

Aerosolization as novel sanitizer delivery system to reduce food-borne pathogens

S.-W. Oh¹, P.M. Gray², R.H. Dougherty² and D.-H. Kang²

¹Food Safety Research Division, Korea Food Research Institute, Korea, and ²Department of Food Science and Human Nutrition, Washington State University, Pullman, WA, USA

2004/0954: received 17 August 2004, revised 13 January 2005 and accepted 14 January 2005

ABSTRACT

S.-W. OH, P.M. GRAY, R.H. DOUGHERTY AND D.-H. KANG. 2005.

Aims: As a preliminary experiment on new sanitizer delivery tools, the efficacy of aerosolized sanitizer on food-borne pathogens was investigated in larger model chamber system.

Methods: Peroxyacetic acid and hydrogen peroxide were aerosolized in a model system against artificially inoculated target micro-organisms on laboratory media. Cultures of four different food-borne pathogens were inoculated and affixed onto three different heights (bottom, wall and ceiling), and three different orientations (face-down, vertical and face-up) inside a commercial semi-trailer cabinet (14.6 × 2.6 × 2.8 m). Sanitizer was aerosolized into 2 µm droplet size fog and treated for 1 h at ambient temperature.

Results: Populations of *Bacillus cereus*, *Listeria innocua*, *Staphylococcus aureus*, and *Salmonella typhimurium* were reduced by an average of 3.09, 7.69, 6.93 and 8.18 log units per plate respectively. Interestingly, *L. innocua*, *Staph. aureus*, and *Salm. typhimurium* showed statistically not different ($P \geq 0.05$) reduction patterns relative to height and orientation that were never expected in a spraying system.

Conclusions: Aerosolized sanitizers diffuse like gaseous sanitizers.

Significance and Impact of the Study: Aerosolization has great potential for use in commercial applications.

Keywords: aerosol, aerosolization, aerosolized, diffusivity, pathogen, reduction, sanitizer.

INTRODUCTION

Sanitation of raw foodstuffs is an important intervention for reducing the occurrence of food-borne outbreaks. Direct application of antimicrobial agents to food is a widely used method and is carried out traditionally by spraying or dipping using aqueous sanitizers. Although these techniques are somewhat effective in reducing levels of pathogens, they can be of limited value because of the interference of surface phenomena, such as cracks, plant hairs and biofilms, which impede contact with pathogens. Viable *Escherichia coli* O157:H7 are present in the inner tissues and stomata of cotyledons of radish sprouts grown from artificially inoculated seeds (Hara-Kudo *et al.* 1998). And significant growth

and multiplication of *E. coli* O157:H7 occurred on injured surfaces of green peppers after incubation for 24 h at 37°C (Han *et al.* 2000). Therefore, aqueous sanitizers could fail to reach and kill pathogens located in those sites. One potential possible way to overcome this disadvantage of aqueous application is to use gaseous sanitizers. Indeed, a number of reports have been published on the efficacy of gaseous sanitizers (Han *et al.* 2000, 2001a,b) and it is thought to be an effective tool in pathogen reduction in foods having surface hindrances. However gaseous sanitizers have several disadvantages such as a sophisticated apparatus is needed for gas generation and the numbers of applicable gaseous sanitizers are limited.

Aerosolization is defined as dispersion in air of a liquid material or a solution in the form of fine mist, usually for therapeutic and sanitary purposes, especially for respiratory medical treatments (Wanner and Rao 1980) and room disinfection (Fišer 1978; Hiom *et al.* 2003). *Pseudomonas*

Correspondence to: Dong-Hyun Kang, Department of Food Science and Human Nutrition, Washington State University, Pullman, WA, USA (e-mail: dhkang@wsu.edu).

aeruginosa (Hashimoto *et al.* 1996), *Staphylococcus aureus* (Maiz *et al.* 1998) and *Aspergillus fumigatus empyema* (Purcell and Corris 1995) are reported target micro-organisms in clinical treatment. Safe room disinfection is another common application for aerosolization. Aerogenic disinfection of poultry houses has been thought to be an effective tool for increasing poultry production (Profè and Steiger 1982; Steiger *et al.* 1982). Fišer (1978) reported that continual disinfection by aerosolization of lactic acid resulted in an improved state of health of chickens. Jarnych (1972) also emphasized the importance of lactic acid aerosol for controlling infectious disease and for improving the health of poultry. Fine aerosol mists have better penetration than trigger spray in assessment of surface bioburden during hospital aseptic processing (Hiom *et al.* 2003).

With its diffusible activity and broad applicable antimicrobial spectrum, aerosolization may be an alternative antimicrobial delivery system. But to date, no studies have reported using aerosolized sanitizers in foods. So, this study was intended to be a preliminary research effort to evaluate aerosolization as a new antimicrobial delivery technique with a very small (2 μm) droplet size in larger model chamber (semi-trailer) system. Peroxyacetic acid plus hydrogen peroxide was chosen for aerosolization for its low corrosiveness, broad killing spectrum and rapid decomposition after use into harmless acetic acid, oxygen and water (Lenahan 1992).

MATERIALS AND METHODS

Bacterial strains

Four bacterial strains, *Bacillus cereus* W-2, *Listeria innocua* (ATCC 33090), *Salmonella typhimurium* (ATCC 363755) and *Staph. aureus* (ATCC 49444), were obtained from the Food Science and Human Nutrition culture collection at Washington State University (Pullman, WA, USA) and maintained by monthly transfers on tryptic soya agar (TSA; Difco Laboratory, Detroit, MI, USA). *Bacillus cereus* was cultured on brain–heart infusion (BHI) agar (Difco) for 3 weeks at 20°C until at least 80% of the bacteria had sporulated. Spores were harvested by depositing 1–2 ml of sterile water onto the surface of BHI culture plates and gently rubbing with a sterile swab. Pooled suspensions were centrifuged at 5 900 $\times g$ for 15 min at 4°C. Spore pellets were washed three times in sterile distilled water, resuspended in a small volume of sterile water and stored at –20°C. All other species were grown in 100 ml tryptic soya broth (Difco) at 37°C for 18 h and centrifuged at 2200 $\times g$ for 25 min at 20°C. The final pellets were resuspended in buffered peptone water, corresponding to *c.* 10⁸ to 10⁹ CFU ml⁻¹. These cell suspensions were used in subsequent experiments.

Experimental design

Each bacterial culture was tenfold serially diluted from 10⁸ cells 100 μl^{-1} to 10⁵ cells 100 μl^{-1} . One hundred microlitres of each culture serial diluents were spread-plated onto duplicate TSA Petri dishes using sterile cotton-tipped swabs to make duplicate dilution sets. One set was used in aerosol treatment and the other was used as the negative control. Petri dishes (with lids removed) were secured to nine locations within a huge model system, commercial semi-trailer (14.6 \times 2.6 \times 2.8 m), using duct tape affixed to the dish bottoms (Fig. 1). Table 1 shows the summary of different placement heights and orientations. Three different sites were selected on the floor, five sites were positioned on the wall, and one site was located on the ceiling (Table 1). All Petri dishes on the floor were placed face-up after removing the lid. Petri dishes were attached to the wall in a vertical position at three different sites. For the remaining sites, two sites on the wall and one site on the ceiling, Petri dishes were positioned in a face-down orientation. The trailer doors were closed and two 10 cm diameter hoses were routed from a commercially fabricated experimental aerosolization generator (Environmental Cleaning Solutions, LLC, Tomahawk, WI, USA) to an interface located in a trailer door at a height of 50 cm. An

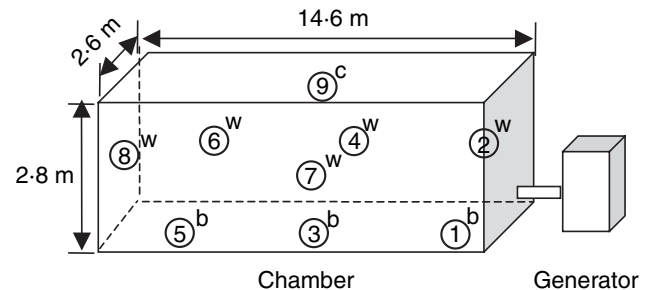


Fig. 1 Schematic diagram of semi-trailer used in aerosol sanitizer experiment. The alphabet indicates the location where inoculated petri dishes were positioned. ①, floor; ②, next to left door; ③, left wall, next to left door, 1.4 m high; ④, floor, next to left wall, 7.3 m inside trailer; ⑤, left wall, 7.3 m inside trailer, 1.4 m high; ⑥, floor, next to right wall, 12.2 m inside trailer; ⑦, left wall, 12.2 m inside trailer, 1.4 m high; ⑧, inverted on right wall, 7.3 m inside trailer, 1.4 m high; ⑨, inverted on inner wall, 14.6 m inside trailer, 1.4 m high; inverted on ceiling ^cceiling, ^wwall, ^bbottom.

Table 1 Height and orientation of positioned Petri dishes

| Height | Petri dish position | Orientation | Petri dish position |
|-----------------|---------------------|-------------|---------------------|
| Floor (0 m) | 1, 3, 5 | Face-up | 1, 3, 5 |
| Wall (1.4 m) | 2, 4, 6, 7, 8 | Vertical | 2, 4, 6 |
| Ceiling (2.8 m) | 9 | Face-down | 7, 8, 9 |

aqueous sanitizing solution consisting of 1800 ppm peroxyacetic acid ($C_2H_4O_3$) and 8800 ppm hydrogen peroxide (H_2O_2) was atomized by the generator into *c.* 2 μm particles using a patented, proprietary process involving electromechanical means and blown into the trailer for 1 h. The doors were opened, 1 h after the generator was turned off, and sanitizer was allowed to dissipate from the trailer before Petri dishes were removed, lids replaced and incubated at 37°C for 24 h. The untreated Petri dishes were used as negative controls.

Statistical analysis

All experiments were repeated three times with duplicate samples. Data were analysed by analysis of variance using the ANOVA procedure of SAS (SAS Institute, Cary, NC, USA). When the effect was significant ($P \leq 0.05$), mean values were separated using Duncan's multiple range test.

RESULTS

The number of *Bacillus cereus* was reduced from the untreated samples ($6.38 \pm 0.09 \log_{10}$ cells plate⁻¹) to sanitizing aerosol treated samples ($3.29 \pm 1.34 \log_{10}$ cells plate⁻¹). The population of *L. innocua* was reduced from $8.66 \pm 0.20 \log_{10}$ cells plate⁻¹ to $0.97 \pm 0.75 \log_{10}$ cells plate⁻¹. *Salm. typhimurium* and *Staph. aureus* were reduced from $8.92 \pm 0.01 \log_{10}$ cells plate⁻¹ to $0.74 \pm 0.513 \log_{10}$ cells plate⁻¹ and $8.46 \pm 0.05 \log_{10}$ cells plate⁻¹ to $1.53 \pm 0.56 \log_{10}$ cells plate⁻¹. By aerosol sanitizer treatment, populations of *B. cereus*, *L. innocua*, *Salm. typhimurium* and *Staph. aureus* were reduced by 3.09, 7.69, 8.18 and 6.93 \log_{10} units from the untreated control respectively (Fig. 2). *Bacillus cereus* showed the lowest reduction and it is thought that spores were not killed as readily as vegetative cells because of their greater resistance to chemical sanitizers. *Listeria innocua* and *Salm. typhimurium* were easily reduced by aerosol carrying peroxyacetic acid and hydrogen peroxide. When data was analysed according to Petri dish height, bottom, wall and ceiling did not differ significantly ($P \geq 0.05$) among strains except *B. cereus* (Fig. 3). Pathogenic reduction depending on Petri dish orientations, i.e. face-up, vertical and face-down, were also analysed (Fig. 4). Like the height data analysis, all strains except *B. cereus* showed similar reduction patterns regardless of their orientation.

DISCUSSION

The aerosol carrying peroxyacetic acid and hydrogen peroxide used in this study, diffuse effectively through different heights and different orientations positioned in huge semi-trailer ($14.6 \times 2.6 \times 2.8$ m). Although aerosol inlet height was only 50 cm from the floor, nearly the same

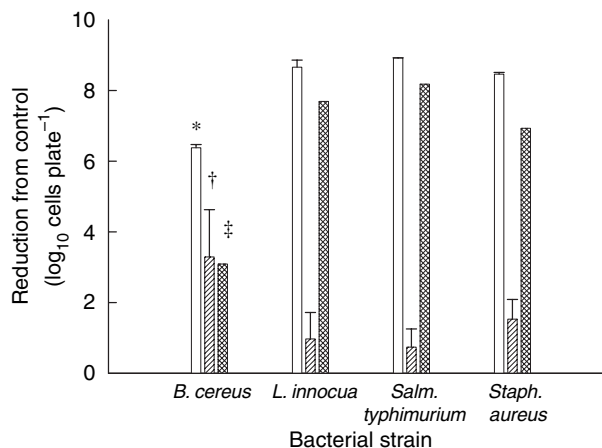


Fig. 2 Microbial reductions of target micro-organism at nine different positioned Petri dishes with aerosolized peroxyacetic acid and hydrogen peroxide in model semi-trailer. *Mean \pm SD before aerosol sanitizer treatment; †Mean \pm SD after aerosol sanitizer treatment; ‡reduction by aerosol sanitizer treatment

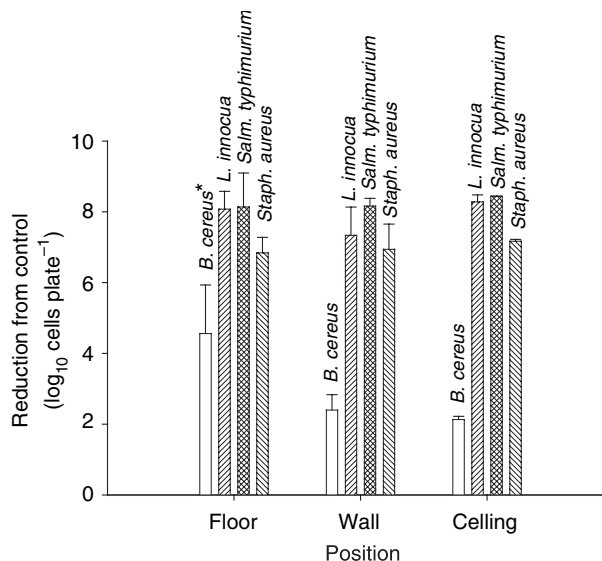


Fig. 3 Microbial reductions of target micro-organism depending on Petri dish heights with aerosolized peroxyacetic acid and hydrogen peroxide in model semi-trailer. *Values followed by different alphabet are statistically different ($P \leq 0.05$)

microbial reduction occurred regardless of Petri dishes height (from 0 to 2.8 m) and orientation (from face-up to face-down). So, it is thought that the aerosol has a powerful diffusiveness characteristic and ability to penetrate all surface irregularities much like gas sanitizers. Unlike earlier experiments (Steiger *et al.* 1982; Steiger 1982) which tested apparatus producing a relatively coarse (8–18 μm) droplet size which settled rapidly, the electromechanical generator

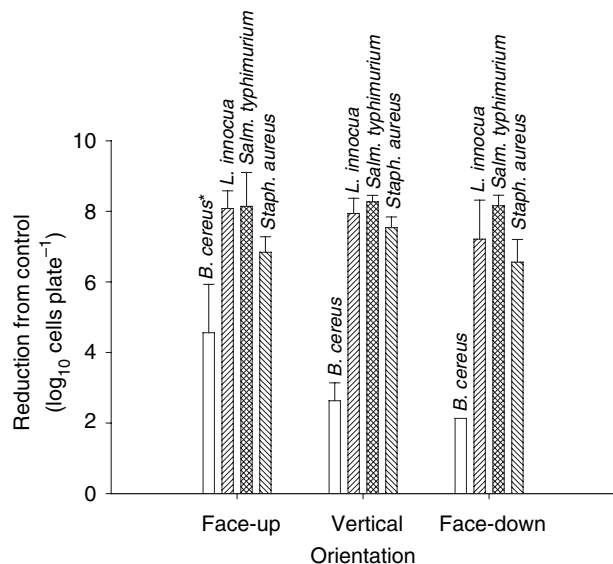


Fig. 4 Microbial reductions of target micro-organism depending on Petri dish orientations with aerosolized peroxyacetic acid and hydrogen peroxide in model semi-trailer. *Values followed by different alphabet are statistically different ($P \leq 0.05$)

used in this study produced a light smoke-like fog ($<2 \mu\text{m}$) which remained suspended and filled the chamber more uniformly.

Micro-organisms can be attached to the surfaces of fruits and vegetables. In that case, aqueous sanitizers may be ineffective at killing micro-organisms. Attachment in inaccessible sites can be a one of the limiting factors of washing efficacy (Sapers 2001). When bacteria attach to the surfaces of fruits and vegetables, they tend to locate in pores, indentations or other natural irregularities where there are protected binding sites (Han *et al.* 2001b). Bacteria are especially inaccessible to aqueous treatments when concealed in injured sites or when present in hydrophobic pockets or folds in leaf surfaces (Lillard 1979). Numerous researchers have investigated the attachment of micro-organisms to food surfaces and their responses to various sanitizer treatments (Villarreal *et al.* 1990; Zottola 1994; Smoot and Pierson 1998). They concluded that the attachment of micro-organisms on such surfaces enhances their resistance to sanitization.

In addition, the formation of biofilms and adhesions to various inert supports may increase the resistance of bacteria to disinfectants (Nguyen-the and Carlin 1994). Colonization by micro-organisms of commodities, and postharvest contact surfaces can provide a protective environment for pathogens by making an extracellular polysaccharide matrix that holds the cells together and glues them to the surface, reducing the effectiveness of sanitizer and other inhibitory agents (Zottola 1994). These biofilms can be produced during transport,

processing, and storage after processing, as well as during growth and maturation of fruits and vegetables by cross-contamination (Beuchat 2002). If pathogens attach to biofilms during transport or storage, their survival and growth may be enhanced. Therefore, it is important to sanitize fruits and vegetables during transport and storage. With the continued development of new aerosol systems in capacity and miniaturization (Newman *et al.* 1986; Coates *et al.* 1997), aerosolization can easily be applicable in trailer cabinets and containers used for transport and of fruits and vegetables.

Sanitizers that are readily soluble in water can be easily delivered to target micro-organisms by using a fine mist aerosol as a carrier. Although gaseous sanitizers are effective, only a limited number of sanitizers can convert into gas form. But, aerosolization can deliver diverse antimicrobial agents. Indeed, in therapeutic applications, various antibiotics are delivered by aerosol ventilation (Purcell and Corris 1995; Griese *et al.* 1998; Melani and Di Gregorio 1998) and in room disinfection applications, organic acids such as lactic acid and acetic acid are delivered by aerosolization. Aerosolization has the advantages of both aqueous and gaseous sanitizers: a wide spectrum of applicable sanitizers, and high penetration power suitable for foods. And, with the continued development of new aerosolization systems, such as the home humidifier with smaller mist droplets, the penetration capacity and diffusiveness will be increased.

This study was intended to be of a preliminary nature. Further research needs to be carried out to optimize sanitation parameters, such as relative humidity, temperature, the composition of the shipping container and so forth. Such factors can impact the effectiveness of antimicrobial aerosol treatment. Therefore, before commercial application of this method can occur, additional studies about environmental effects will need to be investigated.

REFERENCES

- Beuchat, L.R. (2002) Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes Infect* **4**, 413–423.
- Coates, A.L., MacNeish, C.F., Meisner, D., Meisner, S., Kelemen, S., Thibert, R., MacDonald, J. and Vadas, E. (1997) The choice of jet nebulizer, nebulizing flow, and addition of albuterol affects the output of tobramycin aerosols. *Chest* **3**, 1206–1212.
- Fišer, A. (1978) Disinfection of air and dust in fattening houses for chickens by lactic acid aerosol. *Acta Vet Brun* **47**, 173–183.
- Griese, M., Schams, A. and Lohmeier, K.P. (1998) Amphotericin B and pulmonary surfactant. *Eur J Med Res* **3**, 383–386.
- Han, Y., Linton, R.H., Nielsen, S.S. and Nelson, P.E. (2000) Inactivation of *Escherichia coli* O157:H7 on surface-uninjured and -injured green pepper (*Capsicum annuum* L.) by chlorine dioxide gas as demonstrated by confocal laser scanning microscopy. *Food Microbiol* **17**, 643–655.

- Han, Y., Floros, L.D., Linton, R.H., Nielsen, S.S. and Nelson, P.E. (2001a) Response surface modeling for the inactivation of *Escherichia coli* O157:H7 on green peppers (*Capsicum annuum* L.) by chlorine dioxide gas treatments. *J Food Prot* **64**, 1128–1133.
- Han, Y., Linton, R.H., Nielsen, S.S. and Nelson, P.E. (2001b) Reduction of *Listeria monocytogenes* on green peppers (*Capsicum annuum* L.) by gaseous and aqueous chlorine dioxide and water washing and its growth at 7°C. *J Food Prot* **64**, 1730–1738.
- Hara-Kudo, Y., Konuma, H., Iwaki, M., Kasuga, F., Sugita-Konishi, Y., Ito, Y. and Kumagai, S. (1998) Potential hazard of sprouts as a vehicle of *Escherichia coli* O157:H7. *J Food Prot* **60**, 1125–1127.
- Hashimoto, S., Wolfe, E., Guglielmo, B., Shanks, R., Sundelof, J., Pittet, J.F., Thomas, E. and Wiener-Kronish, J. (1996) Aerosolization of imipenem/cilastatin prevents *Pseudomonas*-induced lung injury. *J Antimicrob Chemother* **38**, 809–818.
- Hiom, S.J., Lowe, C. and Oldcorne, M. (2003) Assessment of surface bioburden during hospital aseptic processing. *Int J Pharm Prac*, **September**, R62.
- Jarnych, V.S. (1972) *Aerzoli v verterinariu*. Kolos: Moskva, 350.
- Lenahan, R.J. (1992) Peroxyacetic acid: the new generation sanitizer. *MBAA Tech Q* **29**, 53–56.
- Lillard, H.S. (1979) Levels of chlorine dioxide of equivalent bactericidal effect in poultry processing water. *J Food Sci* **44**, 1594–1597.
- Maiz, L., Canton, R., Mir, N., Baquero, F. and Escobar, H. (1998) Aerosolized vancomycin for the treatment of methicillin-resistant *Staphylococcus aureus* infection in cystic fibrosis. *Pediatr Pulmonol* **26**, 287–289.
- Melani, A.S. and Di Gregorio, A. (1998) Acute respiratory failure due to gentamicin aerosolization. *Monaldi Arch Chest Dis* **53**, 274–276.
- Newman, S.P., Pellow, P.G. and Clarke, S.W. (1986) Choice of nebulisers and compressors for delivery of carbenicillin aerosol. *Eur J Respir Dis* **69**, 160–168.
- Nguyen-the, C. and Carlin, F. (1994) The microbiology of minimally processed fresh fruits and vegetables. *Crit Rev Food Sci Nutr* **34**, 371–401.
- Profé, D. and Steiger, A. (1982) Aerosol distribution in the air on surfaces of the animal house. *Tagungsbericht* **197**, 113–120.
- Purcell, I.F. and Corris, P.A. (1995) Use of nebulised liposomal amphotericin B in the treatment of *Aspergillus fumigatus empyema*. *Thorax* **50**, 1321–1323.
- Sapers, G.M. (2001) Efficacy of washing and sanitizing methods for disinfection of fresh fruit and vegetable products. *Food Tech Biotechnol* **39**, 305–311.
- Smoot, L.M. and Pierson, M.D. (1998) Influence of environmental stress of *Listeria monocytogenes* to Buna-rubber and stainless steel. *J Food Prot* **61**, 1286–1292.
- Steiger, A. (1982) Importance of free air exchange for the aerosol concentration level in the animal house. *Tagungsbericht* **197**, 105–111.
- Steiger, A., Trenner, P. and Profé, D. (1982) Technique of aerogenic disinfection during the service period in large animal houses. *Tagungsbericht* **197**, 93–97.
- Villarreal, M.E., Baker, R.C. and Pegenstein, J.M. (1990) The incidence of *Salmonella* on poultry carcasses following the use of slow release chlorine dioxide (Alcide). *J Food Prot* **53**, 464–467.
- Wanner, A. and Rao, A. (1980) Clinical indications for and effects of bland, mucolytic, and antimicrobial aerosols. *Am Rev Respir Dis* **122**, 79–87.
- Zottola, E.A. (1994) Microbial attachment and biofilm formation: a new problem for the food industry. *Food Tech* **48**, 107–114.