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Note

High fidelity recovery of airborne microbial genetic materials by direct condensation capture into genomic preservatives

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ABSTRACT

A condensation growth tube was adapted to capture bacterial bioaerosols directly into genomic preservatives. As judged by quantitative PCR and direct microscopy, bioaerosol condensation capture conserves airborne microbes' genomes as they exist in the atmospheric environment. This method circumvents the collection stresses bioaerosols experience on air filters, impactors and impingers.

Over the last decade, the aerobiology field has expanded in response to the broadening availability of high-throughput DNA sequencing (Mensah-Attipoe et al. 2017; National Academies of Sciences, Engineering, 2017; Peccia and Hernandez 2006; Peccia and Kwan 2016). The most common approach to characterize airborne microbiomes is to extract DNA from composite particulate matter samples collected on filters or in impactors. This genetic material is amplified and sequenced on semi-automated platforms. Because of the low biomass levels in the atmospheric environment, conventional filter samplers must collect airborne particulate matter for many hours in order to retain the levels of genetic materials required for successful high-throughput sequencing (Adams et al. 2015). Filtration and impaction stresses introduce uncertainties regarding the fidelity of the collected airborne DNA (or RNA).

The physical collection stresses associated with filter collection of bioaerosols, are significant and well documented (Henningson et al. 1997; Macher 1997; Zhen et al. 2018). How these unavoidable sampling stresses manifest in genetic material damage depends on sampling duration, environmental conditions, and sampler type. Despite the potential for imparting physiologic damage to airborne microbes, conventional filtration remains the most widely used aerosol collection method for airborne microbiome surveys. In response, a method that uses humidity to collect airborne microbes from the atmospheric environment was adapted to preserve genetic materials as they exist in aerosols.

In-situ condensation through a laminar-flow growth tube, can be engineered to collect airborne particulate matter with high efficiency (Eiguren-Fernandez et al. 2014). This collection approach preserves microbe physiology by minimizing sample stress through a scenario that includes terminal particle capture directly into genomic preservatives. We demonstrate here, how direct condensation capture can preserve the physiology of bacteria as they are recovered from an airborne state by aerosolizing known quantities of active, pure bacterial cultures into a large particle-free chamber. Deposition rates of these airborne bacteria were independently assessed by juxtaposing aerosol cytometry against time-resolved microscopy and quantitative polymerase chain reaction (qPCR) of cells recovered from the terminal collection reservoirs of condensation growth tubes (CGT) continuously drawing this chamber's air.

Bacillus subtilis (ATCC® 23857™) cells were cultured in 100 mL tryptic soy broth media (TSB) (Hach Company, Loveland, CO) at 37 °C and agitated on an orbital shaker at 180 rpm until late exponential phase (OD_{600} = 0.875 corresponding to a culture concentration of 10^7 cells/mL). Pure cultures were centrifuged at 3,000 × *g* for 5 min, the spent TSB media disposed, and bacteria resuspended in 12 mL of sterile 0.9% NaCl at 37 °C. These washed bacterial cells were immediately introduced into a 6-jet Collison Nebulizer (Mesa Laboratories, Butler, NJ) and aerosolized at 20 psi directly into an 11 m^3 environmental chamber that was prepared with a HEPA filtered atmosphere, maintained at 22 °C and 30% RH. When a concentration of ~10^8 cells/m^3 was achieved in the chamber (defined as $t = 0$), nebulization ceased. Two CGT samplers (Spot Sampler™ particle collector, Aerosol Devices, Fort Collins, CO) were then engaged to collect the aerosol from the environmental chamber in prescribed time-frames (< 3 h). These samplers collect airborne bacteria by creating a region of supersaturation as they travel through a temperature controlled "growth tube" (Fig. 1), such that the cells serve as condensation nuclei that are rapidly engulfed in microdroplets and subsequently collected into liquid preservative or quartz filters (Pallflex® Tissuquartz™, Pall Corporation, New York, NY) saturated with the same. A fluorescence

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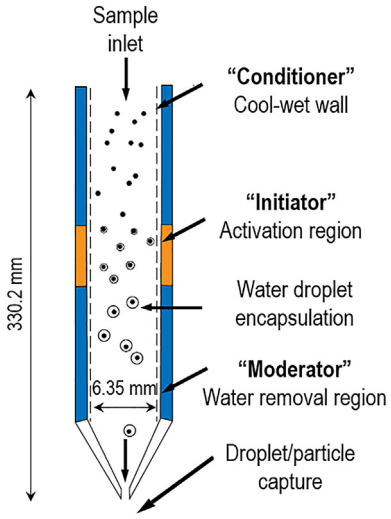

Fig. 1. Condensation growth tube: three-stage humidity condensation system. Particles not to scale.

bioaerosol cytometer (InstaScope, Boulder, CO) was used to monitor, in real time, the bacterial bioaerosols contained in the chamber during the aerosolization and collection periods as previously described (Hernandez et al. 2016).

Three different experimental scenarios were executed by collecting the airborne bacteria from the environmental chamber using these Spot Samplers: 1) Bioaerosol deposition velocity assessment, 2) 16S rRNA gene copy recovery with solid capture collection; and, 3) 16S rRNA gene copy recovery with CGT engagement. Each experiment was replicated a minimum of three times.

Bacterial deposition velocities were experimentally determined from time-series observations of bacterial bioaerosols directed through the CGTs, into the Spot Samplers' liquid collection reservoirs that respectively contained either phosphate buffered saline (PBS), or a genetic preservative (DNA/RNA Shield™, Zymo Research, Irvine, CA). Airborne bacteria, collected in PBS, were immediately assessed by direct epi-fluorescent microscopy using a widely accepted 4′6-diamidino-2-phynylindole (DAPI) staining protocol, adapted for bioaerosol enumeration (Hernandez et al. 1999). DNA was extracted from samples collected in genomic preservative with a ZymoBIOMICS DNA miniprep kit (Zymo Research, Irvine, CA), where 400 µL of supernatant was used according to manufacturer's instructions. The specific content of the 16S rRNA target gene region was then determined by quantitative PCR of these extracts, using the manufacturer's thresholds and customary positive and negative controls (Hospodsky et al. 2010) (QuantStudio 3, Thermo Fisher Scientific, Waltham, MA).

As judged by the recovery of 16s rRNA gene copies, bioaerosols collected directly into liquid preservative were compared to those collected on filters saturated with the same preservative, under otherwise identical conditions. This parallel series of experiments were executed in the environmental chamber with the format described above (i.e., time-series, chamber operation, bacterial preparation and aerosolization), except that two Spot Samplers were co-located and operated in parallel —such that one CGT directed its condensed particle stream into liquid preservative and the other onto preservative saturated filters (5.6 mm quartz).

Additionally, CGT performance was isolated to demonstrate the effects of condensation capture on the efficacy of DNA collection as judged by 16S rRNA gene copies. Under this scenario, the same experimental setup described above, was employed where Spot Samplers were again co-located and operated in parallel; however, only one of them had its growth tube engaged in condensation mode during the sampling period(s).

Fig. 2A presents a typical comparison of real-time cytometric fluorescent particle counts gated for bacteria, against the whole bacterial cells numbers and 16S rRNA gene copies recovered from the CGT reservoirs. Fig. 2B shows good agreement between bacterial bioaerosol deposition rates determined by these independent methods, as judged by their variance after five replicated chamber experiments. There were no significant differences with respect to 16S rRNA gene copy recovery from otherwise identical CGT operations followed by liquid collection or saturated filter collection (Fig. 3A). Growth tube engagement (GT) shows significantly higher recovery of 16S rRNA gene copies than an otherwise identical unit operating without its condensation mechanism engaged (NGT) (Fig. 3B).

CGTs are high efficiency collectors that concentrate airborne

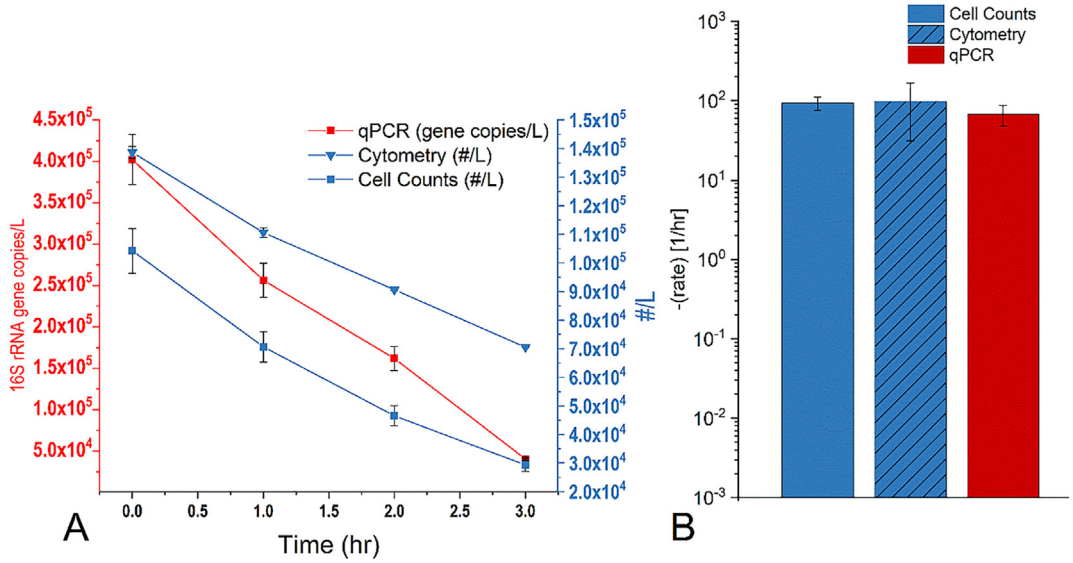

Fig. 2. A. Typical time-series recovery of airborne *Bacillus subtilis* cells from the contents of a CGT (Condensation Growth Tube) reservoir as judged by direct microscopy (–■–) and quantitative PCR (–■–). Aerosol collected by the CGT at the different time points were compared to real-time observations of airborne bacteria using a fluorescence aerosol cytometry (–▲–) during the same collection period. B. Deposition rates (hr^{-1}) using liquid condensation capture: Cell counts as judged by direct microscopy (■), 16S rRNA gene copies (qPCR) (■) and deposition rate for fluorescent cell counts as judged by cytometry (■). Bar height represents average deposition rate calculated from five independent trials; error bars represents one standard deviation.

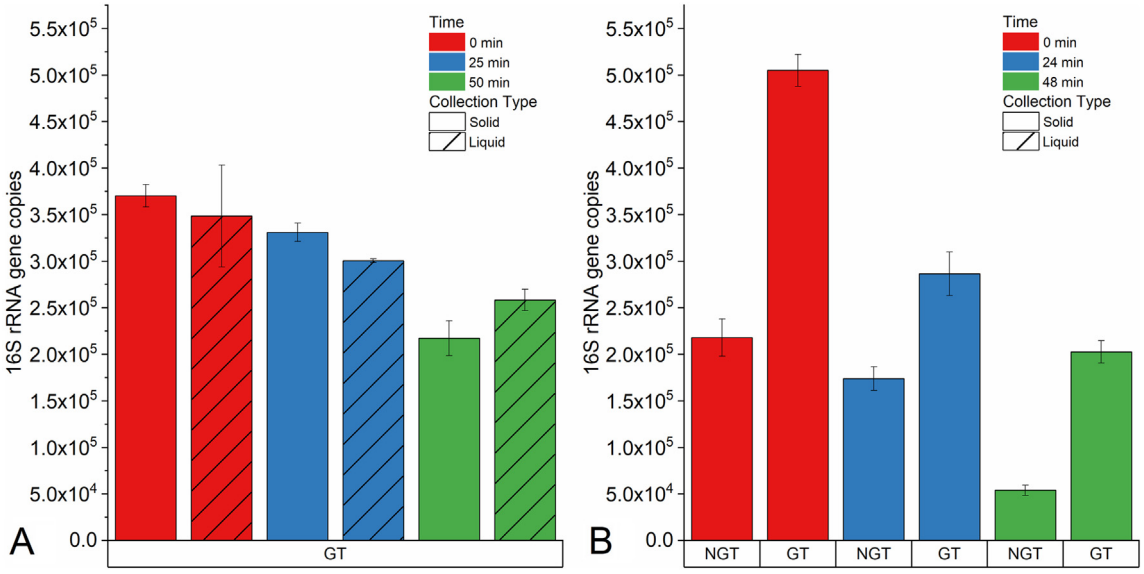

Fig. 3. Time-series recovery of Bacillus subtilis 16 s rRNA gene copies recovered following CGT Condensation Growth collection under the following conditions: A. Comparison of solid (filter) vs. liquid collection with condensation growth tube engaged (GT) for t = 0, 25 and 50 min; B. Comparison of filter collection with (GT) and without (NGT) condensation growth tube engaged for t = 0, 24 and 48 min. Bar heights represent averaged recovery from three independent trials; error bars represent one standard deviation.

particles in very small liquid volumes (500 µL); the CGT in this demonstration study was limited to 1.5 L/min airflow; CGT devices are easily sterilized and volumetrically scalable. Given bioaerosols are concentrated above the detection limits of modern DNA sequencing platforms (c.a. 20 ng), this new CGT collection format is immediately compatible with high-throughput sequencing. These results suggest that terminal CGT capture in genomic preservatives can be leveraged for aerobiological analyses in ways that conventional aerosol collection methods cannot, and thus provide improved confidence in the accuracy of atmospheric microbiome surveys.

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