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Short communication

Effect of carrot residue on the desiccation and disinfectant resistances of food related pathogens adhered to a stainless steel surfaces

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ABSTRACT

To clarify the effects of vegetable residues on the resistances of food-related pathogens to drying and disinfectants, *Salmonella* Thyphimurium was dried with 50% w/w aqueous solutions of seven vegetables and cell survival was assessed. Bacterial suspensions in distilled water or aqueous solutions (0.1 ml of 8 log CFU/ml) were placed on 50 mmφ stainless dishes and dried at room temperature for 120 min. In the absence of aqueous solution, cell numbers decreased from 6.9 to 3.6 log CFU/dish. The solutions of carrot (CR) and green bell pepper (GB) clearly protected the cells. CR and GB also tended to protect the dried *Staphylococcus aureus* cells. The adhered and dried pathogens with CR were treated with 0.1 ml of 0.01% w/v hypochlorous acid (HC), 70% v/v ethanol (ET), 0.2% w/v alkyldiaminoethylglycine hydrochloride (AH) or 0.2% w/v benzalkonium chloride (BC) for 10 min. Adhered *S.* Typhimurium cells were protected from HC and ET treatments by the carrot solution. *S. aureus* was protected from HC, ET, and AH. BC showed clear disinfectant activity under all conditions. These results suggest that trace amounts of carrot sediment protect the bacteria on surfaces from the desiccation and disinfectant treatments, and might also contribute to cross contamination.

1. Introduction

Salmonella is a well-documented pathogen known to occur in a wide range of foods, not only in chicken and egg products (Martelli & Davis, 2012; Zaki, Mohamed, & El-Sheriff, 2015) but also in vegetables (Miller, Davidson, & D'Souza, 2011; Sant'Ana, Landgraf, Destro, & Franco, 2011) and dried products (Kuda, Yazaki, Takahashi, & Kimura, 2013; Mol, Consansu, Alakavuk, & Ozturan, 2010). Most cases of human infection with *Salmonella enterica* subsp. *enterica* serotype Enteritidis result from the consumption of contaminated foods (Mølbak & Neimann, 2002). Furthermore, the incidence of salmonellosis from *S.* Typhimurium has remained relatively stable or increased in Europe and the other countries (Erkmen & Bozoglu, 1995; Gorman & Adley, 2004; Hernandez et al., 2012). *Staphylococcus aureus* is also a leading cause of gastroenteritis resulting from the consumption of contaminated foods. Staphylococcal food poisoning is due to the absorption of staphylococcal enterotoxins that are preformed in various foods, including vegetables (de Barros, da Conceição, Neto, da Costa, Júnior, Junior, & et al, 2009; Seo, Jang, & Moon, 2010).

Adhesion of microorganisms to equipment surfaces has the potential to transmit pathogens to food, and this is apparent in both the food-processing industry (Simões, Simões, & Vieirab, 2010) and in the domestic environment (Hayson & Sharp, 2005). It is known that microorganisms on the inner and/or wet surfaces of food and medical utensils and equipment often form biofilms that exhibit tolerance to various stresses (Weng, van Niekerk, Neethirajan, & Warriner, 2016). Specifically, there are many reports about the biofilms of *Pseudomonas aeruginosa* (Martín-Espada, D'ors, Bartolomé, Pereira, & Sánchez-Fortún, 2014), *Staphylococcus aureus* (Chen, Abercrobie, Jeffrey, & Leung, 2012), *Listeria monocytogenes* (Chaturongkasumrit, Takahashi, Keeratipibul, Kuda, & Kimura, 2011) and *S.* Typhimurium (Nguyen, Yang, & Yuk, 2014), due to their strong resistance to disinfectants and their medical importance in disease, such as nosocomial infections.

We previously reported on the resistance of *S.* Typhimurium and *S. aureus* cells, dried and adhered on stainless steel or glass surfaces with residues of protein and lipid rich foods, such as milk, meat and egg, to drying, surfactant disinfectants and also 254 nm ultraviolet (UV-C) irradiation (Kuda et al., 2011; Kuda et al., 2012; Kuda, Yano, & Kuda, 2008). These protein and lipid rich food residues clearly

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protected the pathogen cells from the stressors. In this study, to clarify the effects of vegetable sediments on the resistance of food related pathogens to drying, *S.* Typhimurium was dried with 50% (w/w) aqueous solutions of seven vegetables and cell survival was determined. Then, the protective effect of selected vegetable on adhered *S.* Typhimurium and *S. aureus* exposed to disinfectants was determined.

2. Materials and methods

2.1. Bacterial culture and vegetables

Salmonella enterica subsp. *enterica* serotype Typhimurium NBRC 13245 and *Staphylococcus aureus* NBRC 12732 were employed to investigate cell attachment and UV-C irradiation treatments on utensil surfaces. To produce cultures, a loop of bacterial cells from Trypticase Soy Agar (TSA; Becton, Dickinson and Co., Sparks, MD) were inoculated into 10 ml of Trypticase Soy Broth (TSB; Becton, Dickinson and Co.) and incubated at 37 °C for 20 h. The cultures reached stationary phase. In generally, stress resistances of the cells of stationary phase are stronger than ones of cells of logarithmic growth phase (Kuda et al., 2008).

Seven raw and fresh vegetables, green bell pepper *Capsicum annuum* var. *grossum*, carrot *Daucus carota* subsp. *sativus*, Japanese white radish *Raphanus sativus* var. *longipinnatus*, black gram sprout *Vigna mungo*, cucumber *Cucumis sativus*, cabbage *Brassica oleracea* var. *capitata* and bok-choy *Brassica rapa* subsp. *chinensis*, were purchased from a retail shop in Tokyo. The fresh vegetables (50 g) were minced with 50 ml of distilled water for 60 s using a blender (at grind mode, 16 Speed Blender, Osaka Chemical, Osaka, Japan) and shaken for 60 min at room temperature. The water extract solution was collected by centrifugation (2500 g for 10 min) and sterilized by filtration with a 0.22 μm pore size filter.

2.2. Surface adhesion of S. Typhimurium and S. aureus

Fifty mmφ stainless steel dishes were purchased from As One Co. (Osaka, Japan) and used as the experimental surface (Kuda et al., 2008). In advance, the dishes underwent ultra-sonication twice for 15 min (Ultra Sonic Automatic Washer, As One Co.), brushing for 60 s and autoclaving at 121 °C for 15 min.

Bacterial cells were placed in the dish and attached according to the method previously reported (Kuda et al., 2011), with slight modifications. Briefly, bacterial suspensions were prepared from TSB cultures (10 ml) in stationary phase. Bacterial cells were centrifuged at 2000 g for 10 min and re-suspended in phosphate buffered saline (PBS: Nissui Pharmaceutical Co., Tokyo, Japan); the washing process was repeated twice. Next, the cells were re-suspended in 10 ml of distilled water (DW) or the vegetable extract solutions at a final cell concentration of about 8 log CFU/ml.

The bacterial suspension (0.1 ml) was dropped in the center of the dish ($n = 3$) and dried for 120 min at room temperature (20–24 °C) in a bio safety cabinet (Class IIA; Airtech Japan Co., Tokyo, Japan) with ventilation. After drying, the adhered cells were scraped and thoroughly re-suspended for 60 s with 5 ml of TSB using a cell scraper (TPP, Zurich, Switzerland). Then, the detached cell suspension (0.1 ml) was immediately diluted with 9.9 ml of PBS, spread on Trypticase Soy Agar (TSA, Becton, Dickinson and Co.), and incubated at 37 °C for 24 h. In a preliminary test, >99% of the adhered cells was detached using this scraping method.

2.3. Disinfectant treatment and enumeration of viable cells on stainless steel dishes

To determine the bactericidal effect of the disinfectants, the dried and adhered *S.* Typhimurium and *S. aureus* cells in carrot extract solution and on the stainless steel surface, prepared as above, were covered with 0.1 ml of 0.01% w/v sodium hypochlorous acid (HC; Wako Pure Chemical, Osaka, Japan), 70% v/v ethanol (ET; Wako Pure Chemical), 0.2% w/v benzalkonium chloride (BC; Wako Pure Chemical) or alkyldiaminoethylglycine hydrochloride (AH; Hygieel, Maruishi Pharmaceutical, Osaka, Japan) solutions, which is the highest recommended concentration indicated by the makers. After 10 min at room temperature, 5 ml of TSB was added. Then, cell viability was determined as above.

2.4. Statistical analysis

The cell viability data was expressed as the mean and standard deviation of log CFU/dish ($n = 3$). Statistical analysis was performed using the software EXCEL Statistic 5.0 (Esumi Co., Ltd., Tokyo, Japan). One-way ANOVA was used to assess differences. Then, individual means were compared using Tukey's test. Significant differences were accepted at $p < 0.05$.

3. Results and discussion

3.1. Effects of drying and vegetable type on bacterial cells on surfaces

The numbers of viable *S.* Typhimurium cells before and after drying on stainless steel surfaces are summarized in Fig. 1A. During the 120 min ventilation at room temperature, the viable cells in DW were thoroughly dried and decreased from 6.9 to 3.7 log CFU/dish.

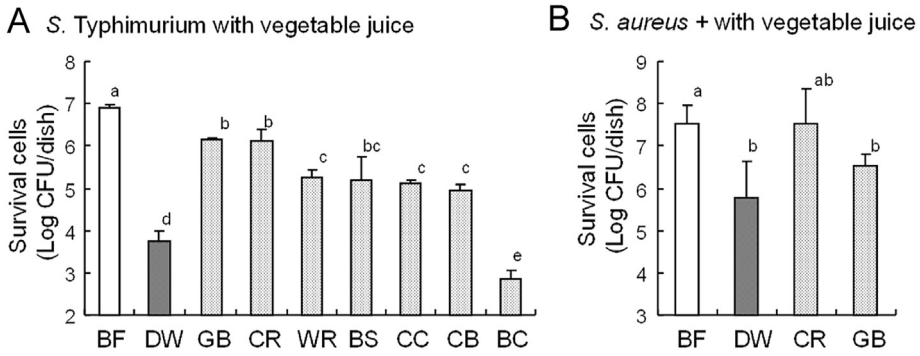

Fig. 1. Survival of *Salmonella* Typhimurium (A) and *Staphylococcus aureus* (B) on stainless steel dishes before (BF) and after 2 h drying at room temperature (20–24 °C) with distilled water (DW), 50% w/w green bell pepper (GB), carrot (CR), Japanese white radish (WR), black gram sprout (BS), cucumber (CC), cabbage (CB) and bok-choy (BC). Values are expressed as mean and SD ($n = 3$). $^{a-e,}$ Different letters indicate significant differences ($p < 0.05$) between groups.