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Short communication

Effect of the quantities of food residues on the desiccation resistance of spoilage lactic acid bacteria adhered to a stainless steel surface

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ABSTRACT

Although lactic acid bacteria (LAB) are regarded as beneficial, these Gram-positive bacteria are considered spoilage microorganisms in various processed foods. In order to study the effect of food residues on the survival of spoilage LAB, *Lactobacillus sakei* and *Leuconostoc mesenteroides* were subjected to drying conditions in the presence of small amounts of meat gravy, dairy milk, soy milk, or egg. Bacterial suspensions (100 μL) were placed on a steel surface (50 mm diameter dish) and dried for 2 h at room temperature in the absence of food residue. Drying reduced bacterial cell counts from about 7 to 8 log CFU/dish to 3–4 log CFU/dish. As little as 10 μL of meat gravy, dairy milk or soy milk, or 1 μL of egg yolk per mL of LAB suspension was sufficient to demonstrate a protective effect on the adhered spoilage LAB, as confirmed by scanning electron microscopy. The results of this study suggest that small sediments of food, where they are protein or carbohydrate rich, can increase the resistance of surface-adherent bacteria to desiccation. Therefore, small amounts of food residue can render sanitization processes ineffective and encourage cross contamination.

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1. Introduction

Microbial adhesion to surfaces is a potential route of transmission for pathogenic and spoilage microorganisms in the food-processing industry (Jiménez-Pichardo et al., 2016; Simões, Simões, & Vieirab, 2010) and in the domestic environment (Azevedo et al., 2005; Haysom & Sharp, 2005). Microorganisms within and/or on wet surfaces such as plants, utensils, and equipment often form biofilms that promote resistance to various kinds of stress (Finn et al., 2013; Maifreni et al., 2015; Wang et al., 2013). In particular, biofilms formed by *Pseudomonas aeruginosa* (Li, Kuda, & Yano, 2014; Long et al., 2016), *Staphylococcus aureus* (Vázquez-Sánchez, Cabo, Ibusquiza, & Rodríguez-Herrera, 2014), *Listeria monocytogenes* (Chaturongkasumrit, Takahashi, Keeratipibul, Kuda, & Kimura, 2011), and *Salmonella* (Nguyen & Yuk, 2013) pose serious threats because of their strong resistance to disinfectants and their roles in nosocomial infections.

Although lactic acid bacteria (LAB) are regarded as beneficial, these Gram-positive bacteria are considered spoilage microorganisms in various processed foods (Pothakos, Stellato, Ercolin, & Devlieghere, 2015). For example, some LABs generate biogenic amines from amino acids in beer (Geissler, Behr, von Kamp, & Vogel, 2016). In the case of wine, some LAB produce not only biogenic amines, but also exopolysaccharide slimes, acetic acid, diacetyl and other undesirable flavours (Petri, Pfannebecker, Fröhlich, & König, 2013). Various LAB cause souring, slime formation, and greening of meat (Borch, Kant-Muermans, & Blixt, 1996; Egan, 1983;). Furthermore, the spoiling and swelling caused by LAB are a big problem for various modified-atmosphere-packaged (MAP) food products (Björkroth & Korkeala, 1997; Rahkila et al., 2015; Vihavainen et al., 2007).

We have previously reported that when food poisoning agents such as the Typhimurium serovar of *Salmonella enterica,* or *S. aureus,* were dried in the presence of nutrient-rich food residues they showed resistance to desiccation, disinfection by surfactants such as benzalkonium chloride, and irradiation with 254-nm ultraviolet (UV)-C light (Kuda, Yano, & Kuda, 2008; Kuda et al., 2011, 2012; Li et al., 2014). This indicates that protein-rich, lipid-rich, and/or carbohydrate-rich food residues can protect pathogens from stressful conditions. Therefore, washing and rinsing prior to sterilization are essential to meet the strictly sterile requirements for food safety. Importantly, the properties of food residues that protect spoilage LAB and other harmful bacteria from stresses such as desiccation, disinfection, or UV-C radiation remain unclear.

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In this study, we investigated the quantity-dependent effects of egg, dairy milk, soy milk, and meat gravy on the survival rates of the food-spoilage bacteria *Lactobacillus sakei* and *Leuconostoc mesenteroides* (Kalschne et al., 2015; Samelis, Kakouri, & Rementzis, 2000;) when desiccated on a stainless steel surface.

2. Materials and methods

2.1. Bacterial culture and food material

L. sakei subspecies *sakei* JCM 1157 and *L. mesenteroides* subspecies *mesenteroides* NBRC 100496 were adhered by drying onto stainless steel dishes. To produce cultures, the bacterial cells were inoculated into 20 mL Gifu Anaerobic Medium (GAM) broth (Nissui Pharmaceutical, Tokyo) and incubated at 30 °C for 72 h. Under these conditions, the cultures reached stationary phase.

Dairy (cow) milk, soy milk, beef, pork, and eggs were purchased from retail shops in Tokyo. The beef and pork were minced separately with an equal amount of distilled water (DW) for 60 s in a blender (16-Speed Blender; Osaka Chemical; Osaka) and shaken for 60 min at room temperature. The gravies were collected by centrifugation (5000 × *g* for 10 min) and sterilized by filtration with a 0.2 μm pore filter (Minisart, Sartorius, Goettingen). The gravies, dairy milk, soy milk, egg albumen, egg yolk, and whole egg samples were serial-diluted by 10-fold increments in DW to a maximum dilution of 10^{-5} (100000-fold).

2.2. Adhesion of food pathogens to surfaces

Fifty-millimeter diameter stainless steel dishes were purchased from As One Co. (Osaka, Japan) and used as experimental surfaces. Prior to use, in order to equalize the effect of the surface conditions on the survival cell count, the steel dishes were ultra-sonicated twice for 15 min, brushed for 60 s, and autoclaved at 121 °C for 15 min. The bacterial cells were placed in the dish and attached as previously reported (Kuda, Shibata, Takahashi, & Kimura, 2015), with slight modifications. Briefly, bacterial cells in the GAM broth culture were washed by centrifugation at 5000 × *g* for 10 min at 4 °C, and re-suspended in phosphate-buffered saline (PBS; Nissui Pharmaceutical). This washing process was repeated twice. The cells were finally re-suspended in 2 mL of DW. Then 50 μl of this cell suspension was added to 1 mL of the diluted food samples or DW (control) that the final cell concentration was approximately 7–8 log CFU/mL.

A bacterial suspension (100 μL) was spread over an area of about 10 mm diameter 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 detached by rubbing for 60 s using a sterile cotton swab and re-suspended in 3 mL PBS containing 0.1% agar (PBS-A). The detached cell suspension (30 μL) was immediately diluted with PBS-A, spread on Plate Count Agar with Bromocresol Purple (Nissui Pharmaceutical) and incubated at 30 °C for 72 h. The viable count of cell suspension with DW on the steel surface before drying was also determined.

2.3. Microscopic observations

For observation, 3 μL of the bacterial cell and food residue suspensions, prepared as described, were adhered by semi-drying onto a cover slip. The bacterial were observed using a tabletop scanning electron microscope (SEM; Miniscope TM3030, Hitachi High-Technologies, Tokyo) in low vacuum and charge reducing mode. The samples were prepared without any vapor deposition or staining treatments.

2.4. Statistical analysis

Bacterial cell viability was expressed in terms of mean and standard deviation of log CFU/dish ($n = 3$). Statistical analysis was performed using EXCEL Statistic 6.0 software (Esumi Co., Ltd., Tokyo, Japan). One-way ANOVA was used to assess differences among groups, and individual means were compared by Tukey's multiple comparison test. Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Effect of drying on the survival of LAB cells on a stainless steel surface

Fig. 1 shows the results of viable counts on the stainless surface before and after drying. In this experiment, the food residue solution was not diluted. After drying for 120 min at room temperature, viable cell count of *L. sakei* in DW significantly ($p < 0.05$) decreased from approximately 6.3–7.6 log CFU/dish to 2.5–4.1 log CFU/dish. The LAB cells were protected from desiccation by beef or pork gravy (Fig. 1A and B), and dairy or soy milk (Fig. 1C and D). Although yolk clearly protected the cells (Fig. 1E and F), the effect was weaker than that of meat gravy or milk. Furthermore, the protective effects of albumen and whole eggs were clearly weaker than that of egg yolk. It can be considered that moisture in food residue and the bacterial cells was not completely removed by the drying, because the drying rate of food residue may be different from that of DW.

3.2. Protective effects of dilute food residues on adhered LAB cells

Fig. 2 shows the viable cell counts of LABs adhered with DW. Both the 100-fold diluted beef and pork gravies affected the survival rate of the two LAB strains (Fig. 2A and B). Although 100-fold diluted dairy milk also protected both LAB strains, the protective effect of soy milk on *L. mesenteroides* was not clear (Fig. 2C and D). In the case of *L. sakei*, the protective effects of yolk and whole egg were clear, even at 1000-fold dilutions (Fig. 2E). Conversely, a protective effect of albumen on *L. sakei* was weak and was only clear upon the diluted one. The protective effect of albumen on *L. mesenteroides* was smaller than that of yolk or whole egg (Fig. 2F).

The results reported here using dairy and soy milk are consistent with those of our previous study where *S. enterica* serovar Typhimurium and *S. aureus* bacteria were used (Kuda et al., 2015). However, differences between the protective effects of yolk, albumen, and whole egg on *S. enterica* serovar Typhimurium or *S. aureus* have not been shown (Kuda et al., 2012). This discrepancy may be due to the antibacterial effect of lysozyme in egg albumen attacking the polysaccharides of the Gram-positive cell wall (Abeyrathne, Lee, & Ahn, 2013), and thereby reducing the survival rates of the LAB used in this study. The sensitivity of Gram-positive bacteria to egg lysozyme differs between strains, and *S. aureus* has been reported to be resistant to egg lysozyme (Masschalck, Deckers, & Michiels, 2002).

Of particular note is the finding that only 1 μL (10^{-2} dilution of 100 μL) of meat gravy, dairy milk, or soy milk could protect one percent or more of the LAB cells. Furthermore, as little as 100 nL (10^{-3} dilution of 100 μL) yolk or whole egg could protect a similar percentage of the LAB cells.

3.3. Microscopic observations of adhered LAB cells

Scanning electron microscopy (SEM) images of dried LAB cells are shown in Fig. 3. In the absence of food residues, the *L. sakei* cells were clearly observable (Fig. 3A). In the case of *L. mesenteroides*

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