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Analysis of airborne microbial communities using 16S ribosomal RNA: Potential bias due to air sampling stress

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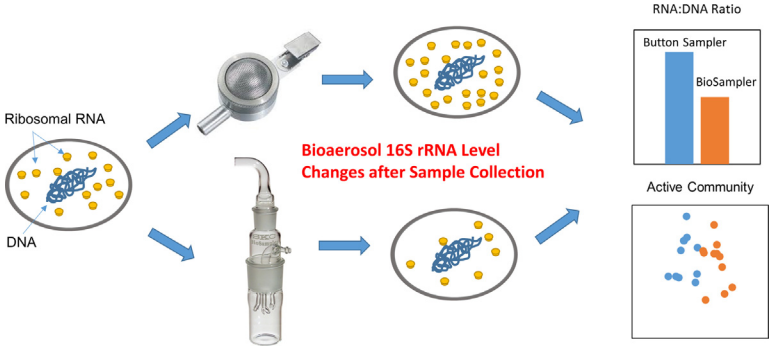
HIGHLIGHTS

- We investigated potential bias caused by air sampling stress on measurement and characterization of 16S rRNA for bioaerosols.
- Liquid impingement-based BioSampler recovered consistently less 16S rRNA than the filtration based Button sampler.
- The 16S rRNA sequences revealed a different bacterial community compared with 16S rRNA gene-based results.
- The difference between 16S rRNA and 16S rRNA gene-based sequencing results depended on the sampling device.
- A number of bacterial taxa were suggested as metabolically active microbes in airborne phase.

GRAPHICAL ABSTRACT



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ABSTRACT

A limited number of studies have been conducted to analyze ribosomal RNA (rRNA, present in the ribosome) in bioaerosol samples to identify currently or potentially active airborne microbes, although its genomic counterpart, the rRNA gene (on the chromosome) has been frequently targeted for airborne microbial community analysis. A knowledge gap still exists regarding whether the bioaerosol rRNA abundances are affected by the bioaerosol collection process. We investigated the effect of air sampling stress on the measurement and characterization of 16S rRNA for bioaerosols in the laboratory and field experiments using quantitative polymerase chain reaction (qPCR) and high-throughput sequencing techniques. In a laboratory study, known quantities of freshly grown *Escherichia coli* cells were spiked onto the filter of a Button Aerosol Sampler and into liquids of BioSampler and SpinCon air samplers and then exposed to sampling stress when the samplers were operated for 2 h. We found that the recovered cellular 16S rRNA abundance as determined by qPCR was dependent on sampler type. Further, two devices (Button Aerosol Sampler and BioSampler) that exhibited markedly different efficiency in preserving 16S rRNA were employed in an outdoor environment to collect bioaerosols simultaneously on eight days in two different seasons. The abundance of 16S rRNA in the outdoor air sample (1.3×10^6–4.9×10^7 copies/m^3) was about two orders of magnitude higher than that of 16S rRNA gene (6.9×10^3–1.5×10^5 copies/m^3). The 16S rRNA sequences revealed a different bacterial community compared with 16S rRNA gene-based results across all samples, and this difference depended on the sampling device. In addition, a number of bacterial taxa exhibited higher abundance in the 16S rRNA gene sequences than in 16S rRNA sequences, which suggests the potential activities of certain microbes in airborne phase. Overall, this study highlights the importance of sampling device selection when analyzing RNA in bioaerosols.

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1. Introduction

The ribosomal RNA (rRNA) gene (on the microbial chromosome), particularly the 16S rRNA gene of *Bacteria* and/or *Archaea*, has been widely utilized in environmental microbiology studies because it is ubiquitous and has low mutation rates throughout *Bacteria* and *Archaea* evolution (Woese, 1987), and it also encompasses hypervariable regions which can be used to distinguish between bacterial taxa (Van de Peer et al., 1996). However, microbial samples often contain 16S rRNA genes from dormant cells (Keer and Birch, 2003; Josephson et al., 1993) or lysed cells (England et al., 1998; Cai et al., 2006), thus precluding the use of 16S rRNA gene when investigating only active microbial community members. In contrast, the rRNA (as part of the ribosome) encoded by this gene is directly linked to cell physiology, e.g. the synthesis of rRNA is growth-rate dependent for a number of bacterial species (Kerkhof and Ward, 1993; Poulsen et al., 1993; Kemp et al., 1993). Thus, analysis of 16S rRNA sequences rather than rRNA gene sequences (DNA) can help reveal those community members who are or have recently been active within a complex microbial community (Pitkänen et al., 2013; Blazewicz et al., 2013). This approach has been employed to analyze microbial samples from water, soil, sediments, and biofilms (Pitkänen et al., 2013; Blazewicz et al., 2013; DeAngelis et al., 2011; Campbell et al., 2011; DeAngelis et al., 2010; Gentile et al., 2006; Yarwood et al., 2013; Perez-Osorio et al., 2010). However, there has been a limited number of studies on rRNA measurements and rRNA-based community analysis in airborne microorganisms (a.k.a. bioaerosols) (Krumins et al., 2014; Klein et al., 2016).

Since airborne biomass is relatively low in abundance compared to soil or water biomass (Lighthart, 2000; Bauer et al., 2003), bioaerosol sampling devices often have to be operated for long time periods which could cause stress on the already collected microorganisms as sample collection continues (Mainelis and Tabayoyong, 2010; Zhen et al., 2013; Wang et al., 2001). Thus, one major concern when studying rRNA in bioaerosols is whether the rRNA abundance in the collected cells remains unchanged during sampling. Although rRNA is a relatively stable RNA type, it could also exhibit variation within a cell under certain changes in environmental conditions (Deutscher, 2003). It has been reported that bacterial rRNA concentrations increase during early exponential growth of cells (Kerkhof and Ward, 1993) and rRNA degrades when cells experience depletion of nutrients and glucose starvation (Deutscher, 2003). A recent study showed that the 16S rRNA abundance in *Sphingomonas aerolata* aerosols in a rotating bioreactor increased when the bacteria were supplied with gaseous growth substrates (Krumins et al., 2014). Certain bacteria, e.g. *Lactococcus lactis* in a non-growth state displayed changes in rRNA content in response to heat shock (Hansen et al., 2001). Since air sampling processes such as impaction, impingement, and desiccation can significantly alter the physiological status of collected bioaerosols, including the loss of viability and impaired cell membrane integrity (Mainelis and Tabayoyong, 2010; Zhen et al., 2013; Wang et al., 2001; Chang and Chou, 2011), it is possible that air sampling also affects the abundance of rRNA in the samples and introduces bias to rRNA-based sample analysis. Thus, if rRNA is to be used to analyze airborne microbial communities, it is important to determine how the sampling process, i.e., stress due to sampling, affects the rRNA of bioaerosol samples and whether the magnitude of the effect depends on a particular bioaerosol collection method or device.

Historically, the growth or metabolic activity of particular bacterial taxa has been investigated by measuring the change in 16S rRNA:16S rRNA gene ratio (Kerkhof and Ward, 1993; Poulsen et al., 1993; Kemp et al., 1993; Rosset et al., 1966). Subsequently, 16S rRNA sequence analysis has been used to identify potentially active members within microbial populations from complex microbial samples (Blazewicz et al., 2013; DeAngelis et al., 2011; Campbell et al., 2011; DeAngelis et al., 2010; Gentile et al., 2006; Yarwood et al., 2013). However, if the sampling process itself, e.g. sampling stress, leads to the increase or decrease in rRNA content of specific bacterial taxa, then their relative abundance within a complex bacterial community could be either overestimated or underestimated. Because the different particle capturing mechanisms of various sampler designs may cause differing effects on cellular rRNA, this potential effect on the sequence abundance of active microbial community members may be air sampling device-dependent.

The objectives of this study were: 1) to study whether the rRNA content of bioaerosol samples changes due to the air sampling process itself, i.e., sampling stress; 2) to assess whether this effect of sampling stress on bioaerosol rRNA is device-dependent; 3) to investigate how this effect impacts the analysis of 16S rRNA sequences from bioaerosols collected in an outdoor environment. First, we investigated the change in 16S rRNA:16S rRNA gene ratio of *Escherichia coli* in response to two-hour aerosol sampling using three different bioaerosol samplers in a laboratory. In the second part of the study, we analyzed microbial communities simultaneously collected from the outdoor air by the same three devices on eight different days in summer and late winter/early spring. The microbial communities represented in the 16S rRNA gene and 16S rRNA were analyzed by pyrosequencing. To the best of our knowledge, this is the first study to investigate the potential effect (bias) of sampling stress on the quantification and characterization of 16S rRNA from bioaerosol samples.

2. Materials and methods

2.1. Bacterial culture in laboratory experiments

A Gram-negative bacterium *E. coli* (ATCC 15597, Manassas, VA) was selected as a test microorganism. This organism has been used as a model microorganism in many bioaerosol studies (Zhen et al., 2013; Chang and Chou, 2011; Hospodsky et al., 2010). The procedures for preparation of *E. coli* suspension were described elsewhere (Zhen et al., 2015) and details are provided in Supplementary Information. Briefly, *E. coli* was precultured in Tryptic Soy broth, harvested by centrifugation, and resuspended in 1 × phosphate-buffered saline (PBS) solution prior to experiments.

2.2. Bioaerosol samplers

A Button Aerosol Sampler (SKC Inc., Eighty Four, PA), later referred to as Button sampler, a BioSampler (SKC Inc.), and a SpinCon wet cyclone (PAS 450-10A, InnovaPrep LLC., Drexel, MO) were used in this study to collect bioaerosols. The Button sampler is a filter-based sampler, and it was used with a 0.8-μm-pore-size polyethersulfone (PES) membrane filter (SUPOR, Pall Life Sciences, Port Washington, NY). Its nominal flow rate is 4 l/min (Aizenberg et al., 2000), but here the sampler was operated at a flow rate of 18 l/min to exacerbate a potential effect of filtration stress on bacterial cells. The two other devices are liquid-based bioaerosol samplers and collect airborne particles by a combination of liquid impingement and cyclonic action. 1 × PBS solution was used as collection fluid for these two devices. The SKC BioSampler with a 5-ml collection cup was operated at its design flow rate of 12.5 l/min. The BioSampler cup was refilled with Milli-Q water approximately every 15 min to replenish the fluid evaporated during its operation. The SpinCon air sampler was operated at a flow rate of 450 l/min and the total sample volume of approximately 10 ml; the device automatically maintains liquid level during its operation from a reservoir of sterile water.

2.3. Experimental procedure

In order to simulate an environment where bacterial cells were continuously exposed to sampling stresses after their initial collection, each device was spiked with a known amount of bacteria and then operated for 2 h at a room temperature by aspirating particle-free air inside a disinfected class II biosafety cabinet (NuAire Inc., Plymouth, MN). The

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