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Biofilm Formation Characteristics of Major Foodborne Pathogens on Polyethylene and Stainless Steel Surfaces

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ABSTRACT - This research was investigated the effects of temperature and time against the formation of biofilms by foodborne pathogens on surfaces of polyethylene and stainless steel. After preliminary experiments with 32 strains from 6 species of foodborne pathogens (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* Typhimurium), one strain from each species with the highest biofilm formation efficiency was selected. All foodborne pathogens showed a tendency toward an increased ability for biofilm formation with increasing temperature, but there was no consistency between the two materials and between foodborne pathogens. At all tested temperatures, the biofilm formation ability of *E. coli* and *P. aeruginosa* on the polyethylene surface was higher than that on the stainless steel surface with significant differences. The foodborne pathogens all formed biofilms immediately upon inoculation, and biofilm formation by *E. coli*, *P. aeruginosa*, and *S.* Typhimurium increased on both the polyethylene and stainless steel surfaces at 1 h after inoculation compared to at 0 h. At 7 days after biofilm formation, the other strains except *S. aureus* showed no difference in survival rates on polyethylene and stainless steel. The ability of these 6 foodborne pathogens to form biofilms showed different trends depending on the type of bacteria and the instrument material, i.e., polyethylene and stainless steel.

Key words: Biofilm, Foodborne pathogens, Polyethylene, Stainless steel, Food contact surface

Because of a diversification of dietary choices and an increase in international interaction, the chances of food contamination and decomposition are on an upsurge. Particularly due to increases in dining out and the increase of school meals, mass foodborne can occur anywhere or at any time[1-3]. Food borne occurs when contaminated food ingredients are used, when proper sterilization and cleaning process are not followed during cooking or through cross-contamination by operators or equipment during the process of production, custody, and storage[1]. These types of cross-contamination by foodborne pathogens are the main cause of mass foodborne in school meals. To prevent this, proper washing and sterilization of not only food ingredients, but also the operators and equipment touching food, must be conducted[4].

When foodborne pathogens touch a food surface, they can produce a biofilm, which has a stronger resistance to sanitizers than floating germs do[5,6]. Biofilms produce a state in which adjacent germs condense with extracellular polymeric substances, which allows pathogens to adhere to the fluid surface, leading to breeding on the surface and additional film formation[6-9]. Germs that exist in biofilms are different from planktonic cells in terms of their growth rate, the structure and construction of their cytoderm, their immunological properties, and their sensitivity toward antimicrobial and enzymatic activity[10]. Specifically, the glycocalyx or biofilm prevents microbes from being affected by detergents, antibiotics, or antibodies, thereby decreasing the efficiency of the antimicrobial agents[11]. Since biofilms on food surfaces allow for significant storage of pathogens during cooking or food processing, the germs inside the biofilm can repopulate to be a main threat to food safety[5,6,12].

Therefore, in this research, we investigated the effects of temperature and time, on the formation of biofilms by foodborne pathogens on the surfaces of polyethylene and stainless steel, which is widely used on food processing equipment.

Materials and Methods

Bacterial strains

Bacillus cereus (5 strains), *Listeria monocytogenes* (5

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strains), *Staphylococcus aureus* (14 strains), *Escherichia coli* (3 strains), *Pseudomonas aeruginosa* (1 strain), and *Salmonella* Typhimurium (4 strains) were obtained from Department of Food Science and Technology of Jeonbuk National University (Jeonju, Korea). All strains were stored at -80°C in each 0.7 mL of tryptic soy broth (Difco, Becton Dickinson, Sparks, MD, USA) and 0.3 mL of 50%(v/v) glycerol. The working cultures were maintained on tryptic soy agar (Difco, BD) slants at 4°C and subcultured monthly. Approximately 10 μL of culture was transferred to fresh agar (3 replicate samples) using a loop and grown at 30°C for 18 to 24 h prior to use in the experiments.

Preparation of polyethylene and stainless steel coupon

Sterile polyethylene coverslips (13-mm diameter, Thermanox® polyethylene coverslip, NUNC™, Rochester, NY, USA) were used as the polyethylene coupons. Stainless steel coupons (1×1 cm) of type 304 with a number 4 finish were also used. Before use, the metal coupons were cleaned in acetone to remove grease, rinsed in distilled water, sonicated (EPS-300x, Sonic & Materials Inc. Newtown, CT, USA) in a 70% ethanol for 30 min to disinfect the surface, and rinsed again with distilled water. The washed stainless steel coupons were then dried, placed in a 100-mL beaker, and autoclaved (Autoclave, WAC-100, DAIHAN Scientific Co., Wonju, Korea).

Evaluation of biofilm-forming ability using cell enumeration

The polyethylene and stainless steel coupons were inoculated on the top surface with 100 μL of *B. cereus* ATCC 13061, *L. monocytogenes* ATCC 19112, *S. aureus* KCCM 11812, *E. coli* KCCM 11234, *P. aeruginosa* ATCC 27853, and *S.* Typhimurium ATCC 11862 at approximately 6 log CFU/mL grown at 30°C for 18 to 24 h in tryptic soy broth and maintained at 30°C in sterile polystyrene petri dishes. After 24 h, the residual medium from the coupon was removed with a pipette, and 100 μL of sterile distilled water was added to the top of these coupons. The coupons were washed three times to remove with a pipette, and the coupons were washed three times to remove all loosely attached cells. The washing step included the three repeated additions and flushing with 100 μL of sterile deionized water on the coupon surfaces.

After washing, the coupons were evaluated for food pathogen biofilm counts. Each polyethylene or stainless steel coupon was placed in a 50-mL polystyrene tube containing 20 mL of peptone water (0.1% peptone and 0.02% Tween 80) with five glass beads (5-mm diameter, Glastechnique Mfg., Lauda-Koenigshofen, Germany) and vortexed (Vortex-2 Genie, Scientific Industries, Inc., Bohemia, NY, USA) for 2 min. The vortexed solution was directly plated using 250 μL for each of four individual plates (detection limit of 1.3 log CFU/coupon) or serially diluted in 0.1% peptone water for spread-plating on tryptic soy agar (Difco, BD) plates.

Effects of temperature on biofilm formation by foodborne pathogens

The polyethylene and stainless steel coupons were inoculated on the top surface with 100 μL of *B. cereus* ATCC 13061, *L. monocytogenes* ATCC 19112, *S. aureus* KCCM 11812, *E. coli* KCCM 11234, *P. aeruginosa* ATCC 27853, or *S.* Typhimurium ATCC 11862 in tryptic soy broth (Difco, BD) and maintained in sterile polystyrene petri dishes. The plates were incubated (Vision Scientific Co., Ltd., Bucheon, Gyoungki, Korea) at 4, 20, or 35°C for 24 h in static conditions[13,14]. After the incubation period, the tryptic soy broth (Difco, BD) growth medium containing planktonic cells was removed, the wells were rinsed three times with sterile deionized water. TSB were added to duplicate wells containing biofilm cells for 24 h of exposure at 22°C for biofilm quantification[15].

Effects of time on biofilm formation by foodborne pathogens

The polyethylene and stainless steel coupons were placed in a 50-mL polystyrene tube containing 20 mL of peptone water with 500 μL of *B. cereus* ATCC 13061, *L. monocytogenes* ATCC 19112, *S. aureus* KCCM 11812, *E. coli* KCCM 11234, *P. aeruginosa* ATCC 27853, or *S.* Typhimurium ATCC 11862. The polystyrene tubes containing the coupons were incubated at 30°C[16]. At specific time intervals (1, 3, 6, and 24 h), the coupons were removed from their respective suspensions and washed with sterile deionized water three times to remove all loosely attached cells, then were evaluated for food pathogen biofilm counts[15].

Survival characteristics of biofilms formed by foodborne pathogens

The polyethylene and stainless steel coupons were inoculated on the top surface with 100 μL of *B. cereus* ATCC 13061, *L. monocytogenes* ATCC 19112, *S. aureus* KCCM 11812, *E. coli* KCCM 11234, *P. aeruginosa* ATCC 27853, or *S.* Typhimurium ATCC 11862 in tryptic soy broth (Difco, BD) and maintained in sterile polystyrene petri dishes. The plates were incubated at 26°C for 7 days in static conditions[13,14]. After 0, 1, 3, 5, or 7 days of incubation, the tryptic soy broth (Difco, BD) growth medium containing planktonic cells was removed, and the coupons were processed for biofilm quantification[15].

Statistical analysis

All statistical analyses were performed using SAS (Statistical Analysis System ver. 6). The means and standard deviations were calculated, and Duncan's multiple range tests were applied[17]. All experiments were performed in triplicate. A probability (p) level of 0.05 was considered statistically significant.

Results and Discussion

Biofilm formation by foodborne pathogens

To select the strains with the highest ability of biofilm formation, we studied the Gram-positive foodborne pathogens *B. cereus* (5 strains), *L. monocytogenes* (5 strains), and *S. aureus* (14 strains) and the Gram-negative foodborne pathogens *E. coli* (3 strains), *P. aeruginosa* (1

Table 1. Evaluation of the biofilm-forming ability of various pathogens on polyethylene and stainless steel surfaces using cell enumeration (Unit: log CFU/coupon)

		Strains	Polyethylene	Stainless steel
Gram positive	*Bacillus cereus*	ATCC 13061	3.21±0.01[a]	3.28±0.01[a]
		ATCC 9634	2.79±0.02[b]	2.79±0.01[c]
		KCCM 11204	2.81±0.03[b]	2.81±0.03[c]
		KFOO 08	2.84±0.03[b]	2.78±0.04[c]
		KFRI 00437	2.82±0.05[b]	2.90±0.03[b]
	Listeria monocytogenes	ATCC 15313	4.87±0.01[d]	4.65±0.02[c]
		ATCC 19111	5.25±0.02[c]	5.01±0.02[b]
		ATCC 19112	6.74±0.01[a]	5.20±0.05[a]
		ATCC 19113	6.47±0.01[b]	4.61±0.07[c]
		ATCC 19114	4.11±0.06[e]	4.22±0.04[e]
	Staphylococcus aureus	KCCM 11335	4.68±0.18[h]	6.07±0.61[b]
		KCCM 11593	4.86±0.04[g]	5.39±0.37[cd]
		KCCM 11640	6.26±0.07[b]	5.24±0.20[de]
		KCCM 11812	6.52±0.07[a]	6.30±0.02[a]
		KCCM 12103	6.46±0.02[a]	5.41±0.06[cd]
		KCCM 12255	5.06±0.08[f]	4.97±0.01[ef]
		KCCM 12256	6.04±0.01[c]	5.10±0.07[de]
		ATCC 25923	5.81±0.08[e]	5.40±0.04[cd]
		KCCM 40510	5.27±0.06[e]	5.79±0.03[b]
		KCCM 40511	4.81±0.12[gh]	4.68±0.04[f]
		KCCM 40881	5.35±0.19[e]	5.13±0.05[de]
		KCCM 40927	5.97±0.08[c]	5.72±0.06[bc]
		KCCM 41291	6.42±0.06[a]	5.03±0.03[def]
		KCCM 41294	5.27±0.07[e]	5.34±0.01[cde]
Gram negative	*Escherichia coli*	KCCM 11234	6.24±0.04[a]	5.55±0.04[a]
		O157:H7 ATCC 43888	5.90±0.02[b]	5.45±0.06[b]
		O157:H7 ATCC 43894	5.45±0.11[c]	5.34±0.02[c]
	Pseudomonas aeruginosa	ATCC 27853	6.02±0.03[a]	5.98±0.01[a]
	Salmonella Typhimurium	ATCC 11862	5.48±0.00[a]	5.69±0.10[a]
		ATCC 14028	5.04±0.05[b]	5.35±0.02[b]
		ATCC 6539	5.02±0.03[b]	4.92±0.10[c]
		KCTC 1025	5.01±0.02[b]	5.55±0.05[d]

Data represent means±standard deviations of three measurements.
There are significant differences (P<0.05) between the samples with different small letters in a column.

strain), and *S.* Typhimurium (4 strains). Table 1 shows the characteristics of biofilm formation for each of foodborne pathogens.

B. cereus, in the facultative anaerobe spore form[18], has been reported to cause food poisoning by contaminating foods, such as fried rice, pasta, boiled meat, boiled vegetables, and salad, and to form biofilms on soil and rice[19]. *B. cereus* has a low ability of biofilm formation on polyethylene and stainless steel surfaces compared to other foodborne pathogens. Among the *B. cereus* strains, ATCC 13061 formed 3.21±0.01 log CFU/coupon on the polyethylene surface and 3.28±0.01 log CFU/coupon on the stainless steel surface, which was the highest ability of *B. cereus* biofilm formation on both surfaces.

L. monocytogenes showed a higher ability of biofilm formation on the polyethylene surface than on the stainless steel surface, except for strain ATCC 19114. Specifically, *L. monocytogenes* ATCC 19112 showed a high ability of biofilm formation of 6.74±0.01 log CFU/coupon on the polyethylene surface. Blackman and Frank[20] showed that biofilms of *L. monocytogenes* could form on various types of food processing surfaces such as stainless steel, Teflon, and nylon. Also, Desai et al.[15] researched methods to control biofilm formation by *L. monocytogenes* on stainless steel and polyethylene surfaces.

Among the 14 species of *S. aureus* tested, *S. aureus* KCCM 40511 formed 4.81±0.12 log CFU/coupon on the polyethylene surface and 4.68±0.04 log CFU/coupon on the stainless steel surface, which was the lowest ability of *S. aureus* biofilm formation on both surfaces. Conversely, *S. aureus* KCCM 11812 formed 6.52±0.07 log CFU/coupon on the polyethylene surface and 6.30±0.02 log CFU/coupon on the stainless steel surface, which was approximately 2 log CFU/coupon higher than the other species of *S. aureus*.

The efficiency of biofilm formation by *E. coli*, which is Gram-negative, was 5.45±0.11-6.24±0.04 log CFU/coupon on the polyethylene surface and 5.34±0.02-5.55±0.04 log CFU/coupon on the stainless steel surface, which was similar. The ability of biofilm formation by *E. coli* KCCM 11234 was slightly higher than that of *E. coli* O157:H7.

P. aeruginosa was used as a representative of the *Pseudomonas* sp. because it does not form Gram-negative aerobacilli spores, grows well at standard refrigeration temperatures, and produces an extracellular toxin that is resistant to heat[14]. *P. aeruginosa* is primarily a deterioration germ that is highly related to the decomposition of meat, milk, marine products, and fresh vegetables and has been reported to harm food safety by forming biofilms on food surfaces and food wrapping materials[18]. The measured efficiency of biofilm formation by *P. aeruginosa* was 6.02±0.03 log CFU/coupon on the polyethylene surface and 5.98±0.01 log CFU/coupon on the stainless steel surface, which was similar to the 6.06 log CFU/cm^2 of biofilm on stainless steel surfaces measured by Emiliane et al.[21].

S. Typhimurium is a Gram-negative, asporogenic bacillus with flagella that provide mobility[18]. It forms a similar amount of biofilm on both polyethylene and stainless steel surfaces. *S.* Typhimurium ATCC 11862 formed 5.48±0.00 log CFU/coupon on the polyethylene surface and 5.69±0.10 log CFU/coupon on the stainless steel surface, which is a significant difference ($P<0.05$) in the ability of biofilm formation.

Effects of temperature on biofilm formation

The effect of temperature was investigated by selecting one of the highest strains of biofilm formation among various foodborne pathogens (Fig. 1).

The ability of biofilm formation of *B. cereus* ATCC 13061 (Fig. 1A) varied significantly according to temperature. Specifically, the ability of biofilm formation on the polyethylene and the stainless steel surface was the highest with 4.63±0.61 log CFU/coupon and 5.20±0.13 log CFU/coupon at 35°C, respectively. At 4°C and 35°C, the ability of biofilm formation on the stainless steel surface were higher than those of the polyethylene surface with no significant differences, but at 20°C the ability of biofilm formation on the polyethylene surface was higher than that of the stainless steel with significant differences.

L. monocytogenes ATCC 19112 (Fig. 1B) is a psychrophilic bacterium that is viable at a range of temperatures from 4°C to 42°C, and its ability for biofilm formation increases with rising temperature with 4.94±0.03-6.24±0.13 log CFU/coupon on the polyethylene surface and 4.68±0.05-6.39±0.04 log CFU/coupon on the stainless steel surface. Additionally, a biofilm of at least 4.5 log CFU/coupon was formed at 4°C. Because *L. monocytogenes* is viable at the refrigeration temperature of 4°C, food hygiene specific to psychrophilic agents is very important, which is required in the checklist for refrigerated and frozen food[18]. At 20°C and 35°C, the ability of biofilm formation of *L. monocytogenes* ATCC 19112 did not show significant differences according to surface type, but did show differences according to temperature ($P<0.05$).

The ability of biofilm formation of *S. aureus* KCCM 11812 (Fig. 1C) was 7.50±0.04 at 20°C and 7.66±0.08 log CFU/coupon at 35°C on the polyethylene surface, which was the highest ability for *S. aureus*, and 7.26±0.04 log CFU/coupon at 35°C on the stainless steel surface. The optimal temperature range for producing enterotoxin by *S. aureus* is 40-45°C. Since the most viable temperature for *S. aureus* is 37°C, its ability for biofilm formation is thought to be the highest at 35°C. At 4°C, the ability of biofilm formation on the stainless steel surface were higher than that of the

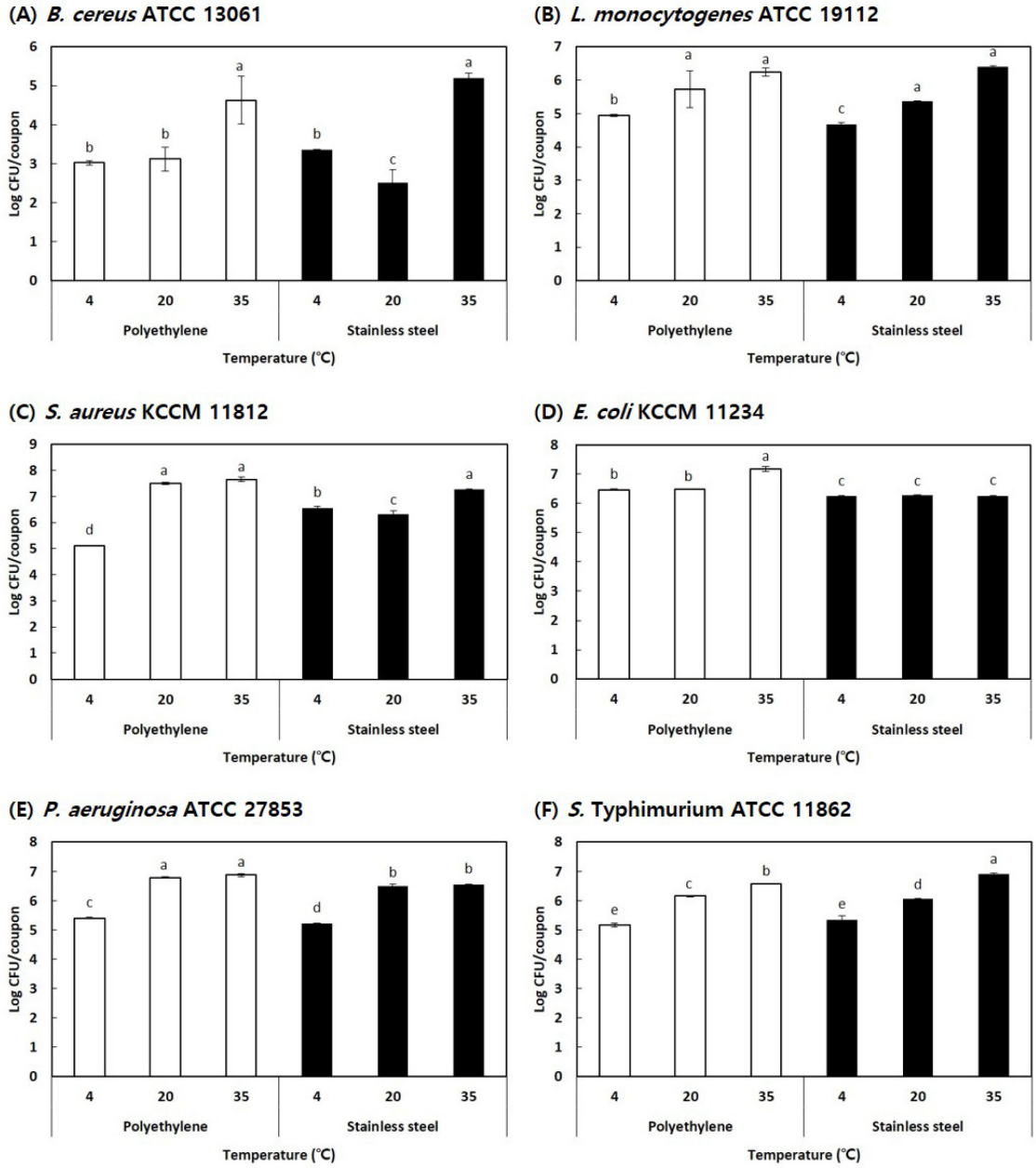

Fig. 1. Effect of temperature on biofilm formation by foodborne pathogens on polyethylene (□) and stainless steel (■) coupon surface**.** Vertical bars represents standard deviation (n=3). There are significant differences (P<0.05) between the samples with different small letters.

polyethylene surface with significant differences, but at 20°C, the opposite appeared.

The ability of biofilm formation of *E. coli* KCCM 11234 (Fig. 1D) on the polyethylene surface was highest at 35°C with 7.17±0.08 log CFU/coupon. There were no differences according to temperature on the stainless steel surface. At all tested temperature, the ability of biofilm formation on the polyethylene surface were higher than those of the stainless steel surface with significant differences.

P. aeruginosa ATCC 27853 (Fig. 1E) had a higher ability of biofilm formation on the polyethylene surface than on the stainless steel surface at each temperature with significant differences, and the ability of biofilm formation increased significantly as the temperature increased. Specifically, the largest amount of biofilm was formed at 35°C on the polyethylene surface (6.88±0.05 log CFU/coupon).

The ability of biofilm formation by *S.* Typhimurium ATCC 11862 (Fig. 1F) significantly increased as the

temperature rose. Specifically, the largest amount of biofilm was formed at 35°C on the stainless steel surface (6.90±0.05 log CFU/coupon). At 20°C, the ability of biofilm formation on the polyethylene surface were higher than that of the stainless steel surface with significant differences, but at 35°C, the opposite appeared.

All microorganisms showed a tendency toward increasing ability of biofilm formation as the temperature rose, but there was no consistent trend between food surfaces and between foodborne pathogens. This corresponds to previous results[13] stating that the ability of biofilm formation of *L. monocytogenes* increased with increasing temperature (from 4 to 37°C) on polystyrene, glass, and stainless steel surfaces. However, another study showed that the efficiency of biofilm formation of *S.* Typhimurium on polystyrene and stainless steel surfaces decreased with increasing temperature, which conflicts with our results[22]. Notably, the ability of biofilm formation of all foodborne pathogen except *B. cereus* ATCC 13061 was at least 4 log CFU/coupon at 4°C. This is presumed to be the cause of food poisoning when foodborne pathogen-contaminated food or food surfaces are stored at refrigeration temperatures.

Effects of time on biofilm formation

Fig. 2 shows the effects of time on biofilm formation by foodborne pathogens on two materials surfaces.

B. cereus ATCC 13061 formed 2.95±0.13 log CFU/coupon of biofilm on the polyethylene surface and 3.07±0.28 log CFU/coupon of biofilm on the stainless steel surface 0 h after pathogen inoculation and formed 3.28±0.02 log CFU/coupon of biofilm on the polyethylene surface and 3.48±0.02 log CFU/coupon of biofilm on the stainless steel surface after 24 h. Although the amount of *B. cereus* ATCC 13061 biofilm increased from 0 h to 24 h with significant differences, it was not highly affected by time, evidenced by its increase of only 0.33-0.41 log CFU/coupon over 24 h.

On the polyethylene surface, *L. monocytogenes* ATCC 19112 formed the highest 5.33±0.02 log CFU/coupon of biofilm after 0 h of inoculation and 5.17±0.07 log CFU/coupon of biofilm after 24 h of pathogen inoculation. Biofilm formation of *L. monocytogenes* ATCC 19112 on the polyethylene surface was not significantly affected by time. On the stainless steel surface, *L. monocytogenes* ATCC 19112 formed the highest 6.14±0.04 log CFU/coupon of biofilm after 1 h of inoculation. Additionally, biofilm formation was decreased after 24 h of pathogen inoculation with 4.60±0.04 log CFU/coupon of biofilm on the stainless steel surface. Up to 5 h of inoculation, the ability to form biofilms on the stainless steel surface was higher than on the polyethylene. Chmielewski and Frank[23] also found that *L. monocytogenes* can adhere to food surfaces in a short time.

S. aureus KCCM 11812 showed a high ability of biofilm formation with 6.64±0.08 log CFU/coupon on the polyethylene surface and 6.85±0.11 log CFU/coupon on the stainless steel surface after 24 h of pathogen inoculation, which indicates that biofilm formation was affected by time.

On the polyethylene surface, *E. coli* KCCM 11234 formed 6.85±0.09 log CFU/coupon of biofilm after 1 h of inoculation, which was the highest ability on the polyethylene surface, and 6.17±0.07 log CFU/coupon of biofilm after 24 h of inoculation, indicating an insignificant effect over time. At tested temperatures except 5 h, the polyethylene surface showed a higher ability of biofilm formation than the stainless steel surface with significant differences.

P. aeruginosa ATCC 27853 typically has a high ability of biofilm formation on the polyethylene surface. Specifically, after 5 h of inoculation, it had formed the highest 7.14±0.02 log CFU/coupon of biofilm on the polyethylene surface. *P. aeruginosa* also formed 5.29±0.04 log CFU/coupon of biofilm after 24 h on the stainless steel surface, which was significantly lower than at 5 h. There were no significant differences in the ability to form biofilms on plastic and stainless surfaces until 1 h after inoculation of *P. aeruginosa*, but significantly higher on plastic surfaces after 5 h.

S. Typhimurium ATCC 11862 formed the highest 6.39±0.01 log CFU/coupon of biofilm on the polyethylene surface and 6.42±0.05 log CFU/coupon of biofilm on the stainless steel surface after 1 h of inoculation. After 24 h, the biofilm formation decreased with 5.88±0.08 log CFU/coupon on the polyethylene surface and 5.60±0.12 log CFU/coupon on the stainless steel surface. There was no time-consistency between the two surfaces.

Mafu et al.[24] reported that cells of *L. monocytogenes* can stick to surfaces of stainless steel, rubber, glass, or polypropylene within 20 min and produce extracellular cell after sticking to the surface. We observed that biofilms were formed by 6 species of foodborne pathogens after 0 h of pathogen inoculation. We also confirmed that biofilm formation by *E. coli* KCCM 11234, *P. aeruginosa* ATCC 27853, and *S.* Typhimurium ATCC 11862 increased at 1 h after pathogen inoculation compared to at 0 h.

Survival characteristics of biofilms formed by foodborne pathogens

To observe the survival characteristics of biofilms formed on polyethylene and stainless steel surfaces, we stored polyethylene and stainless steel coupons containing biofilms at 26°C for 7 days. Fig. 3 shows the estimated survival of the biofilms formed by each of the foodborne pathogens.

B. cereus ATCC 13061 formed 3.41±0.01 log CFU/coupon of biofilm on the polyethylene surface and 4.05±0.11 log CFU/coupon of biofilm on the stainless steel

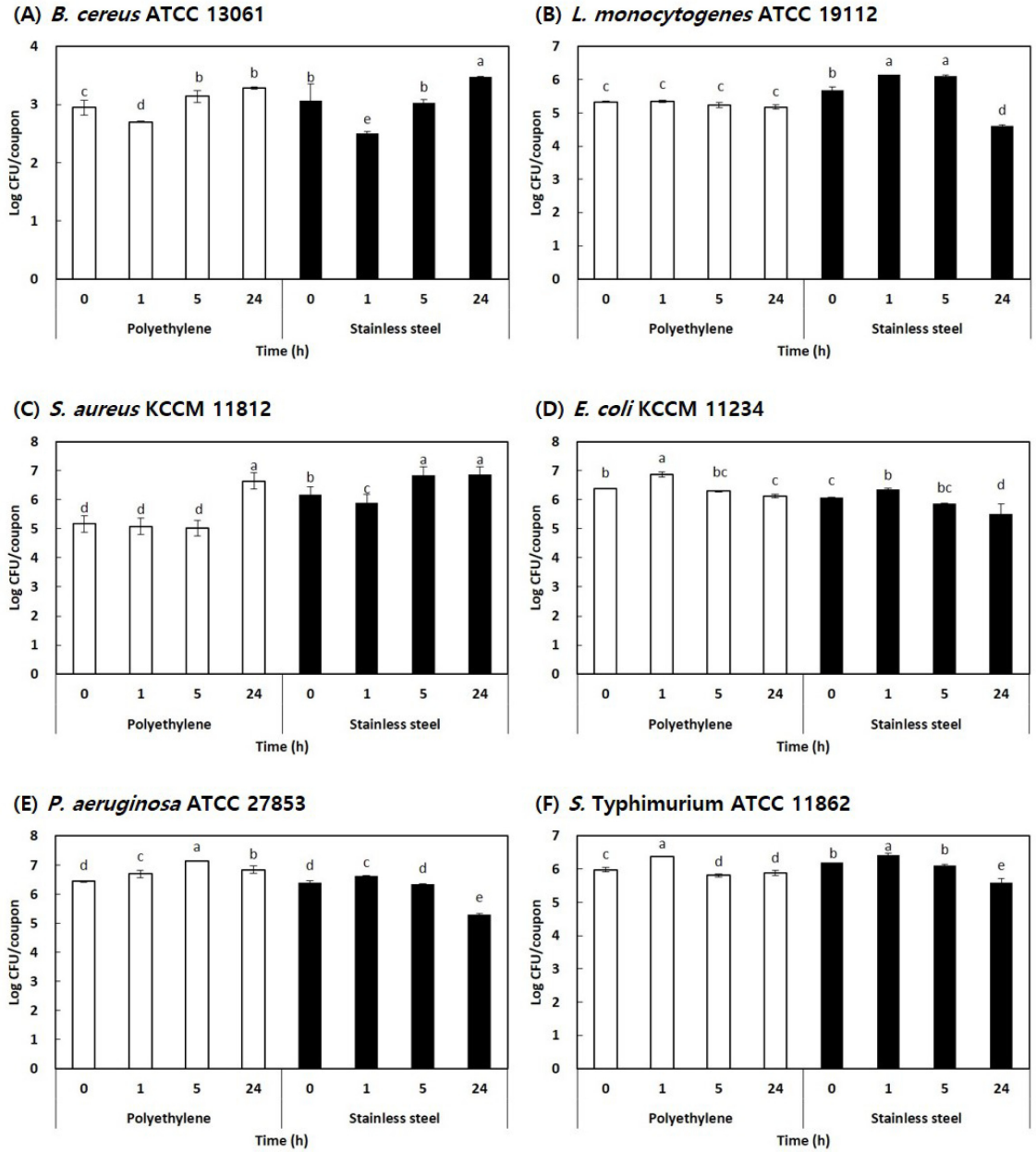

Fig. 2. Effect of time on biofilm formation by foodborne pathogens on polyethylene (□) and stainless steel (■) coupon surface. Vertical bars represents standard deviation (n=3). There are significant differences (P<0.05) between the samples with different small letters.

surface on day 0. It decreased to 2.77±0.02 log CFU/coupon of biofilm on the polyethylene surface and 2.64±0.01 log CFU/coupon of biofilm on the stainless steel surface on day 1. There was a significant difference, which was maintained until day 7.

The biofilm that *L. monocytogenes* ATCC 19112 formed on the polyethylene surface was maintained at over 5 log CFU/coupon (5.92±0.04 log CFU/coupon on day 0; 5.75±0.08 log CFU/coupon on day 5) until slightly decreasing on day 7 (4.97±0.09 log CFU/coupon). The biofilm that formed on the stainless steel surface contained 6.12±0.02 log CFU/coupon on day 0 and decreased to 4.60±0.09 log CFU/coupon on day 3 but did not show any decrease after that. In Henriques and Fraqueza's[6] research on the proliferation and elimination of *L. monocytogenes* adhered to food surfaces, they reported that germ adherence to various types of materials used in tableware is established within 24 h, with a certain amount of germ being maintained after that.

The ability of biofilm formation by *S. aureus* KCCM 11812 on the polyethylene surface was higher than on the

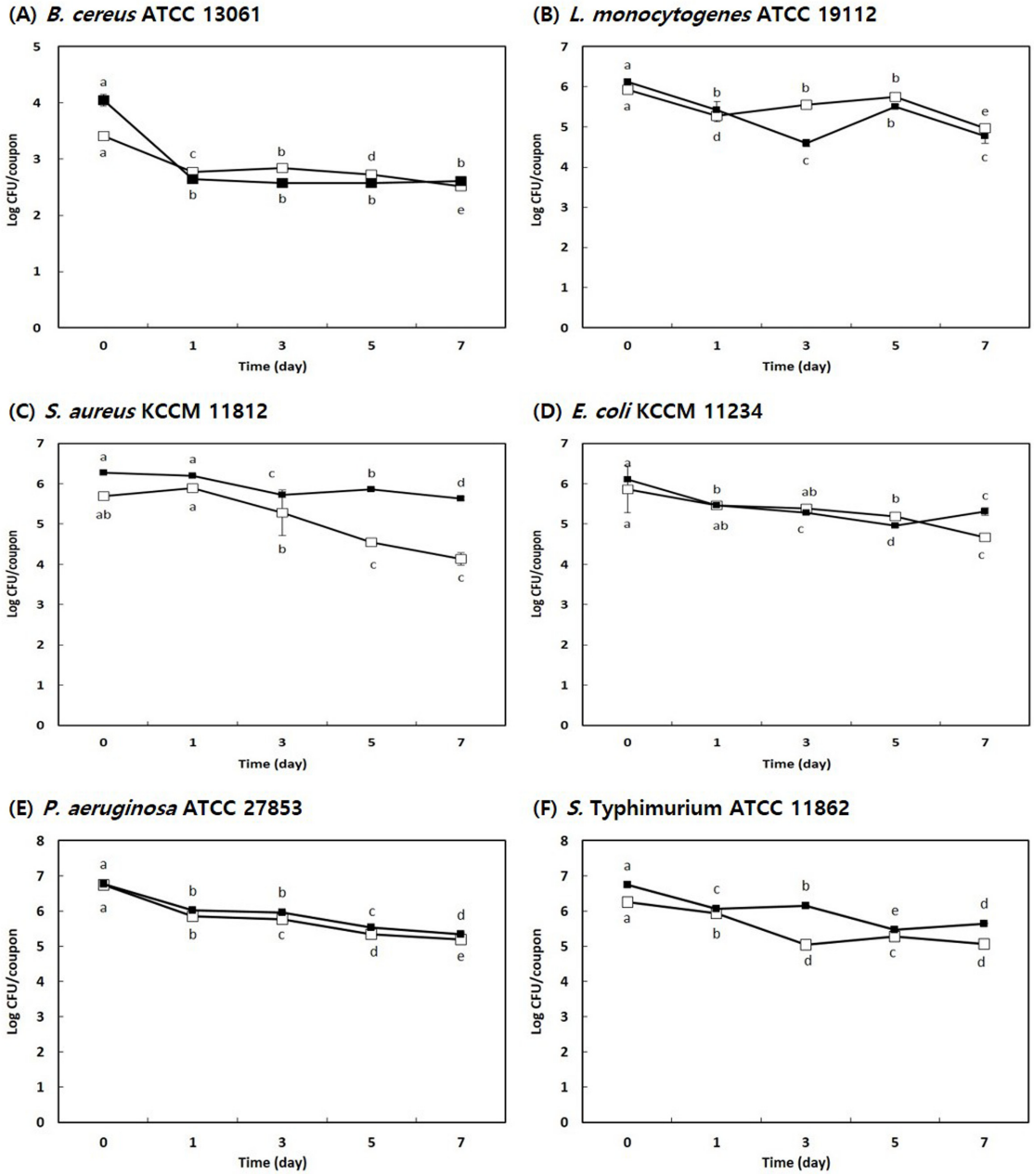

Fig. 3. Survival characteristics of biofilms formed by foodborne pathogens incubated at 26°C for 7 days on polyethylene (□) and stainless steel (■) coupon surfaces using cell enumeration. Vertical bars represents standard deviation (n=3). There are significant differences (P<0.05) between the samples with different small letters.

stainless steel surface during test periods and was maintained at 5.63±0.05 log CFU/coupon after day 7. Biofilms on the polyethylene surface showed a tendency to decrease after day 1.

E. coli KCCM 11234 showed a similar ability of biofilm formation between the polyethylene surface and stainless steel surfaces. It formed the biofilm with 5.86±0.58 log CFU/coupon on the polyethylene surface and 6.11±0.02 log CFU/coupon on the stainless steel surface on day 0, but did show some decrease after that. However, it maintained over 4 log CFU/coupon of biofilm with 4.67±0.03 log CFU/coupon of biofilm on the polyethylene surface and 5.31±0.09 log CFU/coupon of biofilm on the stainless steel surface on day 7.

P. aeruginosa ATCC 27853 did not exhibit any difference in biofilm formation between the polyethylene and stainless steel surfaces. However, the biofilm gradually declined from 6.75±0.01 log and 6.77±0.08 log CFU/coupon on the

polyethylene and stainless steel surfaces, respectively, on day 0 to 5.19±0.01 log and 5.33±0.06 log on the polyethylene and stainless steel surfaces, respectively, on day 7.

The ability of biofilm formation by *S.* Typhimurium ATCC 11862 was higher on the stainless steel surface than on the polyethylene surface during test periods. On the stainless steel surface, the ability of biofilm formation declined from 6.74±0.02 log CFU/coupon on day 0 to 5.64±0.01 log CFU/coupon on day 7.

Excluding *B. cereus* ATCC 13061, which has a low ability of biofilm formation in the early stage, most foodborne pathogens maintained a high amount of biofilm (over 4 log CFU/coupon) over 7 days. Specifically, *S.* Typhimurium ATCC 11862 formed 5.64 log CFU/coupon on the stainless steel surface on day 7. At 7 days after biofilm formation, the other strains except *S. aureus* showed no difference in survival rates in polyethylene and stainless steel.

This amount of biofilm can be detrimental to food safety, and biofilms on food surfaces causing cross-contamination to food is thought to be the main cause of food poisoning. In a previous study[25], *E. coli* O157, *S.* Typhimurium, *Enterobacter sakazakii*, and *B. cereus* were adhered to stainless steel, bronze, copper, and tin surfaces, and most strains survived on the stainless steel and tin surfaces for 72 h. Specifically, the findings on *S.* Typhimurium were similar to our results, in that it maintained approximately 6 log CFU/mL. Additionally, Krysinski et al.[26] found no difference in the amount of *L. monocytogenes* at 24 h and 72 h after applying it to the polyethylene or stainless steel surface, and the type of surface also did not show a significant difference in biofilm formation, and the results was similar to ours. Although biofilms produced by *E. sakazakii* formed on stainless steel surfaces are declining in number, it can survive for up to 60 days. Additionally, when *E. sakazakii* was kept at 25°C for 6 days, it maintained 8-10 log CFU/coupon of biofilm[27].

The ability of 6 foodborne pathogens to form biofilms showed different trends depending on the type of bacteria and the material of the instrument such as polyethylene and stainless steel.

국문요약

식중독 미생물이 polyethylene과 stainless steel의 표면에서 biofilm을 형성하는 특성에 대하여 온도와 시간이 미치는 영향을 조사하였다. 식중독 미생물 6종(*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* Typhimurium) 32균주를 대상으로 예비실험을 하여 각 종별로 biofilm 형성능이 강한 1균주씩을 선발하였다. 시험한 식중독 미생물 6종 모두 온도가 증가함에 따라 biofilm 형성능이 증가하였으며, 식중독 미생물의 종류와 polyethylene 및 stainless steel의 표면에 따른 차이는 일관된 경향을 나타내지 않았다. *E. coli*와 *P. aeruginosa*가 polyethylene 표면에서 biofilm을 형성하는 능력은 stainless steel 표면에서보다 유의적으로 높았다. 식중독 미생물은 표면에 균을 접종했을 때 바로 biofilm을 형성하였으며, *E. coli*, *P. aeruginosa* 및 *S.* Typhimurium은 접종 1시간 후에 모든 표면에서 biofilm을 형성하였다. Biofilm 형성 7일 후, *S. aureus*를 제외한 나머지 균주는 polyethylene과 stainless steel 표면에서 생존률에 차이가 없었다. 시험한 6종의 식중독 미생물의 경우 biofilm을 형성하는 능력은 균의 종류 및 polyethylene과 stainless steel 표면에 따라 다르게 나타났다.

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