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Effect of food residues on efficiency of surfactant disinfectants against food related pathogens adhered on polystyrene and ceramic surfaces

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ABSTRACT

The adhesion of pathogenic bacteria on food contact surfaces increases the risk of cross-contamination in the food industry. However, food-borne disease introduced by the production process can be mitigated by surfactant use. This study investigates the effect of food residues (milk, beef gravy and tuna gravy) on the bactericidal efficiency of benzalkonium chloride (BAC) and alkyldiaminoethylglycine hydrochloride (AGH). The test was conducted on pathogenic bacteria (*Escherichia coli* O26, *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus,* and *B. cereus* spores) dried and adhered to the surfaces of polystyrene and ceramic dishes at room temperature for 1.5 h. The protein and lipid rich food residues protected the bacterial cells from dehydration and from the adverse effects of disinfectants, although bacterial numbers were decreased after drying and the surfaces were clearly sterilized after disinfectant treatment at typical concentrations (0.5 mg/ml–2.0 mg/ml) for 10 min. Following general and proper washing processes, the bactericidal effect of the disinfectants became clearly visible. These results indicate that applying a proper washing process prior to disinfectant treatment can prevent cross-contamination.

1. Introduction

Food-borne disease can be transmitted by surface contamination of equipment and utensils. This risk can be effectively reduced by washing and sterilization with a disinfection agent. A variety of disinfection agents are used in the food industry, such as alcoholic solutions, hypochloric solutions, and quaternary ammonium compounds (QACs). QACs are cationic biocides that have been widely used in both the food and medical fields (Krysinski, Brown, & Marchisello, 1992; Sundheim, Langsrud, Heir, & Holck, 1998). The QAC benzalkonium chloride (BAC) and the amphoteric surfactant alkyldiaminoethylglycine hydrochloride (AGH) are extensively used to sterilize the surfaces of equipment used in the food industry and medical devices used in nosocomial environments (Adair, Geftic, & Justus, 1969; Kawamura-Sato, Wachino, Kondo, Ito, & Arakawa, 2010; Marple, Roland, & Benninger, 2004; Pernak, Mirska, & Kmiecik, 1999).

Bacterial attachment to surfaces and biofilm formation are well recognized in a variety of environments (Carpentier & Cerf, 1993). Biofilm presence on equipment can lead to hygiene problems and productive and economic loss (Bremer, Fillery, & McQuillan, 2006; Verran & Jones, 2000). Adhesion and biofilm development of pathogenic microorganisms, and bacterial resistance, can reduce the effectiveness of disinfection agents (Frank & Koffi, 1990). The tolerance of biofilms formed by *Staphylococcus aureus*, *Bacillus cereus* spores, and *Pseudomonas aeruginosa* has been widely reported (Landry, An, Hupp, Singh, & Parsek, 2006; Lindsay, Brözel, & von Holy, 2006; Saá Ibusquiza, Herrera, & Cabo, 2011; Salgado, Farr, & Calfee, 2003).

Some reports (Barker & Bloomfield, 2000; Scott, Bloomfield, & Barlow, 1982) have indicated that biofilms form most frequently in domestic environments. Since many food containers and utensils are constructed from plastic and ceramic, cleaning these surfaces is a vital part of food safety. This study investigates ways of reducing bacterial adhesion to plastic and ceramic surfaces, and its associated risks. The objective of the study was to clarify (1) the effectiveness of BAC and AGH on actively growing cells, (2) the importance of washing to avoid bacterial adhesion to the surfaces of plastic and ceramic dishes, (3) the effectiveness of BAC and AGH on *B. cereus* spores.

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2. Materials and methods

2.1. Preparation of bacterial strains

The organisms used in this study were *Escherichia coli* O26:HNM (VTI), *P. aeruginosa* IAM1514, *S. aureus* IAM 12544 and *B. cereus* IAM12605 (vegetative cells and spores). Each strain was inoculated into 5 ml Trypto-soya broth (TSB, Nissui Pharmaceutical Co., Tokyo, Japan) and incubated at 37 °C with shaking (120 rpm) for 18 h. Cells were collected by centrifugation (2000 × *g*) for 10 min at RT (room temperature), and re-suspended twice in 5 ml phosphate-buffered saline (PBS, 0.31 mmol/l, pH 7.2). *B. cereus* spores were prepared as follows: *B. cereus* cells were cultivated in TSB and incubated at 37 °C for 2 weeks, then collected by centrifugation (2000 *g* for 10 min at RT), washed with PBS and re-suspended. Vegetative cells were killed by heating at 90 °C for 10 min; the spores were stored at −20 °C for further use.

2.2. Chemicals and samples

Benzalkonium chloride (BAC, 10% solution), alkyldiaminoethylglycine hydrochloride (AGH, 40% solution), soluble starch and BSA (bovine serum albumin) were purchased from Wako Pure Chemical (Osaka, Japan). The neutralized detergent (AES, alkyl ether sulfuric acid ester sodium) was purchased from Kao Corporation (Tokyo, Japan). Food samples, namely, UHT (ultra-high temperature-treated) milk, salad oil (Nisshin Oillio Group. Co., Ltd., Tokyo, Japan), frozen beef (sliced from the outside) and tuna meat, were purchased from a local market (Nonoichi, Japan). Beef and tuna gravies were prepared from the same volumes of the meat and distilled water (50% gravies). These gravies were mixed and then centrifuged at 2000 *g* for 10 min at RT.

2.3. Preparation of dishes

The plastic dishes (diameter: 5 cm, highs: 15 mm; polystyrene, sterilized by ethylene oxide gas) were purchased from As One Co., Ltd. (Osaka, Japan). The ceramic dishes were purchased from a local shop, brushed for 1 min and autoclaved for 15 min at 121 °C prior to experiment.

2.4. Preparation of bacterial adhesion and treatment of BAC and AGH

The strain suspensions (approximately 8–9 log cfu/ml) were mixed with the same volumes of distilled water (DW), salad oil (5%), starch (5%), BSA (5%), milk, 50% beef gravy and 50% tuna gravy. 0.01 ml of each suspension was placed on the centers of the plastic and ceramic dishes, then dried and adhered with ventilation (20 m^3/min) for 90 min at RT in the bio-clean bench (SCB-1300B, Shimadzu Rika Instrument, Tokyo, Japan). The dried spot was approximately 5 mm in diameter. To determine the effects of BAC and AGH, the adhered cells were covered with 0.1 ml of BAC and AGH solutions (0, 0.5, 1.0 and 2.0 mg/ml) for 10 min at RT. Next, 5 ml of PBS was added, and the cells were emphatically brushed for 30 s using cotton swabs (for microbial tests, Nissui Pharmaceutical Co., Tokyo, Japan). The suspensions (1 ml) were serially diluted 10-fold with 9 ml PBS, immediately plated onto Trypto-soya agar (TSA, Nissui Pharmaceutical Co.) and incubated at 37 °C for 48 h.

2.5. Washing treatment combined with BAC and AGH treatment

0.01 ml volumes of each cell suspension were placed in the centers of the plastic and ceramic dishes as described above. Six consecutive treatment steps were implemented: Step (A): pre-drying; Step (B): drying in a bio-clean bench for 90 min with ventilation (20 m^3/min); Step (C): gently washing twice with 4 ml sterilized DW, decanting the DW after each wash; Step (D): infusing the dish with 4 ml AES (0.25 mg/ml) for 10 min, decanting the AES, then gently washing the dish with 10 ml DW (D1). A similar treatment without AES was implemented by gently washing the dish with 4 ml sterilized DW, then decanting the DW (D2); Step (E): treating the dish with 1 ml BAC or AGH (0.5 mg/ml) for 10 min; Step (F): rinsing with 4 ml DW. Following each step of the treatment, the microorganisms that had survived the treatment and had grown on the TSA were counted.

2.6. Image of bacteria adhered on plastic dishes by SEM

Bacterial suspensions mixed with milk and PBS were placed on the plastic dishes in the bio-clean bench and dried for 90 min at RT. The plastic dishes with the adhered bacterial cells were cut by autoclaved scissors into plastic squares, approximately 6 mm × 6 mm in size. Specimens were coated with platinum by ion sputtering (Hitachi Ion Sputter E-1010, Hitachi Co., Tokyo, Japan) and observed under a field emission Scanning Electron Microscope (SEM; Hitachi S-4700, Hitachi Co.) operating at 15 kV.

2.7. Statistical analysis

All experiments were performed in triplicate. Data of surviving cells on the plastic and ceramic dishes were expressed as the mean and SD of log cfu/dish. Statistical analysis was conducted using the software EXCEL Statistic 5.0 (Esumi Co., Ltd., Tokyo, Japan). Differences were assessed by one-way ANOVA. Individual means were compared by Student's *t*-test or Duncan's multiple-range test. Significant differences were defined as $p < 0.05$.

3. Results

3.1. Effect of BAC and AGH on strains dried with organic components on a plastic dish surface

Table 1 shows the effect of BAC on strains dried with organic components on the surfaces of the plastic dishes. In the absence of BAC, *B. cereus* cells were present in fewer numbers than those of the other strains. Following treatment with 0.5 mg/ml BAC, *P. aeruginosa* and *S. aureus* cells dried with organic components failed to grow on the TSA plates. Other studied strains mixed with DW (before drying) and dried with salad oil were also absent on the TSA plates following 0.5 mg/ml BAC treatment. *E. coli* O26 cells dried with BSA and starch were not detected following 1.0 mg/ml BAC treatment. Treatment with 2.0 mg/ml BAC prevented the growth of *B. cereus* cells dried with starch.

Conversely, all of the investigated strains showed greater resistance to AGH (administered for 10 min) than to BAC (Table 2). *E. coli* O26 cells dried with starch and *P. aeruginosa* cells dried with salad oil grew on TSA following treatment with 2.0 mg/ml AGH. Under this treatment, only *B. cereus* failed to grow when dried with any of the organic components. Among the studied strains, *S. aureus* showed the greatest resistance to AGH; *S. aureus* cells dried with DW, BSA and starch were reduced by 3.73, 3.42 and 2.95 log cfu/dish, respectively, following treatment with 2.0 mg/ml AGH.

3.2. Effect of BAC and AGH on strain cells dried with milk, beef gravy and tuna gravy on a plastic dish surface

Table 3 summarizes the effect of BAC on cells dried with milk, beef gravy and tuna gravy on the plastic dishes. The populations of cells dried with milk were higher than those of cells dried with tuna or beef gravy. Although *B. cereus* spores displayed the lowest