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Survival of foodborne pathogens on stainless steel soiled with different food residues

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Abstract Insufficient and ineffective cleaning practices can cause food residues to remain in kitchen and can facilitate bacterial attachment and persistence by protective films. The present study investigated the survival of five major foodborne pathogens on stainless steel coupons, in the presence of cooked rice, whole eggs, and soymilk. Foodborne pathogens showed different survival rates by desiccation and disinfection depending on food residues. Overall, the pathogens showed stronger survival than the control at 0.13–3.97 log CFU/coupon with 5% residues, and at 0.75–5.29 log CFU/coupon with 50% residues. *Staphylococcus aureus* was not affected by the food residue with showing the least difference in concentration, while *Escherichia coli* O157:H7 showed the most significant increase by food residue. The cells with cooked rice were observed using FE-SEM, and demonstrated bacterial binding or embedment. All results suggest that food safety can be practically ensured by food residue types and appropriate cleaning and disinfectants.

Keywords Foodborne pathogens · Food residues · Disinfectant · Cross-contamination · Stainless steel

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Introduction

A large number of foodborne illnesses are associated with food safety failures, such as improper holding times, temperatures, and handling procedures; thus, proper food handling, storage, and preparation are a critical line of defense for preventing these illnesses. Cleaning procedures primarily aim to remove food and other organic soil from food-contact surfaces in order to promote disinfectant processes for the elimination of spoilage and pathogenic bacteria (Kusumaningrum et al., 2003). Outbreaks of food poisoning have increased, with outbreaks occurring in households, restaurants, and other food service facilities (Todd et al., 2009). Unlike food-processing facilities, restaurants, cafeterias, and houses use various foods and ingredients every day from different environments and different food suppliers. Naturally contaminated raw ingredients and contaminants from other environmental sources, such as air, dust, water, human-derived materials (e.g., hair, wounds, nails), and materials from other animals, may contain a wide range of microbial ecologies as potential biological hazards. Therefore, there is a higher

risk of foodborne illness in restaurants, school cafeterias, and houses due to the potential exposure to numerous different types of foodborne pathogens (Sattar et al., 2001; Scott and Bloomfield, 1990; Zhao et al., 1998). During 2009 and 2015 in Korea, 72% of the total foodborne outbreaks and 75% of the total cases were attributed to restaurants and school cafeterias (Ministry of Food and Drug Safety, 2016). The U.S. Food and Drug Administration (FDA) reported over 800 illnesses linked to multistate outbreaks in cereal, romaine lettuce and salads from restaurants in 2018 (U.S. FDA, 2018).

Insufficient and ineffective cleaning practices facilitate bacterial attachment and persistence on food-contact surfaces by creating protective films from organic materials and nutrients and hindering bacterial detachment from the surfaces (Abban et al., 2012; Quan et al., 2017; Verran, 2002). Therefore, the combined effect of proper cleaning and sanitization should be used to ensure food safety in food service facilities (Koo et al., 2013).

Bacterial attachment and biofilm formation have been well-recognized in a variety of abiotic and biotic environments (Giaouris et al., 2014). Microorganisms can survive in the presence of food residues remaining on food processing or handling equipment after use: acid-resistant *Salmonella* Typhimurium and *Staphylococcus aureus* with squid and eggs (Kuda et al., 2012), *S. aureus* with milk (Hamadi et al., 2014), and *Listeria monocytogenes* with ready-to-eat meat products on stainless steel (Somers and Wong, 2004). In addition, the attachment of microorganisms to different surfaces followed by biofilm formation enhanced the resistance of cells to environmental stresses (Burmølle et al., 2006). The factors that affect the attached microorganisms include nutrient availability, type of contact surfaces, and storage conditions, e.g., relative humidity (RH) and temperature (Dourou et al., 2011).

Therefore, we compared the survival of foodborne pathogens on food-contact surfaces, depending on the food residue, storage time and disinfectant. The target food residue materials were selected from ingredients that are frequently used in Korea but that have been investigated by only limited studies to date: cooked rice, whole eggs, and soybeans. The application of real food materials can mimic real food-processing facilities and enable the observation of the microbial responses to the food residue. In addition, this study compared the survival of different bacteria with the same food residue and surface conditions. Stainless steel was selected as the target food-contact surface material because it is the most commonly used surface material in food processing facilities due to its corrosion resistance, hardness, and low cost (Schmidt et al., 2012). The bacterial adherence in the presence of food residues was monitored using quantification analysis and field emission-SEM (FE-SEM).

Materials and methods

Bacterial culture and food materials

Cronobacter sakazakii ATCC 29004, *Escherichia coli* O157:H7 ATCC 43894, *L. monocytogenes* ATCC 19113, *Salmonella* Enteritidis ME14, *Staphylococcus aureus* KCCM 40050 were obtained from the bacterial culture collection of the Korea Food Research Institute. The bacterial cells were incubated in a brain–heart infusion (BHI; MERCK, Darmstadt, Germany) at 37 °C for 18 h with shaking (120 rpm). The cultured cells were harvested via centrifugation for 5 min at 7000×*g*, washed twice, and resuspended in phosphate-buffered saline (PBS) for further use. Instant cooked rice, fresh eggs, and soy milk (100% soybeans with no sugar and no preservatives) were purchased from a retail shop in Seongnam-si, Korea and processed in the following way. Thirty grams of instant rice was aseptically transferred to a blender (SMX-S500, Shinil, Seoul, Korea), and the sample was blended for 2 min with 90 mL of sterile distilled water. The eggshells were washed thoroughly with 70% ethanol and then allowed to dry. The eggs were aseptically broken, transferred to a beaker, and homogenized for 1 min using a hand blender (HM5110, Bomann, Kempen, Germany).

Preparation of stainless steel coupons

Stainless steel coupons (SSCs, SUS304, 2 × 2 × 0.5 cm, As-one Co., Korea) were used in this study. Prior to bacterial adhesion, the coupons were washed with alkaline detergent, rinsed, sonicated for 10 min, and washed with distilled water. The coupons were dried and autoclaved at 121 °C for 15 min.

Survival of adherent cells in the presence of food residues

The food samples were mixed with the resuspended cell culture to obtain a final concentration of approximately 6.0–7.0 log CFU/mL of the bacterial cells and 50%, 25%, and 5% of the food residue. The contaminated SSCs were prepared as follows: 0.05 mL mixture was spread in the center of the SSC and dried under ventilation for 2 h at room temperature in a biosafety cabinet. After drying, the contaminated SSC was washed with 1 mL PBS. For enumeration of the bacteria, the SSCs were transferred to 50 mL sterile conical centrifuge tubes containing 15 mL of PBS and 3 g of beads (glass, 1.5–2.0 mm in diameter). The tubes were vortexed at a maximum speed for 60 s in order to dislodge and disperse the cells from the surfaces of the SSCs. The number of surviving bacteria was determined

using selective agars of the following: eosin methylene blue (EMB, Merck & Co., Kenilworth, New Jersey, USA) for *C. sakazakii, Sal. Enteritidis*; phenylethyl alcohol agar (PEA, MBcell, Seoul, Korea) for *S. aureus*; polymyxin acriflavine lithium chloride ceftazidime aesculin mannitol agar (PALCAM, Oxoid, Basingstoke, England) for *L. monocytogenes*; cefixime tellurite sorbitol MacConkey (CT-SMAC, Oxoid) for *E. coli* O157:H7.

Survival of adherent cells in the presence of food residues and disinfectant

The food samples were mixed with the resuspended cell culture in order to obtain a final concentration of approximately 6.0–7.0 log CFU/mL of the bacterial cells and 25% of the food residue. After drying the contaminated SSCs, 0.2 mL of disinfectant was added and incubated for 10 min at room temperature. The disinfectant concentrations were as follows: 2000 mg/L NaClO (junsei, Tokyo, Japan) and 500 mg/L benzalkonium chloride (BAC, Kukbo Science, Cheongju, Korea). The survival number was determined by plating on each selective agar.

Survival of adherent cells with food residues during storage in high humidity

Instant cooked rice was mixed with each strain in order to obtain a final bacterial concentration of approximately 6.0–7.0 log CFU/mL and a food residue concentration of 25%. After drying the contaminated SSC, the SSCs were stored in a polypropylene storage box. The box was maintained under constant relative humidity (RH) at 68% using saturated potassium iodide (Samchun Pure Chemical Co., Ltd., Korea). The storage boxes were incubated at 25 °C for 10 days. The temperature and RH were monitored using a Thermo TR-71Ui recorder (T&D Corporation, Nagano, Japan). The survival of the pathogens was examined at days 0, 1, 2, 4, 7, and 10. The SSC was washed with 1 mL PBS and transferred to a 50 mL conical centrifuge tube containing 15 mL of sterile PBS and 3 g of beads. The tubes were vortexed for 60 s in order to dislodge and disperse the cells from the surfaces of the coupons. The number of surviving bacteria was determined via plating on each selective agar.

Field emission-scanning electron microscopy

The sample was prepared as described in the earlier section of *Survival of adherent cells in the presence of food residues.* After 2 h of drying, each SSC was fixed in a Karnovsky solution for 2 h at 4 °C and rinsed with 0.05 M sodium cacodylate buffer (pH 7.2) three times. Samples were postfixed with 2% osmium tetroxide in 0.1 M cacodylate buffer for 2 h. After fixation, the coupons were dehydrated with an ethanol solution (30–100%). After dehydration, the SSCs were dried twice with hexamethyldisilazane for 10 min. The dehydrated SSCs were dried in a fume hood, sputter-coated with platinum, and then analyzed via FE-SEM (LEO SUPRA 55, Carl Zeiss, Germany).

Statistical analyses

All experiments were repeated three times with duplicate plates. The data analysis was performed using the PASW Statistics 18.0 program; one-way ANOVA was used to assess differences among the groups, and individual means were compared using Tukey's multiple-range test. Differences were considered to be significant at $p < 0.05$.

Results and discussion

Protective effect of food residues on bacterial adhesion on SSCs

Three different food ingredients that are frequently consumed in Korea were used in this study: cooked rice, whole eggs, and soy milk. In Korea, daily dietary intake of grain, egg, and soy was 289.3 g, 26.8 g, and 32.0 g in 2017, respectively (KCDC, 2018). In particular, rice is the main source of nutrition for Koreans, accounting for 39.8% of daily energy supply and 23.7% of protein supply (Ministry of Health & Welfare, 2013). The nutrient content of the cooked rice was 33.80 g carbohydrate per 100 g of rice, while whole eggs contained 12.58 g of protein and 9.94 g of fat. The soy milk contained 2.70 g of carbohydrates, 3.65 g of protein, and 1.90 g of fat.

In our previous study, 6.85 and 5.90 CFU/cm^2 of total aerobic bacteria were identified in the cold room and apron, respectively (Lim et al., 2017). In addition, the inoculum concentration used in the experiment was determined with the consideration of the reduction result to be over the detection limit for more accurate result. The food samples were mixed with the resuspended cell culture of approximately 6.0–7.0 log CFU/mL of the bacterial cells with 50%, 25%, and 5% of the food residue.

Without food residues, the control exhibited 1.14 to 5.18 log unit reductions after 2 h of initial attachment (Table 1). At 5% food residue, the survival of the bacteria compared with the inoculum level ranged from 0.72 to 3.95 log, from 0.07 to 3.32 log, and from 0.64 to 3.53 log in the cooked rice, whole eggs, and soy milk, respectively (Table 1). *E. coli* O157:H7 exhibited the strongest survival effect with the addition of 5% cooked rice compared to the control with increases of 2.99 and 3.23 log CFU/coupon. The

Table 1 Survival of bacteria on the stainless steel surface in the presence of 0%, 5%, 25% and 50% of cooked rice (log CFU/coupon)

Food-residue	Bacteria	Before drying	After drying*			
			Concentration of food-residue			
			0%	5%	25%	50%
Cooked rice	*C. sakazakii*	6.53 ± 0.03^{Aa1}	3.68 ± 0.28^{Db}	4.88 ± 0.12^{Cb}	5.62 ± 0.07^{Bb}	5.91 ± 0.01^{Bb}
	E. coli O157:H7	6.68 ± 0.00^{Aa}	ND	3.23 ± 0.27^{Cd}	5.27 ± 0.17^{Bc}	5.22 ± 0.18^{Bc}
	L. monocytogenes	6.28 ± 0.13^{Ab}	2.39 ± 0.39^{Dc}	4.13 ± 0.07^{Cc}	5.55 ± 0.01^{Bb}	5.76 ± 0.03^{Bb}
	Sal. Enteritidis	6.65 ± 0.12^{Aa}	3.05 ± 0.12^{Dbc}	4.06 ± 0.24^{Cc}	4.88 ± 0.19^{Bd}	4.77 ± 0.19^{Bd}
	S. aureus	6.69 ± 0.15^{Aa}	5.55 ± 0.04^{Da}	5.68 ± 0.07^{Da}	6.03 ± 0.12^{Ca}	6.30 ± 0.10^{Ba}
Whole eggs	*C. sakazakii*	6.59 ± 0.01^{Aa}	3.27 ± 0.53^{Db}	4.71 ± 0.25^{Cb}	5.36 ± 0.37^{BCc}	5.67 ± 0.29^{ABab}
	E. coli O157:H7	6.65 ± 0.01^{Aa}	1.47 ± 0.58^{Dc}	3.33 ± 0.75^{Cc}	4.95 ± 0.11^{Bc}	5.42 ± 0.31^{Bab}
	L. monocytogenes	6.26 ± 0.26^{Ab}	2.20 ± 0.35^{Cc}	4.97 ± 0.21^{Bb}	5.88 ± 0.12^{Ab}	5.82 ± 0.10^{Ab}
	Sal. Enteritidis	6.50 ± 0.01^{Aa}	3.11 ± 0.54^{Db}	4.40 ± 0.11^{Cb}	5.15 ± 0.28^{Bc}	5.35 ± 0.12^{Bc}
	S. aureus	6.66 ± 0.06^{Aa}	5.25 ± 0.35^{Ca}	6.25 ± 0.13^{Ba}	6.54 ± 0.08^{Aa}	6.63 ± 0.03^{Aa}
Soy milk	*C. sakazakii*	6.53 ± 0.01^{Ac}	2.67 ± 0.16^{Dc}	4.65 ± 0.25^{Cb}	5.00 ± 0.25^{Bb}	5.47 ± 0.24^{Bbc}
	E. coli O157:H7	6.74 ± 0.04^{Aa}	ND	3.97 ± 0.14^{Cc}	5.14 ± 0.11^{Bb}	5.29 ± 0.05^{Bc}
	L. monocytogenes	6.06 ± 0.05^{Ad}	2.98 ± 0.10^{Eb}	4.28 ± 0.20^{Dc}	5.29 ± 0.12^{Cb}	5.58 ± 0.01^{Bb}
	Sal. Enteritidis	6.53 ± 0.01^{Ac}	2.09 ± 0.08^{Dd}	3.50 ± 0.19^{Cd}	3.69 ± 0.52^{Cc}	4.70 ± 0.06^{Bd}
	S. aureus	6.67 ± 0.01^{Ab}	5.36 ± 0.01^{Da}	6.03 ± 0.09^{Ca}	6.47 ± 0.02^{Ba}	6.58 ± 0.05^{ABa}

*Cell suspensions were placed onto a stainless steel surface and dried in a bio safety cabinet for 2 h with ventilation

ND not detected

[1]Values with different alphabetic notations are statistically different ($p < 0.05$). Uppercase letters designate statistical difference of values within each row, whereas lowercase letters describe statistical difference of values within each column

viable cell count of *S. aureus* on SSC was the highest among the five foodborne pathogenic bacteria used in this study. There was no significant difference in the *S. aureus* with 5% and 0% cooked rice. *S. aureus* showed no reduction from the inoculum in the 25% whole eggs. *S. aureus* was the bacterium least affected by the presence of food residue among the bacteria tested.

Allan et al. (2004) demonstrated that stainless steel does not support the survival of *L. monocytogenes*, and this property was also observed in this study. The survival rate of *L. monocytogenes* on stainless steel without food residue was relatively low. However, significant increases in survival were observed in the presence of food residues, particularly with whole eggs from 2.20 log CFU/coupon without eggs to 4.97 log CFU/coupon with 5% whole eggs. Once the bacterium was attached to the residue, extensive measures should be taken for the removal of *L. monocytogenes* in order to prevent foodborne illness. Koo et al. (2013) tested the efficiency of removing *L. monocytogenes* from a turkey luncheon meat on food-contact surfaces using cleaning cloths. Without disinfectant, *L. monocytogenes* was reduced by 0.92–2.62 and 2.21–3.44 log CFU/cm^2, depending on the cloth fabrics on stainless steel and Formica laminate surfaces, respectively (Koo et al., 2013).

Sal. Enteritidis exhibited an increased viable cell count as the concentration of food residue increased but showed a lower survival rate than other strains used in the experiment. Allan et al. (2004) also demonstrated that *Salmonella* and *Yersinia* were not affected by stainless steel soiled with porcine serum, while significantly higher numbers of *L. monocytogenes* survived with the serum. In contrast, *S. aureus* recovered to the inoculum level in food residues at 25% and 50%, which is in agreement with the results of Kuda et al. (2012). Overall, the result was varied depending on the pathogens and food type. *C. sakazakii* was the most resistant to cooked rice than other pathogens, while other pathogens were more resistant with the addition of whole eggs. Whole eggs contain a relatively higher fat and protein content than the other food residues, which increases the protective effect that can interfere with disinfectant activity (Kuda et al., 2012). Protein- and carbohydrate-rich soymilk was also able to protect foodborne pathogens from desiccation (Kuda et al., 2015). Therefore, the results of this study demonstrate that bacteria can attach to contaminants, such as protein residues, carbohydrates, and other food residues and survive on abiotic surfaces and in dry environments.

Survival of adhered cells in the presence of food residues and disinfectant

As a subsequent study, the bactericidal effect of disinfectant on various food residues formed on SSC was evaluated. Figure 1 shows the survival of pathogens in the presence of food residues and disinfectant. Bacteria without food residues were completely killed by using the BAC and NaClO solution (data not shown). *C. sakazakii* had a high survival rate of 6 logs or more in cooked rice and soy milk. The survival rate of cooked rice residue treated with BAC and NaClO was higher than that of PBS. *E. coli*

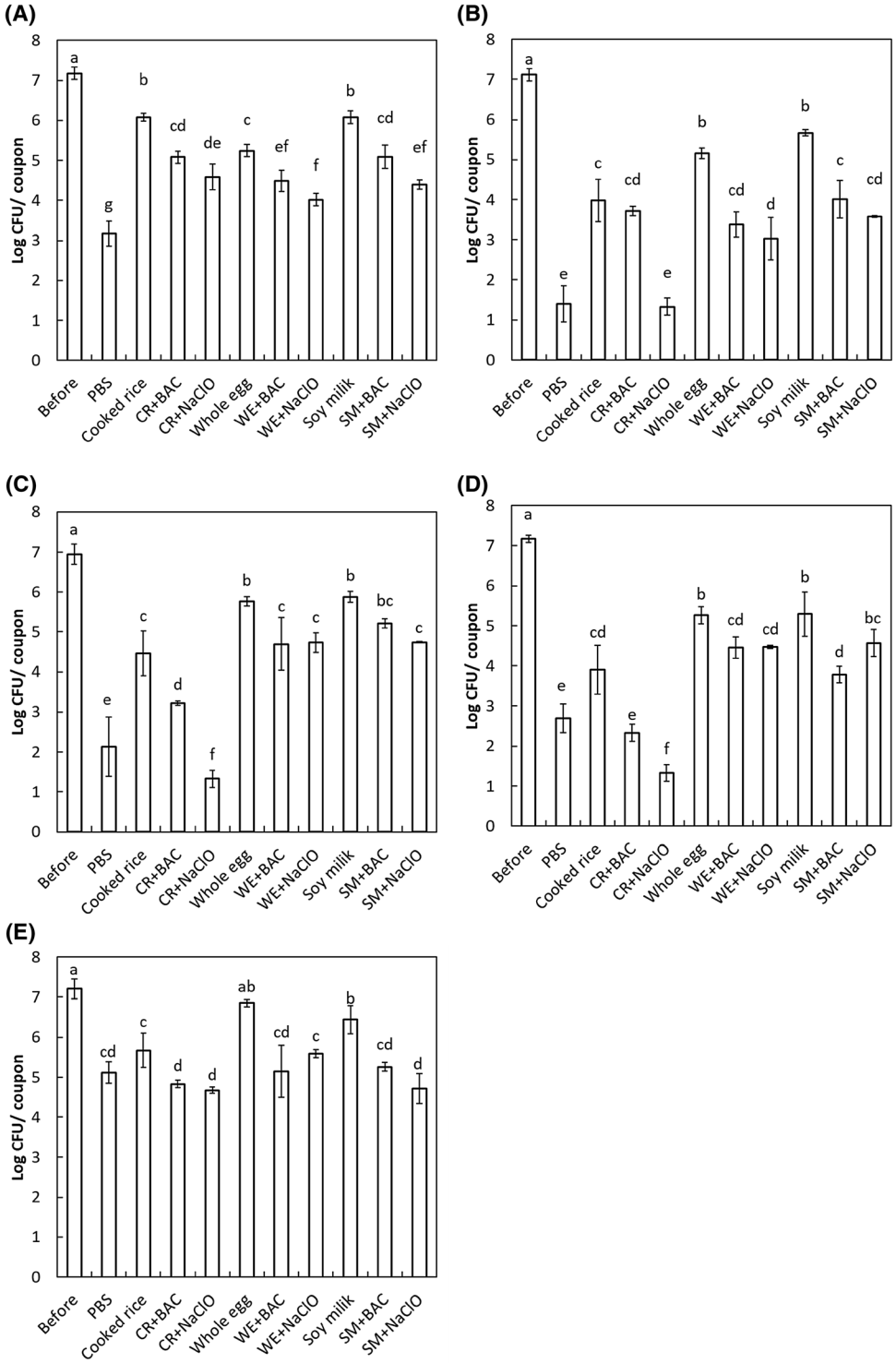

Fig. 1 Efficiency of disinfectant on stainless steel surfaces in the presence of 25% food residue. (**A**) *C. sakazakii*; (**B**) *E. coli* O157:H7; (**C**) *L. monocytogenes*; (**D**) *Sal.* Enteritidis; (**E**) CR, cooked rice; WE, whole eggs; SM, soy milk. Means with different superscripts (a–g) in the same column are different from each other ($p < 0.05$, Tukey's multiple range test)

O157:H7 showed a higher survival rate treated with disinfectants in the egg and soy milk residues, but the cooked rice-treated NaClO was similar to that observed without residues. *L. monocytogenes* showed the same survival rate to the disinfectants treated with egg and soy milk residues. Cooked rice had the lowest resistance to disinfectants. For *Sal.* Enteritidis and BAC were more effective on soy milk and NaClO on cooked rice. *S. aureus* showed a high survival rate against both BAC and NaClO with all residues. *S. aureus* was 4.82 log CFU/coupon, which was similar to that of *C. Sakazakii* in cooked rice residue. When BAC was applied to soy milk, *L. monocytogenes, Sal.* Enteritidis and *E. coli* O157:H7 showed 5.21 log CFU/coupon, 3.78 log CFU/coupon, and 4.01 log CFU/coupon of surviving cells, respectively. However, Fazlara and Ekhtelat (2012) reported that BAC was more effective on Gram-positive bacteria (*S. aureus*, *L. monocytogenes*) than Gram-negative bacteria (*Sal.* Typhimurium, *E. coli* and *Pseudomonas aeruginosa*). In Gram-negative bacteria, the resistance is due to the outer membrane of a lipopolysaccharide layer (Brul and Coote, 1999). Many authors have demonstrated that biofilm formation or bacterial adhesion increases resistance to physicochemical stresses, such as disinfectant and pH (Gibson et al., 1999). Kuda et al. (2015) reported that carotenoids and water-soluble polysaccharides affected the survival rate in the desiccation of pathogens with food residue. In this study, we showed that planktonic cells can be resistant to desiccation and disinfectant by the nutrients in food residues.

Survival of foodborne pathogens on stainless steel during storage

The survival of *S. aureus*, *L. monocytogenes*, *E. coli* O157:H7, and *Sal.* Enteritidis in the presence of 25% cooked rice on stainless steel at 68% RH and 25 °C for up to 168 h is depicted in Fig. 2. The initial inoculum concentration was between 6.41 and 6.78 log CFU/coupon. Without food residue, a rapid decrease in bacteria was observed in all five pathogens during the first 24 h of storage. *L. monocytogenes*, *E. coli* O157:H7, and *Sal.* Enteritidis resulted in nearly complete reduction after 48 h, while *S. aureus* remained constant at 1.64 log CFU/coupon. This result demonstrates that *S. aureus* can remain viable on stainless steel to become a potential risk through persistence in the environment even without food residue. Chaibenjawong and Foster (2011) reported that *S. aureus* can survive on dry plastic surfaces for more than 1097 days. Moon et al. (2017) reported that *S. aureus* rapidly diminished and became undetectable in dried filefish after 14 days. In the presence of cooked rice, *C. sakazakii* remained viable at the highest concentration among all pathogens to 3.82 log CFU/coupon, which was approximately 2.71 log units higher than the control. Previous studies have demonstrated that *C. sakazakii* can survive in various dry foods: the pathogen has been detected in rice starch and flour (Osaili et al., 2009). Other pathogens exhibited increases of 1.26 to 2.18 log units compared to the control with cooked rice. Cooked rice could protect the bacteria from dehydration by creating a carbohydrate coating (Fig. 2). However, this coating did not support the survival of *Sal.* Enteritidis, which exhibited

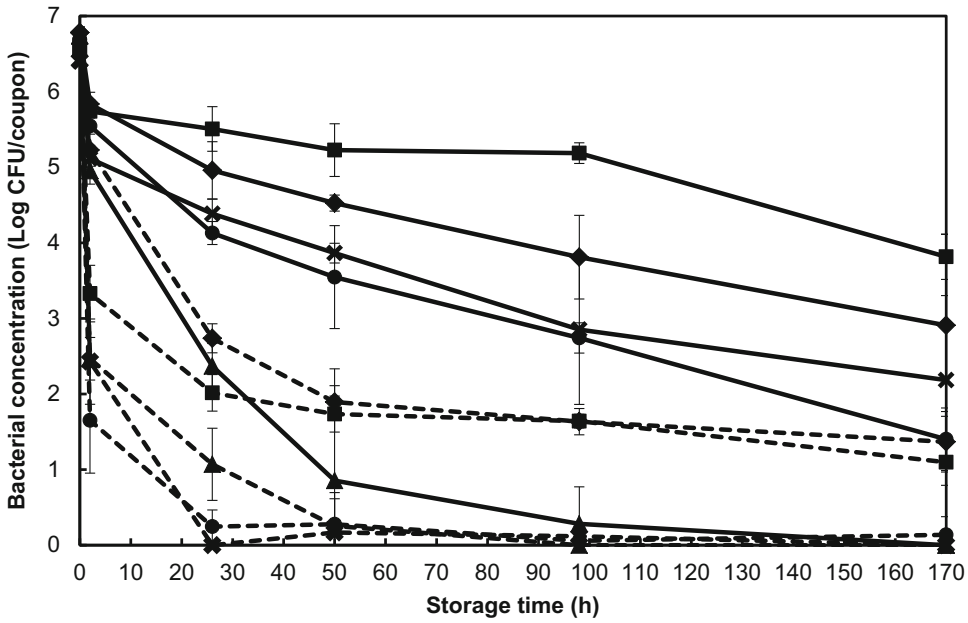

Fig. 2 Survival of bacteria attached to stainless steel surfaces in the presence (solid line) and in the absence (dotted line) of 25% cooked rice. *C. sakazakii* (filled square), *E. coli* O157:H7 (filled circle), *L. monocytogenes* (filled cross), *Sal.* Enteritidis (filled triangle) and *S. aureus* (filled diamond) were inoculated with cooked rice on the stainless steel and stored at 25 °C for one week. The values are expressed as mean and SD (n = 3)

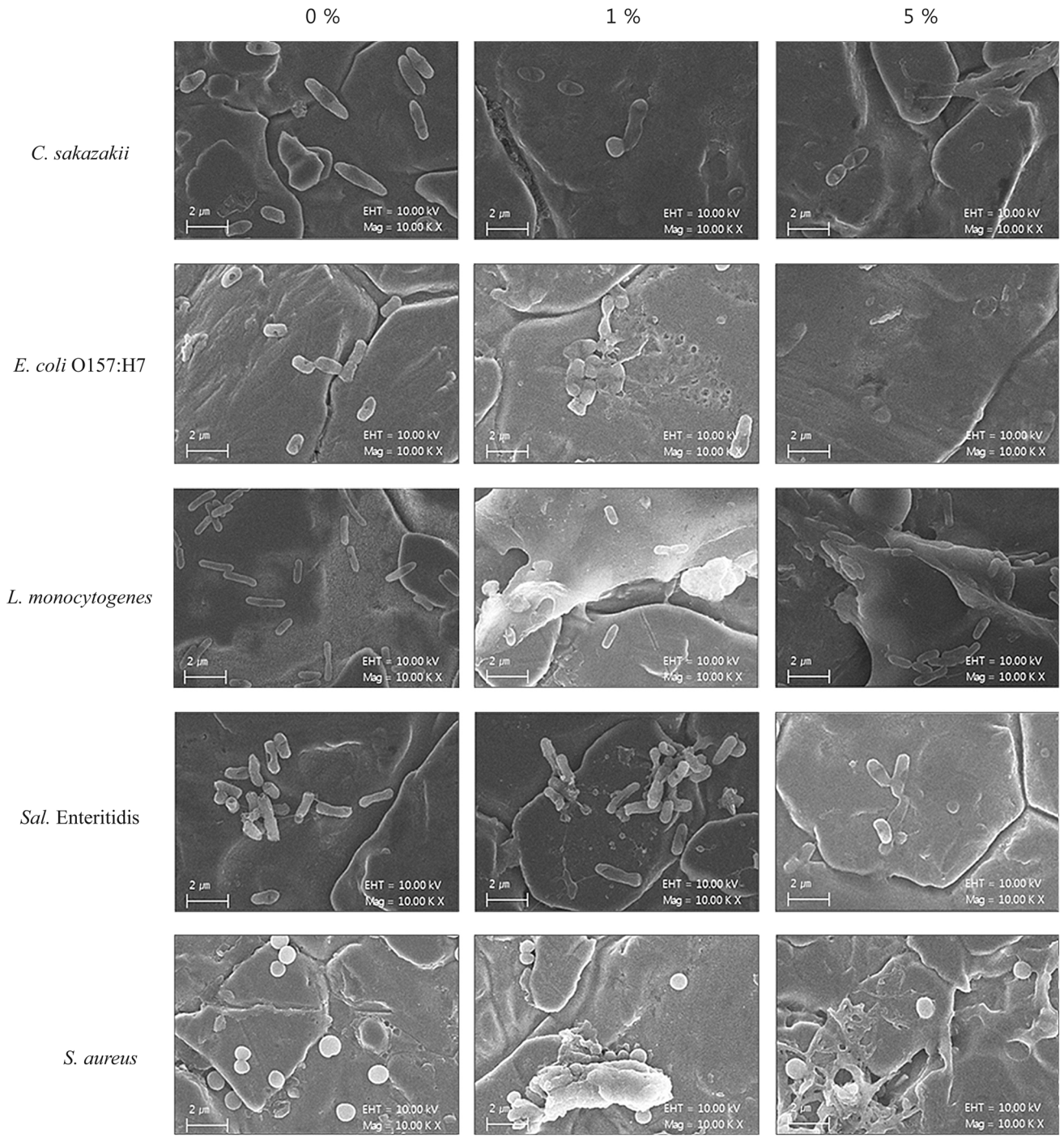

Fig. 3 Field emission-scanning electron microscopy images of *C. sakazakii*, *E. coli* O157:H7, *L. monocytogenes*, *Sal*. Enteritidis and *S. aureus* on stainless steel in the presence of 0%, 1%, and 5% cooked rice. Scale bar shows 2 µm of distance

a complete reduction after 96 h. Bea et al. (2012) reported that *S. aureus* and *C. sakazakii* slowly decreased after storage at 23%, 43%, 68%, and 85% relative humidity for 5 days compared with *E. coli* O157:H7, *L. monocytogenes*, and *Sal*. Typhimurium.

To further understand the adherence of the pathogens, the attached cells were analyzed using FE-SEM on a stainless-steel surface with 0%, 1%, and 5% cooked rice (Fig. 3). Since 25% of food residue was too thick to monitor the bacterial attachment on the FE-SEM, we have selected lower concentration in this study. In the presence of cooked rice, the pathogens exhibited binding to the particulates or embedding in the food residues. The higher the concentration of cooked rice was, the lesser and more

indistinct the bacterial images were seen to exhibit potential embedment. Brown and others have observed the preferential binding of *Campylobacter jejuni* to chicken juice particulates, rather than the coverslips (Brown et al., 2014). This phenomenon indicates that the attachment of bacteria bound to food residues becomes as strong as that of the food residues on abiotic surfaces, which causes the removal of bacteria from the surfaces to become more challenging (Abban et al., 2012). When food residue is present on abiotic surfaces, the residue becomes a new surface for bacteria to attach such that the abiotic surface material does not affect the attachment. Protein or other nutrient content in aqueous extracts of chicken fillets decreased the contact angle and the surface hydrophobicity in order to alter the electrostatic interaction through increasing the bacterial attachment and hindering the removal process (Bernbom et al., 2009; Jahid and Ha, 2012). In conclusion, foodborne pathogens were investigated on stainless steel surfaces soiled with food residue in order to confirm the importance of eliminating food residue from utensils and kitchen surfaces in cooking environments. From these results, the survival of the pathogens contaminating the surfaces of food processing substrates can vary depending on the nutrients and contents of food contamination, and thorough cleaning and drying are strongly recommended to ensure the food safety of food processing environments.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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