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New specific indicators for qPCR monitoring of airborne microorganisms emitted by composting plants

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ABSTRACT

Bioaerosols emitted from composting plants are an issue because of their potential harmful impact on public or workers' health. There is a major lack of knowledge concerning the dispersal of airborne microorganisms emitted by composting plants and the consequent potential exposure of nearby residents. This inadequate knowledge is partly due to the fact that there is currently no method for specifically tracing these microorganisms in the air. The objective of this study was to validate the use of microbial groups as indicators of composting bioaerosols by comparing their concentration in air samples, whether impacted by composting activity or not. Three potential microbial indicators were chosen among the core species of composting bioaerosols. They belong to the genus *Saccharopolyspora*, to the *Thermoactinomycetaceae* and to the fungus *Thermomyces*. Quantitative PCR systems using TaqMan probes were designed to quantify each of the three phylotypes in air samples collected outdoors in natural environments and at composting plants. Compost-turning operations at industrial plants resulted in an increase in the concentration of the three phylotypes of at least 2 orders of magnitude when compared to the concentration measured in control samples collected upwind, and of at least 1 order of magnitude compared to the background concentration measured in natural environments unaffected by industrial activity. In conclusion, these three thermophilic phylotypes can be used as indicators of airborne microorganisms emitted by industrial composting plants. They may be particularly relevant in studying the dispersal of bioaerosols around composting plants and the exposure of nearby residents. This is the first time that indicators of compost bioaerosols have been validated by comparing their concentrations in impacted samples to their background levels in natural environments.

1. Introduction

Bioaerosols generated at composting plants are emitted into the atmosphere during operational activities involving movement of the waste (waste delivery, shredding, compost pile turning and compost screening) (Sanchez-Monedero et al., 2005; Taha et al., 2006; Schlosser et al., 2009). These biological emissions (fungi, bacteria, actinomycetes, endotoxins and 1–3-β-glucans) from composting installations may have a potential impact on the health of workers and of residents living in close proximity (Herr et al., 2003; Swan et al., 2003; Sykes et al., 2007; Schlosser et al., 2009). If the effects on respiratory health of exposure to composting bioaerosols has been shown for workers (Bünger et al., 2000, 2007; Schlosser et al., 2009), the impacts on residents is still not well documented. This is partly due to the fact that indicators of exposure to composting bioaerosols have been lacking. In the same way, the lack of specific composting bioaerosol markers makes dispersal studies more difficult since it is not possible to distinguish aerosols produced by composting activities from others sources of bioaerosol present around the site.

The airborne microorganisms usually monitored in composting aerosols are cultivable bacteria or fungi (mesophilic and/or thermophilic), Gram-negative bacteria or more definite microbial taxons such as *Aspergillus fumigatus* and actinomycetes (Hryhorczuk et al., 2001; Sanchez-Monedero et al., 2005; Fischer et al., 2008; Schlosser et al., 2009; Pankhurst et al., 2011). These microbial groups are not specific to compost aerosols and are widespread in the environment. Furthermore, most studies on composting bioaerosols have been carried out using cultural methods. However, the culturability of bacteria occurring in the environment is generally low (Amann et al., 1995) and culture methods underestimate considerably the number of bacteria present in air samples (Peccia and Hernandez, 2006; Albrecht et al.,

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2007). Molecular techniques based on DNA analysis enable us to quantify non-cultivable bacteria. In particular, qPCR is sensitive, rapid and has been applied for quantifying microbial groups in various environmental matrices, including air (Stetzenbach et al., 2004).

In an earlier study, the microbial diversity in composting bioaerosols was characterized using molecular inventories (16S and 18S rDNA libraries) (Le Goff et al., 2010). This study showed the presence of core bacterial and fungal species common to bioaerosols collected at five different composting plants during the turning of composting piles in the thermophilic phase. Several criteria were then considered in order to select potential indicators of composting bioaerosols. The indicators should: a) be thermophilic, since thermophilic species multiply during the composting process but are not thought to occur naturally at high concentrations in air (Neef et al., 2003); b) be present in at least four of the five molecular inventories; c) have microbial relatives already identified in compost in previous studies. Finally, the indicator should be present in air during composting activities at a higher concentration than in natural environments not impacted by composting activity. In fact, a good indicator need not necessarily be absent from other environments but its background level should be several orders of magnitude below its concentration in emitted bioaerosols.

The objective of the present study was to define novel microbial indicators specific to compost bioaerosols and to design qPCR systems for each of them in order to be able to trace airborne microorganisms emitted by composting plants. Among the core species identified by Le Goff et al., three phylotypes (two bacterial and one fungal) were selected as potential indicators of composting bioaerosols because they met the first three criteria defined above. The two bacterial phylotypes belong to the genus *Saccharopolyspora* and to the *Thermoactinomycetaceae*. The fungal phylotype is related to *Thermomyces lanuginosus*. These three thermophilic microbial groups are recognized components of compost microflora, and have the closest SSU ribosomal DNA sequences present in public databases associated with the compost biotope.

In this paper, we first present the development of quantitative real-time PCR systems specific to each of these three phylotypes. Their relevance to monitoring bioaerosols specific to compost was then tested at real scale by comparing their concentration at composting plants during one emitting operation (the turning of composting piles) with both the concentration in upwind control samples and the background concentration observed in natural environments. To our knowledge, this is the first time that qPCR has been used to monitor composting bioaerosols. This also constitutes the first validation of specific indicators obtained by comparing levels in affected air samples to background concentrations observed in natural environments.

2. Materials and methods

2.1. Air sample collection and DNA extraction

Air samples were collected outdoors in two types of context: environments not affected by composting activity (natural environments); and composting plants. The natural ecosystems included coastal areas, woods, moor, private gardens and rural areas. The five composting plants studied were operating in open windrows and were treating different types of waste. For each composting plant, sampling was carried out on the same day at two locations: outside the plant (upwind) and close to a pile undergoing turning (downwind). Both samples were collected in the absence of any other emitting activity at the plant (no shredding, screening or (un)loading of waste) and the upwind sample was collected before the start of the turning. Table 1 gathers the characteristics of each compost pile which was manipulated (waste, age, temperature), the distance between the sampling point and the turning zone, as well as the meteorological conditions (temperature, moisture and wind speed) measured using a testo 445 (testo Inc, Sparta, USA). The samples collected during the turning operations were the same as those used for the 16S rDNA and 18S rDNA libraries from the study of Le Goff et al. (2010). The turning was performed on composting piles in thermophilic phase, either by a wheel loader or a windrow turning machine. The compost piles were in trapezoidal cross-sectional windrows with an average height of three meters. The size of compost piles at site B, treating solid municipal waste, was smaller (two meters on average) so several compost piles were turned to get enough biological material for PCR analysis. For this site, the waste was first put for two-three days in a rotating drum (DANO® drum tube), screened and then formed into piles.

Air samples were collected through the air biocollector Coriolis®μ (Bertin Technologies, Montigny-le-Bretonneux, France)

Table 1
Characteristics of compost windrows and meteorological conditions during air sampling.

Plant	Sample	Waste	Compost age (weeks)	Compost temperature (°C)	Distance to the turning (m)	Meteorological conditions		
						Wind speed ($m\,s^{-1}$)	Temperature (°C)	Moisture (%)
A	Upwind				175	1	21	60
	Downwind	Green waste	8	50	1–5	2	26	57
B	Upwind				50	1	18	100
	Downwind	Solid municipal waste	3–4[a]	56–67[a]	5–20	4	21	72
C	Upwind				40	3	20	78
	Downwind	Green waste and sludge	3	60	15	1	25	61
D	Upwind				80	2	2	100
	Downwind	Green waste and biowaste[b]	6	55	1–5	4	6	100
E	Upwind				100	1	18	100
	Downwind	Green waste and sludge	6	60	10	3	23	80

[a] Several compost windrows were turned.
[b] Industrial biowaste, manure, mycelium, fats, waste from the cosmetic industry.