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Factors Affecting Adhesion of *Staphylococcus epidermidis* to Stainless Steel Surface

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Adhesion behavior of *Staphylococcus epidermidis* from its suspension onto stainless steel (SUS304) surface was studied, focusing on the effects of such factors as initial cell concentration of suspension, suspending medium, and roughness of stainless steel surface. For all cell concentrations tested (10^2–10^4 CFU/ml), adhesion occurred at low levels within 0.5 h of exposure to the cell suspension, and reached maximum levels in 3 h. The number of adherent cells per surface area was approximately proportional to the cell concentration of the suspension. Significantly higher adhesion was observed for cells suspended in peptone saline than those suspended in physiological saline. Significantly higher adhesion was also observed on roughly polished surface (R_a=1.37 μm) than on smoother surfaces ($R_a \leq 0.14 \mu$m). Shear force application by whirlpool rinsing removed only 50–82% of the adherent cells. The presence of cells still remaining on the surface indicated that the adhesion of *S. epidermidis* cells was irreversible in part.
Key words: microbial adhesion, stainless steel, *Staphylococcus epidermidis*

1. Introduction

In the food industry, microbial adhesion to equipment surfaces and subsequent development of biofilm are very serious issues because of potential to cause cross-contamination, which leads to lowered shelf-life, food spoilage, and transmission of disease [1-4]. Adhesion and subsequent growth on surfaces is a common strategy of bacteria for survival in both natural habitats and man-made environments [5]. Upon adhesion, bacterial cells could become less susceptible to cleaning and sanitizing agents, particularly after they have developed mature biofilm structure, wherein the matrix of organic polymers could provide additional protection. Reduced efficacy has been reported for a number of sanitation procedures against attached microorganisms [6]. Schwach and Zottola [7] showed that *Pseudomonas fragi, Salmonella montevidio,* and *Bacillus cereus* on stainless steel surface were not completely inactivated by treating with up to 150 ppm sodium hypochlorite. Stone and Zottola [8] demonstrated that *P. fragi* cells were not completely removed from stainless steel tube surface through a CIP cycle including alka-

line (potassium hydroxide/potassium hypochlorite) and acid (phosphoric and citric acids) treatments. *Listeria monocytogenes* cells adherent to glass surface exhibited increased resistance to benzalkonium chloride, anionic acid sanitizer, and heat [9]. Therefore, to control the risk of microbial cross-contamination, further knowledge on the adhesion of microorganisms to abiotic surfaces is desired.

Microbial adhesion to an abiotic surface is probably governed by complex interactions between the microorganism and the substrate surface involving physical, chemical, and biochemical factors. Several studies have been conducted on microbial adhesion onto different types of food contact surface [10-12]. However, the effects of factors on microbial adhesion have not yet been fully clarified or sometimes have been reported with inconsistency. For example, a milk-fouled stainless steel surface was reported to attract far more bacterial cells than a clean stainless steel surface [13], whereas adhesion of microorganisms suspended in milk to stainless steel surface was reported to be less than those suspended in phosphate buffered saline [14]. Opposing results have been reported also for the effect of roughness of stainless steel surface on microbial adhesion: positive correlation and independence between microbial adhesion and sur-

face roughness [15]. A reason for those conflicting results may be variation in cell surface characteristics and adhesion mode of different microbial species. Therefore, further compilation of adhesion data for various species is needed for better understanding of microbial adhesion.

Despite the research efforts devoted on microbial adhesion, very little information is available on the adhesion behavior of *Staphylococcus epidermidis* onto food contact surfaces. Coagulase−negative staphylococci (CNS), particularly *S. epidermidis*, have been implicated in increased cases of human infections in recent years [16, 17]. Although these reported cases were mainly of infections originating from implanted medical devices, its implications on sanitation issues in the food industry could not be disregarded. CNS are commonly found on human skin [18] and could be transferred to food or food contact surfaces by food handlers and are thus frequently found in food processing environments [17, 19]. In fact, Sharma and Anand [4] reported that *Staphylococcus* spp. was a predominant microorganism in biofilms found in commercial and experimental dairy plants.

The objective of this study is to provide better understanding on adhesion behaviors of *S. epidermidis* to stainless steel surfaces. Stainless steel is chosen as the substrate material because it is a commonly used material for food manufacturing equipment and has been the material of choice in the food industry for many years because of its mechanical strength, corrosion resistance, longevity, and ease of fabrication [20]. The factors considered here are initial concentration of cell suspension, suspending medium, and roughness of stainless steel surface. Removability of *S. epidermidis* cells from the stainless steel surfaces under the application of shear force is also taken as a subject of this study.

2.　Materials and Methods

2.1　Microbial strain

S. epidermidis NBRC 12993, maintained as a glycerol stock at −80℃, was inoculated in Trypticase soy broth (TSB) (Becton, Dickinson and Co., Maryland, USA) and grown at 37℃ for 18−20 h. The culture was then plated on Trypticase soy agar (TSA), which was made by the addition of 15 g/L agar to TSB. Following incubation at 37℃ for 48 h, the TSA plate was stored at 4℃ and used as a working stock.

2.2　Stainless steel surfaces

Three types of stainless steel (SUS304) plates (50×50× 3 mm) A, B, and C with different degrees of surface roughness were purchased from Toste (Osaka, Japan). The surface roughness qualities of each type of the plate, as expressed in roughness parameters R_a, R_q, R_y, and R_z, are listed in Table 1. Because the four parameters are approximately proportional to each other, we refer only to R_a value as the representative roughness parameter hereafter. All the stainless steel plates were cleaned by soaking in an alkali detergent (SCAT 20−X, Dai−ichi Kogyo Seiyaku Co. Ltd., Kyoto, Japan) for 24 h, rinsed with water, dried in clean ventilated oven at 60℃ for 1 h, and stored in 70% ethanol. They were dried under UV light in a clean bench just before use in every experimental run.

2.3　Preparation of microbial suspension

For every run of adhesion experiment, *S. epidermidis* cells from the working stock were sub−cultured in 5 ml of TSB at 37℃ for 18−20 h. The stationary−phase microbial cells thus obtained were harvested by centrifugation at 3,500×g for 10 min and resuspended in peptone saline (1 g/L peptone and 8.5 g/L NaCl). The resuspended cells were then serially diluted in peptone saline to achieve a desired initial cell concentration. In some experiments, to study the effect of suspending medium on adhesion, phosphate buffered saline (PBS) and physiological saline (0.85% NaCl) were also used instead of peptone saline, with an additional centrifugation−resuspension procedure before dilution.

Table 1　Roughness parameters of the stainless steel plates.

Roughness parameter (μm)		Plate type		
		A	B	C
R_a	Arithmetic mean of vertical deviation from mean line (average roughness)	0.04	0.14	1.37
R_q	Root mean square of vertical deviation from mean line	0.05	0.18	1.76
R_y	Maximum peak-to-valley height	0.35	1.20	10.20
R_z	Average maximum peak-to-valley height	0.30	0.70	7.20

2.4 Microbial adhesion

A stainless steel plate was soaked in 25 ml of the microbial suspension at 25℃. The contaminated stainless steel plate was withdrawn after a designated period of exposure to the microbial suspension and rinsed twice with 20 ml of peptone saline to remove loosely adherent cells. Preliminary tests showed that further rinsing did not reduce the cells left on the surface (data not shown). The number of cells on the stainless steel plate was enumerated as described in 2.5. Change in the cell concentration of the microbial suspension during the exposure period was also monitored by colony counting on TSA.

2.5 Enumeration of microbial cells adherent to stainless steel surface

The number of microbial cells adherent to a unit area of the stainless steel surface (surface density) was enumerated by swab-vortex method as follows. The entire surface of the stainless steel plate was swabbed twice with a sterile cotton swab. The cotton tip was then cut off and soaked in 1 ml of peptone saline and subjected to vigorous vortex-mixing for 1 min. The swabbing and mixing were expected to disperse microbial cells individually. The cell suspension thus obtained was adequately diluted if necessary and plated on TSA. The number of *S. epidermidis* cells recovered by swabbing (X_1) was determined from colony count on the TSA plate after incubation at 37℃ for 48 h. To confirm the number of *S. epidermidis* cells still remaining on the stainless steel plate after swabbing (X_2), the plate was made in contact with TSA for 1 min. The number of colonies emerged on the TSA after incubation at 37℃ for 48 h was taken as X_2. The surface density of *S. epidermidis* cell was calculated from the sum of X_1 and X_2. In general, X_2 accounted for less than 10% of the sum (X_1+X_2).

2.6 Removability of adhered cells

To study the removability of cells adhered to the stainless steel surface, the artificially contaminated plates obtained as described in 2.4 was rinsed with shear force application using either sterile distilled water or peptone saline solution as a rinsing medium. A 2-L capacity stainless steel vessel (135 mm in diameter, 145 mm in height) containing 1 L of a rinsing medium was placed in a water bath kept at 25℃. The contaminated plate and a 3-blade impeller were placed in the rinsing medium such that the distance between the plate and the center of impeller was 45 mm. After the rinsing medium was stirred at 2,000 rpm for a designated period of time, the number of cells remaining on the plate surface was enumerated as described in 2.5.

2.7 Scanning electron microscopy (SEM)

Stainless steel plates ($10 \times 10 \times 1$ mm) with different degrees of surface roughness ($R_a = 0.04$ and $0.55\,\mu$m) were soaked in 10^4 CFU/ml microbial suspension in peptone saline at 25℃ for 3 h. After rinsing twice with 20 ml of peptone saline to remove loosely adherent cells, the contaminated plates were freeze-dried (JFD-310, JEOL, Tokyo, Japan). The stainless steel plates were then mounted on aluminium stubs, coated with Pt/Pd for 90 s (Ion sputter coater E-1030, Hitachi, Tokyo, Japan), and subjected to observation with a scanning electron microscope (S-4000, Hitachi).

2.8 Data analysis

Every experimental run was replicated in three trials. Values of the surface density of adherent cell in CFU/cm^2 and the cell concentration of microbial suspension in CFU/ml were converted to $\log_{10}$ values for statistical analysis by Student's *t*-test (to examine two samples assuming an equal variance for each) or analysis of variance (ANOVA) followed by Tukey's multiple comparison test (to examine more than two samples). Statistical significance was set at *P*-value less than 0.05.

3. Results

3.1 Adhesion as affected by initial cell concentration of microbial suspension

Adhesion behavior of *S. epidermidis* cells from suspension onto the surface of stainless steel plate was studied at the initial cell concentrations of approximately 10^2, 10^3, and 10^4 CFU/ml to simulate low to medium degrees of microbial contamination. Figure 1 shows adhesion courses of *S. epidermidis* cells suspended in peptone saline at varying initial concentrations onto type A stainless steel surface ($R_a = 0.04\,\mu$m) at 25℃. For each microbial suspension tested, adhesion onto the stainless steel surface occurred at a low level within 0.5 h of exposure to the microbial suspension, while the surface cell density reached a maximum and approximately unchanged after 3 h of exposure. Results also showed that the cell concentration of the microbial suspension remained unchanged within 6 h for each case, indicating that no growth of initially inoculated cells occurred during the experimental run. Thus, it was confirmed that the increase in surface density of *S. epidermidis* cell observed in 3 h of exposure
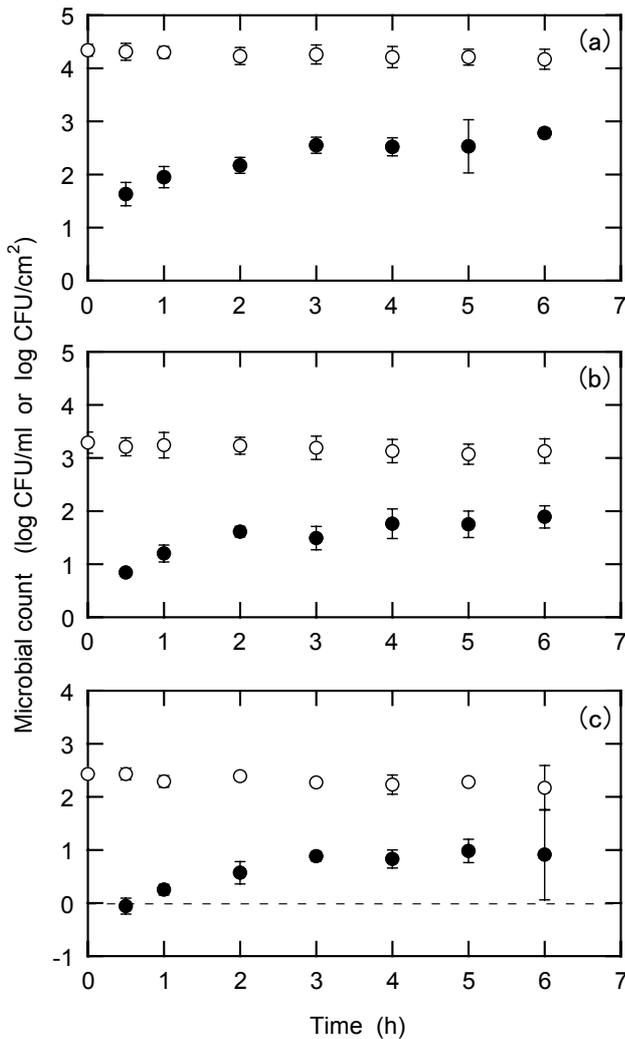
Fig. 1 Viable cell numbers of *S. epidermidis* in suspension (○) (log CFU/ml) and on type A stainless steel plate (●) (log CFU/cm^2) at varying levels of initial cell suspension concentration: (a) 10^4 CFU/ml, (b) 10^3 CFU/ml, and (c) 10^2 CFU/ml. Each value is a mean of three tests in duplicate.

was ascribed to adhesion, but not to growth. For each case of initial cell concentration, the final number of *S. epidermidis* cell adherent to the surface was about 1/10 of the cell number in suspension. Thus, the surface density of cells adherent to the stainless steel surface was approximately proportional to the suspension concentration. Based on these results, the time for the exposure of the test surfaces to microbial suspensions was fixed at 3 h hereafter.

3.2 Adhesion as affected by suspending medium

Figure 2 compares adhesion behaviors of *S. epidermidis* cells suspended in peptone saline, PBS, and physiological saline. The microbial suspensions were made in contact with type A stainless steel plates ($R_a = 0.04\,\mu$m) at 25℃ for 3 h. For all concentrations of the cell suspension tested (10^2–10^4 CFU/ml), significantly higher adhesion was observed for the cells suspended in peptone saline than those suspended in physiological saline. The cells suspended in PBS tended to show medium adhesion, though significant differences from those suspended in others were not detected statistically.

3.3 Removability of adherent cells

Removability of the cells adherent to type A stainless steel surface ($R_a = 0.04\,\mu$m) was studied under shear force application using whirlpool rinsing treatment. The stainless steel surface was preliminary contaminated by soaking in 10^4 CFU/ml microbial suspension in peptone saline at 25℃ for 3 h. Figure 3 shows the surface density of *S. epidermidis* cell remaining on the surface after whirlpool rinsing at 2,000 rpm for 0–15 min either with peptone saline solution (with the same composition as the suspending medium used in adhesion experiment) or with distilled water. After 5 min of the whirlpool treatment, the number of *S. epidermidis* cell remaining on the surface was reduced to 1/5 of that before whirlpool rinsing for both the cases with peptone saline and distilled water. Further treatment up to 15 min did not cause significant reduction in the number of remaining cells. Viability tests for *S. epidermidis* cells suspended in distilled water showed no lethal effect of distilled water on the microbial cells within 15 min (data not shown). Therefore, the decrease in the surface density of *S. epidermidis* cell with whirlpool rinsing was ascribed to removal by the application of shear force, but not to inactivation of the microbial cells. Furthermore, removal of adhered cells using peptone saline was not significantly different from that using water as rinsing medium.

3.4 Adhesion as affected by surface roughness

Figure 4 compares the surface densities of *S. epidermidis* cell adherent to stainless steel surfaces with varying surface roughness ($R_a = 0.04$–$1.37\,\mu$m) before and after whirlpool water rinsing (2,000 rpm, 15 min). Statistical analyses indicated significantly higher adhesion on the stainless steel surface having the lowest degree of polish ($R_a = 1.37\,\mu$m). Accordingly, results showed a significantly higher number of *S. epidermidis* cell still remaining on the same type of (roughest) stainless steel surface ($R_a = 1.37\,\mu$m) after the whirlpool rinsing treatment. For the surfaces of type A plate and type B plate ($R_a \leq 0.14\,\mu$m), minor differences in the surface cell density were
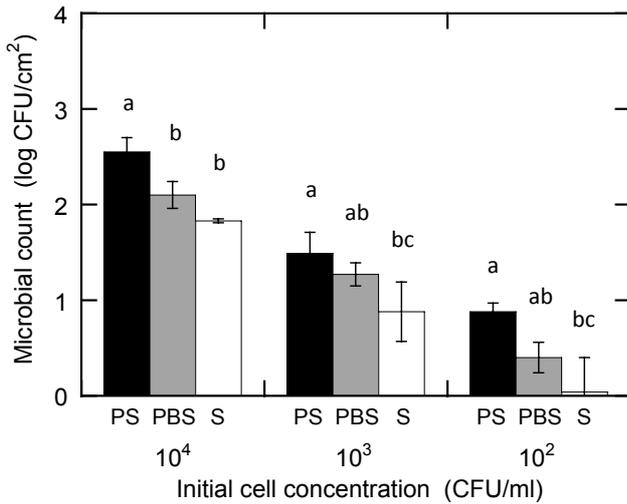
Fig. 2 Comparison of numbers of *S. epidermidis* cells adherent to type A (R_a=0.04 μm) stainless steel surface from cells suspended in different suspending media. Stainless steel plates were exposed for 3 h to cells suspended in peptone saline (PS), phosphate buffered saline (PBS), and physiological saline (S). Each value is a mean of three tests in duplicate.
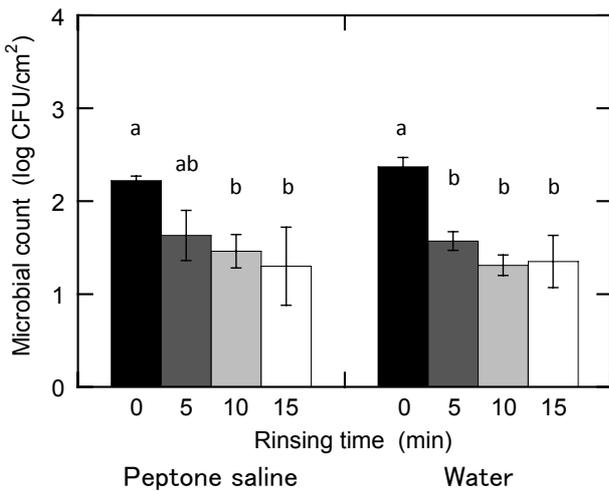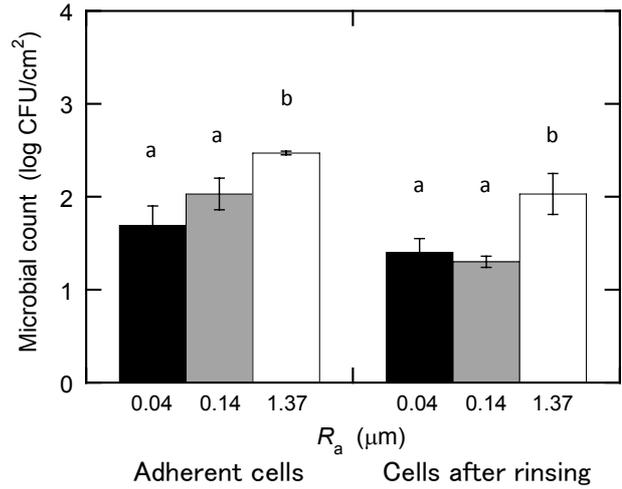
Fig. 4. Comparison of numbers of *S. epidermidis* cells on stainless steel plate of varying surface roughness before and after whirlpool water rinsing (2,000 rpm, 15 min). Each value is a mean of three tests in duplicate.

Fig. 3 Microbial counts on type A stainless steel plate surface (R_a=0.04 μm) after whirlpool rinsing as an index of removability of adhered *S. epidermidis* cells. Artificially contaminated stainless steel plates were rinsed with shear force application (2,000 rpm) using peptone saline or water for 0–15 min. Each value is a mean of three tests in duplicate.

0.55 μm. The images show differences in the topography of stainless steel surface according to the average surface roughness: deeper abrasion lines on surface with the higher R_a value (0.55 μm) than on the smoother surface (R_a=0.04 μm). Although the cells on plates were not enumerated, the SEM images help us to roughly confirm the result of adhesion experiments: higher cell adhesion on the rougher surface. On the rougher surface (R_a= 0.55 μm), *S. epidermidis* cells were observed in deep crevice areas (a1) as well as on relatively smooth areas (a2) of the stainless steel plate surface. Majority of the cells adhered to the surface in more or less grouped fashion. Especially in deep crevices, they were found as large clusters (a1). In relatively smooth area, the cells were found as tiny planar clusters or single cells (a2). On the smoother surface (R_a=0.04 μm), adherent *S. epidermidis* cells were observed mostly as tiny planar clusters or single cells (b). Thus a feature of adhesion of *S. epidermidis* cells to rough stainless steel surfaces was the adhesion in crevices as large clusters.

4. Discussion

Microbial cell attachment to surfaces is generally considered to take place in two stages: a reversible stage followed by an irreversible stage [21]. In the reversible stage, there is a weak interaction between bacteria and substrate, involving van der Waals, electrostatic, and hydrophobic interactions. In this stage, the bacteria can still exhibit Brownian motion and to be easily removed by

observed especially prior to whirlpool water rinsing. However, these differences were found statistically insignificant. In general, about 20–50% of the attached cells still remained on the surfaces after the whirlpool rinsing.

3.5 SEM analysis

Figure 5 shows SEM images of *S. epidermidis* cells on the stainless steel surfaces with R_a values of 0.04 and

mild shear force. The irreversible stage starts as a result of anchoring by appendages and/or production of extracellular polymers. This study focused on the first or so−called reversible stage of microbial attachment.

Related studies reported that microbial adhesion was observed after 5 s to 2 h of exposure to microbial cell suspensions [6, 10, 11, 21, 22]. In those studies, however, the reported exposure times were fixed and the course of adhesion over a period of time was not investigated. Figure 1 showed that the adhesion of *S. epidermidis* cells to stainless steel surface reached a maximum in 3 h of exposure regardless of the cell concentration of suspension. Considering that we determined the counts of

cells adherent to the surface after mild rinsing with peptone saline solution to remove loosely adherent cells, it may take a certain period of time, 3 h in the case of *S. epidermidis*, for the cells to make their attachment firm enough to withstand the mild rinsing. However, further study is needed to clarify the mechanism.

The maximum surface density of adherent *S. epidermidis* cells was found to be approximately proportional to the cell concentration of suspension. Thus *S. epidermidis* cells apparently showed a feature of reversible adhesion. However, it was also found that whirlpool rinsing treatment removed only 50−82% of the adherent cells; the rest remained on the surface (Figs. 3 and 4). The application of
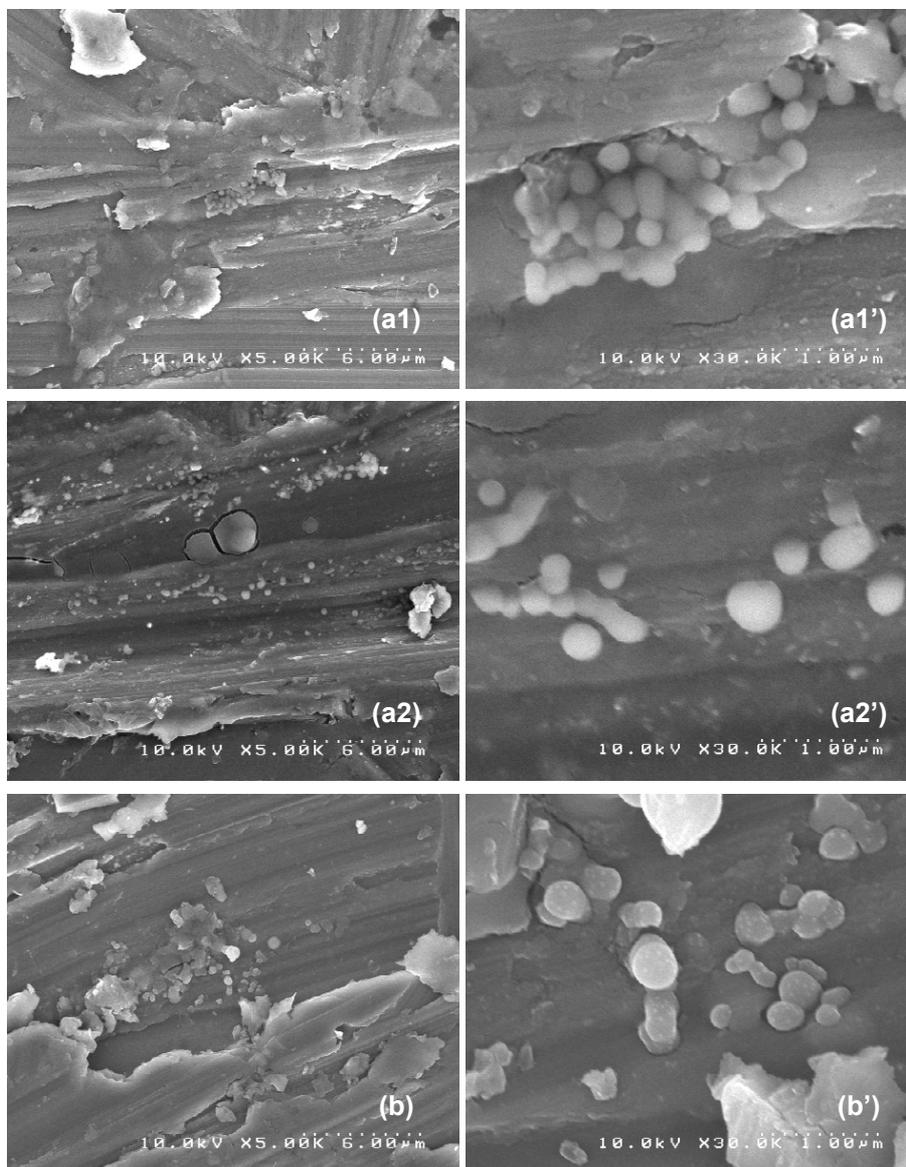

Fig. 5   Scanning electron microscope images of *S. epidemidis* cells adherent to stainless steel plates of (a1 and a2) R_a＝0.55 μm and (b) R_a＝0.04 μm. Stainless steel plates were artificially contaminated with *S. epidermidis* cell suspension (10^4 CFU/ml) for 3 h at 25℃. Images a1', a2' and b' were magnified images of a1, a2, and b, respectively.

high shear stress in the whirlpool rinsing was expected to overcome weak interactions between adherent cells and the surface. Thus some cells can be recognized as reversibly attached, and the other as already irreversibly attached. This suggests that microorganism can irreversibly adhere to stainless steel surfaces even with little possibility of anchorage by appendages or extracellular polymers.

As shown in Fig. 2, *S. epidermidis* cells adherent to the stainless steel surface in a greater surface density when suspended in peptone saline than in physiological saline. This indicates that the presence of organic components in the suspending medium enhanced cell adhesion, or its strength, to the stainless steel surface. However, this study also provides data (Fig. 3) indicating that the use of peptone saline as a whirlpool rinsing medium did not significantly affect detachment of adherent *S. epidermidis* cells compared with water whirlpool rinsing. As described by Bos *et al*. [23], when microorganisms and substrate surface are in aqueous environment containing organic molecules, the surface may be first become covered with a layer of the organic molecules prior to adhesion of microorganisms. The results of this study indicate that adhesion of *S. epidermidis* is greatly influenced by the organic molecules on the surface but scarcely influenced by those in solution, though further study is necessary to clarify the details.

To summarize the previous works on the effects of suspending medium and/or surface pre−conditioning, both enhancing and inhibiting effects on microbial adhesion have been reported. In terms of suspending medium, presence of whey protein was reported to increase microbial adhesion to stainless steel, rubber, and glass surfaces [24]. Similarly, Flint *et al*. [13] found that milk−fouled surfaces attracted 10−100 times more vegetative cells and spores of *Bacillus stearothermophilus* compared to clean stainless steel surface. On the other hand, adhesion of *L. monocytogenes* and *Salmonella typhimurium* suspended in whole milk, skim milk, or diluted milk onto stainless steel and Buna−N chips were found to be less than those suspended in PBS [14]. Barnes *et al*. [25] reported similar observation of reduced adhesion of *Staphylococcus aureus*, *L. monocytogenes*, and *Serratia marcescens* to stainless steel coupons pre−treated with skim milk. Because physico−chemical properties of bacterial cell surfaces may also be factors which influece the adhesion [21], further compilation, as well as systematic analysis, of data for the combination of microbial species and the media components is needed for better understanding of the effect of organic

molecules.

As for the influence of surface roughness, a significantly higher adhesion of *S. epidermidis* cells from their suspension, as well as higher retention of them through the whirlpool rinsing treatment, were observed for the roughest surface of stainless steel ($R_a=1.37\,\mu$m), as shown in Fig. 4. Earlier studies on the effect of surface roughness of stainless steel to microbial adhesion and/or removal have demonstrated opposing observations. A greater number of *S. aureus* was observed to adhere to stainless steel 2B finish (0.412 μm) compared to the No. 8 mirror finish (0.035 μm) [25]. Previous works conducted by Leclercq−Perlat and Lalande [26] and by Wirtanen *et al*. [27] demonstrated a positive correlation between cleanability and increased surface smoothness in the removal of biofilms. In contrast, for *Pseudomonas* sp., *L. monocytogenes*, and *Candida lipolytica*, Hilbert *et al*. [28] found that surface roughness did not significantly affect the attachment to and removal from stainless steel surface in the range of R_a value from 0.01 to 0.9 μm. For stainless steel surfaces having R_a values between 0.015 and 1.04 μm, no clear relationship was established between the roughness parameter and the number of viable *Streptococcus thermophillus* adherent to the surface [29]. Flint *et al*. [15] also showed that the adhesion of thermo−resistant streptococci was almost independent from surface roughness ($R_a=0.5-3.3\,\mu$m). The effect of surface roughness might depend on the microbial species, possibly due to difference in adhesion manner and/or cell surface characteristics.

The higher adhesion and retention of *S. epidermidis* cells on the roughest stainless steel surface shown in this study may be attributed first to a greater surface area available for cell adhesion compared to other plates having smoother surface. However, as shown in Fig. 4, significant differences in adhesion and detachment of cells were observed only for type C plate ($R_a=1.37\,\mu$m). The increase in surface area due to surface roughness may be insignificant when $R_a \leq 0.14\,\mu$m. Another explanation for the enhanced adhesion on the roughest surface may be entrapment of microbial cells in crevices of the surface. Schwach and Zottola [7] suggested that the apparent involvement of adhesion and entrapment of *B. cereus* were apparently responsible for maintaining the intact cells on the stainless steel surface. SEM images shown in Fig. 5 provided information on adhesion manner of *S. epidermidis* cells on the stainless steel surface. To summarize the results shown in Fig. 5, *S. epidermidis* cells adhered to the stainless steel plates as large clusters in deep crevices and

as tiny planar clusters as well as single cells in relatively smooth area. Enhanced adhesion of *S. epidermidis* cells to the surface of type C plate may be ascribed to the presence of deep crevices. Adhesion mainly occurs and concentrates in the crevices probably on such rough surfaces. It was thus confirmed that the surface roughness indeed plays an important role in *S. epidermidis* adhesion. Kusumaningrum *et al.* [30] also observed the presence of microbial cells in clumps on stainless steel surface for *S. enteritidis*, *S. aureus*, and *Campylobacter. jejuni* during their survival on the surface. They found some cells in the crevices of the stainless steel surface. The significantly higher retention of adherent cells observed on the surface of type C plate after whirlpool rinsing (Fig. 4) might also be ascribed to the possible protection provided by the deep crevices.

Stainless steel surfaces having such a high R_a values as type C plate are not typical for use in the food industry. However, even though the surfaces are initially smooth, they may be abraded upon repeated use and abuse. Scratches, abrasions and other surface damage, just like the crevices, could provide the niche for microbial adhesion and retention. Microorganisms concealed in the cracks and crevices of the substrate surface may not be efficiently removed during cleaning and disinfecting treatments and could potentially be a source of cross-contamination of food products during processing [28].

In conclusion, this work revealed that the adhesion of *S. epidermidis* on stainless steel surface was significantly affected by surface roughness (presence of crevices), presence of organic components in the suspending medium, and cell concentration of contaminants. Adhesion of cells on the surface occurred even in short contact periods of time but it increased gradually and reached a maximum within 3 h regardless of the concentration of the cell suspension. Not a small portion of *S. epidermidis* cells were shown to irreversibly adhere to the surface. The results of this study provide better understanding on the factors influencing the adhesion behavior of *S. epidermidis* on stainless steel surface, as well as the strength of cell attachment.

5.　Acknowledgement

6.　References

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◇◇◇◇ 和文要約 ◇◇◇◇

Staphylococcus epidermidis のステンレス鋼表面に対する付着に及ぼす諸因子の影響

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　食品製造機器表面に対する微生物の付着は，一般に除去および殺菌が困難とされるバイオフィルム形成の初期段階ともなるため，食品製造現場の衛生を保つ上で極めて重要な研究課題である．しかし，これまでの研究報告では対立する結果が報告されている例も少なくなく，微生物の機器表面への付着に対する各種因子の影響を統一的に解釈できる状況には至っていない．菌種による表面特性や付着様式の差異が影響している可能性もあり，より多くの菌種に対する検討結果の蓄積が望まれる．本研究では，食品製造現場で検出されることが少なくない表皮ブドウ球菌 *Staphylococcus epidermidis* を対象としてとりあげ，そのステンレス鋼表面に対する付着挙動について検討した．

　付着実験は，表皮ブドウ球菌の懸濁液にステンレス鋼板を浸漬する形で行い，拭き取り法とコンタクトプレート法を合わせた方法によりステンレス鋼板上に付着した菌数を計数した．なお，表皮ブドウ球菌の付着に影響を及ぼす因子として，菌懸濁液の初期菌濃度，懸濁媒体の成分，ステンレス鋼表面の粗さに着目した．

　初期菌濃度 10^2–10^4 CFU/ml の範囲では，付着実験開始 30 分後には低レベルながら菌の吸着が見られた．付着菌数はその後徐々に増加し，3 時間後までには最大値となり，それ以降は有意な付着菌数の変化は見られなかった．この間，懸濁液中の菌濃度は変化することなく，したがって付着菌数の増加は増殖によるものではないことが示された．また，ステンレス鋼表面に付着した菌の表面密度が懸濁液中の菌濃度にほぼ比例していた．懸濁媒体の影響については，有機物の存在が付着菌の表面密度を増加させることが示された．さらに，表面粗さの大きなステンレス鋼表面（R_a=1.37 μm）に付着する菌数は，滑らかな表面（R_a≤0.14 μm）に比べて有意に多いことが明らかになった．なお，このように付着した菌に対して撹拌槽を用いた脱離実験を行ったところ，撹拌によるせん断力を加えても，脱離した菌の割合は 50–82％ に留まり，少なくとも付着菌の一部は不可逆的に付着していることが示された．さらに，菌の付着状況を走査電子顕微鏡で観察したところ，とくに表面粗さの大きなステンレス鋼表面では，比較的深い研磨痕（クレバス）に表皮ブドウ球菌がクラスタを形成して付着している割合の高いことが判明し，研磨痕や損傷による表面の凹凸の深さが表皮ブドウ球菌の付着に大きな寄与をすることが示唆された．