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Preacclimation alters *Salmonella* Enteritidis surface properties and its initial attachment to food contact surfaces

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ABSTRACT

Exposure of *Salmonella* to environmental stress, prior to its adherence to a food contact surface, may change the cell surface properties and consequently affect its initial attachment and biofilm formation. This study investigated the influence of temperature and pH preacclimation on the initial attachment of *Salmonella* Enteritidis to acrylic and stainless steel. Besides, changes in physicochemical properties of cells were examined; and their surface attachment was modeled by xDLVO theory. Results showed that control cells pre-grown at 37 °C had significantly ($P < 0.05$) higher initial attachment, followed by those pre-grown at 25, 42, and 10 °C. The initial attachment of cells pre-grown at pH 5.3 or 6.3 was not significantly ($P > 0.05$) different from control cells pre-grown at pH 7.3, but they were significantly higher compared to cells pre-grown at pH 8.3 and 9.0. No significant difference was observed between cell attachment to acrylic and stainless steel, although they had different physicochemical properties. The xDLVO theory successfully explained higher attachment for cells pre-grown at optimal condition on both contact surfaces. However, the xDLVO theory could not explain the similar attachment of cells to acrylic and stainless steel. This study elucidates that commonly used intervention technologies including cold storage, thermal treatment, and alkaline antimicrobial agents might alter the physicochemical properties of *S.* Enteritidis cells and result in varied initial attachment levels.

1. Introduction

Salmonella spp., which leads to more than 1.2 million illnesses, with approximately 23,000 hospitalizations and 450 deaths every year, is the major cause of foodborne disease in the United States [1]. Among more than 2500 identified *Salmonella* serotypes, *Salmonella enterica* serotype Enteritidis has been reported as the most commonly isolated serotype, responsible for 18% of laboratory-confirmed *Salmonella* infections in 2012 [2].

Salmonella spp. has been found to exist predominantly as biofilm in nature instead of free-living planktonic form [3]. Biofilm is an aggregation of bacteria enclosed within a self-produced matrix of extracellular polymeric substances (EPS) [4]. The biofilm forming ability of bacteria has gained much attention over the last decade because cells within a biofilm can better withstand the chemical agents and environmental stresses than their planktonic counterparts [5,6], resulting in greater difficulty in inactivating them during cleaning and food processing. The persistence of *Salmonella* spp. in food processing environments might lead to cross contamination of food products, causing economic losses due to foodborne illness and food recall [7].

The biofilm forming ability of *Salmonella* on various surfaces, such as glass, stainless steel, polycarbonate, concrete, tile, acrylic and polystyrene, has been well documented [8–12]. However, most of these studies have focused on the impacts of environmental conditions on the later stage of *Salmonella* biofilm formation (at least after incubation for 24 h) and relatively little attention has been paid to its initial attachment which is one of the critical steps in the biofilm development. To our knowledge, bacterial attachment to surfaces has not been fully understood and it is thought to be mediated by the properties of both bacterial cells and contact surfaces, such as hydrophobicity, surface charge, surface roughness, the presence of fimbriae, flagella, and extracellular polymeric substances [13].

xDLVO (extended Derjaguin-Landau-Verwey-Overbeek) theory assumes that particle adhesion is driven by the sum of Lifshitz-van der Waals (LW), Lewis acid–base (AB), and electrostatic double layer (EL) interaction energies as a function of the separation

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distance between a particle and a hard surface. Several studies have used this theory to explain bacterial attachment to contact surfaces due to the similarity between the dimension of a bacterial cell and a colloidal particle. For example, some investigators have found that bacterial attachment could be predicted by the xDLVO model [14,15], while others have reported that xDLVO model could not explain bacterial attachment in all cases [16,17].

Prior growth conditions, such as pH, temperature, nutrient, etc., have been shown to affect the physicochemical characteristics of *Listeria monocytogenes* and its initial adhesion to food contact surfaces [18,19]. However, there is a lack of information regarding the adhesion of *Salmonella* spp. pre-grown under different environmental conditions to food contact surfaces. A better understanding of how environmental pre-growth conditions affect initial adhesion of bacterial pathogens to the food contact surfaces will help to design better strategies to prevent bacterial attachment and thus inhibit biofilm formation. Therefore, the objective of the present study was to investigate the effect of previous growth temperature and pH on the initial adhesion of *S.* Enteritidis to two food contact surfaces (acrylic and stainless steel 304). In addition, surface properties of *S.* Enteritidis grown under different growth conditions were determined and their roles in bacterial initial attachment to contact surfaces were predicted by xDLVO theory.

2. Materials and methods

2.1. Bacterial strain and culture conditions

Salmonella Enteritidis (ATCC13076) was purchased from the American Type Culture Collection (Manassas, VA, USA) and preserved on porous beads in a cryoinstant vial (DeltaLab, Barcelona, Spain) at −80 °C. Frozen culture was activated by transferring one bead into a test tube containing 10 mL of sterile tryptic soy broth (TSB) (Oxoid, Basingstoke, UK), followed by incubating the culture at 37 °C for 18 h. After two consecutive transfers at 37 °C for 18 h, cells were used as inoculum for further experiments.

2.2. Initial attachment

The effect of previous growth temperature and pH on bacterial initial attachment was investigated by inoculating *S.* Enteritidis into TSB (pH 7.3) and incubating at different temperature conditions (10, 25, 37, and 42 °C) or TSB with different pH conditions (pH 5.3, 6.3, 7.3, 8.3, and 9.0) adjusted by lactic acid and trisodium phosphate followed by incubation at 37 °C. Cells grown into stationary phase were harvested (3500 × *g*, 4 °C for 10 min) and washed twice with 0.15 mol/L NaCl (Goodrich Chemical Enterprise, Singapore). The stationary phase was reached after 192, 25, 18, and 18 h for cells grown at 10, 25, 37, and 42 °C (pH 7.3), respectively; while it took 24, 18, 18, 18, and 24 h for cells grown at pH 5.3, 6.3, 7.3, 8.3, and 9.0 (37 °C), respectively. The final number of cells reached stationary phase was around 10^9 CFU/mL. Working cell suspensions were prepared by re-suspending the washed cells in TSB to achieve an initial concentration of 10^7 CFU/mL. Cells grown at 37 °C, pH 7.3 were used as control.

Acrylic and stainless steel (grade 304) coupons were used as the models of food contact surfaces. Prior to use, the coupons (2.5 cm × 1 cm × 0.2 cm) were soaked in a detergent solution (Teepol Multipurpose Detergent, Supply Trade Ltd., Kent, UK) and sonicated for 30 min (57H, Ney Dental International, CT, USA). Thereafter, the coupons were immersed in 70% ethanol and sonicated for another 15 min. After each sonication step, the coupons were rinsed with tap water and deionized water to remove any remaining residues. All coupons were air-dried and autoclaved at 121 °C for 15 min. Each sterile coupon was transferred to a test tube containing 10 mL of cell suspension (10^7 CFU/mL) and incubated at 25 °C under static condition. The coupons were removed at appropriate time intervals and the number of cells attached to the coupons was counted as described below.

2.3. Enumeration of attached cells

Each coupon bearing attached cells was carefully transferred from the test tube to a sterile petri dish. The coupon was gently tapped against the side of the petri dish using sterile forceps to remove excess liquid droplets and washed three times with 10 mL of phosphate buffered saline (PBS) (Vivantis Inc., CA, USA) to remove any loosely attached cells. The coupon was then transferred to a sterile test tube containing 9 mL of 0.1% peptone water (w/v) (Oxoid). Detachment of cells from the coupons was performed by sonication (48 kHz) for 3 min. After sonication, the cell suspension was diluted with 0.1% peptone water (w/v) and spiral plated on tryptic soy agar (TSA) (Oxoid) plates. Enumeration of cells was conducted by an automated colony counter (aCOLyte, Synbiosis, Frederick, MD, USA) after incubation of TSA plates at 37 °C for 24 h.

2.4. Evaluation of biofilms with a fluorescence microscope

To evaluate the biofilm formation, coupons were stained with the LIVE/DEAD® *Bac*Light Bacterial Viability Kit (Molecular Probes Eugene, OR, USA). Briefly, 3 μL of SYTO®9 stain and 3 μL of propidium iodide (PI) stain were added to 1 mL of sterilized deionized water to prepare the working solution. Each biofilm sample was stained with 200 μL of working solution and incubated in dark for 30 min. After incubation, samples were gently washed with sterilized deionized water and air-dried. The biofilms were observed under 40× or 100× objective lens using a Olympus BX51 fluorescence microscope (Olympus corporation, Tokyo, Japan) equipped with an Olympus DP71 camera, an U-RFL-T mercury lamp, and appropriate filter cubes for SYTO®9 (WB, dichroic mirror DM500, excitation filter BP450-480, barrier filter BA515) and PI (WG, dichroic mirror DM570, excitation filter BP510-550, barrier filter BA590).

2.5. Zeta potential measurement of bacteria and coupons

Zeta potential measurement of *S.* Enteritidis and the coupons was performed as previously described with slight modification [16]. Briefly, bacterial cells in the stationary phase were harvested, washed twice with 0.15 mol/L NaCl and re-suspended in TSB to obtain a cell density of around 10^8 CFU/mL. Zeta potential of the bacterial cells was measured at pH 7.0 using a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK). Zeta potential of acrylic or stainless steel surface was measured using a Surpass Electrokinetic Analyzer (Anton Paar GmbH, Graz, Austria) at pH 7.0 with 0.001 mmol/L KCl (Goodrich Chemical Enterprise) as buffer solution.

2.6. Contact angle measurements of bacteria and coupons

Contact angle measurements were carried out based on sessile drop method using a goniometer (VCA optima, AST Products Inc., Billerica, MA, USA) at room temperature. The three liquids used for contact angle measurements were water, ethylene glycol (Sigma–Aldrich, St Louis, MO, USA) and hexadecane (Alfa Aesar, Ward Hill, MA, USA). For bacterial cells, cells prepared as described above were adjusted to a concentration of 10^9 CFU/mL. Bacterial lawns were prepared by filtering 25 mL of cell suspension onto a mixed cellulose membrane filter with 0.22 μm pore size using negative pressure. The membrane was attached to a glass slide using double-sided adhesive tape and air dried for 1 h. One microliter of

each liquid was deposited on the membrane. For the coupons, the measurement was performed by directly placing 1 μL of each liquid onto the surface. The droplet image was automatically captured by a high-resolution video camera and the contact angle was calculated by AutoFAST contact angle calculation software (AST Products Inc.).

2.7. Calculation of surface tension components and hydrophobicity

The surface tension components of a solid surface were calculated using Young's equation [20]:

$$(1+\cos\theta)\gamma_L = 2\left(\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^{+}\gamma_L^{-}} + \sqrt{\gamma_S^{-}\gamma_L^{+}}\right) \quad (1)$$

where θ is the contact angle, $\gamma_L$is the surface energy of the liquid, γ^{LW}, γ^{+}, and γ^{-} are the Lifshitz-van der Waals, electron-acceptor and electron-donor components of the surface tension for solid (S) and liquid (L). The surface tension parameters (γ_L, γ_L^{LW}, γ_L^{+}, γ_L^{-}) of the three liquids used were obtained from literature [21].

The hydrophobicity was calculated from the surface tension component values using the equation below [20]:

$$\Delta G_{iwi} = -2\left(\sqrt{\gamma_S^{LW}} - \sqrt{\gamma_L^{LW}}\right)^2 - 4\left(\sqrt{\gamma_S^{+}\gamma_S^{-}} + \sqrt{\gamma_L^{+}\gamma_L^{-}} - \sqrt{\gamma_S^{+}\gamma_L^{-}} - \sqrt{\gamma_S^{-}\gamma_L^{+}}\right) \quad (2)$$

If $\Delta G_{iwi} < 0$, the surface is considered hydrophobic; if $\Delta G_{iwi} > 0$, the surface is considered hydrophilic. The surface tension components and hydrophobicity for bacteria was determined using Eqs. (1) and (2) by replacing γ_S^{LW}, γ_S^{+}, and γ_S^{-} with γ_B^{LW}, γ_B^{+}, and γ_B^{-}.

2.8. Calculation of free interaction energies based on xDLVO theories

The xDLVO free energy of interaction was ascertained as the sum total of Lifshitz-van der Waals (LW), Lewis acid–base (AB), and electrostatic double layer (EL) free energy of interaction [22]:

$$\Delta G^{xDLVO} = \Delta G_{LW} + \Delta G^{AB} + \Delta G^{EL} \quad (3)$$

The Lifshitz-van der Waals (LW) interaction was calculated with the following equation [14]:

$$\Delta G^{LW} = -24\pi l_0^2 \gamma \left(\sqrt{\gamma_B^{LW}} - \sqrt{\gamma_L^{LW}}\right)\left(\sqrt{\gamma_S^{LW}} - \sqrt{\gamma_L^{LW}}\right)/6l \quad (4)$$

where l is the separation distance, l_0 is the minimum separation distance (0.157 nm), γ is the radius of the cell (2.07 μm) that was measured with Zetasizer Nano ZS using dynamic light scattering.

The Lewis acid–base interaction was determined with the equations below [14]:

$$\Delta G^{AB} = 2\pi\gamma\lambda\Delta G_{slb}^{AB}\exp\left(\frac{l_0 - l}{\lambda}\right) \quad (5)$$

$$\Delta G_{slb}^{AB} = 2\left[\sqrt{\gamma_L^{+}}\left(\sqrt{\gamma_B^{-}} + \sqrt{\gamma_S^{-}} - \sqrt{\gamma_L^{-}}\right) + \sqrt{\gamma_L^{-}}\left(\sqrt{\gamma_B^{+}} + \sqrt{\gamma_S^{+}} - \sqrt{\gamma_L^{+}}\right) - \sqrt{\gamma_B^{-}\gamma_S^{+}} - \sqrt{\gamma_B^{+}\gamma_S^{-}}\right] \quad (6)$$

where λ is the characteristic decay length of AB interaction in water (0.6 nm for hydrophilic bacteria).

The electrostatic double layer interaction was calculated as follows [14]:

$$\Delta G^{EL} = \pi\varepsilon\gamma\{(\varphi_B + \varphi_S)^2 \ln[1 + \exp(-kl)] + (\varphi_B - \varphi_S)^2 \ln[1 - \exp(-kl)]\} \quad (7)$$

where ε (7.08×10^{-10} F/m) is the electrical permittivity of the medium, $\varphi_B\varphi_S$ are the zeta potential of the bacteria and the coupon, k (9.09×10^{8} m^{-1}) is the reciprocal Debye length.

2.9. Statistical analysis

Initial attachment and zeta potential were determined from independent triplicate experiments with technical duplicate. Contact angle measurements were performed on three independent bacterial lawns or coupons with at least five droplets. Statistical analysis was carried out by one-way analysis of variance (ANOVA) and means were compared by Duncan's multiple range test using SPSS software (Statistical Package for the Social Sciences, version 18.0, IBM, NY, USA). P value below 0.05 was considered as significant.

3. Results

3.1. Effect of pre-growth temperature and pH on initial attachment

The effect of pre-growth temperature on the attachment of *S.* Enteritidis to acrylic and stainless steel is shown in Fig. 1. The results indicated that *S.* Enteritidis attached to the tested surfaces in a very short time. After 0.17 h of incubation, the population of attached cells ranged from 3.52 to 4.97 log CFU/cm^2 and 3.27 to 5.17 log CFU/cm^2 on acrylic and stainless steel, respectively, depending on their previous growth temperature. During the first hour, increasing the growth temperatures from 10 to 37 °C significantly ($P < 0.05$) enhanced the initial attachment, whereas increasing the growth temperature further to 42 °C resulted in significantly lower level of attachment compared to the cells grown at 25 or 37 °C. Compared to cells grown at 10 °C, the attachment of cells grown at 42 °C to acrylic or stainless steel was approximately 0.5 or 1.0 log CFU/cm^2 higher in the first 0.33 h, respectively. However, similar cell population ($P > 0.05$) was found on both tested surfaces from 0.5 h onward.

The effect of pre-growth pH on attachment of *S.* Enteritidis to acrylic and stainless steel is shown in Fig. 2. During the first 6 h, cells grown in acidic (pH 5.3 and 6.3) and neutral conditions (pH 7.3) exhibited greater attachment than cells grown in alkaline conditions (pH 8.3 and 9.0). For example, the population of attached cells at 0.17 h on acrylic was 5.21, 5.25, 4.97, 4.54, and 4.18 log CFU/cm^2 for cells grown at pH 5.3, 6.3, 7.3, 8.3 and 9.0, respectively. There was no significant difference ($P > 0.05$) in the level of attachment between control cells and cells previously cultivated at acidic pHs. However, the attachment of cells pre-grown at pH 8.3 was significantly higher compared to those pre-grown at pH 9.0 in the first 0.17 h of incubation.

Regardless of the previous growth temperature and pH conditions, a small increase in the number of attached cells was seen as incubation time increased and the maximum population of attached cells was achieved at 6 h of incubation. However, a slight decrease in the population of attached cells occurred at 12 h and the population remained the same until 24 h. To confirm that the lower attachment was not due to the incomplete removal of biofilm at a later stage using the sonication method, biofilms formed by control cells at 6 h or 24 h before and after sonication were stained with SYTO 9 and PI dyes (Fig. 3). The results showed that higher population density was observed at 6 h compared to 24 h.

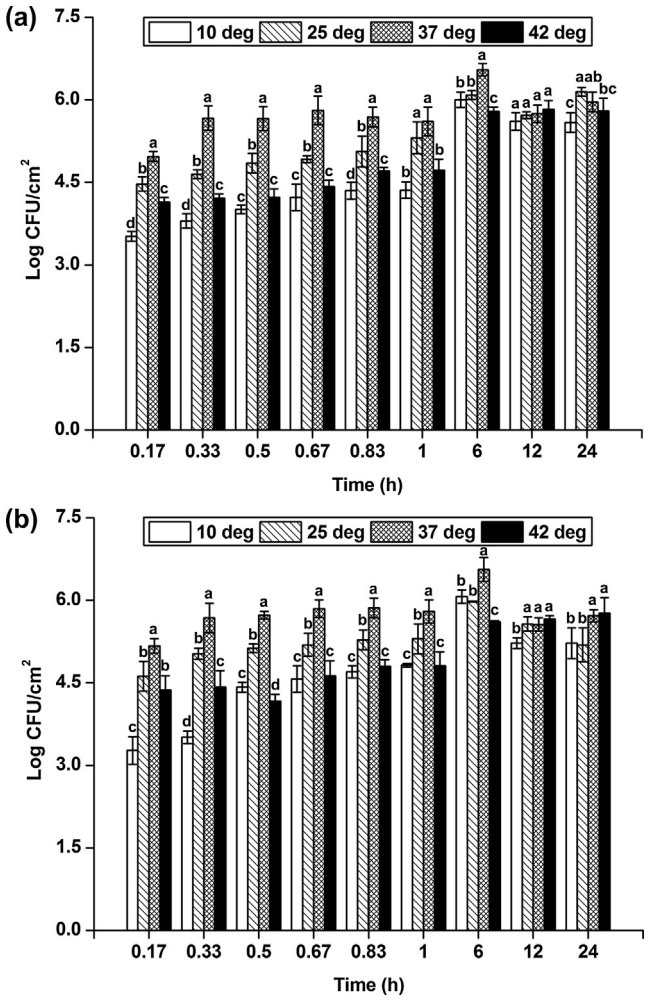

Fig. 1. Attachment of *Salmonella* Enteritidis previously grown under various temperatures (10, 25, 37 and 42 °C) to (a) acrylic and (b) stainless steel immersed in TSB (pH 7.3) at 25 °C. Values followed by different letters indicate that means are significantly different ($P < 0.05$).

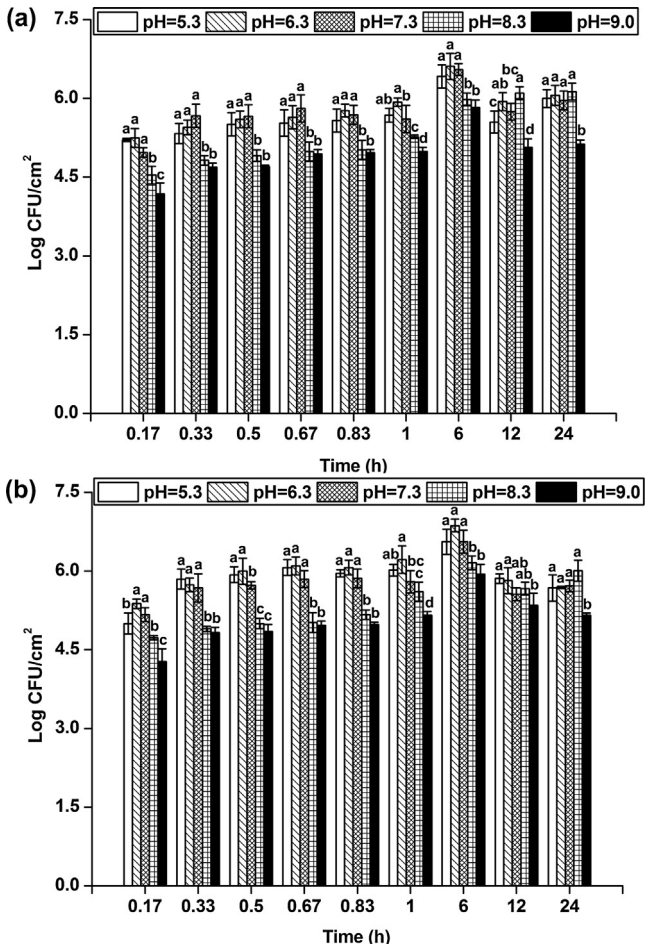

Fig. 2. Attachment of *Salmonella* Enteritidis previously grown under various pHs (5.3, 6.3, 7.3, 8.3 and 9.0) to (a) acrylic and (b) stainless steel immersed in TSB (pH 7.3) at 25 °C.

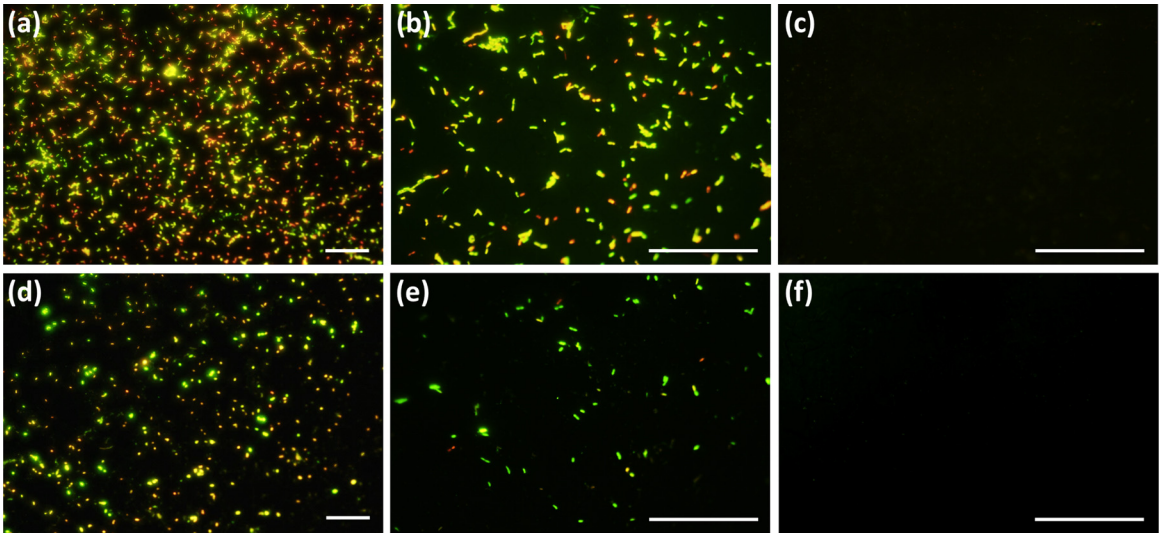

Fig. 3. Fluorescence microscopy images of *Salmonella* Enteritidis biofilms growing on stainless steel surface stained by SYTO 9 and PI dyes. (a and b) biofilms formed in TSB for 6 h at different magnifications before and (c) after sonication, and (d and e) 24 h before and (f) after sonication. Scale bars represent 50 μm.

In addition, all biofilm cells were removed from the contact surface after 3 min of sonication. The complete removal of biofilm cells from acrylic surface was also observed (data not shown). No significant difference between the number of cells attached to acrylic and that attached to stainless steel was observed under all test conditions.

3.2. Zeta potential of bacteria and coupons

The surface charges of bacterial cells under different growth conditions are presented in Table 1. The results showed that *S.* Enteritidis cells had a net negative charge on their cell surface for all conditions examined. However, the magnitude of the charge was significantly ($P < 0.05$) affected by its growth condition. For the temperature effect, the most negative surface charge was found for the cells grown at 10 °C, with a potential of −6.27 mV; while the least negative one was obtained for the control cells grown at 37 °C, with a potential of −3.77 mV. For the pH effect, the highest and the lowest negatively charged cells were those cultivated at alkaline pHs, with a potential of −4.59 and −2.94 mV for cells grown at pH 8.3 and 9.0, respectively. However, no significant difference ($P > 0.05$) in the surface charge was observed for control cells grown at pH 7.3 and cells grown in acidic conditions. The surface charges of the contact surfaces are shown in Table 2. Both surfaces were negatively charged, and acrylic was more negatively charged compared to stainless steel.

3.3. Surface tension components and hydrophobicity

The contact angle values of the bacterial cell lawns as well as the calculated surface tension components and hydrophobicity based on contact angles are shown in Table 1. Regardless of the growth conditions, all cells had higher γ_B^- values compared to γ_B^+ values, indicating that all cell surfaces had higher electron donating than electron accepting properties. In addition, the ΔG_{iwi} values of all cells were above zero and thus the cells were considered as hydrophilic under all tested conditions. Cells grown at 10 °C had the highest electron donating property (78.63 mJ/m^2) and the most hydrophilic surface (77.05 mJ/m^2) among the cells grown at tested temperatures, while control cells grown at 37 °C had the lowest electron donating property (55.10 mJ/m^2) and the least hydrophilic surface (46.85 mJ/m^2). Growth in acidic conditions (pH 5.3 and 6.3) did not significantly ($P > 0.05$) modify *S.* Enteritidis surface physicochemical characteristics, but cells grown in alkaline conditions (pH 8.3 and 9.0) were more hydrophilic and more likely to donate electrons, compared to control cells grown in neutral condition (pH 7.3).

The contact angles, surface tension components, and hydrophobicity of acrylic and stainless steel are presented in Table 2. Similar to the bacteria, both acrylic and stainless steel had surfaces that were predominantly electron donors (high values of γ_S^-). However, both materials were hydrophobic due to the negative values of ΔG_{iwi}. Comparing the hydrophobicity of the materials, stainless steel was found more hydrophobic than acrylic, with ΔG_{iwi} value of −44.45 mJ/m^2 and −31.18 mJ/m^2, respectively.

3.4. Adhesion prediction based on xDLVO theory

Components of total interaction energy for *S.* Enteritidis previously grown at pH 7.3, 37 °C on acrylic and stainless steel are presented in Fig. 4. It was found that ΔG^{LW} and ΔG^{AB} were always negative, implying attractive LW and AB interactions between cells grown at pH 7.3, 37 °C and acrylic (Fig. 4a). However, at a relatively long separation distance, repulsive EL interaction was observed and it reached a maximum value of about 60 kT at 1.6 nm. As the separation distance became shorter, the negative influence of EL interaction diminished, and became attractive when the separation distance was smaller than 1 nm. Similar to acrylic, LW interaction between the cells grown at pH 7.3, 37 °C and stainless steel was always attractive while EL interaction was attractive at a shorter separation distance (<0.5 nm) but repulsive at a long separation distance (Fig. 4b). In contrast, at separation distance less than 5 nm, AB interaction with stainless steel was repulsive with a maximum value of 5980 kT at the minimum separation distance.

The profiles for LW, EL, and AB interaction energy between acrylic or stainless steel and *S.* Enteritidis grown at other conditions as a function of separation distance were very similar to cells grown at pH 7.3, 37 °C. Nevertheless, ΔG^{AB} were positive between acrylic and the cells grown at 10, 25, and 42 °C, as well as, for the cells

Table 1
Contact angles, surface tension components, hydrophobicity, and zeta potential measurements of *Salmonella* Enteritidis grown under various conditions.

Growth condition[a]		Contact angle (°)[b]			Surface tension component (mJ/m^2)			ΔG_{iwi} (mJ/m^2)	Zeta potential (mV)
		θ_w	θ_E	θ_H	γ_B^{LW}	γ_B^+	γ_B^-		
Temperature (°C)	10	31.8 ± 1.0^c	53.4 ± 0.8^a	32.9 ± 2.6^a	23.33 ± 0.64^b	0.00 ± 0.00^b	78.63 ± 1.93^d	77.05 ± 2.22^d	−6.27 ± 0.43^d
	25	35.7 ± 1.2^b	51.4 ± 0.8^a	20.6 ± 2.8^{bc}	25.84 ± 0.44^a	0.00 ± 0.00^b	69.79 ± 0.82^c	66.39 ± 0.91^c	−5.55 ± 0.27^c
	37	45.5 ± 0.9^a	52.6 ± 2.1^a	19.2 ± 1.1^c	26.08 ± 0.16^a	0.02 ± 0.03^b	55.10 ± 1.52^a	46.85 ± 2.94^a	−3.77 ± 0.27^a
	42	37.0 ± 2.1^b	47.9 ± 1.4^b	23.5 ± 1.4^b	25.35 ± 0.26^a	0.08 ± 0.05^a	64.71 ± 3.88^b	57.01 ± 5.59^b	−4.43 ± 0.12^b
pH	5.3	43.9 ± 2.9^{ab}	49.2 ± 3.6^{ab}	24.6 ± 2.7^a	25.14 ± 0.52^c	0.13 ± 0.09^{ab}	55.03 ± 2.47^b	44.29 ± 2.90^b	−3.89 ± 0.23^b
	6.3	40.6 ± 2.3^{bc}	44.5 ± 1.1^c	14.3 ± 1.4^c	26.75 ± 0.16^a	0.18 ± 0.05^a	55.91 ± 3.10^b	44.42 ± 4.23^b	−3.87 ± 0.05^b
	7.3	45.5 ± 0.9^a	52.6 ± 2.1^a	19.2 ± 1.1^b	26.08 ± 0.16^b	0.02 ± 0.03^c	55.10 ± 1.52^b	46.85 ± 2.94^b	−3.77 ± 0.27^b
	8.3	39.4 ± 0.9^c	48.2 ± 2.2^{bc}	14.5 ± 0.7^c	26.72 ± 0.08^a	0.05 ± 0.05^{bc}	60.95 ± 1.85^a	53.61 ± 3.57^a	−4.59 ± 0.38^c
	9.0	37.5 ± 2.5^c	47.3 ± 1.7^{bc}	20.6 ± 1.8^b	25.85 ± 0.29^b	0.07 ± 0.03^{bc}	63.28 ± 2.93^a	55.22 ± 3.74^a	−2.94 ± 0.24^a

[a] Different letters within a column (a–d) indicate that means are significantly different among different temperatures or pHs ($P < 0.05$).
[b] θ_w, θ_E, θ_H represent contact angles measured by water, ethylene glycol, and hexadecane.

Table 2
Contact angles, surface tension components, hydrophobicity, and zeta potential measurements of acrylic and stainless steel.

Material[a]	Contact angle (°)[b]			Surface tension component (mJ/m^2)			ΔG_{iwi} (mJ/m^2)	Zeta potential (mV)
	θ_w	θ_E	θ_H	γ_S^{LW}	γ_S^+	γ_S^-		
Acrylic	75.4 ± 1.5^b	46.1 ± 1.4^b	19.1 ± 1.9^b	26.09 ± 0.30^a	1.73 ± 0.38^a	8.96 ± 1.70^a	−31.18 ± 3.10^a	−32.21 ± 0.20^b
Stainless steel	88.1 ± 1.6^a	73.6 ± 1.2^a	29.7 ± 1.3^a	24.10 ± 0.29^b	0.00 ± 0.01^b	8.09 ± 0.93^a	−44.45 ± 3.68^b	−16.51 ± 0.20^a

[a] Different letters within a column (a, b) indicate that means are significantly different ($P < 0.05$).
[b] θ_w, θ_E, θ_H represent contact angles measured by water, ethylene glycol, and hexadecane.

Table 3
Interaction energies (10^3 kT) between *Salmonella* Enteritidis and contact surfaces according to the xDLVO theory at the minimum separation distance (0.157 nm).

Growth condition		Acrylic				Stainless steel			
		ΔG^{LW}	ΔG^{EL}	ΔG^{AB}	ΔG^{xDLVO}	ΔG^{LW}	ΔG^{EL}	ΔG^{AB}	ΔG^{xDLVO}
Temperature (°C)	10	−0.06	−0.48	15.88	15.34	−0.03	0.13	32.24	32.33
	25	−0.16	−0.60	8.84	8.07	−0.09	0.07	22.69	22.67
	37	−0.17	−0.92	−3.29	−4.38	−0.09	−0.08	5.98	5.81
	42	−0.14	−0.80	6.45	5.51	−0.08	−0.02	18.88	18.78
pH	5.3	−0.13	−0.89	−1.63	−2.66	−0.07	−0.07	7.73	7.60
	6.3	−0.20	−0.90	−0.24	−1.33	−0.10	−0.07	9.47	9.29
	7.3	−0.17	−0.92	−3.29	−4.38	−0.09	−0.08	5.98	5.81
	8.3	−0.20	−0.77	2.58	1.61	−0.10	−0.01	13.78	13.66
	9.0	−0.16	−1.06	5.24	4.02	−0.09	−0.15	17.22	16.99

ΔG^{LW}, ΔG^{EL}, ΔG^{AB}, ΔG^{xDLVO} represent Lifshitz-van der Waals, electrostatic double layer, Lewis acid–base, and total xDLVO interaction energies.

grown at pH 8.3 and 9.0, which was similar to the AB interactions between stainless steel and the cells grown at pH 7.3, 37 °C. The interaction energy of each component and total interaction energies between *Salmonella* cells and acrylic or stainless steel at the minimum separation distance (0.157 nm) are shown in Table 3.

The xDLVO interaction energy profiles, which were the summation of ΔG^{LW}, ΔG^{AB}, and ΔG^{EL}, between *S.* Enteritidis grown at different conditions, and acrylic or stainless steel are shown in Table 3 and Fig. 5. xDLVO theory predicted repulsive force between acrylic and cells grown at most of the tested conditions, as indicated by the positive values of ΔG^{xDLVO}. Only the control cells grown at 37 °C, pH 7.3 and cells grown at pH 5.3 and 6.3 were favorable for attachment to acrylic. For stainless steel, positive ΔG^{xDLVO} values were found for all tested conditions, suggesting repulsive interaction between the bacteria and stainless steel irrespective of the treatment applied. Compared to stainless steel, acrylic was predicted to be a more favorable attachment surface for *S.* Enteritidis due to the lower ΔG^{xDLVO} values (Table 3). For example, ΔG^{xDLVO} value between the control cells grown at 37 °C and acrylic was −4380 kT, while it was 5810 kT between the cells and stainless steel. In addition, the attachment of control cells grown at 37 °C to acrylic or stainless steel was predicted to be higher than cells grown at 42 °C, followed by those grown at 25 and 10 °C. For different pHs, control cells grown at pH 7.3 was predicted to attach to acrylic or stainless steel more than cells grown at pH 5.3, followed by those grown at pH 6.3, pH 8.3, and pH 9.0.

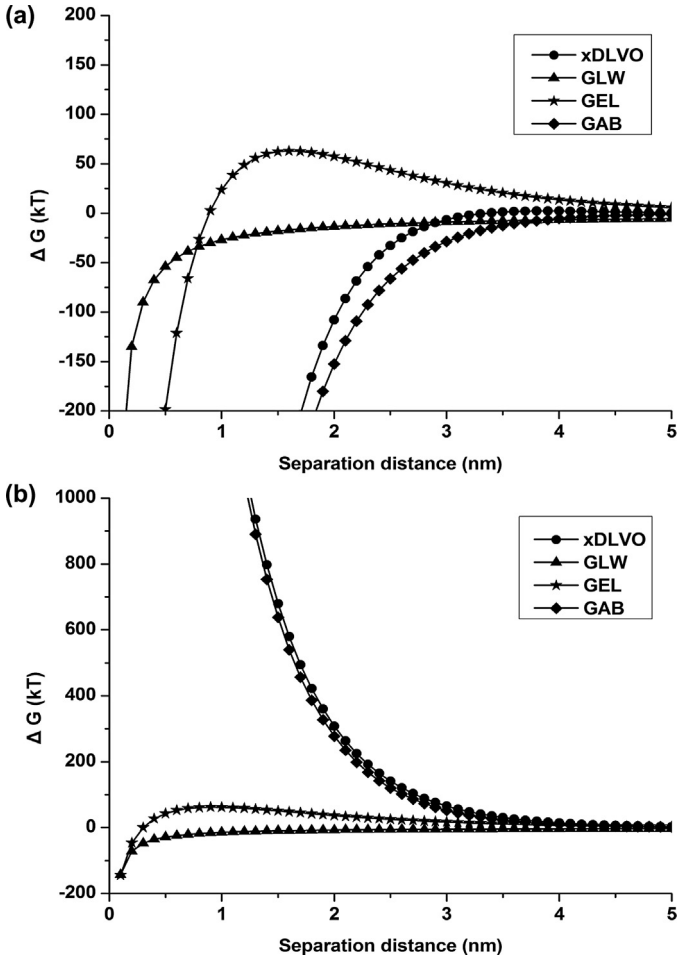

Fig. 4. Components of total interaction energy between *Salmonella* Enteritidis previously grown at pH 7.3, 37 °C and (a) acrylic and (b) stainless steel. xDLVO, total xDLVO interaction; GLW, Lifshitz-van der Waals interaction; GEL, electrostatic double layer interaction; GAB, Lewis acid–base interaction.

4. Discussion

Adhesion is the first step in the establishment of foodborne pathogens in the food-processing environment, and is critical for their subsequent biofilm development and persistence on food contact surfaces. Before contact with the surfaces, *Salmonella* might have been exposed to different environmental stresses, such as heat, cold, acidic or alkaline conditions, due to the physical and chemical treatments used to eliminate or control them. These environmental stresses might influence the physicochemical properties of *Salmonella* and thus adhesion and biofilm formation. Therefore, *S.* Enteritidis grown under different temperatures or pHs were used as inoculum and the initial attachment was investigated at room temperature to simulate the real food processing environment.

The results obtained from the present study demonstrated that the growth conditions of *S.* Enteritidis prior to the exposure to the test surfaces affect the level of attachment. The higher attachment levels were achieved by control cells pre-grown at 37 °C, pH 7.3, or pre-grown at acidic pH (pH 5.3 and 6.3). Similar to the present results, Smoot and Pierson [18] reported that optimum growth conditions (30 °C, pH 7.0) promoted the attachments of *L. monocytogenes* to Buna-N rubber and stainless steel. However, they found that previous growth at acidic condition (pH 5.5) inhibited the bacterial attachment, which was even lower than the attachment by cells grown at alkaline conditions (pH 8.5). Moreover, preculturing in a lactic acid-supplemented medium (pH 6.0) has been reported to result in better adhesion of *L. monocytogenes* to stainless steel [23]. The different effects of acidic growth condition on subsequent attachment to tested surface observed in these studies might be due to the differences in the experimental conditions, such as different bacterial strains and culturing procedures.

Two surfaces, acrylic and stainless steel 304, were used in this study due to their popularity in the food industry [24]. The results showed that the attachment of *S.* Enteritidis to both contact

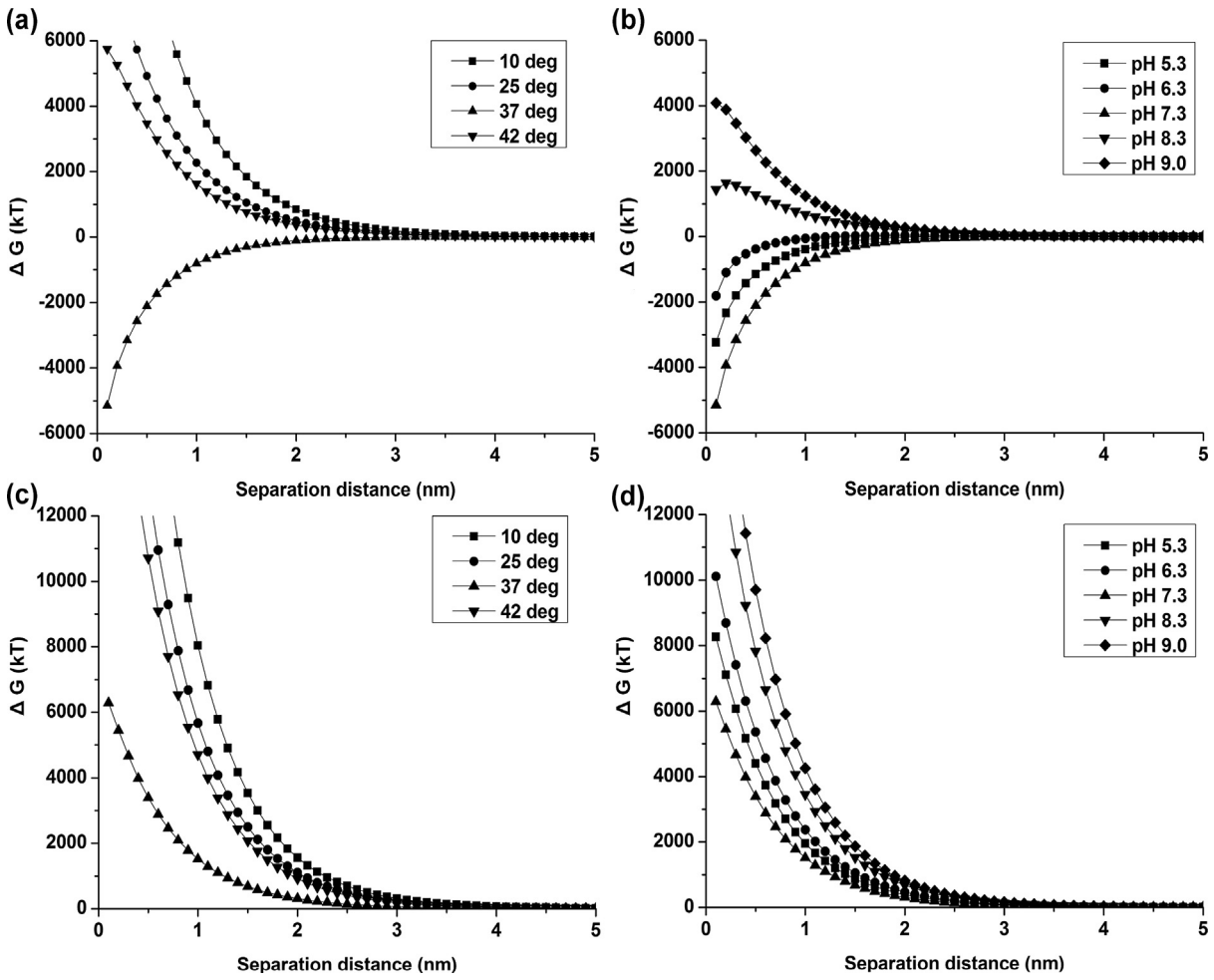

Fig. 5. Total interaction energy calculated based on xDLVO theory between *Salmonella* Enteritidis previously grown at different temperatures or pHs and (a and b) acrylic, or and (c and d) stainless steel.

surfaces was not significantly different at all tested temperature and pH conditions. By contrast, Nguyen et al. [12] used the same tested surfaces and reported that *S. Typhimurium* was more adherent to stainless steel than to acrylic. The different results indicate that attachment of *Salmonella* spp. to food contact surfaces might be serovar-dependent and it might be imprudent to conclude that one food contact surface is superior to the other or others based on the observation from only one or a few *Salmonella* strains.

To explore if the differences in initial attachment resulting from different growth conditions were due to the alterations in physicochemical properties, the hydrophobicity was determined using contact angle measurement. The results indicated that although all cells were hydrophilic, cells grown at optimal growth temperature and pH, and acidic pH were more hydrophobic than at other conditions. Similarly, Moorman et al. [25] and Giotis et al. [26] found that alkaline pH and low temperature could decrease the hydrophobicity of *L. monocytogenes* and *L. innocua*, respectively. However, it has been reported that acid condition could increase the hydrophobicity of *L. innocua* and S. Typhimurium compared to neutral pH [25,27]. Although some studies found positive correlations between cell surface hydrophobicity and bacterial attachment to surface [28,29], other investigations indicated that there was a lack of correlation [30,31]. The present study showed that adhesion of *S.* Enteritidis with higher hydrophobicity to the tested surfaces was usually significantly higher than those with lower hydrophobicity.

Surface charge has also been suggested to play a role in bacterial attachment [15,28]. The results of bacterial surface charge demonstrated that all *S.* Enteritidis cells were negatively charged, with values ranging from −2.94 to −6.27 mV, which were comparable to those reported by Chia et al. [16] for other *Salmonella* serovars. The negative charge of cell surface might be attributed to the excessive carboxyl and phosphate groups located in the cell walls [32]. As the tested surfaces were also negatively charged, the electrostatic double layer interactions between *S.* Enteritidis and surfaces were repulsive. Therefore, less negatively charged cells would experience lower electrostatic repulsion. Surface charge and attachment were generally correlated in this study, except that the cells grown at pH 9 with least negative charge had a poor attachment capability. These contradictory results indicated that factors other than surface charge might be involved in determining bacterial initial attachment.

Due to the complexity of bacterial initial attachment to surfaces, xDLVO theory, which takes Lifshitz-van der Waals, Lewis acid–base, and electrostatic double layer interaction energies between the surface and the bacterium into consideration, was used in this study to predict the attachment of *S.* Enteritidis grown under various conditions to the tested surfaces. In general, there were good agreements between the theoretical predictions and the experimental data. For example, the theory successfully predicted that the attachment of control cells grown at 37 °C was more favorable than those grown at other temperatures. This was also true for the pH study; control cells grown in neutral conditions and cells grown at acidic

conditions were predicted to be more favorable for attachment than cells grown at alkaline conditions. The successful prediction of poor attachment capability of cells pre-grown at pH 9 by xDLVO was due to that xDLVO theory assumes that Lewis acid–base interaction plays the dominant roles in bacterial attachment to surfaces (Table 3). However, the theory incorrectly predicted that the attachment of *S*. Enteritidis grown at 42 °C would be greater than that of cells grown at 25 °C. The failures of xDLVO theory in predicting the adhesion of microorganisms to surfaces have been also reported in several studies [16,17,33]. One reason why the theoretical predictions were not in accordance with the experimental data could be due to that the theory assumes smooth and uniform surfaces and does not take account of the roughness and the heterogeneity of the bacterial cells as well as the surfaces [34]. In addition, xDLVO theory assumes direct contact between the bacteria and the substrate, but cell adhesion may occur from a distance [35]. Bacterial surface is covered by a variety of polymers, including lipopolysaccharides, appendages, and extracellular polysaccharides for Gram-negative bacteria. Attractive polymer interactions may occur when the surface polymers have a high affinity for the surface and long enough to bridge the distance between the surface and bacteria [36]. At large separation distance (>5 nm), the energy barrier for bacteria to overcome is very small and cells may be attracted towards the surface due to the attractive Lifshitz-van der Waals force alone. Therefore, new models for the prediction of bacterial attachment should take biological properties of bacteria and the surface roughness into account.

The theory predicted repulsive force between all cells and stainless steel, and between most cells and acrylic. At 0.157 nm, the xDLVO interaction energies between the cells and acrylic were lower compared to those between the cells and stainless steel and hence acrylic would be a better surface for cell attachment. However, the attachments of *S*. Enteritidis to the two surfaces were not significantly different ($P > 0.05$) in this study. This is probably because the xDLVO prediction was made with the zeta potential and contact angle measurements on the bare surfaces. On the contrary, the attachment of bacteria to surfaces in this study took place in TSB. The presence of organic compounds in the suspension would result in the development of a conditioning film on the surface of the coupons, which may reduce the difference in the surface properties of stainless steel and acrylic for cell attachment.

5. Conclusion

This is the first study to investigate the effect of pre-growth conditions, on the bacterial cell surface properties as well as on the subsequent attachment of *S*. Enteritidis to acrylic and stainless steel. The results showed that the initial attachments were higher for control cells grown at optimal temperature and pH in addition to cells grown at acidic pH conditions. All cells were hydrophilic and negatively charged, but the magnitude varied with the growth conditions. Generally, cells that achieved higher attachment had higher hydrophobicity and less negative charge. Although stainless steel was more hydrophobic and less negatively charged compared to acrylic, no significant difference between them in the attachments was observed. The different attachment capabilities of cells grown under different conditions were explained by xDLVO theory in most cases. However, other determinants (such as surface roughness and heterogeneity of both the bacteria and the contact surface, as well as, the presence of extracellular polymeric substances on bacterial surface) should be taken into consideration when designing new models to predict bacterial attachment. The present study indicated that current control measures including cold storage, thermal treatment, or alkaline antimicrobial might be also effective in inhibiting the initial attachment of *S*. Enteritidis to food contact surfaces; however, acidic antimicrobial should be used with caution due to its promotion of *S*. Enteritidis attachment.

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