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ORIGINAL PAPER

Collection of airborne bacteria and yeast through water-based condensational growth

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Abstract One limitation in air sampling of airborne microorganisms is their inactivation by forceful impaction and/or dehydration during the collection process. Proper inhalation risk assessments require proof of viability, as non-viable microorganisms cannot cause infectious diseases. In this study, laboratory-generated aerosols of a vegetative bacterium (*E. coli*) or yeast (*S. kudriavzevii*) were collected by a laminar-flow water-based condensational "growth tube collector (GTC)," and the GTC's collection efficiencies were compared with those using an industry standard BioSampler. Collection efficiencies resulting from two types of collection media, phosphate-buffered saline (PBS) and nutrient media (Nutrient Broth, NB, for *E. coli,* and Yeast Tryptone Glucose Broth, YTGB, for *S. kudriavzevii*) were also assessed. Both the GTC and the BioSampler performed equally when PBS was used as the collection medium for *E. coli*, whereas more viable *E. coli* cells were collected in the GTC than the BioSampler with NB. For *S. kudriavzevii,* the GTC outperformed the BioSampler using either PBS or YTGB. This is likely because aerosolized *E. coli* cells can better survive impaction than *S. kudriavzevii* under the conditions used, and the BioSampler has a much higher collection efficiency for particles in the size range of single-celled *E. coli* than *S. kudriavzevii*. Moreover, the GTC had a detection limit one order of magnitude lower for yeast aerosols compared with that of the BioSampler. These results indicate that the GTC is a promising device for sampling viable aerosolized gram-negative bacteria and yeast, as it is less damaging to these types of microorganisms during the collection process.

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1 Introduction

Inhalation of aerosolized pathogenic microorganisms (bacteria, fungi, viruses) is a well-known route for acquiring respiratory and other infections. Indeed, inhalation of bacteria, such as *Streptococcus pyogenes* (the major agent of streptococcal pharyngitis) and *Streptococcus pneumoniae* (a major agent of bacterial pneumonia), can lead to the development of severe infections. Furthermore, some bacteria such as *Bacillus anthracis* (which causes anthrax) have been weaponized into bioweapons that when dispersed as aerosols in high population areas can lead to mass fatalities (Burton et al. 2007; CDC 2006). Less understood are the potential health hazards posed by aerosolized yeast cells. For example, inhalation of certain *Candida* and *Sporobolomyces* spp. can lead to human allergies (Burge 1985). A variety of bioaerosol samplers based on impingement, filtration, and electrostatic precipitation have previously been tested and utilized for the collection of airborne bacteria and fungi (Dungan and Leytem 2009; Engelhart et al. 2007; Prussin et al. 2016). The widely used Andersen Cascade Impactor (ACI) and the electrostatic precipitator (ESP) can collect aerosols of bacteria and fungi on agar or liquid media, but they are only appropriate for short-term sampling (Andersen 1958; Thorne et al. 1992). Noteworthy, deactivation of infectious microbes due to the high impaction force of the Anderson Impactor and ozone formation in the ESP are deleterious to microorganisms (Buttner and Stetzenbach 1991; Castle et al. 1969; Stewart et al. 1995).

The AGI-30 and the BioSampler are liquid-based impingers that have been widely used for bioaerosol sampling, as the liquid collection medium used in those samplers makes it convenient for the enumeration of microorganisms or for downstream usage in conjunction with modern analytical methods (Willeke et al. 1998; Xu et al. 2011). Ding and Wang (2001) tested the AGI-30 for the collection of *E. coli* aerosols, and found that the collection efficiency of the system was less than 70% at the recommended flowrate of 12.5 L/min. The more popularly used commercially available BioSampler has been used as a reference sampler for many bioaerosol studies (Dybwad et al. 2014; Fennelly et al. 2015). The BioSampler is an improvement over the AGI-30; its performance is better as it minimizes the effects of particle bounce and reaerosolization, and its use allows for extended sampling time periods (Lin et al. 1999; Willeke et al. 1998).

Though the BioSampler has clear advantages over the AGI-30, it has proven to be inefficient for the collection of particles smaller than 300 nm and particles larger than 5 µm. The physical collection efficiency is less than 10% for particles smaller than 100 nm due to ineffective impaction for nanoparticles, and less than 80% for particles larger than 5 µm due to inlet loss (Hogan et al. 2005; Lin et al. 1999). In addition, many microorganisms are subject to killing during the impaction process due to high impaction forces (Li et al. 1999). Even though the AGI-30 and the BioSampler are widely used samplers, both can damage or kill aerosolized vegetative bacteria such as *E. coli*, *B. globigii* and *P. fluorescens* during their collection due to either sonic jet velocity of the collection air flows or impaction of the bacteria onto their glass walls (Kassab 2009; Lin et al. 2000).

In order to increase collection efficiencies, and allow longer sampling periods, different collection media have been recommended. Liquids with similar viscosity, like phosphate-buffered saline (PBS), are recommended for short-term air sampling of aerosolized bacteria and fungus spores, mycelia, or yeast by the impingement methods, while non-evaporating liquids like mineral oil are suggested for long-term sampling (Dungan and Leytem 2016; Li et al. 1999; Lin et al. 2000). However, air samples collected with mineral oil are difficult to analyze and require post-sampling process, as both molecular analytical and culture methods require aqueous solutions (Xu et al. 2011). Several studies have been conducted to study the effect that the nature of the collection media has on the recoveries of living microorganisms in the BioSampler. Dungan and Leytem (2016) showed that the recoveries of culturable *E. coli* were substantially greater with phosphate-buffered saline (PBS) as collection media, compared with deionized (DI) water, and the incorporation of peptone, antifoam B, and betaine would increase the recoveries for long-term sampling. DI water was shown to be superior to Tween mixture for the recoveries of viable *L. pneumophila* due to decreased bounce and reaerosolization

(Zhen et al. 2013). Damage caused by different collection liquids varies. Thus, different collection fluids used in a liquid-based sampler can influence the collection efficiency of viable bacteria

Filter-based samplers have also been widely used for sampling aerosolized bacteria and fungi in epidemiological studies (Durand et al. 2002; Uhrbrand et al. 2017). By comparing the particle concentrations upstream and downstream of filters, the physical collection efficiencies of polytetrafluoroethylene and gelatin filters for *Bacillus atrophaeus* endospores were shown to be more than 93% (Burton et al. 2007). However, filter-based samples are more suitable for molecular analysis but less adequate for assessing infectivity; desiccation, extraction and post-sampling processes can significantly deactivate a large fraction of the collected microorganisms (Burton et al. 2005; Tseng and Li 2005). Moreover, a study by Agranovski et al. (2002) showed that different microorganisms responded to stress differently and thus collection efficiency of different microorganisms with the same sampler varies considerably. In this study, the collection efficiency of stress sensitive *P. fluorescens* bacteria was 61%, but for the stress resistant *B. subtilis* bacteria and *A. verscicolor* fungal spores collection efficiencies were 95 and 97% (Agranovski et al. 2002), respectively. Indeed, many factors need to be considered in selecting a proper bioaerosol sampler to avoid inaccurate results, including the sensitivity of the microorganisms, collection media, sampling flow, and sampling time (Kesavan and Sagripanti 2015; Li et al. 1999). Considering the various limitations described above for existing bioaerosol samplers, development efforts for new samplers are needed to improve our capability in collecting viable bioaerosols reliably. Such capabilities are critically important in enabling our better understanding of transmission mechanisms and infectivity of bioaerosols.

In this study, a laminar-flow water-based condensational "growth tube collector (GTC)" was evaluated for the collection of viable bacteria and yeast. The GTC mimics what happens in human lungs on a cold day by introducing cold aerosol particles into a warm growth tube saturated with water vapor. The process encapsulates small particles into larger droplets, thus enabling efficient collection of these enlarged particles through gentle impaction (Hering and Stolzenburg 2005; Hering et al. 2005). It has already been demonstrated that the GTC is efficient at the collection of labortory-generated aerosols of bacteriophage MS2 and influenza viruses, and airborne viruses in a student infirmary (Lednicky et al. 2016; Pan et al. 2016, 2017). For MS2 containing particles that are smaller than the cutoff size of the BioSampler, the collection efficiency of the GTC is more than 10 times higher than the BioSampler (Pan et al. 2016). For laboratory-generated H1N1 influenza virus-containing particles that are larger than the cutoff size of the BioSampler, the GTC's collection efficiency was more than 74%; in comparison, the BioSampler collected less than 10% of aerosolized H1N1 (Lednicky et al. 2016). Although theoretically the GTC is also expected to be very efficient for the collection of viable bacteria and yeast, there exists no verification yet.

The objectives of this study were to determine whether the GTC could provide a new tool for the efficient collection of airborne bacteria and fungi, and compare its performance with the industry standard, the SKC BioSampler. Two microorganisms were selected, vegetative gram-negative bacteria and vegetative yeast, due to their difference in responding to environmental stress. Three different collection fluids were also studied to evaluate how the nature of the collection media may affect the viability of the collected microorganisms.

2 Materials and methods

2.1 Bioaerosol samplers

The water–vapor based GTC tested in this study consists of 8 parallel growth tubes with a total flow rate of 8 L per minute (LPM). The GTC's operating mechanism was described previously (Pan et al. 2016): briefly, incoming particles first get cooled in the conditioner that is maintained at 6 °C, and then get amplified to droplets in the micron range in the initiator kept at 45 °C. To minimize surface disruption and impaction damage, the amplified particles are delivered through 32 nozzles to a 35 mm Petri dish that holds the collection medium.

The BioSampler (SKC Inc., Eighty-Four, PA, USA) is an impinger wherein incoming airborne particles moving in a swirling motion are captured through contact with swirling liquid collection medium. The SKC's recommended flowrate for the BioSampler is 12.5 LPM, at which flowrate the cutoff

size (50% efficiency) of the BioSampler is about 300 nm (Willeke et al. 1998).

2.2 Test microorganisms

Overall, the biological properties such as survivability upon impaction of aerosolized *E. coli* are dependent on both the particular strain used for experimentation and the culture method (Griffiths et al. 1996; Li et al. 1999). For this work, *Escherichia coli* strain K-12 (Cat. #15597), obtained from the American Type Culture Collection (ATCC, Manassas, VA), was used. *E. coli* is a Gram-negative rod-shaped bacterium that is approximately 0.5–1.5 μm in width and 2–6 μm in length (Choi et al. 2015; Tille 2013; Willey et al. 2009). To initiate cultivation, a loopful of lyophilized *E. coli* was inoculated onto Difco Nutrient Agar (Sigma–Aldrich, Inc., St. Louis, MO, USA), then incubated overnight at 35 °C. Bacteria growing on the plate were thereafter streaked for isolation. An *E. coli* suspension was next prepared by inoculating a loopful of bacteria from an isolated colony into 50 mL of sterile Difco Nutrient Broth (NB, Sigma–Aldrich, Inc., St. Louis, MO, USA), and the flask incubated overnight at 37 °C before use (Shiloach and Fass 2005).

Saccharomyces kudriavzevii (ATCC Cat. #2601™) is an ellipsoid-shaped yeast with a diameter of 5–6 μm in the *Saccharomyces* sensu stricto complex (Naumov et al. 2000). It has been widely used in the production of alcoholic beverages (Stribny et al. 2015). Stress sensitivities of *Saccharomyces* species also vary (Kvitek et al. 2008). To initiate cultivation of the *S. kudriavzevii,* a loopful of lyophilized yeast was aseptically inoculated onto Yeast Tryptone Glucose Agar (YTGA, Sigma-Aldrich, Inc., St. Louis, MO, USA). After incubation at 27 °C for 48 h, the yeast was streaked for isolation, then 48 h later, an isolated colony was incubated for 48 h at 27 °C before use.

2.3 Experimental system and procedure

A schematic diagram of the bioaerosol testing system that was used for the collection of aerosolized *E. coli* and *S. kudriavzevii* is shown in Fig. 1. *E. coli* or *S. kudriavzevii* aerosols were generated using a six-jet Collision nebulizer (Model CN25, BGI Inc., Waltham, MA) operated at 3.5 LPM for *E. coli* and 4 LPM for *S. kudriavzevii.* These conditions were chosen to reduce frothing. The aerosols produced were then sent through a dilution dryer to eliminate the excess water content in the aerosols. Afterwards, the aerosols were split into three flows: one for the GTC, one for the BioSampler, and one for an Optical Particle Counter (OPC, Model 1.108, GRIMM Technologies Inc.) A relative humidity (RH) sensor (Model HX94C, OMEGA Engineering Inc., Stamford, CT) was used to measure the RH of the aerosols before they entered the samplers. The RH was maintained at 38 ± 5% during the experiment. The GTC was operated at a flow rate of 8 LPM according to previous studies (Pan et al. 2017), while the BioSampler was operated at the manufacturer recommended flow rate of 12.5 LPM. Exhaust air flows were passed through HEPA filters before release into a biosafety cabinet. A sampling time of 15 min was used for all collections for easy comparison between the GTC and the BioSampler. The OPC, which measures particles larger than 500 nm up to 32 μm, was used to measure the count size distribution of the microorganisms after they passed through the dilution dryer.

The *E. coli* suspension in the Collison Nebulizer was prepared by aseptically transferring 5 mL of *E. coli* stock suspension to 35 mL of sterile phosphate-buffered saline (PBS, no Mg^{2+} or Ca^{2+}), and vortexed for 20 s before use. For the *S. kudriavzevii* suspension, the yeast was first grown as a lawn on a 100 mm Petri dish of Nutrient Agar at 27 °C for 48 h. Afterward, the *S. kudriavzevii* lawn was gently scraped off the agar surface and transferred to 40 mL of sterile PBS solution using an inoculation loop. The sample was then vortexed for 20 s prior to use.

2.4 Viability assessment of microorganisms collected on different media

The following collection media were evaluated: (1) PBS for both *E. coli* and *S. kudriavzevii*; (2) Nutrient Broth (NB) for *E. coli*; (3) and Yeast Tryptone Glucose (YTG) broth for *S. kudriavzevii.* PBS is the collection liquid recommended by SKC Inc. for bioaerosol collection because it is non-nutritive and thus replication is inhibited in the collection medium, and that is important for accurate enumeration, and it diminishes cell killing by providing isotonic conditions. NB and YTG are culture media for *E. coli* and *S. kudriavzevii*, respectively. The PBS solution was prepared by diluting 10X PBS stock solution (Fisher Scientific) to 1X using sterile Deionized (DI) Water.

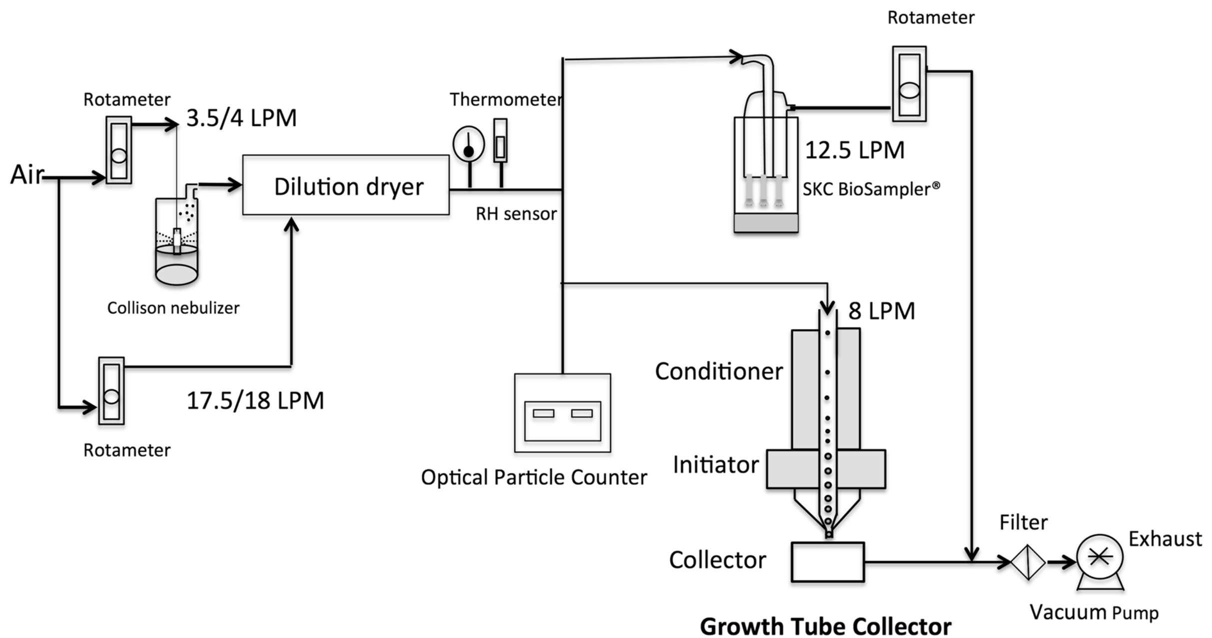

Fig. 1 Schematic diagram of the Bioaerosol testing system

NB was prepared according to the manufacturer's instructions by dissolving 30 g of the Difco Nutrient Broth powder into 1 L DI water. The YTG solution was composed of 1 g yeast extract, 1 g tryptone, and 1 g glucose in 100 mL DI water. All the collection liquids were autoclaved at 120 °C for 20 min at a pressure of 15 psi. Twenty mL of collection liquid was used for the BioSampler, and 1.5 mL for the GTC.

Immediately after the sampling, serial dilutions were performed with the same liquid for collection, and 0.1 mL aliquots directly spread over Petri dishes containing MacConkey agar (Fisher Scientific) for *E. coli* and Yeast Tryptone Glucose Agar (YTGA) with 100 mg/L ampicillin (Sigma-Aldrich, Inc., St. Louis, MO, USA) for *S. kudriavzevii*. Afterwards, the Petri dishes were incubated for 24 h at 37 °C for *E. coli* and at 27 °C for *S. kudriavzevii, respectively*. To test the effects of temperature on the recovery of potentially damaged *E. coli*, additional 30 min storage of the collected *E. coli* by the BioSampler at different temperatures (25 ± 2, 38 ± 2 °C) were performed before serial dilutions.

The efficiency of the GTC compared to the BioSampler was determined using the following Eq. (1):

$$\text{Performance} = \frac{C_{\text{GTC}} \times V_{\text{GTC}}/(Q_{\text{GTC}} \times t)}{C_{\text{BioSampler}} \times V_{\text{BioSampler}}/(Q_{\text{BioSampler}} \times t)} \quad (1)$$

where Performance is the ratio of the collection efficiency of the GTC to the BioSampler, C (CFU/mL) is the microorganism titer in the collection sampler over sampling time t, V (mL) is the liquid volume in the collection sampler over time t, Q (L/min) is the aerosol flow rate, and subscripts GTC and BioSampler stand for the respective sampler. A t test was used to compare the results of Performance values obtained for different collection media, while a Chi squared test was used to compare the experimental data with the expected results.

3 Results and discussion

3.1 Aerosol Sizes of Nebulized *E. coli* and *S. kudriavzevii*

Aerosol size distributions measured at the outlet of the dilution dryer, with *E. coli* and *S. kudriavzevii* in PBS solution for aerosolization, are shown in Fig. 2a. Particle size distributions of aerosolized DI water and PBS solution alone (i.e., without the microorganisms)

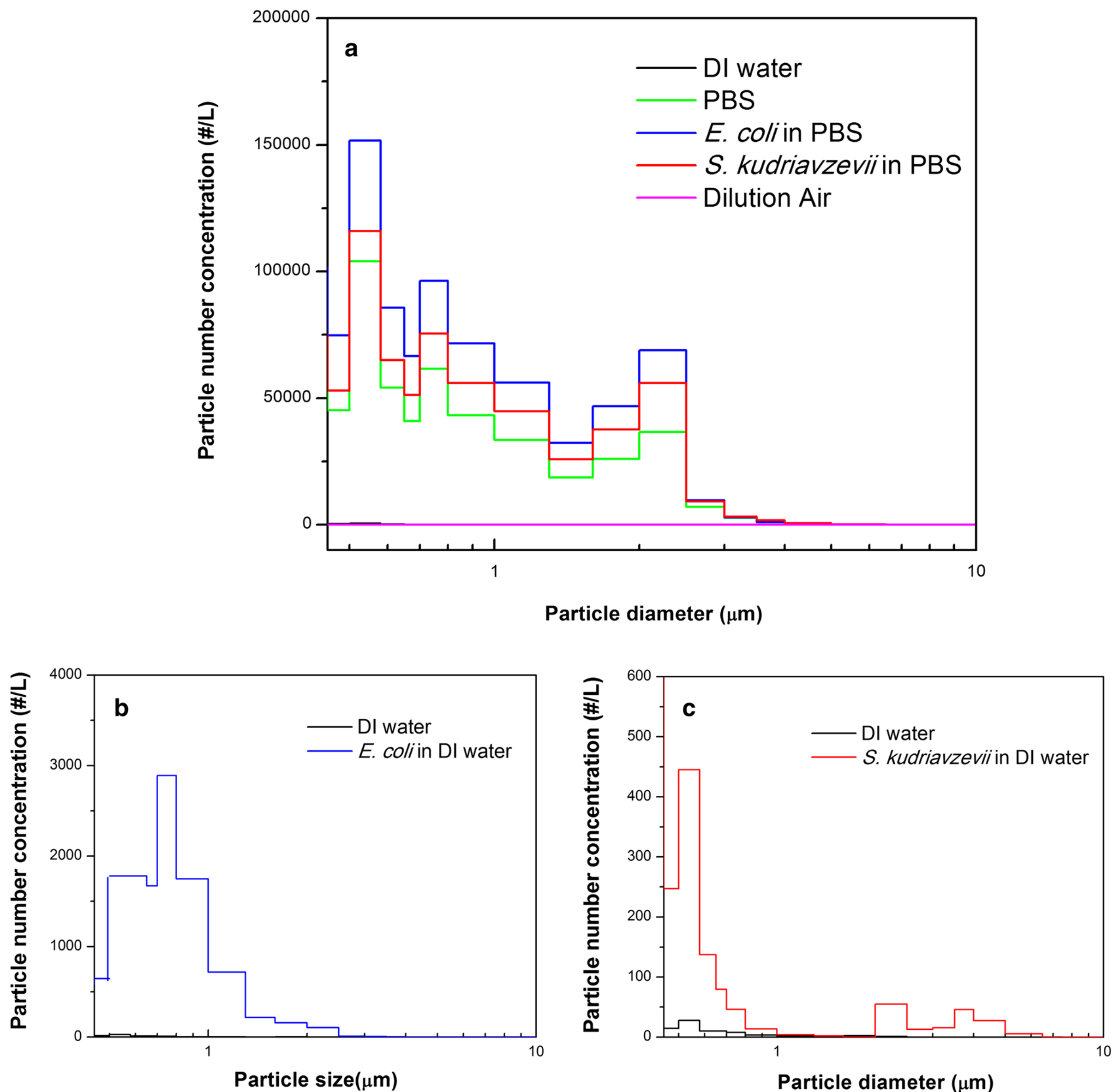

Fig. 2 **a** Particle size distributions of nebulized droplets using PBS solutions containing *E. coli, S. kudriavzevii* or just PBS solutions, measured by the OPC at the outlet of the dilution dryer; **b** Particle size distributions of nebulized droplets using DI water containing *E. coli*; **c** Particle size distributions of nebulized droplets using DI water containing *S. kudriavzevii*

at the outlet of the dilution dryer are also plotted for comparison. As shown, particle sizes with and without microorganisms do not differ in diameter. The majority of the particles generated from the Collison nebulizer with PBS as the suspension solution are from PBS components, and it is hard to distinguish microorganism-containing particles from those with PBS components alone. In comparison, only negligible amount of particles were generated from the Collison nebulizer with DI water as the suspension media. Thus, DI water was used as the suspension liquid for *E. coli* and *S. kudriavzevii* in the following experiment to enable identification of the particle size of those microorganism-containing aerosols.

With DI water as the suspension medium for the aerosolization of *E. coli,* particle size distribution after the dilution dryer as shown in Fig. 2b has a mode size of 0.5–1.6 μm. Generally, a single *E. coli* cell is

approximately 0.5 µm in width by 2 µm in length as characterized by optical microscopic measurement (Choi et al. 2015). The result in Fig. 2b matches the size dimension of *E. coli*. Comparing the concentration levels in Fig. 2a with Fig. 2b, it can be seen that *E. coli* cells account for only a minor portion of the nebulized particles.

Particle size distribution after the dilution dryer for *S. kudriavzevii*, with DI water as the suspension solution, is shown in Fig. 2c. As illustrated, a bimodal size distribution is observed for *S. kudriavzevii* aerosols after the dilution dryer, with a maximum peak at 0.5–0.8 µm and a smaller and broader peak between 2 and 6.5 µm. *S. kudriavzevii* cells used in this study is a budding species with particle size approximately 5–7 µm in diameter (Naumov et al. 2000). Therefore, the mode size of 2–6.5 µm likely was the viable fungus or the spores, while that of 0.5–0.8 µm might be the cell debris caused by the high pressure inside the Collison nebulizer. Comparison of the concentration levels in Fig. 2a, c shows that yeast cells account for a tiny portion of the nebulized particles.

3.2 Biological collection efficiency for *E. coli*

Figure 3a shows the comparison between the GTC and the BioSampler for the collection of viable *E. coli*, with PBS and NB as the collection media. The GTC outperformed the BioSampler when NB was used the collection media; all the Performance values were larger than 1. A Chi squared test analysis (value = 2.95 < 3.84) confirmed the significant difference between the collection efficiencies of the two systems with the NB as the collection media. On the other hand, using PBS as the collection media, the Performance values varied widely, from 0.2 to 1.4 and overall the BioSampler visually seems to perform better than the GTC. However, Chi squared test (value = 9 > 3.84) showed no significant difference with the PBS as the collection media. As most of the *E. coli* containing particles are larger than 0.5 µm (that is, larger than the cutoff size of the BioSampler), the physical collection efficiencies of these two samplers are expected to be comparable. Therefore, the differences between their collection efficiencies of the viable bacteria may be contributed by the stress during the sampling processes, the *E. coli* growth in the collection media, and the recovery of the damaged bacteria cells.

As *E. coli* does not grow in PBS solution, the results are determined by the sampling stress and the recovery of the injured cells (Koseki and Yamamoto 2006). Compared with the gentle particle deposition in the GTC, the swirling motion of the BioSampler can induce higher stress and subsequently more damage to the *E. coli* cells. On the other hand, the lower temperature of the BioSampler (temperature of the collection media is 17 °C after sampling while room temperature is 25 °C), compared with 37 °C at the

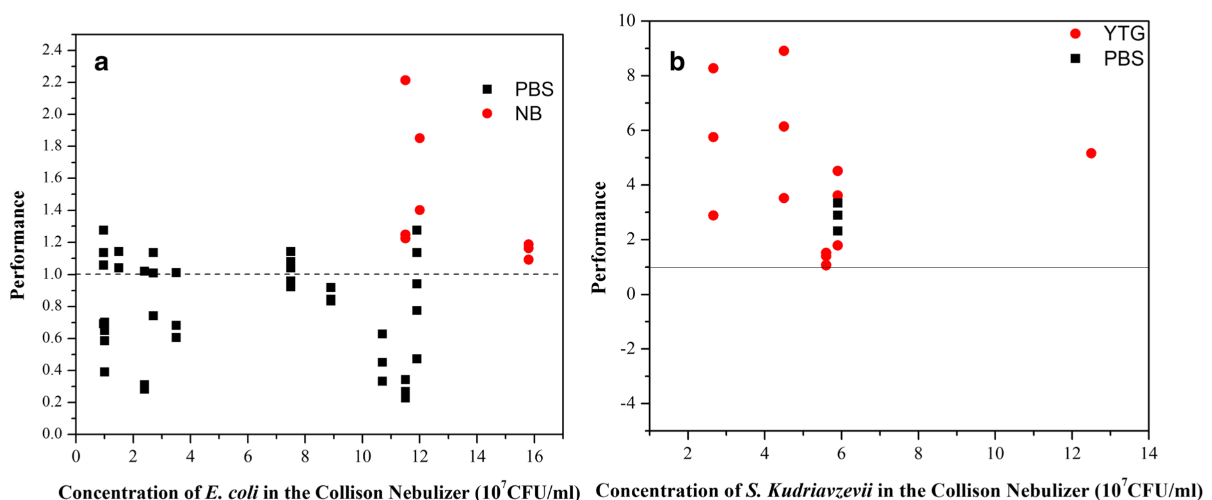

Fig. 3 **a** Comparison of the GTC with the BioSampler for the collection of viable *E. coli*; **b** Comparison between the GTC and the BioSampler for the collection of viable *S. Kudriavzevii*

GTC's collector, favors the recovery of damaged cells. Koseki and Yamamoto (2006) showed *E. coli* in PBS solution suffering from high hydrostatic pressure recovered well if incubated at 25 °C, but did not do so when incubated at 37 °C. To verify the effect of temperature on recovery in our system, *E. coli* collected by the BioSampler were stored at different temperatures for 30 min before culturing. The results showed that the number of viable *E. coli* stored at room temperature (25 ± 2 °C) was 1.3 ± 0.2 times of the number of the viable *E. coli* stored at 38 ± 2 °C. The results also imply that lowering the temperature of the GTC's collecting chamber may further improve the recovery and inhibit the replication of the collected *E. coli*.

Compared with PBS that inhibits *E. coli*'s growth, NB is a nutrient medium for *E. coli*. The doubling time of *E. coli* in rich medium is around 20 min depending on the strains (Sezonov et al. 2007). Thus, the replication of bacteria in NB solution needs to be considered. In this study, at the same initial concentration in the Collison Nebulizer, the number of cells collected by the GTC with NB as the collection media (1396 ± 106 CFU/L_{air}) was higher than the number of cells collected with PBS as the collection media (785 ± 215 CFU/L_{air}); *P* value is 0.003, which indicates a significant difference for these two collection media. This might be due to *E. coli* replication, since the stress caused by the GTC can be minor. However, for the BioSampler, the replication process of *E. coli* is less evident than the GTC; the BioSampler collected 1863 ± 790 CFU/L_{air} cells with the PBS as the collection media compared with 978 ± 349 CFU/L_{air} with the NB, with the P value (0.114) indicating no significant difference between the two collection media for the BioSampler. It implies that there was no replication following extra stress caused by collection (Griffiths et al. 1996). This might be due to the much stronger swirling motion with the sonic jet velocity inside the BioSampler that inhibited the bacteria growth in NB solution compared with the PBS solution. Besides, peptone solutions like NB have been found to foam for impingement-based sampling. In our study, NB solution lost 1.4 mL after 15 min of sampling for the BioSampler, which was greater than the loss of the PBS solution (0.9 mL). This larger loss of collection liquid results in fiercer particle bounce during sampling, as well as reaerosolization caused by bubbles breaking up (Zhen et al. 2013). Moreover, we noticed white solids formed on the wall of the BioSampler after air sampling with NB as the collection media, which implies that wall loss for NB solution might be more serious than the PBS solution; this observation agrees with a previous study showing that the adhesion of the particles to the inner wall of the BioSampler could account for particle losses as high as 30% (Han and Mainelis 2012). All these drawbacks may explain why the BioSampler performed better with PBS than with NB.

3.3 Biological collection efficiency for *S. kudriavzevii*

Figure 3b shows the comparison between the GTC and the BioSampler for the collection of viable *S. kudriavzevii* cells, with PBS and YTG as the collection media. As illustrated, the GTC outperformed the BioSampler, for both collection media; all the Performance values are > 1. Chi square test showed a statistically significant difference for these two samplers (value = $18 > 3.84$), indicating that the GTC outperformed the BioSampler, for the collection of viable *S. kudriavzevii* regardless of the collection medium. Since the yeast's replication time is around 90 min, that is longer than the 15 min sample collection time of this study, and thus its growth in the collection media can be ignored (Griffiths et al. 1996). This is corroborated by our results: the number of cells collected using YTG is similar to that obtained for PBS, for both samplers (Table 1). Thus, the results suggest that the better performance of the GTC is partially due to the gentler collection, as the GTC deposited the yeast aerosols at a velocity of 12.2 m/s (at 25 °C) compared with 313 m/s (at 37 °C) for the BioSampler (Willeke et al. 1998). The other possible reason might be the loss of particles at the inlet of the BioSampler. Similar to the AGI-30 impinger, which has the same inlet diameter of 0.9 cm, the overall physical collection efficiency of the BioSampler inlet is $> 98\%$ for 1 μm particles, but is substantially reduced to around 80% for particles larger than 5 μm due to inlet loss (Grinshpun et al. 1994; Seshadri et al. 2009). On the other hand, the physical collection efficiency of the GTC is nearly 100% for particles from 6 nm to 10 μm (Lednicky et al. 2016).

The detection limit of the GTC and the BioSampler for the collection of viable *S. kudriavzevii* cells is shown in Fig. 4. Because the size of single yeast cell is

Table 1 Number concentration of *S. kudriavzevii* cells collected by the BioSampler and the GTC with different collection media

	BioSampler (CFU/L)	GTC (CFU/L)
YTG	147 ± 20	471 ± 175
PBS	146 ± 7	415 ± 61

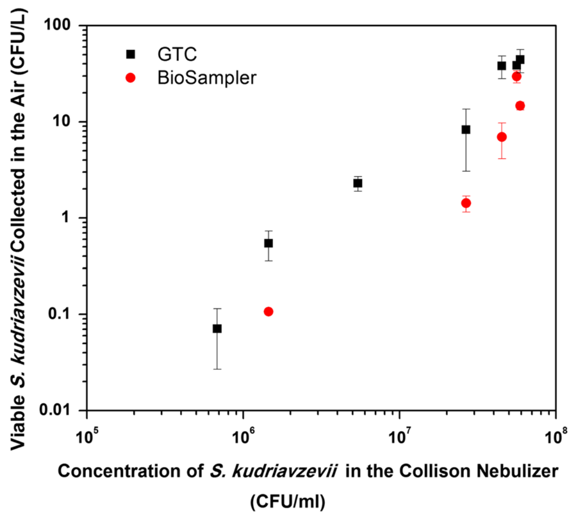

Fig. 4 Viable *S. Kudriavzevii* aerosol concentration per liter of air collected by the BioSampler and by the GTC as a function of the concentration of *E. coli* and *S. kudriavzevii* cells in the nebulizer

equivalent to or even larger than the volume median droplet size (2–4 μm) generated from Collison Nebulizer (Chen 1993), aerosolization efficiency for yeast cells is low. Therefore, a conversion value for the yeast was not calculated for aerosol concentration in the air. Nevertheless, the GTC collected a higher number of cells than the BioSampler, and the number of viable *S. kudriavzevii* cells collected increased with the concentration in the Collison Nebulizer for both the GTC and the BioSampler. For number concentrations less than 1.5×10^6 CFU/mL in the Collison nebulizer, no viable cells could be observed in the samples collected with the BioSampler. On the other hand, viable cells were collected with the GTC for concentrations in the nebulizer as low as 7×10^5 CFU/mL. Besides the gentle impaction of the GTC that protects the viability of the yeast, another possible factor for the lower detection limit is its 1.5–2.0 mL collection volume, compared with 20 mL collection media used for the BioSampler. The lower volume allows for collection of airborne bacteria and fungi into a more concentrated suspension.

Numerous studies have been conducted to determine the size distribution and/or the characteristics of airborne viable bacteria or fungi; however, the samplers used in these studies present considerable limitations in comparing the collection efficiency. For the collection of viable microorganisms, the overall sampling efficacy of the sampler is composed of two parts: the physical collection efficiency, which includes both the inlet sampling efficiency and the collection efficiency of the sampling media, and the biological efficiency that is sampling without affecting their biological activity (Nevalainen et al. 1993). For example, Albrecht et al. (2007) sampled airborne microbes with an AGI-30 and an MD8 gelatin filter sampler, and concluded that cultivation based methods underestimated the number of airborne microbes. However, that study did not take into consideration the inlet and inner wall losses of the AGI-30, the desiccation problem of the filters, and deactivation of viable microorganisms by these two samplers. Chang et al. (2001) quantified the levels of culturable microorganisms in swine farms with filters and impingers, and Adam et al. (2015) assessed the fungal and bacterial composition of air in a chamber study with open-face filters. As the samplers they used might significantly deactivate collected microorganisms or can collect only certain size range of the bioaerosols, results from these samplers can be biased.

Figures 3a shows that the GTC has similar performance with the BioSampler for the collection of *E. coli*, whereas Fig. 3b displays that the GTC outperformed the BioSampler for the collection of S. *kudriavzevii*. The difference between the collection efficiencies for these two microorganisms might be caused by the differences in particle size distribution, stress sensitivity and recovery of the damaged microorganisms. The particle size of *E. coli* is 0.5–1.6 μm, a size for which the physical collection efficiency of the BioSampler is almost 100% with no inlet loss (Ding and Wang 2001). However, the particle size of S. *kudriavzevii* is 2–6.5 μm; in this size range, the inlet loss of the BioSampler can be as high as 20%. Besides, the bacterium *E. coli* is less sensitive to physical forces compared with yeast S. *kudriavzevii* (Gross et al. 1994). Thus, the swirling motion of the BioSampler might have less effect on *E. coli* compared with S. *kudriavzevii*. Moreover, as the doubling

time of S. *kudriavzevii* is much longer than 15 min, the recovery of the damaged yeast cells can be negligible in our study, whereas recovery of *E. coli* at room temperature can be considerable.

Figure 3a, b suggests that the performance of both the GTC and the BioSampler depends on the types of collection liquids and the types of microbes chosen. Water-based nutrient broths that can maintain the viability of the microbes have long been used for air sampling for long sampling periods; however, replication of the microorganisms in these media may result in the overestimation of the number of viable microbes collected. In this study, we used NB and YTG as the collection liquids for the sampling of viable *E. coli* and *S. kudriavzevii*, respectively, and we verified that *E. coli* could replicate in the NB solution, while *S. kudriavzevii* didn't grow in YTG solution during the 15 min sampling. Thus, for bacteria with short replication time like *E. coli*, PBS solution provides a more accurate count of microorganisms, while for fungi with long replication time, both the culture media and the PBS solution are acceptable. However, as the viable fraction of the collected bacteria decreases over the time the microorganisms are kept in solution (Griffiths et al. 1996), nutrient media may be useful as the collection fluid for the GTC for the identification of viable microorganisms under the conditions that bioaerosol concentration in the air is really low. On the other hand, the formation of froth on the surface of the collection medium may deactivate a considerable number of the microbes (Springorum et al. 2011). Solutions for the problems observed in this study may include lowering the temperature of the sampling liquid, shortening sampling time, and adding antifoams. During collection, considerable foam was formed in the BioSampler when the nutrient solution was used as collection media; on the contrary, no foam was observed while collecting bacteria or fungus with the GTC. The elimination of foam forming in the GTC is due to the low aerosol impaction velocity in the surface of the liquid media, as no swirling motion is required for deposition. As froth formation can also lead to higher reaerosolization rate and particle bounce, which subsequently result in the underestimation of the collected microbes as well as higher deactivation rate, the results suggest that gentle deposition of the GTC is advantageous for sampling bioaerosol and also for better preservation of the microorganism's viability. With no froth formation in the GTC, culture media might also be a good choice for infectious agents that are sensitive to antifoams.

In summary, samplers that can collect a wide size range of microorganisms with less stress are in great need. The novel GTC based on laminar-flow water-based condensational particle growth technology, which has been demonstrated to have more than 90% physical collection efficiency for particles from 6 nm to 100 μm, might be a promising alternative to conventional bioaerosol samplers. Aside from viable *E. coli* and S. *kudriavzevii,* it has also been shown to be highly efficient at the collection of viable bacteriophage MS2 and influenza H1N1 viruses that are much smaller in particle size.

4 Conclusions

The performance of the GTC for the collection of viable bacterium *E. coli* and yeast *S. kudriavzevii* in comparison with the industry standard BioSampler was evaluated in the study. Results showed that the collection efficiency of the GTC for viable *E. coli* is equivalent to or better than the BioSampler depending on the collection media, while for *S. kudriavzevii,* the GTC outperformed the BioSampler regardless of the collection media. Advantages of the GTC resulting in better performance are: (1) reduced inlet loss; (2) minimization of wall loss; (3) reduced sampling impaction velocity (12.2 vs. 313 m/s); reduced collection volume (1.5 vs. 20 ml); and (4) minimal to no formation of froth during collection. The results also suggest that collection media should be carefully chosen for different kinds of microbes. For microorganisms with short doubling time like *E. coli,* PBS solution might work better, while for those with long replication time such as *S. kudriavzevii*, both the nutrient media and the PBS solution work well. This flexibility in using nutrient media as the collection fluid for the GTC may be important for the detection of viable microorganism when its concentration in air is low. Meanwhile, an improvement of the GTC by decreasing the temperature of the collection chamber may further favor the preservation of the viable microbes.

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Compliance with ethical standards

Conflict of interest The underlying water-based condensation growth technology is patented by the employer of two of the authors (S. Hering and A Eiguren-Fernandez), and has been licensed for commercial use in the field of airborne particle collection.

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