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Recovery of culturable *Escherichia coli* O157:H7 during operation of a liquid-based bioaerosol sampler

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ABSTRACT

Collection fluids used in liquid-based bioaerosol samplers can influence the viability of microorganisms. In this study we determined the recovery efficiency of vegetative *E. coli* O157:H7 cells that were spiked into low viscosity evaporating collection fluids during operation of a BioSampler® for up to 90 min at room temperature. The collection fluids tested were deionized (DI) water, DI water with 0.1% (w/w) antifoam B (AFB), phosphate-buffered saline (PBS), and osmoprotectants consisting of peptone (with and without AFB) or betaine at 0.1% (w/w) in DI water. Using DI water, there was a rapid decline in the recovery of culturable *E. coli*, with only 11, 3, and 0% being recovered after 30, 60, and 90 min, respectively. Recoveries were substantially greater with use of PBS (53, 25, and 16%, respectively) but not as high as with use of the osmoprotectants or AFB. Peptone and AFB alone or together allowed for the greatest recovery of *E. coli*, with average values ranging from 87 to 98% at 90 min. Betaine was also determined to be an effective osmoprotectant for runtimes of 30 and 60 min, with respective *E. coli* recoveries of 101 and 77% from the impingers. The results from this study support the incorporation of peptone, AFB, and betaine in collection fluids for BioSampler runtimes from 30 to 90 min. Runtimes longer than the recommend 30 min with low viscosity collection fluids are sometimes necessary when the airborne concentration of a target microorganism is low and one is trying to increase the probability of detection.

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1. Introduction

In an attempt to measure the concentration of airborne microorganisms, a variety of systems based on impingement, impaction, filtration, and gravitational settling have been utilized (Dungan and Leytem 2009). During air sampling campaigns with such bioaerosol samplers, the objective is the efficient collection of the airborne microorganisms without affecting their cellular integrity and subsequent detection. While culture-independent techniques (e.g., quantitative PCR) offer low limits of detection, they cannot be used to differentiate between viable and nonviable microorganisms, thus culture-dependent techniques are still commonly used by researchers. Culture-dependent techniques, however, are not without disadvantage, as not all microorganisms are culturable even though they may be viable. This can result in an underestimation of the microorganism concentration in the bioaerosol sample (Stewart et al. 1995; Terzieva et al. 1996). In addition, another concern is that the microorganisms become stressed or damaged during sampling, making it difficult sometimes to cultivate them.

One widely used liquid-based bioaerosol sampling device, called the BioSampler® (SKC Inc., Eighty Four, PA, USA), traps airborne microorganisms in a swirling liquid (Lin et al. 1999). For short-term sampling events, use of sterile distilled water or liquids of similar viscosity (e.g., peptone water, phosphate-buffered saline [PBS], physiological saline, nutrient broth) are recommended by SKC Inc. in their operating instructions. Non-evaporating liquids (e.g., mineral oil, glycerol) can also be used, especially for long-term sampling, but use of glycerol has been shown to greatly decrease microorganism viability possibly due to changes in osmotic pressure (Lin et al. 1999). Because peptone solutions have been documented to foam during impinger operation (Springorum et al. 2011), antifoaming agents (e.g., antifoam A and B) have been utilized (Brooks et al. 2004; Chi and Li 2006; Dungan et al. 2010).

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In the present study, our objective was to assess the recovery of vegetative *E. coli* O157:H7 cells when low viscosity collection fluids were used in the BioSampler for up to 90 min. *Escherichia coli* is an organism of interest in aerosol monitoring studies (Chinivasagam et al. 2009; Dungan 2010) due to the pathogenic nature of some strains and its usefulness as an indicator of fecal contamination. The specific objectives were to determine if use of phosphate-buffered saline (PBS), osmoprotectants (i.e., peptone, betaine), or antifoam B (AFB) in the collection fluid would result in enhanced recoveries of culturable *E. coli* O157:H7 when compared to deionized (DI) water. It was not within the scope of this study to assess the collection efficiency of airborne *E. coli*, reaerosolization, and temperature effects on the recovery of vegetative cells.

2. Materials and methods

2.1. *Preparation of* Escherichia coli

Escherichia coli O157:H7 strain B6-914 was obtained courtesy of Dr. Pina Fratamico (USDA-ARS, Wyndmoor, PA, USA). This *E. coli* strain does not produce Shiga-like toxins I and II, but was engineered to contain a green fluorescent protein (gfp) gene (Fratamico et al. 1997). Stock cultures of the bacterium were maintained in a sterile glycerol solution (10%, v/v) at −70°C. To initiate cultivation of the bacterium, a loopful of the cryopreservation was used to inoculate Tryptic soy agar (Becton, Dickinson, and Co., Franklin Lakes, NJ, USA) slants containing ampicillin (Sigma Aldrich, St. Louis, MO, USA) at 100 mg/L. After growth was established at 35°C, a loopful of the culture was subsequently used to inoculate 50 mL aliquots of Tryptic soy broth (Becton, Dickinson, and Co.) containing ampicillin (100 mg/L), which was incubated overnight at room temperature with shaking. The bacterium was harvested by centrifugation at 4,500 *g* for 10 min at 5°C. Afterwards, the pellet was washed two times with cold PBS, then resuspended in buffer to an optical density of approximately 2.0 at 600 nm. The suspension was diluted 100-fold with PBS and stored for no longer than 0.5 h at 5°C prior to being used as the inoculum. The titer of the inoculum was approximately 5×10^5 colony forming units (CFU)/mL.

2.2. *Biosampler experiments*

The collection fluids tested were the following: (i) deionized (DI) water; (ii) PBS; (iii) betaine; (iv) peptone; (v) AFB; and (vi) peptone and AFB. The PBS was prepared according to that of Sambrook and Russell (2001). Betaine, peptone, and AFB were obtained from Sigma Aldrich and dissolved in DI water at 0.1% (w/w). The collection fluids were autoclaved at 121°C for 20 min. For each experiment six BioSamplers were used, as well as three controls which consisted of 125-mL Erlenmeyer flasks. A total of 19.9 mL of collection fluid was added to each vessel, followed by the addition of 0.1 mL of the *E. coli* inoculum. The initial titer was approximately 2.5×10^3 CFU/mL or 5×10^4 cells/impinger or flask. Each BioSampler was then connected to a VAC-U-GO pump (SKC Inc.) and the flasks were subjected to orbital shaking at 120 rev/min. The VAC-U-GO pump maintains a flow rate of about 12.5 L/min in the BioSampler.

All experiments were conducted at room temperature (20 ± 2°C) and samples were collected at 0, 15, 30, 60, and 90 min, with weighing of the vessels before and after sample collection to determine the amount of collection fluid that evaporated. The samples were then serially diluted in PBS and 0.1 mL aliquots of the dilutions were spread directly onto Petri plates containing Tryptic soy agar (TSA) with ampicillin (Sigma Aldrich) at 100 mg/L. The plates were incubated aerobically overnight at 35°C. To screen for any potential contamination, colonies were verified as *E.coli* strain B6-914 by exciting them with an ultraviolet lamp at 365 nm. The total number of culturable *E. coli* cells in each vessel was determined by multiplying the CFU/mL by the appropriate dilution factor and volume of collection fluid (mL) at the time of sampling. The % recovery of *E. coli*, as presented in Figure 1, was determined by dividing the total cell counts at each sampling period by the total count at time zero, then multiplying by 100.

3. Results and discussion

The BioSampler manufacturer recommends a short-term sampling period of ≤30 min when using low viscosity fluids to maintain recovery efficiency, but longer sampling periods have been used during field campaigns (Dungan et al. 2011). Figure 1a shows the recovery of culturable *E. coli* O157:H7 from the BioSamplers over a 90 min operating period when the collection fluid was DI water. In contrast to the controls, there was a rapid decline in the recovery of bacteria, with only 33, 11, and 3% being recovered after 15, 30, and 60 min, respectively. At 90 min about 50% of bacteria were recovered from the controls, while none were recovered from the BioSamplers. In the former case, the DI water likely caused osmotic stress and, subsequently, affected their ability to be cultivated. The results also clearly suggest an additional effect of the swirling motion of the collection fluid injured the cells, causing death or putting them into a viable but nonculturable (VBNC) state. It is suspected that the reduced recovery of *E. coli* with increased

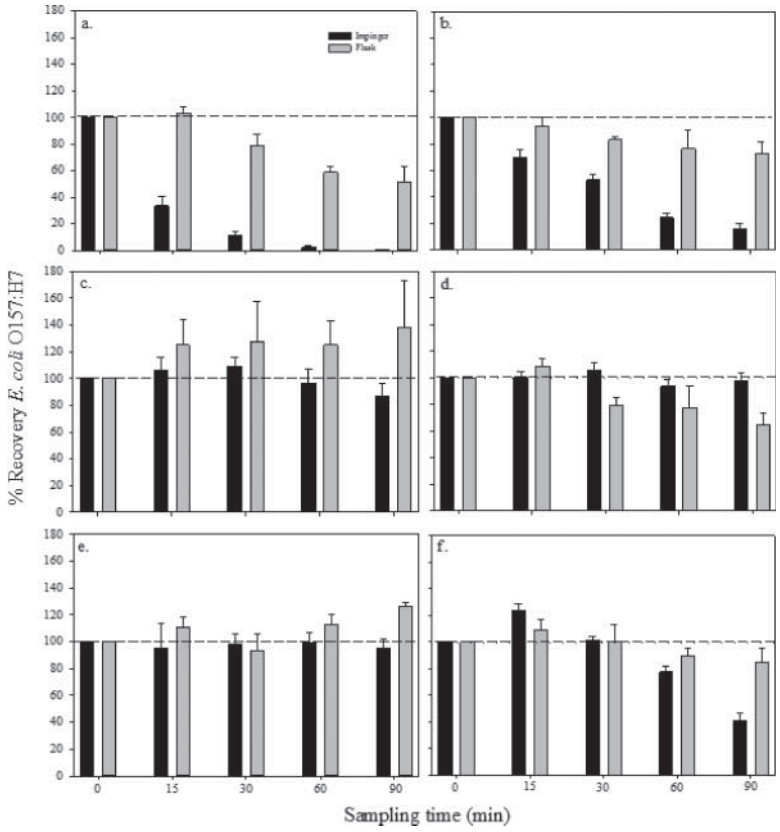

Figure 1. Recovery of culturable *E. coli* O157:H7 from the impingers and flasks (controls) with (a) deionized water, (b) phosphate-buffered saline, (c) peptone [0.1%, w/w], (d) antifoam B [0.1%, w/w], (e) peptone and antifoam B [each at 0.1%, w/w], and (f) betaine [0.1%, w/w] as collection fluids. The dashed line in each window is set at 100% recovery. Error bars represent the SEM ($n = 6$ or 3 for impingers and flasks, respectively).

impinger runtime was not a result of reaerosolization. This is supported by the fact that 87 to 98% of *E. coli* was recovered after 90 min with the use of peptone and/or AFB (Figure 1c–e). In addition, Willeke et al. (1998) and Lin et al. (2000) found negligible reaerosolization of polystyrene latex particles and *Pseudomonas fluorescens* (0.3 to 2.0 μm) from BioSamplers when the collection fluid was DI water, respectively.

The recovery of culturable *E. coli* O157:H7 from the impingers with PBS was greater over that of DI water, with recoveries of 70, 53, 25, and 16% at 15, 30, 60, and 90 min, respectively (Figure 1b). Phosphate-buffered saline is commonly used in microbiological procedures to maintain physiological pH and isotonicity, thus reduces cellular stress. While PBS performed much better than DI water at 15 and 30 min, use of PBS for BioSampler operating times $\geq$30 min is not recommended, and DI water should not be used as a collection fluid due to overall poor recovery efficiencies.

Water-based nutrient solutions have also been used as collection fluids to help maintain the viability of microorganisms (Hameed and Khodr 2001; Chi and Li 2006; Van Droogenbroeck et al. 2009). Peptone solution is commonly used as an evaporating collection fluid (Pillai et al. 1996; Tanner et al. 2005; Dungan et al. 2011), which is believed to maintain the viability of microbial cells by preventing osmotic shock (Agranovski et al. 2003). As shown in Figure 1c, the recovery of *E. coli* was greater from the impingers when peptone was dissolved in DI water. On average, 100% of the *E. coli* was recovered at 30 min, while 96 and 87% were recovered at 60 and 90 min, respectively. Based on the slight positive slope of *E. coli* recovery in the control flasks, it is possible that VBNC cells in the inoculum were being reactivated by the presence of peptone. Terzieva et al. (1996) found that higher cell counts could be obtained on TSA compared to growth on MacConkey agar, indicating that cells injured during the impingement process could be recovered.

To determine the effect of an antifoaming agent on the recovery of *E. coli*, AFB was added to DI water (Figure 1d). What was most interesting was that *E. coli* recovery was lower from the control flasks at 30, 60, and 90 min. At 90 min, 64% of *E. coli* was recovered from the flasks versus 98% from the impingers. The active ingredient in AFB (i.e., polydimethylsiloxane) is generally considered to be inert and non-toxic, thus we are unable to explain this trend. When both peptone and AFB were

used together the trend was similar to that of peptone alone (Figure 1e).

Betaine is an osmoprotectant that aides in the survival of bacteria in marine and other saline environments, where high concentrations are accumulated in cells (Imhoff and Rodriguez-Valera 1984; Ken-Dror et al. 1986; Ghoul et al. 1990; Kets et al. 1996). Roth et al. (1988) demonstrated that osmotically stressed *E. coli* cells could be cultivated when exposed to betaine. The recovery of airborne bacteria from a municipal wastewater treatment plant was found to be 70% greater when betaine was incorporated into collection fluid (at 0.06%, w/w) than without (Marthi and Lighthart 1990). In this study, 101% of *E. coli* was recovered from the impingers with betaine at 30 min, while respective recoveries were 77 and 41% at 60 and 90 min (Figure 1f). Interestingly, at 15 min, *E. coli* recoveries from the impingers and control flasks were 124 and 109%, respectively, suggesting that a reactivation of VBNC cells occurred. Because recoveries were greater from the controls at 60 and 90 min, fluid swirling or other related factors in the impinger are likely causing cell injury beyond natural die-off. Compared to use of DI water or PBS as collection fluids, recovery of *E. coli* was substantially greater with betaine, but not as high when peptone and/or AFB was used.

The pH of the collection fluid, which is rarely considered in bioaerosol research studies, could also have affected bacteria survivability in this study. The pH of the DI water was 4.3, while the PBS and peptone and/or AFB solutions ranged from 6.8 to 7.3, and the betaine solution was 5.0. Because DI water alone or with osmoprotectants is not buffered, pH control is not possible as with the PBS.

Operators should also be aware that significant quantities of collection fluid will evaporate during a 90-min run. This will most certainly prevent long runtimes at high temperatures and low humidity. In this study about 50% of the collection fluid evaporated at room temperature, and rates were nearly identical for each of the fluids (data not shown). One item that was not addressed in this study was the temperature of the collection fluid, which is highly influenced by ambient temperature and humidity. Springorum et al. (2011) attempted to address this issue by tempering the collection fluid, but the results did not provide a definitive answer as to its effectiveness. While temperature control of liquid-based bioaerosol samplers is not an option available from manufacturers to date, it is a topic that should be considered along with the use of osmoprotectants in future studies.

In conclusion, the results from this study support the incorporation of osmoprotectants (peptone, betaine) or AFB in collection fluids, but not use of DI water or PBS, to enhance the recovery of culturable *E. coli* cells from the BioSampler. The greatest recoveries (87–98%) occurred at 90 min when DI water was amended with peptone (with or without AFB) or AFB, thus we recommend their use during extended impinger operations. Runtimes greater than 30 min are sometimes necessary when the airborne concentration of a target organism is low and one is trying to increase the probability of detection. However, because our results are specific to one *E. coli* strain, it should not be assumed that similar recoveries will occur with other *E. coli* strains or bacterial genera and species. It is recommended that additional testing be conducted with the targeted organism(s) of interest to verify the effectiveness of the collection fluids.

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