward 46–92 elementary charge units.

Biological particles are complex structures that are naturally electrically charged. For example, the cell surface of gram-negative bacteria contains ionizable amino, phosphate and carboxyl groups of proteins, and lipopolysaccharides are present in the outer membrane. Such structures contribute to the overall natural net negative electrical surface charge carried by this type of microorganism when airborne (Mainelis, Willeke, Baron, Grinshpun, & Reponen,

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2002b; Mainelis et al., 2001; Noyce & Hughes, 2002). Because of differences in shape and cell-wall structure, the number of electrical charges carried on outer-cell envelopes varies from strain to strain.

Previous experiments showed that laboratory-generated bioaerosols had a wide, bipolar electrical charge distribution (Mainelis et al., 2001, 2002a), and that microorganisms aerosolized under these conditions also carry an overall net negative electrical charge (Kim, Yoon, Park, & Hwang, 2011; Mainelis et al., 2002b). Cells in a liquid may carry thousands of elementary charge units (Sherbet & Lakshmi, 1973), so water-borne microorganisms are probably also electrically charged. For example, Mainelis et al. (2001) showed that some bacteria aerosolized using a nebulizer could carry more than 10,000 elementary charge units; however, less than 1% of the aerosolized bacteria were found to carry more than 2000 negative units. This was confirmed by Xie, Shen, and Yao (2011), who showed that most of the bacterial cells contained in a nebulized aerosol carry less than 200 elementary charge units. Mainelis et al. (2001) also showed that aerosolized bacterial cells carry higher levels of electrical charge than inert non-biological airborne particles (e.g. NaCl, which has less than 20 elementary charges). They suggested that the electrical charge carried by laboratory-generated bacteria consists of two components: their own natural charge, which can be high, and the charge imparted by the aerosolization process. Inert non-biological particles, in contrast, carry only the charges imparted by the dispersion process. Thus, the width of the electrical charge distribution and number of electrical charges carried by laboratory-generated bioaerosols partly depends on the aerosolization method. *Pseudomonas fluorescens* bacteria aerosolized by a bubbling generator carry fewer electrical charges than the same cells aerosolized by a Collison nebulizer (Mainelis et al., 2001). This is because liquid-disrupting forces and the contact-charging effect (mechanical friction between particles) in a bubbling generator are far weaker than those exerted by a nebulizer. In wet-dispersion methods, the electrical charges carried by airborne particles after droplet drying may also depend on the initial size of the droplets containing particles and the initial electrical charge of the droplets themselves (Forsyth, Liu, & Romay, 1998; Gu & Li, 1998). The precise roles of the different physical factors and charging mechanisms causing airborne microorganisms to become electrically charged have not yet been studied.

Because of these effects, freshly generated aerosols may require neutralization to standardize laboratory assays and improve reproducibility while avoiding possible bias in results. All neutralizer types (radioactive and non-radioactive) ionize the surrounding atmosphere by producing positive and negative ions; airborne particles carrying electrical charges can discharge by interacting with ions of opposite polarity. Once the charge state of particles has reached equilibrium, the aerosol shows a bipolar distribution described by the Boltzmann equation (Flagan, 2011). In the case of airborne microorganisms, high positive and negative electrical charges present on their membranes have been shown to affect the culturability or viability of sensitive organisms through physical or chemical processes (Fletcher et al., 2007; Kim et al., 2011; Lee, Hyun, Hwa Lee, & Hwang, 2014; Mainelis et al., 2002a, 2002b).

We previously developed, patented, and characterized the performance of a liquid sparging aerosolizer-type bioaerosol generator that disperses microorganisms by bubbling compressed air through a film of microbial suspension (Simon et al., 2011). This liquid-based gentle-bubbling aerosolization method minimizes stress and damage to microorganisms during aerosol generation. We hypothesized that the microorganisms generated by this generator would carry few electrical charges. If this proved true, a subsequent neutralization step, which is likely to have a negative effect on the viability of sensitive microorganisms, would not be necessary.

The main objective of this work is to measure the electrical charges on laboratory-generated airborne bacteria using our single-pass bubbling aerosolizer, which is a recognized device that causes less cell damage than a Collison nebulizer. The fraction of neutral particles and number of elementary charges per particle as a function of the aerodynamic diameter of the bacteria generated under different operating conditions are measured. Based on our measurements, the need for and feasibility of neutralizing the bacteria generated is discussed.

Materials and methods

Experimental setup and real-time devices

The experimental setup allows aerosolization of microorganisms from a liquid suspension, optional electrostatic precipitation, transport through a custom-built sampling vessel, and parallel real-time measurement of airborne particle properties, as shown in Fig. 1. The components of this setup are described below.

Generation, microbial strains and preparation of liquid bacterial cultures

The bubbling generator (Fig. 1) and all of the materials used for aerosol generation have been described elsewhere (Simon, Betelli, Koehler, Coulais, & Duquenne, 2013; Simon & Duquenne, 2013; Simon et al., 2011). A brief description and diagram of the generator are available in the online supplementary data (Section S1).

Escherichia coli (*E. coli*; CRBIP ATCC 8739) and *Enterococcus hirae* (*E. hirae*; CRBIP ATCC 10541) were the microorganisms investigated in this study as representative sensitive bacteria. *E. coli* is a rod-shaped gram-negative bacterium, ranging in diameter from 0.3 to 1.0 μm and in length from 1.0 to 6.0 μm. This bacterium is frequently used in laboratory-based bioaerosol assays and is present in the air in many indoor and occupational environments. The gram-positive bacterium *E. hirae* has spherical to ovoid cells, ranging from 0.5 to 1.0 μm in diameter. The protocol used to prepare liquid bacterial cultures has been fully described elsewhere (Simon & Duquenne, 2013; Simon et al., 2011) and is detailed in the online supplementary data (Section S2). The pH of liquid suspensions was measured before aerosol generation using a pH meter (Mettler-Toledo AG, SevenMultiTM S47, Viroflay, France), and was close to 6.6 for both model microorganisms. The liquid culture was fed into the generator by a peristaltic pump prior to aerosolization of bacteria (Fig. 1).

Electrostatic precipitator (ESP)

The electrostatic precipitator (ESP) was sized and manufactured at the Institut National de Recherche et de Sécurité (INRS), France. The ESP was composed of two concentric electrodes between which an electrical field was created by applying continuous voltage to the central electrode. This electrical field caused the more mobile charged particles to deviate from their trajectory and precipitate on the walls of the device. At the highest voltage applied (7.5 kV), only neutral particles exit the ESP.

Sampling vessel and real-time devices

The homogenization and sampling vessel was developed previously (Simon et al., 2011) to condition and sample the bioaerosol generated (Fig. 1). This 12-L sampling vessel includes a conical inlet (around 30-cm long) and cylindrical sampling zone (30-cm long and 20-cm in diameter) equipped with six sampling probes (length = 20 cm, i.d. = 10 mm) for simultaneous connection