Doctoral thesis

Study of transition and sterilization of microorganism on the plastic surface

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Menu

Introduction

Cross-contamination of bacterial pathogens in the home and in food service establishments is thought to be a major contributing factor for food-borne illness during food handling and preparation, microorganisms on raw foods can be transferred to various surfaces, such as cutting boards and dishes. Bacteria on the contaminated surfaces may transfer to other food by contacting. The transfer rate of bacteria from contaminated surface to ready-to-eat food may affect by many factors, such as initial inoculums size, type of bacteria, contacting time.

Escherichia coli, *Pseudomonas aeruginosa*, *Staphylococcus aureus, Bacillus cereus,* and *B. cereus spores* were main bacteria strains caused food-borne illness. Bacteria mixed with food residues adhered on surface may form biofilm and made them hard to clean. Also biofilm were found resistant to some sanitizers and could survived after cleaning.

Proper washing has been recognized as one of the most effective step to prevent cross-contamination and minimize transfer of microorganisms to ready-to-eat foods in modern homes and institutional kitchens.

In this research, influence factors to transfer rate of *E. coli* O157:H7 from plastic chopping boards surface to ready-to-eat food was clarified. To reduce the bacteria on plastic surface, effect of surfactant disinfectant (benzalkonium chloride, BAC and alkyldiaminoethylglycine hydrochloride, AGH) to bacteria mixed with food component dried on plastic and ceramic surface was also studied. Bacteria was detected after BAC and AGH treatment, but BAC and AGH following general and proper washing treatment could reach the sterilization requires. These results indicated that washing is an important step in food safety and could reduce the risk of cross-contamination on plastic surface.

Chapter 1 evaluated the influence factors to affect transfer rate of *E. coli* O157:H7 from contaminated

chopping boards to ready-to-eat ham. The effect of initial inoculums size, food component, pressure, contacting time, number of scratches, and semi-dried surface of ham to transfer rate were studied.

Chapter 2 evaluated effect of food residues (milk, beef gravy and tuna gravy) on efficiency of surfactant disinfectants (BAC and AGH) against food related pathogens (*E. coli* O26, *P. aueruginosa*, *S. aureus*, *B. cereus*)adhered on plastic and ceramic dishes surfaces. In this study, the importance of washing to avoid bacterial adhesion to the surfaces of plastic and ceramic dishes was also clarified.

Chapter 1

Transfer rate of *Escherichia coli* O157:H7 from contaminated chopping boards to ready-to-eat ham

1. Introduction

Cross-contamination is recognized as a major cause of food-borne illness (Bloom and Scott, 1997; Guzewich and Ross, 1999; Knabel 1995) and kitchen incidences have increased as the availability of ready-to-eat food increases (Cogan et al., 2002; Gorman et al., 2002). Home environments are the final link in the food chain. Chopping boards contaminated by bacteria during food preparation may continue to harbor the bacteria, which may later transfer to other foods. Therefore, avoiding cross-contamination between chopping board and food is crucial for reducing the risk of food-borne diseases.

Chopping boards are indispensable for food preparation in the home. Some studies have reported that cut or scratched plastic chopping boards are more difficult to clean than new or lightly scratched boards (Scuderi et al., 1996; Barker et al., 2003). The transfer of bacteria from one surface to another is influenced by type of bacteria (Mackintosh and Hoffman. 1984; Rusin et al., 2002); surface material (Chen et al., 2001; Gill and Jones, 2002); and post-inoculation time (Scott and Bloomfield, 1990).

The kitchen environment has been identified as a major source of pathogenic bacteria, including *E. coli* O157:H7 (Scuderi et al., 1996; Barker et al., 2003), which produces symptoms at infection doses of less than 10 cells (Buchanan and Doyle, 1997). Several studies have implicated human contact or contact between kitchen surfaces and foods in bacterial survival and cross-contamination (Chen et al., 2001; Montville et al., 2001; Kusumaningrum et al., 2003). In recent years, ready-to-eat food has become fresh, nutritious and easily prepared, and its consumption has increased accordingly. However, ready-to-eat food is readily cross-contaminated by contact with preparation surfaces harboring pathogenic bacteria. One source of food-borne disease may caused by ham prepared on unclean chopping boards. The surface of ham is soft, smooth, moist and hydrophilic, and therefore easily attached to chopping boards. However, the transfer rate (TR) of *E. coli* O157:H7 from contaminated plastic chopping boards to ham has been little investigated.

The objective of this study is to clarify the influence of initial inoculum size, pressure, number of scratches and other effecting factors on the transfer rate of *E. coli* O157:H7 from contaminated chopping boards to ready-to-eat ham.

2. Materials and methods

2.1. Samples and chemicals

Ready-to-eat ham (aseptically packaged), and UHT milk were purchased from a local supermarket (Nonoichi, Japan). Each ham portion weighed approximately (18±1.0) g. Soluble starch was purchased from Wako Pure Chemical(Osaka, Japan). The neutralized detergent was purchased from Kao Corporation (Tokyo, Japan). Rifampicin was purchased from Nacalai Tesque (Kyoto, Japan).

2.2. Preparation of chopping boards

Plastic chopping boards purchased from a local supermarket (Nonoichi, Japan) were cut into (5×5) cm^2 squares, and either scored with scratches at the center (3×3 cm^2) or left intact. Prior to experiment, all board coupons were sterilized under UV light for 10 min in the bio clean-bench (SCB-1300B, Shimadzu Rika Instrument, Tokyo, Japan).

2.3. Preparation of bacteria

This experiment employed a rifampicin resistant strain of *Escherichia coli* O157:H7 (isolated from cow dung, a non-virulent strain that does not produce verocytotoxins VT1 and VT2). The strain was prepared as described in Adelberg et al (1965), cultured in 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 centrifuging (2000 g) for 10 min at room temperature (RT), re-suspended and washed twice with 5 ml phosphate-buffered saline(PBS, 0.31 mmol/l, pH 7.2), then suspended in 1 ml PBS.

2.4. *Preparation of inoculation.*

The suspensions for spreading were prepared as follows: 0.5 ml of the *E. coli* O157:H7 suspension was mixed with 0.5 ml PBS and 1ml neutral detergent solution (0.55%, to facilitate homogeneous spreading of the bacteria). When the bacteria were supplemented with food components, 0.5 ml of the bacterial suspension was mixed with 0.5 ml neutral detergent and 1ml milk or 10% soluble starch. A drop of mixed suspension (0.02 ml) was then deposited onto the center of the chopping board sections and spread with an autoclaved writing brush across a 9 cm^2 area. The sections of chopping board were covered with ham

and placed in sterile stomacher bags for bacterial detection. The ham-coated board sections were placed in plastic dishes (diameter:9 cm, highs:15 mm, sterilized by ethylene oxide gas, As One Co., Ltd, Osaka, Japan), and 10 ml of PBS was added. Bacteria were detected by swabbing the boards with a cotton swab (for microbial test, Nissui Pharmaceutical Co., Tokyo). The suspensions (1 ml) were 10-fold serially diluted with 9 ml PBS and immediately plated onto Trypto-soya agar(TSA, Nissui, Tokyo) with rifampicin (1 mg/ml). The plates were incubated at 37 °C for 48 h.

The *E. coli* O157:H7 cells in the initial suspensions (bacterial population less than 10^5 cells) were counted by the three-tube MPN (most probable number) method. The chopping board coupons were contaminated as described above. Ham was left on the boards for 1 min, then placed in stomach bags containing 40 ml PBS and immediately homogenized in the stomacher. The board sections were placed in the stomach bags containing 40 ml PBS and cells were detached by pulsification (PUL 100 Pulsifier for food pathogen detection, Microgen Bioproducts. Ltd, Surrey, UK) for 1 min. Suspensions of ham and chopping boards were tested in TSB with rifampicin(1 mg/ml) incubated at 37 °C for 48 h.

2.5. Effect of number of scratches on the transfer rate of E. coli O157:H7 from contaminated chopping boards to ham.

Boards were scored with 10, 20 or 30 scratches (crossed or uncrossed) using a knife. The bacteria transferred to the ham from the contaminated chopping boards during 1 min contact time were enumerated by the plate method as described above.

2.6. Effect of drying on the transfer rate of E. coli O157:H7 from chopping boards to ham

Contaminated chopping boards were prepared as above and dried for 1 h in a clean-bench at RT. Moisture was absorbed from the ham samples by placing the ham on a kimtowel (Nippon Paper Crecia Co. Ltd. Tokyo, Japan) that had been sterilized in an oven at 140°C for, 4 h. Next, the ham samples were either dried in a clean-bench (ventilation 20 m^3/min; SCB-1300B, Shimadzu Rika Instrument, Tokyo, Japan) for 30 min at RT or left moist. Following this procedure, the ham placed on the chopping boards for 1 min was immediately enumerated by the plate method as described above.

2.7. Transfer rate of E. coli O157:H7 from contaminated chopping boards to ham samples

The cell population in the initial suspension was about 10^6cfu/coupon. Chopping boards were contaminated as described above. In succession, five pieces of ham were placed on the chopping board, each for 1 min, and the cell populations on the ham and chopping boards were enumerated by the plate method.

2.8. Effect of pressure on the transfer rate of E. coli O157:H7 from contaminated chopping boards to ham

The chopping boards were contaminated as described above. The cell population in the original suspension was about 10^6cfu/coupon. Pressures of 3 g/cm^2, 36 g/cm^2 or 70 g/cm^2 were applied to the ham samples on the boards for1 min. The number of *E. coli* O157:H7 cells transferred to the ham was calculated by immediately plating the cells after each pressure application, and enumerating the colonies as described above.

2.9. SEM of bacteria adhered to the chopping boards

Bacterial suspensions mixed with milk and PBS were placed on scratched or unscratched chopping board coupons in a bio-clean bench and dried for 90 min at RT. The board coupons were cut by autoclaved scissors into 1 cm^2 sub-sections. 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 20 kV.

2.10. Microbiological analyses and enumeration

The transfer rate was calculated as follows:

TR = cell population in ham/ cell population in the initial inoculum spread across the chopping boards

The cell population in the ham is the number of *E. coli* O157:H7 transferred from the contaminated chopping boards to the ham.

The TR data collected from the plastic chopping boards are expressed as the mean and SD of the measurements. Statistical analysis was conducted by the EXCEL Statistic 5.0 software (Esumi Co., Ltd., Tokyo, Japan).Significant differences were distinguished by $p<0.05$. All experiments were performed in triplicate ($n = 3$).

3. Results and Discussion

3.1. Effect of initial inoculum size on the transfer rate of E. coli O157:H7 from contaminated chopping boards to ham

The TR from the contaminated surface to ham is expected to depend on the concentration and initial load of pathogenic cells on the chopping boards. The effect of initial inoculum size on the TR was evaluated by the plating and MPN methods, and the results are summarized in Table 1-1. As the initial inoculum size decreases, the TR of *E. coli* O157:H7 reduces on both scratched and unscratched chopping boards (from 32.3% to 18.1% and from 34.5% to 18.6%, respectively). The TRs of scratched and unscratched chopping boards were not significantly different ($p<0.05$). According to this results, the higher the initial population, the higher the TR. By contrast, Fravalo et al (2009) reported that *Campylobacter* transfer from chicken thighs to chopping boards is inversely related to initial load. Montville and Schaffner (2003) similarly found that TR is inversely related to initial size. This discrepancy maybe caused by the different bacteria, materials and methods used in the present study. On the other hand, our results are consistent with those of Takeuchi and Frank (2000), who found that higher initial loads of *E. coli* O157:H7 are better attached to lettuce leaves.

Table 1-1 Transfer rate of *E. coli* O157:H7 from contaminated chopping boards to ham, varying the initial inoculum size

Unscratched chopping boards			Scratched chopping boards		
Initial inoculum size (log cfu/coupon)	Cell population in ham (log cfu/ham)	TR (%)	Initial inoculum size (log cfu/coupon)	Cell population in ham (log cfu/ham)	TR (%)
7.54±0.06	7.04±0.07	32.3±3.2	7.67±0.01	7.08±0.02	34.5±1.8
6.60±0.05	6.09±0.04	31.0±1.4	6.74±0.10	6.19±0.04	26.6±7.9
5.54±0.02	4.84±0.17	23.4±4.3	5.56±0.06	4.90±0.11	22.0±3.6
5.17±0.03	4.27±0.23	18.1±1.2	4.55±0.08	3.82±0.04	18.6±1.8
3.31±0.22*	2.71±0.11*	21.3±0.9*	3.34±0.00*	2.77±0.00*	21.8±0.0*
2.47±1.10*	1.85±0.15*	24.2±4.8*	2.71±0.12*	2.03±0.00*	21.5±6.2*

Initial inoculum size: population of *E. coli* O157:H7 spread on chopping boards
Cell population in ham: estimated number of *E. coli* O157:H7 cells transferred to ham
Values are expressed as mean and SD (n=3)
Time of contact with contaminated surface: 1 min
*: count by MPN (most proble number method)
Scratched chopping boards: 5 scratches were scored using a knife

Low initial inoculum size were counted by the MPN method. As the initial inoculum size was varied, the TR evaluated by this method did not significantly. However, the TR evaluated by the MPN method was lower than that obtained by plate counting at the highest initial inoculum size (7.04 versus 7.67 log cfu/ham), reinforcing that lower initial inoculum size lowers the TR in this study. Similarly, no significant differences were found between scratched and unscratched boards (p>0.05). Bacterial transfer from surfaces can be quantified in many ways. In this study, the cells were detached from the surfaces of the chopping boards by a cotton swab, and enumerated by plate counting or pulsification (in the MPN method). Like pulsification, sonication reportedly causes cell damage, and cotton swabbing is a preferable technique for detaching cells from surfaces (Chavant et al., 2004). In this study, however, both techniques yielded acceptable results.

3.2. Effect of contact time on the transfer rate of E. coli O157:H7 from contaminated chopping boards to ham

During preparation, food may be placed on chopping boards for a short time. The time for which the

ham remains on the chopping board may also affect the TR. To investigate this effect, ham samples were placed on sections of chopping board for 10s, 30s, 60s, 3 min or 5 min. The TR of *E. coli* O157:H7 from chopping boards to ham did not significantly depend on contact time (p>0.05; data not shown), and ranged from 21.2% to 26.4%,and from 21.7% to 26.7% on unscratched and scratched chopping boards, respectively (Table 1-2). However, Dawson et al., (2007), who implemented long-term contact (exceeding 8 h), reported that TR does increase with contact time. Dickson (1990) also suggested that the longer the contact time, the higher the transfer rate. In this study, longer contact time was not attempted because food is likely to be rapidly prepared in home kitchens, and probably remains on the chopping board for only a few minutes.

Table 1-2 Effect of contact time on the transfer rate of *E. coli* O157:H7 from contaminated chopping boards to ham

	unscratched chopping boards		scratched chopping boards	
contact time	cell population in ham (log cfu/ham)	TR (%)	cell population in ham (log cfu/ham)	TR (%)
10s	5.09±0.04	21.2±1.1	5.04±0.06	22.7±2.3
30s	5.19±0.02	26.4±0.4	5.07±0.07	24.4±2.7
60s	5.16±0.11	25.1±4.7	5.02±0.09	21.7±3.0
180s	5.12±0.11	24.3±4.0	5.11±0.05	26.7±1.6
300s	5.12±0.12	24.4±4.0	5.10±0.08	26.2±3.5

Initial inoculum size of unscratched chopping boards: 5.77±0.03 log cfu/coupon
Initial inoculum size of scratched chopping boards: 5.69±0.02 log cfu/coupon
Values are expressed as mean and SD (n=3)
Scratched chopping boards: 5 scratches were scored using a knife

3.3. Transfer rate of food-supplemented E. coli O157:H7 from contaminated chopping boards to ham.

During food preparation, a variety of foods can be placed on chopping boards, and contaminating microorganisms may become mixed with food residues. The influence of food components on the TR was evaluated, and the results are summarized in Table 1-3. Again, the TR was found to increase with increasing initial inoculum size. The TR of bacteria mixed with starch ranged from 38.5% to 18.9% on unscratched boards, and from 34.7% to15.3% on scratched boards. The TR of cells mixed with milk

ranged from 34.8% to 22.1% and from 38.5% to 34.0% on scratched and unscratched boards, respectively. However, the TRs of cells mixed with starch did not significantly differ on scratched and unscratched chopping boards. In general, the TRs of *E. coli* O157:H7 mixed with milk were slightly higher than those of cells mixed with starch. These differences probably arise from the components of milk, such as lipid and protein, and their concentrations. The TR of *E. coli* O157:H7 mixed with milk was more than 30% on scratched higher than on unscratched chopping boards. We consider that the milk occupied the space opened by the scratches, bringing the cells closer to the ham.

Table 1-3 Transfer rate of *E. coli* O157:H7 mixed with food components from contaminated chopping boards to ham

E. coli O157:H7 mixed with strach					
Unscratched chopping boards			Scratched chopping boards		
Initial inoculum size (log cfu/coupon)	cell population in ham (log cfu/ham)	TR (%)	Initial inoculum size (log cfu/coupon)	cell population in ham (log cfu/ham)	TR (%)
8.53±0.04	8.10±0.13	38.5±10.9	8.67±0.05	8.20±0.07	34.7±2.2
7.27±0.01	7.11±0.19	22.7±4.3	6.81±0.22	6.35±0.03	24.1±1.5
5.95±0.02	5.23±0.05	18.9±1.3	6.16±0.08	5.35±0.02	15.4±2.0
E. coli O157:H7 mixed with milk					
Unscratched chopping boards			Scratched chopping boards		
Initial inoculum size (log cfu/coupon)	cell population in ham (log cfu/ham)	TR (%)	Initial inoculum size (log cfu/coupon)	cell population in ham (log cfu/ham)	TR (%)
7.90±0.13	7.43±0.01	34.8±9.7	7.66±0.04	7.25±0.08	38.5±4.3
6.84±0.23	6.39±0.15	33.6±10.5	6.57±0.07	6.13±0.10	36.8±2.0
5.81±0.02	5.16±0.08	22.1±4.2	5.55±0.03	5.09±0.03	34.0±0.9

Initial inoculum size: population of *E. coli* O157:H7 spread on chopping boards
Cell population in ham: estimated number of *E. coli* O157:H7 cells transferred to ham
Values are expressed as mean and SD (n =3)
Time of contact with contaminated surface: 1 min
Scratched chopping boards: 5 scratches were scored using a knife

3.4. Effect of pressure on the transfer rate of E. coli O157:H7 from contaminated chopping boards to ham

During preparation, the knife used to slice the ham introduces pressure to the ham. Pressure encourages close contact with the chopping board and might thereby affect the TR. The impact of pressure on the TR is shown in Figure 1-1. The TR of *E. coli* O157:H7 from contaminated unscratched chopping boards to unweighted ham (a single piece of ham) was 29.31%. Under pressures of 3 g/cm^2, 36 g/cm^2, and 70 g/cm^2,the TR increased to 39.4 %,44.2% and 54.3%, respectively. Similar results were obtained on scratched chopping boards. The TR under 70 g/cm^{2}pressure was twice that of unweighted ham. Kusumaningrum et al., (2003) and Vorst et al., (2006) similarly reported that TR increases at higher pressures. These results may be attributed to the soft, smooth surface of ham. Higher pressure might reduce the surface space between the ham and the board and increase the contact area, thereby raising the TR. Bower et al.,(1996) also suggested that higher pressures bring surfaces into closer contact. This result indicates that pressure exerts a major influence on the TR and should be noted in food safety investigations.

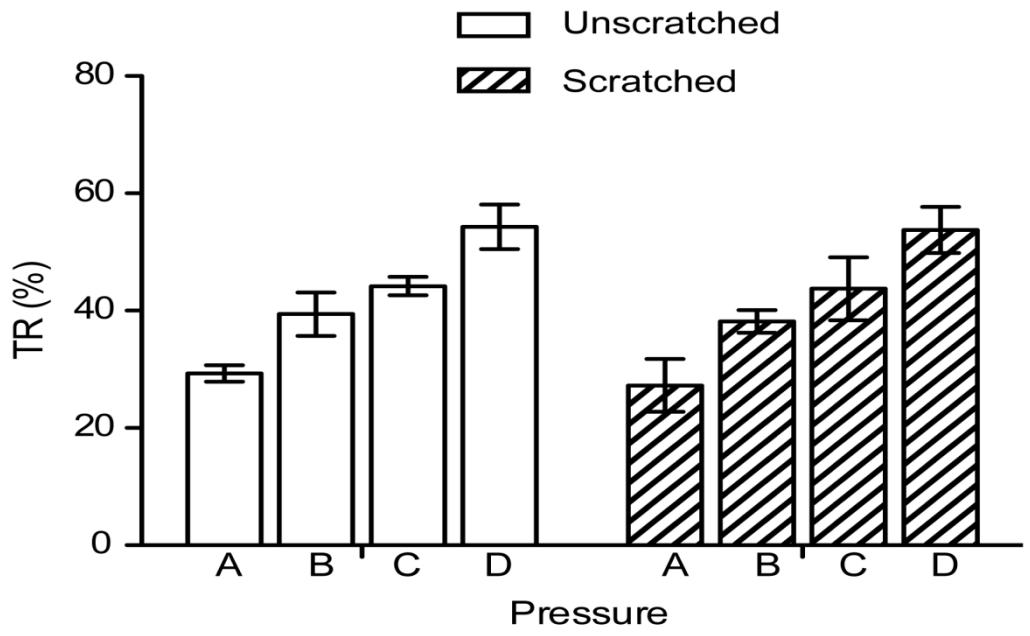

Figure1-1 *Effect of pressure on transfer rate of E. coli O157:H7 from contaminated chopping boards to ham*

A: Unweighted ham B:Weighted by 3 g/cm^2

C: Weighted by 36 g/cm^{2}D: Weighted by 70 g/cm^2

TR: transfer rate

Time of contact with contaminated surface: 1 min

Scratched chopping boards: 5 scratches were scored using a knife

Values are expressed as mean and SD (*n*=3)

3.5. Transfer rate of E. coli O157:H7 from contaminated chopping boards to successive ham samples

To determine the incidence of pathogenic cell transfer after repeated contact with the same contaminated surface, five ham samples were successively placed on a specified contaminated region of the board. The result is shown in Figure 1-2. The initial inoculum size deposited onto unscratched chopping boards was 5.24 log cfu/coupon. With successive placements, the population of *E. coli*

O157:H7 transferred to the ham steadily decreased from 4.82 to 3.81 log cfu/ham. The TR similarly decreased from 27.7% to 3.9%. The same results were obtained on scratched chopping boards; from the first to the last ham placement, the TR reduced from 23.7% to 3.9%. The population of cells transferred to the ham reduced 4.78 and 4.89 log cfu/ham on unscratched and scratched boards, respectively. Similarly, Wachtel et al., (2003) found that *E. coli* transfers to pieces of lettuce successively placed on a contaminated surface. After contact with 5 successive ham samples, the residual cell populations were 4.27 log cfu/coupon (9.0%) on unscratched boards and 4.88 log cfu/coupon (19.5%) on scratched boards (data not shown). These results indicate that once chopping boards have been contaminated by pathogen cells, successive food placements on the same surface may disseminate food-borne infections.

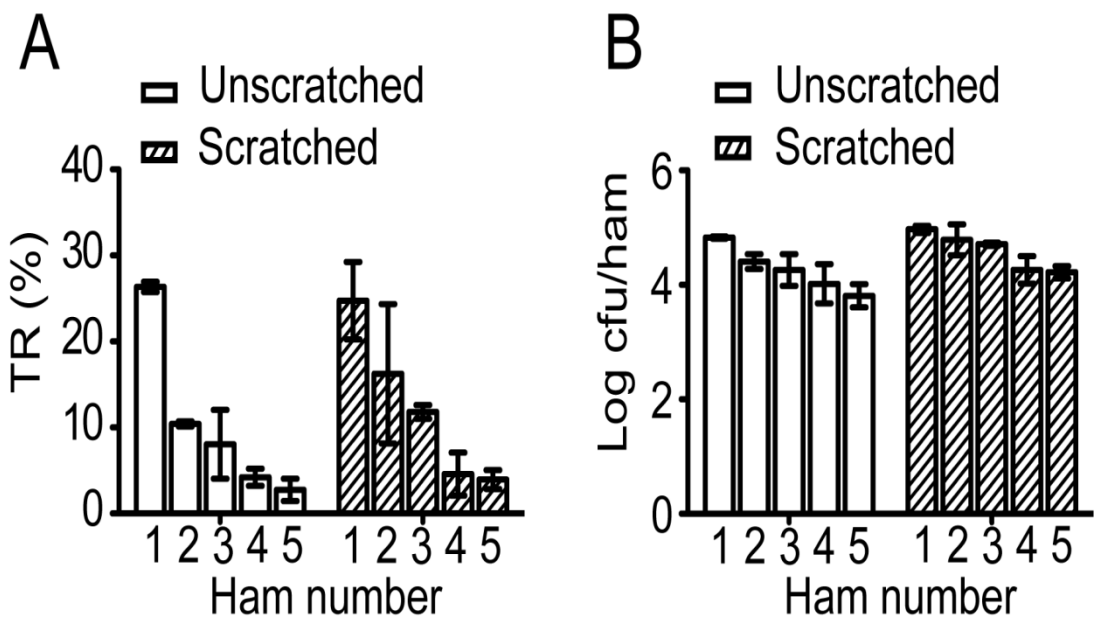

Figure 2-2.*Transfer rate of E. coli O157:H7 from contaminated chopping boards to 5 ham samples successively placed on the contaminated area.*

A: Results of transfer rate; B: Results of population of bacteria

Ham number: 5 pieces of ham were successively placed on a contaminated region of the chopping board.

Time of contact with contaminated surface: 1 min

Scratched chopping boards: 5 scratches were scored using a knife

Values are expressed as mean and SD (*n*=3)

3.6. Effect of number of scratches on the transfer rate of E. coli O157:H7 from contaminated chopping boards to ham

When used for a long period of time, chopping boards will likely become scored by knife incisions. The resulting scratches will complicate the surface topography, and may embed or trap bacteria, thereby affecting the TR. For this reason, the influence of number of scratches on the TR was investigated. Table1-4 shows the effect of varying scratch number on the TR of *E. coli* O157:H7 from contaminated chopping boards to ham samples. In this experiment, the TR ranged from 20.1% to26.6%, and the number of cells transferred to ham ranged from 5.25 log cfu/ham to 5.54 log cfu/ham. The number of scratches exerted no significant influence on the results. Some previous studies reported a clear correlation between TR and surface roughness (Dawson et al., 2007; Knobben et al., 2007); the higher the roughness, the lower the TR. However, consistent with our results, Flint et al.,(2000) found no relationship between bacterial TR and surface roughness. In the present study, the effect of scratch number on TR might have been attenuated by the wet surface and the relative simplicity of the scored surface. Although the number of scratches did not influence the TR in this study, pathogenic cells could nonetheless take refuge in the scratches, increasing the risk of cross-contamination.

Table 1-4 Effect of number of scratches on transfer rate of *E. coli* O157:H7 from contaminated chopping boards

Number of scratches	Initial inoculum size (log cfu/coupon)	Cell population in ham (log cfu/ham)	TR (%)
0	6.07±0.01	5.46±0.02	24.5±0.5
10A	6.05±0.04	5.42±0.03	24.4±0.6
10B	6.08±0.01	5.39±0.03	20.3±1.2
20A	6.12±0.05	5.54±0.04	26.6±2.5
20B	6.02±0.10	5.41±0.06	24.9±3.5
30A	6.06±0.02	5.42±0.12	22.7±1.9
30B	6.04±0.02	5.25±0.14	20.1±1.0

A: Parallel scratches
B: Criss-crossed scratches
Control: Unscratched chopping boards
Values are expressed as mean and SD (n=3)
Time of contact with contaminated surface: 1 min
Scratches were scored using a knife

3.7. Effect of semi-dried ham on the transfer rate of E. coli O157:H7 from contaminated chopping boards to ham.

The surface of ham may loss moisture according to the keeping or producing method. Such drying surface might influence the TR between board and food. The influence of semi-dried ham surface on the TR from contaminated chopping boards to ham samples is summarized in Table 1-5. When semi-dried ham was placed on chopping board (0.02 ml suspension spread) surfaces, the TR of *E. coli* O157:H7 increased to 34.7% and 31.5% on unscratched boards and scratched boards, respectively. This result was significantly higher than ham contact to board (the same as above, ham placed on suspension spread chopping board surface). This result is opposite to the report of Pérez-Rodríguez et al., (2007). Also, Sattar et al., (2001) reported an opposite result; that bacterial exchange between donor and recipient fabrics was enhanced when both materials were moist. Semi-dried in this study, reduced moisture of ham surface only, when semi-dried ham contact to the chopping boards, cell suspensions of board surface maybe absorbed to ham surface for supplying the moisture loss of ham, and increased bacterial population to ham. These results indicate that dried surfaces of food may also elevate the bacterial transfer rate.

Table 1-5 Effect of semi-dried ham on the transfer rate of *E. coli* O157:H7 from contaminated chopping boards to

	Un-scratched chopping boards			Scratched chopping boards		
	Initial inoculum size (log cfu/coupon)	Ham (log cfu/ham)	TR (%)	Initial inoculum size (log cfu/coupon)	Ham (log cfu/ham)	TR (%)
A	5.72±0.09	5.13±0.05	25.0±3.2	5.68±0.02	5.04±0.06	22.7±2.3
B	5.69±0.05	5.24±0.10	34.7±4.4	5.87±0.05	5.38±0.06	31.5±2.6

A: Ham placed on chopping boads as before

B: Ham semi-dried for 30 min in a clean-bench at RT and then placed on chopping boards

Scratched chopping boards: 5 scratches were scored using a knife

values are expressed as mean and SD (n =3)

Time of contact with contaminated surface: 1 min

Moisture of ham without dried and semi-dried ham was 73% and 70%, respectivelly.

3.8. SEM of chopping boards

Figure 1-3 shows SEM images of *E. coli* O157:H7 cells adhered to the chopping boards. Under the microscope, *E. coli* O157:H7 cells were clearly visible (A), indicating that cells on chopping boards can directly transfer to food. Cells mixed with milk are obscured, and possibly protected, by the milk (B). This image also suggests that cells mixed with milk can readily attach to chopping boards. *E. coli* O157:H7 cells clearly reside in the scratches(C) and mixed with milk (D). As shown in the images, significant numbers of *E. coli* O157:H7 cells embed in the scratches. Although such embedded cells may not directly contact food placed on the board surface, they may transfer to the food via mixing with liquid or moisture. The image of cells mixed with milk and inhabiting the scratches supports the results of Table 1-3, in which the TR of cells mixed with milk is higher in scratched boards than in unscratched boards. Cells mixed with milk occupied the edge and the interior of scratches; having contacted with food, such cells may transfer to the food and cause food-borne diseases. Surface scratches may provide refuges for cells mixed with food components, enabling the cells to establish bacterial layers similar to biofilms. Within these layers, bacteria are more robust to cleaning and are therefore more likely to cross-contaminate food.

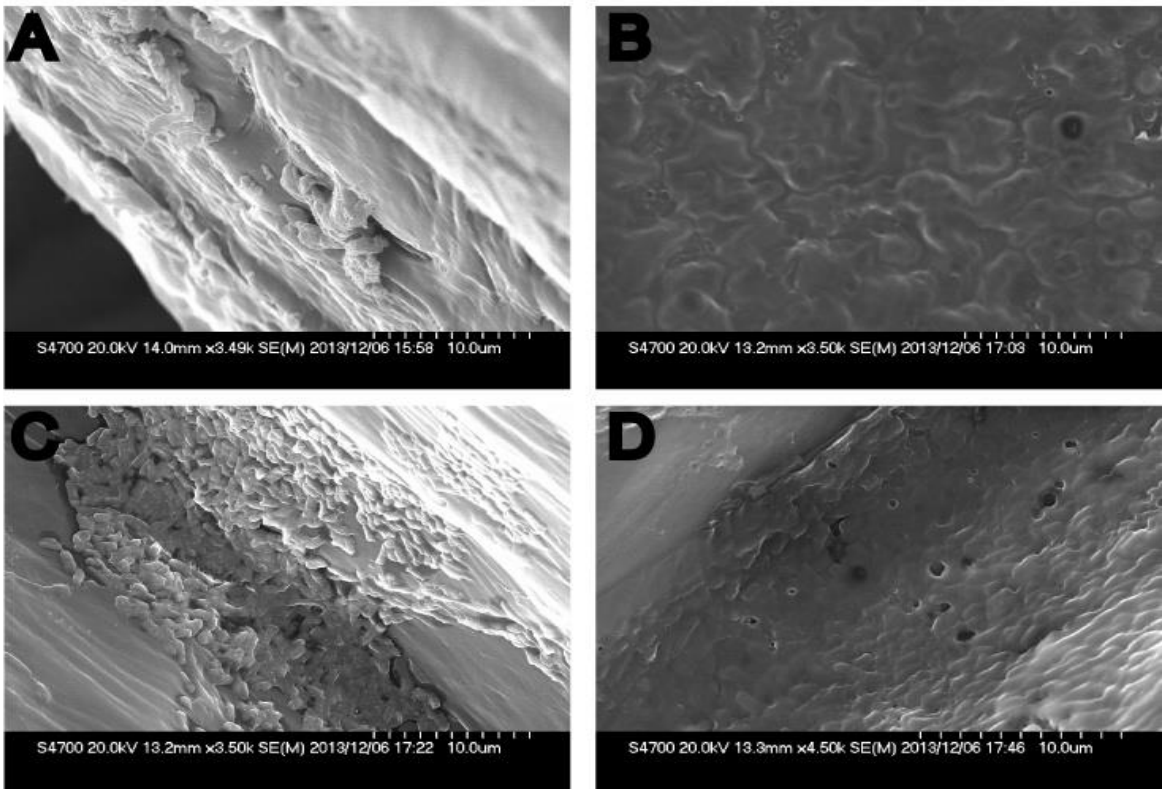

Figure 1-3 *SEM images of E. coli O157:H7 adhered to chopping boards*

A: *E. coli* O157:H7 on chopping board

B: *E. coli* O157:H7 mixed with milk on chopping board

C: *E. coli* O157:H7 occupying the scratches on a chopping board

D: *E. coli* O157:H7 mixed with milk and occupying the scratches on a chopping board

4. Conclusion

This study has investigated several factors that potentially affect the transfer rate of *E. coli* O157:H7 from contaminated chopping boards to ready-to-eat ham. According to the results, transfer rate is significantly influenced by initial inoculum size, pressure, and surface moisture. Contaminated chopping boards can transfer bacteria to successive food items placed on the contaminated region. Furthermore, bacterial cells mixed with food components and occupying surface scratches may pose an important hidden problem in food safety. Clean chopping boards are crucial for food safety, since the initial population of pathogenic cells transferred to the food is reduced by the cleaning process. Cleaning also removes many of the residual cells on chopping boards that are responsible for cross-contamination. This results indicate that adequate cleaning of chopping boards is central to preventing food-borne diseases.

References

Adelberg, E. A., Mandel, M. and Chen, G. C. C. (1965). Optimal conditions for mutagenesis by*N*-methyl-*N*'-nitro-*N*-nitrosoguanidine in *Escherichia coli* K 12. *Biochemical and Biophysical research Communications, 18,* 788-795.

Barker, J., Naeeni, M., and Bloomfield, S. F. (2003). The effects of cleaning and disinfection in reducing *Salmonella* contamination in a laboratory model kitchen. *Journal of Applied Microbiology, 95,* 1351-1360.

Bloomfield, S. F., and Scott, E. (1997). Cross-contamination and infection in the domestic environment and the role of chemical disinfectants. *Journal of Applied microbiology, 83,* 1-9.

Bower, C. K., McGuire, J., and Daeschel, M. A. (1996). The adhesion and detachment of bacteria and spores on food-contact surfaces. *Trends in Food Science and Technology, 7,* 152-156.

Buchanan, R., and Doyle, M. (1997). Food born disease significance of *Escherichia coli* O157:H7 and other Enterohemorrhagic *E. coli*. *Food Technology, 51,* 69–76.

Chavant, P., Gaillard-Martinie, B., and Hebraud, M. (2004). Antimicrobial effects of sanitizers against planktonic and sessile *Listeria monocytogenes* cells according to the growth phase. *FEMS Microbiology. Letters. 236,* 241-248.

Chen, Y., Jackson, M., Chea, F. P., and Schaffner, D. W. (2001). Quantification and variability analysis of bacterial cross contamination rates in common food service tasks. *Journal of Food Protection, 64,* 72-80.

Cogan, T. A., Slader, J., Bloomfield. S. F., and Humphrey, T. J. (2002). Achieving hygiene in the domestic kitchen: the effectiveness of commonly used cleaning procedures. *Journal of Applied Microbiology,* 92, 885-892.

Dawson, P., Han, I., Cox, M., Black, C., and Simmons, L. (2007). Residence time and food contact time effects on transfer of Salmonella Typhimurium from tile, wood and carpet: testing the five-second rule. *Journal of Applied Microbiology, 102,* 945-953.

Dickson, J. S. (1990). Transfer rate of *Listeria monocytogenes* and *Salmonella typhimurium* between beef tissue surfaces. *Journal of Food Protection, 53,* 51-55

Flint, S, H., Brooks, J, D., and Bremer, P, J. (2000). Properties of the stainless steel substrate, influencing the adhesion of thermo-resistant streptococci. *Journal of Food Engineering, 43,* 235-242.

Fravalo, P., Laisney, M. J., Gillard, M. O., Salvat, G., and Chemaly, M. (2009). *Campylobacter* Transfer from Naturally Contaminated Chicken Thighs to Cutting Boards Is Inversely Related to Initial Load. *Journal of Food Protection, 72,* 1836-1840.

Gill,C, O., and Jones, T. (2002). Effects of wearing kintted or rubber gloves on the transfer of Escherichia coli between hands and meat. *Journal of Food Protection, 65,* 1045-1048.

Gorman,R., Bloomfield, S., and Adley, C. C. (2002). A study of cross-contamination of food-borne pathogens in the domestic kitchen in the Republic of Ireland. *International Journal of Food Microbiology, 76,* 143-150.

Guzewich,, J. J., and Ross, M. P.(1999). Evaluation of risks related to microbiological contamination of ready-to-eat food by food preparation workers and the effectiveness of interventions to minimize those risks. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition.

Knabel, S. J. (1995). Foodborne illness: role of home food handling practices. *Food Technology, 49,* 119-131.

Kobben, B. A. S., Van der Mei, H. C., Van Horn, J. R., and Busscher, H. J. (2007). Transfer of bacteria between biomaterials surfaces in the operating room – an experimental study. *Journal of Biomedical Materials Research A, 80A,* 790-799.

Kusumaningrum, H. D., Riboldi, G., Hazeleger, W. C., Beumer, R. R. (2003). Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. *International Journal of Food Microbiology, 85,* 227– 236.

Mackintosh, C. A., and Hoffman, D. N. (1984). An extended model foe transfer of micro-organisms via the hand: differences between organisms and the effect of alcohol disinfection. *Journal of Hygiene,*

Cambridge, 92, 345-355.

Montville, R., Chen, Y., and Schaffner, D. W. (2001). Glove barriers to bacterial cross-contamination between hands and food. *Journal of Food Protection, 64,* 845-849.

Montville, R., and Schaffner, D. W. (2003). Inoculum size influences bacterial cross contamination between surfaces. *Applied and Environmental Microbiology, 69,* 7188-7193.

Pérez-Rodríguez, F., Valero, A., Todd, E. C. D., García-Gimeno, R. M., and Zurera, G. (2007). Mathematical modeling the cross-contamination of Escherichia coli O157:H7 on the surface of ready-to-eat meat product while slicing. *Meat Science, 76,* 692-699.

Rusin, P., Maxwell, S., and Gerba, C. (2002). Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. *Journal of Applied Microbiology, 3,* 585-592.

Sattar, S. A., Springthorpe, S., Mani, S., Gallant, M., Nair, R. C., Scott, E., et al. (2001). Transfer of bacteria from fabrics to hands and other fabrics: development and application of a quantitative method using *Staphylococcus aureus* as a model. *Journal of Applied Microbiology, 90,* 962-970.

Scott, E. and Bloomfield, S. (1990). The survival and transfer of microbial contamination via cloths, hand, and utensils. *Journal of Applied Bacteriology. 68,* 271-277.

Scuderi, G., fantasia, M., Fileticl, E., and Anastasio, M. P. (1996). Foodborne outbreaks caused by salmonella in Italy. 1991-1994. *Epidemiology and Infection, 116,* 257-265.

Takeuchi, K., and Frank, J. F. (2000). Penetration of Escherichia coli O157:H7 into lettuce tissues as affected by inoculums size and temperature and the effect of chlorine treatment on cell viability. *Journal of Food Protection, 63,* 434-440.

Vorst, K. L., Todd, E. C., and Ryser, E. T. (2006). Transfer of *Listeria monocytogenes* during mechanical slicing of turkey breast, bologna, salami. *Journal of Food Protection, 69,* 619-626.

Wachtel, M. R., McEvoy, J. L., Luo, Y. G.., Williams-Campbell, A. M., and Solomon, M. B. (2003).

Cross-Contamination of Lettuce (*Lactuca sativa* L.) with Escherichia coli O157:H7 via Contaminated Ground Beef. *Journal of Food Protection, 66,* 1176-1183.

Chapter 2

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

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 (Krysinskiet al., 1992; Sundheim et al., 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 et al.,1969;Kawamura-Sato et al.,2010;Marpleet al., 2004;Pernak et al., 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 et al., 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 *Stapylococcus aureus, Bacillus cereus* spores, and *Pseudomonas aeruginosa* has been widely reported (Landry et al., 2006; Lindsay et al., 2006; Saá Ibusquiza et al., 2011; Salgado, 2003).

Some reports (Barker et al., 2000; Scott et al., 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.

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 in5 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 for1 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 90min at RT in the bio-clean bench (SCB-1300B, Shimadzu Rika Instrument, Tokyo, Japan). The dried spot was approximately5 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 for90 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 15kV.

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. Images of bacterial cells adhered on plastic surfaces by crystal violet staining

At first, it has been observed for bacterial adhesion state on the plastic surface using 96 wells plate by crystal violet staining (Figure 2-1). *E. coli* O26 were gathered on the plastic surface, cells of *S. aureus* and *P. aeruginosa* were evenly distribute on the plastic surface. Cells of *P. aeruginosa* gathered and form a multi-layer may form biofilm (arrow). Also, cell population of *S. aureus* and *P. aeruginosa* were more than that of *E. coli* O26. Cells of *B. cereus* (vegetable cells) seemed hard to adhered on plastic surface. As the results showed in washing treatment, after washing twice, cells of *E. coli* O26, *S. aureus* and *P. aeruginosa* dried with distilled water were still detected but *B. cereus* were not found.

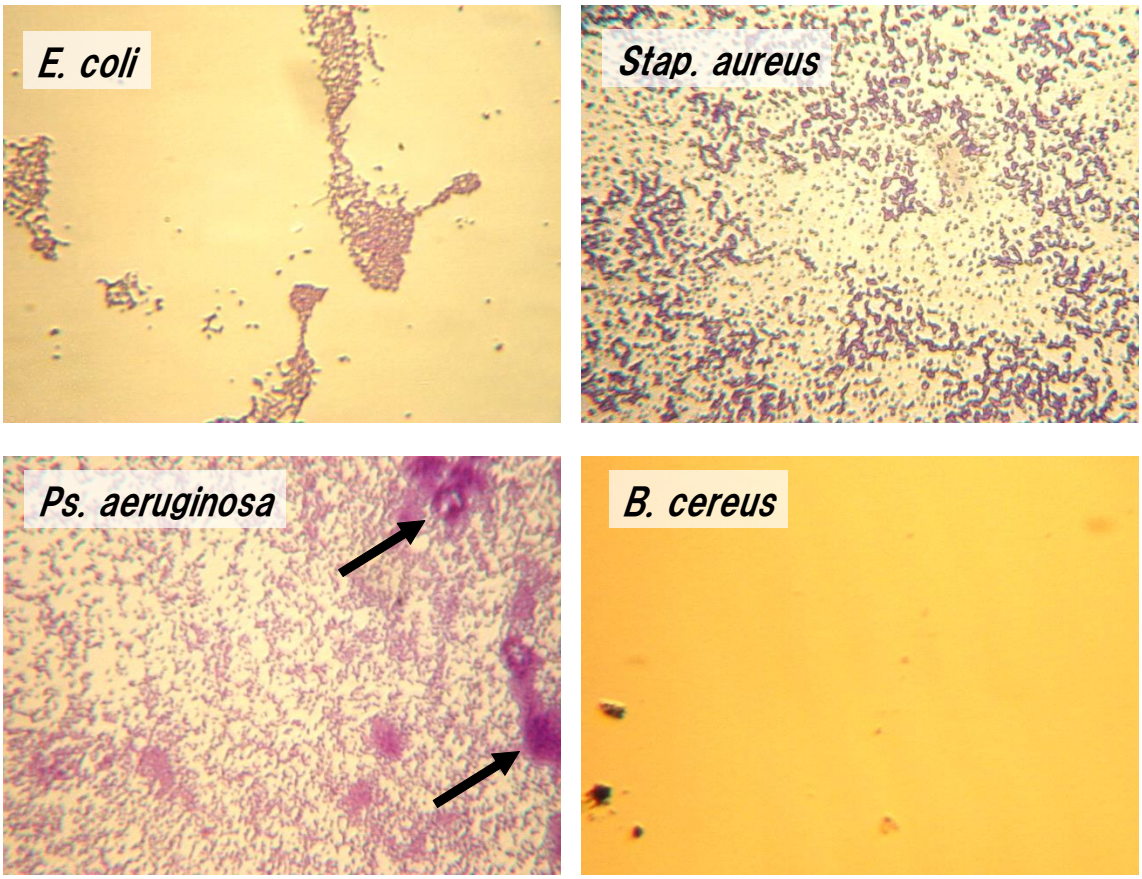

Figure 2-1.Images of bacterial cells adhered on plastic surfaces by crystal violet staining

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

Table2- 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.

Table 2-1 Effect of BAC on strains dried with organic components on a plastic dish surface(log cfu/dish)

Bacterial strains	BAC concentration (mg/ml)	Before drying	Dried with			
		DW	DW	BSA	Starch	Salad Oil
E. coli O26	0	7.77±0.11	6.59±0.13	8.14±0.11 a	7.45±0.18 a	5.81±0.01
	0.5	NG	NG	3.99±0.13 b	5.29±0.21 b	NG
	1.0	NG	NG	NG	NG	NG
	2.0	NG	NG	NG	NG	NG
P. aeruginosa	0	8.71±0.03	7.47±0.11	5.03±0.16	7.05±0.18	5.97±0.29
	0.5	NG	NG	NG	NG	NG
	1.0	NG	NG	NG	NG	NG
	2.0	NG	NG	NG	NG	NG
S. aureus	0	7.54±0.18	6.51±0.28	7.02±0.11	7.32±0.30	5.03±0.29
	0.5	NG	NG	NG	NG	NG
	1.0	NG	NG	NG	NG	NG
	2.0	NG	NG	NG	NG	NG
B. cereus	0	7.09±0.14	5.52±0.11 a	4.80±0.14	5.43±0.15 a	3.93±0.20
	0.5	NG	2.54±0.21 b	NG	4.53±0.08 b	NG
	1.0	NG	NG	NG	2.78±0.12 c	NG
	2.0	NG	NG	NG	NG	NG

Cell suspensions (0.01 ml) were placed on a plastic dish surface and dried for 90 min
Values are mean and SD (n =3)
DW, distilled water; NG; not grown (<2.0)
BAC treatment for 10 min

Conversely, all of the investigated strains showed greater resistance to AGH (administered for 10 min) than to BAC (Table 2-2). *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.

Table 2-2 Effect of AGH on strains dried with organic components on a plastic dish surface(log cfu/dish)

Bacterial strains	AGH concentration (mg/ml)	Before drying DW	Dried with DW	Dried with BSA	Dried with Starch	Dried with Salad Oil
E. coli O26	0	7.74±0.14 a	6.59±0.13 a	8.01±0.02 a	7.56±0.12 a	5.73±0.56 a
	0.5	50.5±0.03 b	5.44±0.05 b	6.85±0.49 b	7.31±0.24 a	5.01±0.14 a
	1.0	NG	NG	NG	6.84±0.02 b	NG
	2.0	NG	NG	NG	6.47±0.25 b	NG
P. aeruginosa	0	8.63±0.05 a	7.55±0.24	4.97±0.01	6.91±0.16 a	5.82±0.04 a
	0.5	5.85±0.53 b	NG	NG	5.56±0.06 b	5.51±0.09 b
	1.0	4.55±0.21 c	NG	NG	NG	5.26±0.26 b
	2.0	NG	NG	NG	NG	4.16±0.11 c
S. aureus	0	7.68±0.09 a	6.62±0.03 a	7.13±0.14 a	7.41±0.09 a	4.94±0.08 a
	0.5	4.36±0.05 b	4.48±0.12 b	6.12±0.32 b	7.01±0.05 b	3.12±0.17 b
	1.0	3.94±0.11 c	3.59±0.17 c	5.76±0.40 b	6.79±0.06 b	NG
	2.0	NG	2.89±0.42 d	3.71±0.26 c	4.46±0.08 c	NG
B. cereus	0	7.18±0.01	5.48±0.10	4.80±0.14	5.46±0.04 a	4.08±0.12 a
	0.5	NG	NG	NG	4.48±0.13 b	3.42±0.25 b
	1.0	NG	NG	NG	NG	3.08±0.30 b
	2.0	NG	NG	NG	NG	NG

Cell suspensions (0.01 ml) were placed on a plastic dish surface and dried for 90 min
Values are mean and SD (n=3)
DW, distilled water; NG; not grown (<2.0)
AGH treatment for 10 min

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

Table 2-3 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 population numbers among the studied strains, they showed strong resistance to BAC. Spores dried with milk were reduced only by 0.39 log cfu/dish following treatment with 2.0mg/ml BAC. *P. aeruginosa* was also strongly resistant to BAC. Populations

of *E. coli* O26,*S.aureus* and *B. cereus* were reduced but remained above the detection limit. Administration of 2.0 mg/ml BAC to *S. aureus* cells dried with milk and gravies reduced their populations by less than 3 log cfu/dish. Under the same treatment, the populations of *E. coli* O26 dried with beef and tuna gravies were reduced by about 5.07 and 4.50 log cuf/dish, respectively, while *B. cereus* dried with milk, beef gravy and tuna gravy was reduced by more than 3 log cfu/dish. These strains (*E. coli* O26 and *B. cereus*) were much more sensitive to BAC than the other strains in this study.

Table 2-3 Effect of BAC on strain cells dried with milk,beef gravy and tuna gravy on a plastic dish surface (log cfu/dish)

Bacterial strains	BAC concentration (mg/ml)	Dried with		
		Milk	Beef gravy	Tuna gravy
E. coli O26	0	9.32±0.02 a	9.25±0.02 a	9.24±0.02 a
	0.5	8.81±0.10 a	7.29±0.59 b	7.95±0.29 b
	1.0	8.33±0.14 ab	6.83±0.30 b	6.96±0.30 c
	2.0	7.36±0.35 b	4.18±0.43 c	4.74±0.42 d
P. aeruginosa	0	8.13±0.10 a	7.23±0.07 a	7.53±0.03 a
	0.5	7.99±0.01 b	7.11±0.10 a	7.48±0.04 a
	1.0	7.88±0.03 b	6.58±0.26 b	7.32±0.02 ab
	2.0	7.78±0.02 c	5.40±0.32 c	6.51±0.07 b
S. aureus	0	8.77±0.01 a	8.29±0.03 a	8.33±0.14 a
	0.5	8.49±0.01 a	6.24±0.18 b	7.43±0.12 b
	1.0	7.52±0.22 b	5.97±0.04 c	6.32±0.32 c
	2.0	6.92±0.02 b	5.80±0.12 c	5.55±0.21 d
B. cereus	0	6.71±0.01 a	6.29±0.12 a	6.85±0.05 a
	0.5	5.46±0.17 b	5.11±0.41 b	6.52±0.06 a
	1.0	4.59±0.32 c	3.70±0.30 c	5.99±0.28 b
	2.0	3.45±0.21 d	3.16±0.10 c	3.55±0.21 c
B. cereus spores	0	4.92±0.03 a	4.83±0.03 a	4.87±0.01 a
	0.5	4.89±0.28 a	4.54±0.21 a	4.29±0.04 b
	1.0	4.78±0.12 a	4.06±0.06 ab	3.78±0.12 c
	2.0	4.53±0.13 b	3.44±0.17 b	3.63±0.33 c

Cell suspensions (0.01ml) were placed on a plastic dish surface and dried for 90 min
Values are mean and SD (n =3)
BAC treatment for 10 min

AGH exerted less bactericidal effect than BAC on strains dried with milk, beef gravy and tuna gravy (data not shown). Under AGH treatment, the population reduction was judged to be insignificant. Especially, cells of all strains dried with milk were reduced by less than 1 log cfu/dish following 2.0 mg/ml AGH treatment. As found for BAC, *P. aeruginosa* and *B. cereus* spores were more resistant to AGH than the other strains.

3.4. Effect of washing treatment combined with BAC and AGH treatment on cells dried with milk on the surfaces of plastic and ceramic dishes

Table 2-4 summarizes the effect of washing treatment combined with BAC and AGH treatment on cells dried with milk on the surfaces of the plastic dishes. Following the washing step (step D2) and 0.5 mg/ml BAC treatment (step E1, E2) for 10 min, both *E. coli* O26 and *S. aureus* cells dried with milk failed to grow. Although cells of *E. coli* O26 and *S. aureus* were still detected after AGH (step E3, E4) treatment, cells were not found after rinsing. *B. cereus* spores were similarly not detected following the washing treatment. In this study, *B. cereus* spores were considered to be more resistant than their cellular counterparts. The washing step also eliminated *P. aeruginosa* cells (data not shown).

Table 2-4 The effect of washing and surfactant treatment on strains dried on a plastic dish surface

	on the plastic dish *E. coli* O26 dried with		on the plastic dish *S. aureus* dried with		on the plastic dish *B. cereus* spores dried with	
washing process	DW	Milk	DW	Milk	DW	Milk
A: Before drying	8.09±0.12		7.99±0.13		4.89±0.08	
B: After drying	7.11±0.02	8.43±0.21	5.39±0.20	8.22±0.06	4.21±0.02	4.72±0.07
C: After water washing twice	5.46±0.42	8.18±0.05	4.99±0.22	8.01±0.16	3.02±0.21	3.49±0.02
D1: C+washing treatment	4.87±0.22	6.28±0.12	4.88±0.36	7.86±0.01	NG	3.15±0.05
D2: C+detergent treatment	NG	5.94±0.11	NG	4.92±0.01	NG	NG
BAC and AGH treatment						
E1: D1+0.5 mg/ml BAC (1ml) 10min	NG	NG	NG	NG	NG	NG
E2: D2+0.5 mg/ml BAC (1ml) 10min	NG	NG	NG	NG	NG	NG
E3: D1+0.5 mg/ml AGH (1ml) 10min	NG	5.84±0.20	NG	6.09±0.10	NG	NG
E4: D2+0.5 mg/ml AGH (1ml) 10min	NG	4.40±0.08	NG	4.02±0.09	NG	NG
F: E3+water rinsing	NG	NG	NG	NG	NG	NG
G: E4+water rinsing	NG	NG	NG	NG	NG	NG

Cell suspensions (0.01ml) were placed on a plastic dish surface and dried for 90 min
Values are mean and SD (n=3)
DW, distilled water; NG; not grown (<2.0)
D2: 0.285mg/ml alkyl ether sulfuric acid ester sodium treatment for 10 min

The effects of washing treatment combined with BAC and AGH on the ceramic dish surfaces are presented in Table 2-5. Following washing combined with0.5 mg/ml BAC (step E1, E2), neither *E. coli* O26 nor *S. aureus* cells dried with milk were detected on the plates. Similarly, the rinsing step prevented the survival of cells treated with 0.5 mg/ml AGH. After washing with detergent, no *B. cereus* spores were detected. Similar results were obtained for the ceramic dishes.

Table 2-5 The effect of washing and surfactant treatment on strains dried on a plastic dish surface

	on the plastic dish *E. coli* O26 dried with		on the plastic dish *S. aureus* dried with		on the plastic dish *B. cereus* spores dried with	
washing process	DW	Milk	DW	Milk	DW	Milk
A: Before drying	8.27±0.01		7.82±0.11		4.93±0.04	
B: After drying	6.11±0.05	8.43±0.21	5.63±0.33	7.87±0.04	4.71±0.02	4.58±0.13
C: After water washing twice	5.51±0.21	8.17±0.02	5.19±0.42	7.12±0.04	NG	4.24±0.01
D1: C+washing treatment	3.93±0.08	7.69±0.39	4.42±0.21	7.07±0.14	NG	4.17±0.04
D2: C+detergent treatment	NG	6.80±0.23	3.93±0.01	5.68±0.12	NG	NG
BAC and AGH treatment						
E1: D1+0.5 mg/ml BAC (1ml) 10min	NG	NG	NG	NG	NG	NG
E2: D2+0.5 mg/ml BAC (1ml) 10min	NG	NG	NG	NG	NG	NG
E3: D1+0.5 mg/ml AGH (1ml) 10min	NG	6.51±0.06	NG	5.38±0.03	NG	NG
E4: D2+0.5 mg/ml AGH (1ml) 10min	NG	5.80±0.03	NG	4.81±0.14	NG	NG
F: E3+water rinsing	NG	NG	NG	NG	NG	NG
G: E4+water rinsing	NG	NG	NG	NG	NG	NG

Cell suspensions (0.01ml) were placed on a plastic dish surface and dried for 90 min
Values are mean and SD (n =3)
DW, distilled water; NG; not grown (<2.0)
D2: 0.285mg/ml alkyl ether sulfuric acid ester sodium treatment for 10 min

The effects of washing treatment combined with BAC and AGH on scratched plastic surfaces are showed in Table 2-6. After washing treatment, cells of *E. coli* O26 were reduced gradually on scratched plastic surface. After 0.05% BAC treatment (step E1, E2), population of cells on scratched surface were

not found. Cells could found after washing and 0.05% AGH treatment (step E3), even after rinsing (step F), *E. coli* O26 cells were still detected on the scratched surface. This result indicated scratched plastic surface was difficult to sterilize and the risk of scratched surface should be notice. On the other hand, after AGH combined with neutralized detergent treatment (step E4), cells were not found on the scratched plastic surface, this result showed washing effect of neutralized detergent was better than washing with water. Scratched plastic surface washing by neutralized detergent could get a better washing effect.

Table 2-6 Effect of washing and surfactant treatment on *E. coli* O26 dried on unscartched and scratched plastic surfac

washing process	*E. coli* O26 dried with milk on unscratched plastic surface	*E. coli* O26 dried with milk on scratched plastic surface
A: Before drying	8.90±0.09	8.34±0.06
B: After drying	8.40±0.21	7.93±0.24
C: After water washing twice	8.28±0.05	7.23±0.27
D1: C+washing treatment	6.28±0.12	6.59±0.32
D2: C+detergent treatment	5.94±0.11	5.94±0.49
BAC and AGH treatment		
E1: D1+0.5 mg/ml BAC(1ml) 10min	NG	NG
E2: D2+0.5 mg/ml BAC(1ml) 10min	NG	NG
E3: D1+0.5 mg/ml AGH(1ml) 10min	5.84±0.20	5.46±0.15
E4: D2+0.5 mg/ml AGH(1ml) 10min	4.40±0.08	NG
F: E3+water rinsing	NG	3.27±0.04
G: E4+water rinsing	NG	NG

Cell suspensions (0.01ml) were placed on a ceramic dish surface and dried for 90 min
Values are mean and SD (n =3)
DW, distilled water; NG; not grown (<2.0)
D2: 0.285mg/ml alkyl ether sulfuric acid ester sodium treatment for 10 min
5 criss-crossed scratches were scored using a knife

Figure 2-2 shows SEM images of bacterial cells adhered to the plastic dishes. Under the microscope, cells of strains dried with PBS were clearly visible, and easily distinguishable from salt crystals. By

contrast, cells of strains dried with milk were not clearly observed because they were obscured by milk, and therefore could not be discriminated from salt crystals.

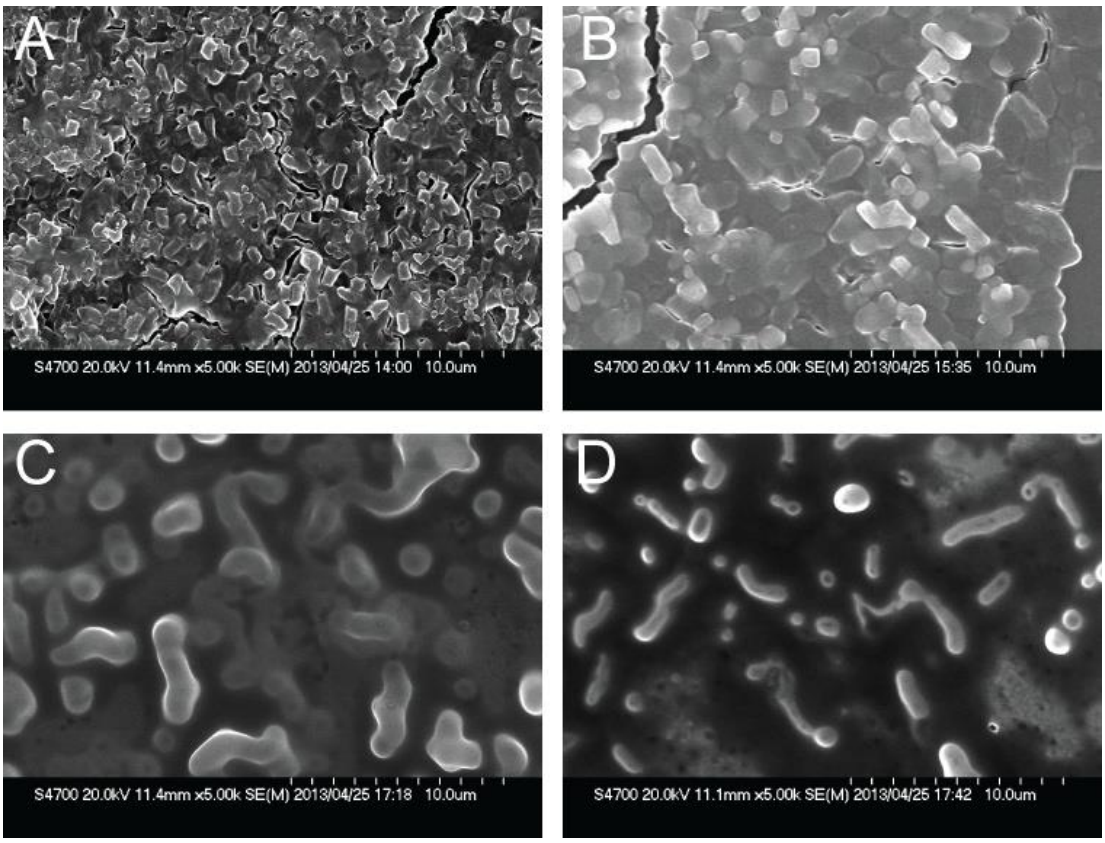

Figure 2-2. *Image of bacteria adhered on plastic dishes by SEM*

A *E. coli* O26 dried with 0.85% salt water;

B *B. cereus* dried with 0.85% salt water;

C *E. coli* O26 dried with milk;

D *B. cereus* dried with milk.

4. Discussion

This study assessed the effects of BAC and AGH on several pathogenic strains that adhered to the surfaces of plastic and ceramic surfaces. Organic components and food residues are known to protect bacteria from heat or detergents (Kusumaningrum et al., 2002; Line et al., 1991). They also provide nutrients for bacteria and are potential sources of cross-contamination to utensils (Sta˚hlet al., 2004). According to Rusell (1992), organic layers can prevent chemicals from reaching the cytoplasm of the cells. In this experiment, strains were mixed with DW, starch, BSA and salad oil, followed by drying. Treatment with BAC at commonly-used concentrations (0.5–2.0 mg/ml) effectively prevented the growth of pathogenic cells adhered to plastic surfaces in the presence of organic compounds (Table 1). Similarly, Houari & Martino(2007) found that typical concentrations of BAC inhibit biofilm formation of various bacterial cells. However, with the exception of *B. cereus*, strains dried with organic components were highly resistant to 2.0 mg/ml AGH (Table 2). Under this treatment, *P. aeruginosa* dried with salad oil and *E. coli* O26dried with starch were both reduced by less than 2 log cfu/dish. Following treatment with 2.0 mg/ml with AGH, the population of *S. aureus* were reduced by approximately 3 log/dish. As revealed in Table 2, AGH exerted much smaller bactericidal effect on the studied strains than BAC, when the strains were dried with organic component and adhered to plastic surfaces.

Conversely, biofilm formation largely blocked the penetration of both BAC and AGH into the pathogenic cells in this study. At high concentration (2.0 mg/ml), neither agent reduced the populations of cells dried with milk, beef gravy and tuna gravy to low levels on the plastic surfaces (Table 3). The same strains dried with milk, beef gravy and tuna gravy adhered to stainless surfaces are similarly resistant to BAC (Kuda et al., 2008). Wirtanenet al. (1996) reported that detergent exerts limited cleansing effect on surface biofilms. In the present study, the greatest resistance was exhibited by *P. aeruginosa*, whose resistance to BAC has been previously reported (Sakagami et al., 1989). Other reports have shown that pathogens are BAC-resistant in the following order: *P. aeruginosa>E. coli>S. aureus* (Reuda et al., 2003).

In our study, consistent with the results of Heinzel (1998), *B. cereus* spores were also highly resistant to BAC and AGH treatments.

E. coli has demonstrated resistance to some sanitizers (Sundheim et al., 1998). The most sensitive organism in our study was *B. cereus*, consistent with the report of Fazlara & Ekhtelat (2012).Although many studies have found that BAC is more effective against Gram positive than Gram negative bacteria(Brill et al., 2006; Fazlara, A., &Ekhtelat, M. 2012), our results suggest otherwise. We further found that while AGH reduces the populations of the studied strains adhered to solid surfaces, it is much less effective than BAC administered at the same concentration, as also reported by Kajiuraet al., (2001) and Shimizu et al., (2002).

In strains exposed to the washing treatment (Tables 2-4 and 2-5), we found no significant differences between adhesion to plastic and ceramic surfaces. *E. coli* O26 and *S. aureus* dried with DW, *B. cereus* spores, *P. aeruginosa* and *B. cereus* dried with DW and milk were eliminated by the washing step, even without BAC or AGH treatment (Table 2- 4). Following the washing treatment plus 0.5 mg/ml BAC(step E1, E2), *E. coli* O26 and *S. aureus* dried with milk failed to grow, but washing plus the same concentration of AGH (step E3, E4) yielded viable cells of both strains. *E. coli* O26 and *S. aureus* subjected to rinsing followed by 0.5 mg/ml AGH were not detected. However, 0.5 mg/ml BAC and AGH treatment without washing exerted negligible effect on cells dried with milk (Table 2-3). Since milk is rich in protein, carbohydrates, phosphatide and fat, milk-coated bacterial surfaces will be largely protected from the disinfectant. Cells of *E. coli* O26 dried with milk on scratched plastic surface were hard to sterilized (Table 2-6). After washing by water and 0.05% AGH treatment, plus rinsing (F), cells were still found, and cells were not detected by neutralized detergent combined with 0.05% AGH (G). Cells hide in the scratches on surface could be a risk to food safety.

The above results suggest that the populations of bacterial cells dried with PBS are markedly reduced following disinfectant treatment plus washing. However, the same disinfectants (BAC and AGH) without

washing did not affect the growth of bacterial cells dried with milk. The washing treatment likely re-dissolved the milk particles and removed them, enabling the disinfectants to penetrate the exposed bacterial cells and thereby exert their sterilizing effect.

From the SEM images and the results of Tables 2-4 and 2-5, we can infer that once the food residues have been washed away, the exposed bacterial cells become vulnerable to the sterilizing effects of the disinfectants. This result confirms the importance of washing in the food industry. Food residues can prevent surfactant disinfectants from penetrating the interiors of cells, but can be removed by washing. The beneficial effect of washing concurs with the results of Kuda et al. (2008). Similarly, Peng et al. (2002) reported that rinsing reduced the numbers of *B. cereus* cells in biofilms by 4.5 log cfu/chip. A greater than 5-log reduction in *B. cereus* numbers was achieved after subjecting contaminated eating utensils to washing plus minimal concentrations of sanitizers (Lee et al., 2007). These studies suggest the importance of adequate washing with detergents during food processing. This suggestion is consistent with the reports of Barker et al. (2003) and Wirtanen et al. (1996).

Swabbing is one of the methods for the detachment of bacterial cells from biofilm (Gibson et al., 1999; Holahet al., 1988;Moltz et al.,2005;Vorst et al., 2004) and used in this study. Other methods, such as vortexing with glass beads (Stopforth et al., 2002; Trachoo & Frank, 2002; Zhao et al., 2004) and sonication (Chavant et al., 2004) can damage the cells and obscure the effects of the disinfectant. Pan et al. (2006) reported no obvious correlation between the presence of aggregates and the sampling time or treatment conditions following swab detachment.

In conclusion, this study reveals that high concentrations of BAC and AGH may effectively reduce adhesion of pathogens to plastic and ceramic surfaces. In particular, these surfaces could be effectively sterilized by BAC and AGH treatment plus washing. We advocate washing and rinsing prior to and following sterilization, respectively, as important steps in meeting the strict sterilization requirements of food safety. Also, the cells on scratched plastic surface were hard to sterilized and cells in scratches could

be a risk to food safety and should be noticed.

References

Adair, W. F., Geftic, G. S., and Justus, G. J. (1969).Resistance of *Pseudomonas* to quaternary ammonium compounds I. growth in benzalkonium chloride solution.*Applied Microbiology*, *18*, 299-302.

Barker, J., and Bloomfield, S. F.(2000).Survival of*Salmonella*in bathrooms and toilets in domestic homes following salmonellosis. *Journal of Applied Microbiology*,*89*, 137–44.

Barker, J., Naeeni, M.,and Bloomfield.S. F. (2003).The effects of cleaning and disinfection inreducing Salmonella contamination in a laboratory model kitchen. *Journal of Applied Microbiology, 95*, 1351–1360.

Bremer, P. J., Fillery, S., and McQuillan, A. J. (2006).Laboratory scale clean-in-place (CIP) studies on the effectiveness of different caustic and acid wash steps on the removal of dairy biofilms. *International Journal of Food Microbiology, 106*, 254–262.

Brill,F.,Goroncy-Bermes,P., and Sand,W.(2006). Influence of growth media on the sensitivity of *Staphylococcus aureus* and *Pseudomonas aeruginosa* to cationic biocides. *International Journal of Hygiene and Environmental Healthy, 209,*89-95.

Carpentier, B., and Cerf, O. (1993).Biofilms and their consequences with particular reference to hygiene in the food industry. *Journal of Applied Bacteriology,75,*499–511.

Chavant, P., Gaillard-Martinie, B., and Hebraud, M. (2004). Antimicrobial effects of sanitizers against planktonic and sessile *Listeria monocytogenes* cells according to the growth phase. *FEMS Microbiology.Letters.236,*241–248.

Fazlara, A., and Ekhtelat, M. (2012). The disinfectant effects of benzalkonium chloride on some important foodborne pathogens. *American-Eurasian Journal of Agricultural & Environmental Science*, *12*, 23-29.

Frank, J. F., and Koffi R. A. (1990). Surface-adherent growth of *Listeria monocytogens* is associated with increased resistance to surfactant sanitizers and heat. *Journal of Food Protection, 53,* 550-554.

Gibson, H., Taylor, J. H., Hall, E. K., and Holah, J. T.(1999). Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms. *Journal of Applied Microbiology*, *87*, 41–48.

Heinzel, M. (1998). Phenomena of biocide resistance in microorganisms. *International Biodeterioration & Biodegradation*, *41*, 225–234.

Holah, J. T., Betts, R. P., and Thorpe, R. H. (1988). The use of direct epifluorescent microscopy (DEM) and the epifluorescent filter technique (DEFT) to assess microbial populations of food contact surfaces. *Journal of Applied Bacteriology*,*65***,** 215–221.

Houari, A., and Martino, P. Di.(2007). Effect of chlorhexidine and benzalkonium chloride on bacterial biofilm formation. *The Society for Applied Microbiology, Letters in Applied Microbiology, 45*,652–656.

Kajiura, T.,Tanaka, M.,Wada, H.,Ito, K., Koyama, Y.,and Kato, F.(2001). Effects of disinfectants on shiga-like toxin converting phage from enterohemorrhagic *Escherichia coli* O157: H7. *Journal of Health Science*,*47*, 203–207.

Kawamura-Sato, K., Wachino, J., Kondo, T., Ito, H., and Arakawa, Y. (2010). Correlation between reduced susceptibility to disinfectants and multidrug resistance among clinical isolates of *Acinetobacter* species. *Journal of Antimicrobial Chemotherapy*,*65*, 1975–1983.

Krysinski, E. P., Brown, L. J., and Marchisello, T. J. (1992). Effect of cleaners and sanitizers on *Listeria monocytogenes* attached to product contact surfaces. *Journal of Food Protection*,*55*,246-251.

Kuda, T., Yano,T., and Kuda, M. T. (2008). Resistances to benzalkonium chloride of bacteria dried with food elements on stainless steel surface. *LWT -Food Science and Technology*,*41*,988-993.

Kusumaningrum, H. D., van Putten, M. M., Rombouts, F. M., and Beumer, R. R. (2002). Effects of antibacterial dishwashing liquid on foodborne pathogens and competitive microorganismsin kitchen sponges. *Journal of Food Protection*, *65*, 61–65.

Landry, M. R., An, D., Hupp, T. J., Singh, K. P., and Parsek, R. (2006). Mucin-*Pseudomonas aeruginosa*

interactions promote biofilm formation and antibiotic resistance. *Molecular Microbiology, 59,* 142-151.

Lee, J., Cartwright, R., Grueser, T., and Pascall, M. A.(2007). Efficiency of manual dishwashing conditions on bacterial survival on eating utensils. *Journal of Food Engineering,80,* 885-891.

Lindsay, D.,Brözel, V.S., and von Holy, A. (2006). Biofilm-Spore Response in *Bacillus cereus* and *Bacillus subtilis* during Nutrient Limitation. *Journal of Food Protection*, *69,* 1168-1172(5).

Line, J. E., Fain, A. R., Moran, A. B., Martin, L. M., Lechowich, R. V., and Carosella, J. M. (1991). Lethality of heat to *Escherichia coli* O157:H7: D-value and Z-value determinations in ground beef. *Journal of Food Protection*, *54*, 762–766.

Marple, B., Roland, P., and Benninger, M. (2004). Safety review of benzalkonium chloride used as a preservative in intranasal solutions: an over view of conflicting data and opinions .*Otolaryngology - Headand Neck Surgery,130*, 131–141.

Moltz, A. G., and Martin. S. E. (2005). Formation of biofilms by *Listeria monocytogenes* under various growth conditions. *Journal of Food Protection*，*68***,** 92-97.

Pan, Y., Breidt, F. Jr., and Kathariou, S. (2006). Resistance of *Listeria monocytogenes* biofilms to sanitizing agents in a simulated food processing environment. *Applied and Environmental Microbiology*, *72*, 7711–7717.

Peng, J. S., Tsai, W.C., and Chou, C. C. (2002). Inactivation and removal of *Bacillus cereus* by sanitizer and detergent. *International Journal of Food Microbiology*, *77*, 11–18.

Pernak, J., Mirska, I.,and Kmiecik, R.(1999). Antimicrobial activities of new analogues of benzalkonium chloride. *European Journal of Medicinal Chemistry***,** *34*, 765-771**.**

Rueda, J., Amigot, Lázaro, J. A., and Ducha, J.(2003). Evaluación de desinfectan- tes de amoniocuaternariosobrece- pas bacterianas de origen animal. *Revue Scientifiqueet Technique,22,* 1097-1104.

Russell, A. D. (1992). Plasmids and bacterial resistance. In A. D. Russell, W. B. Hugo, & A. J. Ayliffe

(Eds.), *Principles and practice of disinfection* (second ed., pp. 225–229). Oxford, UK: Blackwell Scientific Publications.

Saá Ibusquiza, P., Herrera, J. J. R., and Cabo, M. L. (2011). Resistance to benzalkonium chloride, peracetic acid and nisin during formation of mature biofilms by *Listeria monocytogenes. Food Microbiology,28*, 418–425.

Sakagami, Y., Yokoyama, H., Nishimura, H., Ose, Y., and Tashima, T. (1989). Mechanism of resistance to benzalkonium chloride by *Pseudomonas aeruginosa. Applied and Environmental Microbiology*, *55*, 2036-2040.

Salgado, C. D., Farr, B. M ., and Calfee, D. P. (2003). Community-acquired methicillin-resistant *Staphylococcus aureus*: a meta-analysis of prevalence and risk of factors. *Clinical Infections Diseases, 36*, 131-139.

Scott, E., Bloomfield, S. F., and Barlow, C.G. (1982). An investigation of contamination in the home. *Journal of Hygiene,89*, 279–93.

Shimizu, M., Okuzumi, K.,Yoneyama, A.,Kunisada, T.,Araake, M., Ogawa, H., and Kimura, S. (2002). Invitro antiseptic susceptibility of clinical isolates from nosocomial infections. *Dermatology*, *204*, 21-27.

Ståhl, W. E., Johansson, E., and Håkanson, H. (2004). Cross contamination in dishwashers. *Journal of Hospital Infection,56*,312–317.

Stopforth, J. D., Samelis, J., Sofos, J. N., Kendall, P. A.,and Smith, G. C. **(**2002). Biofilm formation by acid-adapted and non-adapted *Listeria monocytogenes* in fresh beef decontamination washings and its subsequent inactivation with sanitizers. *Journal of Food Protection.65***,** 1717–1727.

Sundheim, G., Langsrud, S., Heir, E., and Holck, A. L. (1998). Bacterial resistance to disinfectants containing quaternary ammonium compounds. *International Biodeterioration and Biodegradation*, *41*, 235–239.

Trachoo, N., and Frank, J. F. (2002). Effectiveness of chemical sanitizers against *Campylobacter jejuni*-containing biofilms. *Journal of Food Protection*,*65***,** 1117–1121.

Verran, J., & Jones, M. (2000). *Industrial biofouling*. New York: John Wiley and Sons Ltd.

Vorst, K. L., Todd, E. C. D., and Ryser, E.T. (2004). Improved quantitative recovery of *Listeria monocytogenes* from stainless steel surfaces using a one-ply composite tissue. *Journal of Food Protection, 67*, 2212-2217.

Wirtanen, G.,Husmark, U.,and Mattila-Sandholm, T. (1996). Microbial evaluation of the biotransfer potential from surfaces with *Bacillus* biofilms after rinsing and cleaning procedures in closed food-processing systems. *Journal of Food Protection*,*59*,727-733.

Zhao, T., Doyle, M. P., and Zhao, P. (2004). Control of *Listeria monocytogenes* in a biofilm by competitive-exclusion microorganisms. *Applied and Environmental Microbiology, 70*, 3996–4003.

Conclusion

Chapter 1 studied the transfer rate (TR) of *E. coli* O157:H7 from chopping boards coupons to ready-to-eat ham. The significant influence factors to TR includes initial inoculum size, pressure on ham, and semi-dried surface of ham. The higher the initial inoculum size, the higher the TR. TR of initial inoculums size of 10^8cfu/coupon was 32.3% and 34.5% on un-scratched boards and scratched boards, respectively, higher than TR of lower initial inoculums size. When pressure increased to 70g/cm^2, the TR increased two times than no-pressure on ham. Also, TR of semi-dried surface of ham was higher than ham without drying. The influence of number of scratches and contacting time in this study, did not affect the TR significantly. Similarly, same experiment on scratched (5 scratches) and un-scratched chopping boards were found no significant to TR. Ham attached on the same contaminated place of chopping boards in succession also caused bacteria transfer. According to the image of SEM, food component mixed with bacteria hide in scratches also affect the TR and could be a risk factor for food safety. These results indicated cleaning of chopping boards is important during food process and could reduce the risk of food-borne disease caused by cross-contamination.

Chapter 2 investigates the effect of food residues (milk, beef gravy and tuna gravy) on the bactericidal efficiency of benzalkonium chloride (BAC) and alkyldiaminoethylglycine hydrochloride (AGH). Test bacteria (*E. coli* O26, *S. aureus*, *P. aeruginosa*, *B. cereus* and *B. cereus* spore) were mixed with food element (starch, salad oil and BSA) and food components (milk, beef gravy and tuna gravy) dried on plastic surfaces for 90 min, then treated with BAC and AGH in different concentration (0.05%, 0.10% and 0.20%). Bacteria mixed with water, starch, salad oil and BSA after treatment by 0.20% BAC were not detected on the TSA plates. AGH could reduced bacteria dried on plastic surface but still found after treat

to *E. coli* O26 mixed with starch and *P. aeruginosa* mixed with salad oil. *S. aureus* showed great resistance to AGH. BAC showed higher disinfecting effect on studied strains than AGH in the same concentration, but these two surfactant disinfectants were not found sterilization effect to studied strain mixed with milk, beef gravy and tuna gravy. After combination treatment with proper washing process, bacterial cells were not found on the dishes surfaces. These results showed the importance of washing in food safety, and surfactant disinfectants following with washing could reduce the risk of cross-contamination.

This research evaluated the risk of contaminated plastic surface in food process and in food safety. Bacteria adhered on chopping boards surface could transfer to food with a low inoculums size and at a very short time. The TR between scratched and un-scratched boards surface was insignificant in this study, but food component mixed with bacteria could hide in the scratches and made them difficult to clean. Higher initial inoculums size and higher pressure caused higher TR, bacteria on the same contaminated place could transfer to ham in succession. These results suggested plastic surface used in food process or kitchen need adequately clean and sterilize. After BAC or AGH treatment, bacteria dried on plastic surface were reduced to a low level but still detected. 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%– 2.0%) for 10 min. Following general and proper washing processes, the bactericidal effect of the disinfectants became clearly visible.

In conclusion, chopping boards contaminated by pathogen bacterial cells could transfer to food by contacting and cause food-borne illness. Even a very low initial inoculums size or very short contacting time could cause the transfer. Bacterial cells hide in the scratches on chopping boards could also transfer to food. BAC and AGH used in this study could reduce the bacterial cells, but when cells mixed with milk,

meat gravy and tuna gravy dried on plastic surface, the effect of BAC and AGH on cells adhered on plastic surface (unscratched) were not significantly found, combination with washing and neutralized detergent treatment could reach the sterilization require. On scratched plastic surface, after washing treatment and AGH treatment, cells still could found. This result indicated scratches on plastic surface is an important risk to food safety and should be noticed.

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