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Predominance of single bacterial cells in composting bioaerosols

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HIGHLIGHTS

- Composting bioaerosols were collected by an Electrical Low Pressure Impactor.
- Bioaerosols analyzed by combining culture, flow cytometry and qPCR.
- Single bacteria were predominant in composting bioaerosols.
- Predominance of single cells is relevant in terms of dispersal.

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ABSTRACT

Bioaerosols emitted from composting plants have become an issue because of their potential harmful impact on public or workers' health. Accurate knowledge of the particle-size distribution in bioaerosols emitted from open-air composting facilities during operational activity is a requirement for improved modeling of air dispersal. In order to investigate the aerodynamic diameter of bacteria in composting bioaerosols this study used an Electrical Low Pressure Impactor for sampling and quantitative real-time PCR for quantification. Quantitative PCR results show that the size of bacteria peaked between 0.95 μm and 2.4 μm and that the geometric mean diameter of the bacteria was 1.3 μm. In addition, total microbial cells were counted by flow cytometry and revealed that these qPCR results corresponded to single whole bacteria. Finally, the enumeration of cultivable thermophilic microorganisms allowed us to set the upper size limit for fragments at an aerodynamic diameter of ~0.3 μm. Particle-size distributions of microbial groups previously used to monitor composting bioaerosols were also investigated. In collected the bioaerosols, the aerodynamic diameter of the actinomycetes *Saccharopolyspora rectivirgula*-and-relatives and also of the fungus *Aspergillus fumigatus*, appeared to be consistent with a majority of individual cells. Together, this study provides the first culture-independent data on particle-size distribution of composting bioaerosols and reveals that airborne single bacteria were emitted predominantly from open-air composting facilities.

1. Introduction

Bioaerosols are continuously released into the air as a consequence of wind-borne dust from soil, oceans and vegetation. A wide range of industrial activities are also a source of bioaerosols when processes involve the disturbance of biological materials. Bioaerosols have been linked to harmful effects on human health: airborne bacteria and moulds are important contributors to allergic or respiratory diseases in humans (Douwes, 2003; Simon-Nobbe et al., 2008). It is thus essential to determine the area impacted by such risk-bearing emissions. The size of biological particles, which can vary from 1 nm to approximately 100 μm, is a key parameter in defining their dynamics behavior and distances traveled (Després et al., 2012; Swan et al., 2003). Knowledge about the predominance of microbial cells as clumps or as unbound free

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entities is critical for modeling dispersion and improving risk assessment (Drew et al., 2006). Depending on particle size, bioaerosols have been viewed either as dropout particles or as a gas, given that particles with a diameter <3 μm essentially do not settle (Tellier, 2006). Models based on particles (aggregates) result in a more rapid drop-out of the concentrations. The particle-size distribution has also to be taken in consideration when evaluating sanitary risks. Particle size has a big influence on where, in the respiratory tract, deposition occurs and on the survival of the microbe in the aerosol (Darquenne, 2012; Thomas et al., 2008).

Bioaerosols generated at composting plants are released during processes that involve such vigorous movement of material such as shredding, compost pile turning or compost screening. The study reported here aimed to determine if airborne particles released from composting facilities are:

(i) single-cell bacteria ranging from 0.5 to 2 μm in diameter (Madelin and Johnson, 1992; Madsen et al., 2009; Reponen et al., 1998). In which case, bacteria could have a long atmospheric residence time and could be transported over long distances;
(ii) mainly large-sized particles composed by debris (compost or leaf fragments) which serve as "rafts" for microorganisms (Lighthart and Shaffer, 1995; Lighthart et al., 1993; Shaffer and Lighthart, 1997).

Studies on the particle-size distribution in bioaerosols emitted by composting facilities are rare (Byeon et al., 2008; Chiang et al., 2003). This one is the first presenting culture-independent data. To carry out the researches, composting bioaerosols were collected using an 11-stage electrical low pressure impactor and microbial cells were analyzed by quantitative PCR, flow cytometry and culture. Airborne microbe size distribution was investigated using qPCR on total bacteria and on two microbial groups: thermophilic actinomycetes (*Saccharopolyspora rectivirgula*-and-relatives) and *Aspergillus fumigatus,* both of which are usually monitored when studying emission and dispersal of bioaerosols from composting facilities (Albrecht et al., 2008; Le Goff et al., 2011; Millner et al., 1977; Neef and Kampfer, 2002).

2. Materials and methods

2.1. Air sampling

Air samples were collected outdoors at one composting plant in the South of France operating in the open air and treating green waste (30 kt/year). Air samples were collected during disturbance of the compost windrow. Sampling was done at 100 m and 50 m distance in two directions from the compost windrow, upwind and downwind respectively. Aerosols were sampled between the 10th and 20th of September 2012. Wind speed (m s^{-1}), temperature (°C), relative humidity and solar radiation (W m^{-2}) during sampling were recorded using a Prosensor weather station (Amanvillers, France). Temperature averaged between 22 and 26 °C and mean wind speeds between 0.5 and 3.5 m s^{-1} Table 1 shows the meteorological data, the type of operational activity and the distances from the activity for each sampling day.

2.2. Size distribution of total airborne particles and bioaerosols

The aerosols were monitored using an Optical Particle Counter (OPC) (Model 1.108, GRIMM Technologies, Inc, Germany). The Grimm OPC measures particles of ambient aerosols in the range of 0.3–20 μm. Particle counting and sizing were performed with a 15-channel real-time aerosol spectrometer using a light-scattering technology for single-particle counts. The flow rate was 1.2 L.min-1. The samplings by the GRIMM were performed on 8 different days at upwind and downwind during 90min (Table 1). Bioaerosols were collected through a 11-stages low pressure impactor (ELPI™ Electrical Low Pressure Impactor, Dekati Ltd., Tampere, Finland). The ELPI™ was used to determine the aerodynamic particle-size distribution; particles were collected in the different impactor stages according to their aerodynamic diameter (d_a). The ELPI™ operates at a flow-rate of 10 L min^{-1}. The sampling system collected the particles in the size range 3 nm – 10 μm with polyethersulfone filters (0.2 μm pore size, Ø 25 mm, Supor®200 Pall corporation). The collection filters were coated with glycerol to prevent particle bounce and the corona charger unit was switched off.

The 50% cut diameters for the 11 impactor stages, which denote the particle size at which 50% of the particles are collected, were 0.03, 0.05, 0.09, 0.15, 0.26, 0.38, 0.61, 0.95, 1.6, 2.4 and 4 μm. The samplings by the ELPI™ were performed on 3 different days at 50 m distance downwind during 90min (Table 1).

2.3. Calculation of the geometric mean and geometric standard deviation of aerodynamic diameters

The geometric mean of the aerodynamic diameter d_a, d_g, depends on the concentration of the bacterial 16S rRNA gene copies on the geometric midpoint of the interval (based on Yamamoto et al., 2012).

Table 1
Parameters of measurements for each sampling location.

Sampling location	Date of sampling	Activity	OPC (Grimm)	Impactor (Dekati)	mean wind speed (m/s)	mean solar radiation (W/m²)	mean temperature (°C)	mean relative humidity (%)
Upwind 130m	10-Sep-12	turning	√		2,7	591	25,3	61
Downwind 50 m	11-Sep-12	turning	√	√	2,5	674	25,6	59
Downwind 50 m	12-Sep-12	turning	√	√	4,8	472	23,6	48
Downwind 50 m	13-Sep-12	turning	√		3,6	719	22,1	30
Upwind 100m	17-Sep-12	screening	√		0,5	432	23,6	49
Downwind 50 m	18-Sep-12	turning	√	√	2,1	644	23,2	69
Downwind 50 m	19-Sep-12	turning	√		2,9	706	22,9	44
Downwind 50 m	20-Sep-12	piling	√		3,1	497	22,4	51