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Bacterial cell attachment, the beginning of a biofilm

Jon Palmer · Steve Flint · John Brooks

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Abstract The ability of bacteria to attach to surfaces and develop into a biofilm has been of considerable interest to many groups in numerous industries, including the medical and food industry. However, little is understood in the critical initial step seen in all biofilm development, the initial bacterial cell attachment to a surface. This initial attachment is critical for the formation of a bacterial biofilm, as all other cells within a biofilm structure rely on the interaction between surface and bacterial cell for their survival. This review examines what are believed to be some of the most important aspects involved in bacterial attachment to a surface.

Introduction

Many bacteria have been shown to exist predominantly attached to surfaces in contact with liquids [25]. The advantage gained by the bacteria in living attached to a surface are thought to include higher concentration of nutrients close to a surface, promoted genetic exchange and, for a pathogen, increased protection from the host's immune system [27]. In certain industrial situations, bacterial cell attachment to metallic surfaces may result in biocorrosion, resulting in the damage to pipelines and other important metallic surfaces and costing millions of dollars in repairs [6]. The dominating factor involved in the initial attachment of a bacterial cell to a surface has remained elusive and today it is thought that a multitude of factors are involved, including surface conditioning, mass transport, surface charge, hydrophobicity, surface roughness and surface micro-topography.

J. Palmer (✉) · J. Brooks
Institute of Food Nutrition and Human Health,
Massey University, Palmerston North, New Zealand
e-mail: J.S.Palmer@massey.ac.nz

S. Flint
Fonterra Research Centre, Palmerston North, New Zealand

Conditioning of a surface

During the first stage, molecules present in the bulk flow, both organic and inorganic are carried toward the surface either by diffusion or turbulent flow. This accumulation of molecules at the solid–liquid interface on surfaces found in many food industries is commonly called a conditioning film and leads to a higher concentration of nutrients at the surface compared with the liquid phase [59].

The adsorption of organic molecules such as proteins to surfaces could play an important role in bacterial attachment, as this conditioning of the surface may alter the physical–chemical properties of the surface. Factors affected can include surface free energy, hydrophobicity and electrostatic charges [26].

Conflicting opinions exist on the importance of a conditioning film in initial bacterial attachment, with Fletcher [31] reporting that the presence of proteins such as albumin, gelatin and fibrinogen inhibited attachment of a marine *Pseudomonas* to polystyrene. Parkar et al. [78] demonstrated that the presence of skim milk on a surface of stainless steel, even at concentrations as low as 1%, reduced the attachment of spores and vegetative cells of thermophilic bacilli. Skim milk was also found to reduce the attachment of *S. aureus*, *Listeria monocytogenes* and *Serratia marcescens* to stainless steel [5]. Even individual milk components such as casein and β-lacto globulin were

reported by Helke et al. [41] to reduce attachment of *L. monocytogenes* and *Salmonella typhimurium* to stainless steel. One reason for the reduced attachment reported above may be that the proteins in the bulk fluid phase may act as competition for binding sites on the surface of the stainless steel, reducing the ability of bacteria to attach. However, Speer and Gilmour [99] reported that stainless steel and rubber surfaces treated with either whey proteins or lactose demonstrated an increase in attachment of milk-associated micro-organisms. Holah and Gibson [45] claimed that Johal [51] observed a reduction in the surface charge of stainless steel after conditioning of the stainless steel in meat juices and suggested that this was "enhancing the potential accumulation of bacteria on the surfaces". Also Jeong and Frank [50] suggested that the presence of proteins on a surface favours biofilm formation, as attached proteins could be a source of nutrients for bacteria. More recently, Verran and Whitehead [120] reported that the presence of proteinaceous material such as bovine serum albumin (BSA) on an inert surface retained bacterial cells at a higher rate, during a cleaning cycle, than cells on a clean surface. The shearing off of bacterial cells from a surface may result in bacterial footprints or bacterial surface polymers remaining behind on the surface and this may also play a role in further bacterial cell attachment. The conflicting observations reported on the importance of a conditioning film on bacteria attachment may be the result of different laboratory conditions, different bacterial strains, surfaces and differing organic molecules used to create a conditioning film.

The presence of primary colonisers that allow different species of oral bacteria to adhere to teeth has been observed for many years in the dental industry and is commonly called co-aggregation [123]. Sasahara and Zottola [94] reported co-aggregation between two species of bacteria not usually associated with the oral cavity (*L. monocytogenes* and *Pseudomonas fragi*) on a glass surface. In pure culture form, *L. monocytogenes* showed sparse adherence to a glass surface; however, when grown with *Pseudomonas fragi*, significant adherence of *L. monocytogenes* was observed. They concluded that *Pseudomonas fragi* was the primary coloniser and the exopolysaccharide produced by the *Pseudomonas fragi* was responsible for the observed increase in *L. monocytogenes* adherence. Corpe [21] also reported that *Caulobacter* spp. appeared to attach to glass surfaces at a higher rate in the presence of *Pseudomonas* spp. than in their absence. It seems logical to think that co-aggregation must also occur in many different environments outside the oral cavity of humans and may have an important role to play in the attachment of bacteria to surfaces in the food industry and in other medical or environmental areas. Interestingly, Trachoo and Brooks [106] demonstrated that *Campylobacter* can co-aggregate with *Enterococcus* to form biofilms and these were found extensively in the poultry industry in Thailand.

Mass transport

Mechanisms by which bacteria are transported to a surface can include Brownian motion, sedimentation due to differences in specific gravity between the bacteria and the bulk liquid, or convective mass transport, by which cells are physically transported towards the surface by the movement of the bulk fluid. Yang et al. [125] demonstrated that the convective mass transport towards a substratum surface can occur in a stainless steel pipe. This convective mass transport produces a higher cell attachment rate in the presence of a T-junction than when the convective mass transport is parallel to a substratum, or in this case a stainless steel surface. This suggests that convective mass transport towards a surface may help facilitate the close approach of bacteria to a surface and thus play a role in initial bacterial attachment. The convective mass transport results reported by Yang et al. [125] also reinforce the importance of plant design in controlling microbial contamination, growth and ease of cleaning. In turbulent flow, the creation of multiple, small turbulent eddies may drive small particles including bacterial cells towards surfaces which may in turn override the Gibbs energy barrier required by bacteria to come into contact with a surface. The Gibbs energy barrier is the sum of the van der Waals interactions, commonly attractive and electrostatic interactions, usually negative, due to both bacteria and substratum surfaces being negatively charged [109]. Balancing this increase in contact of bacteria with the substratum is the reduced thickness of the boundary layer, which would allow increased scouring and thus reduce the time available for bacteria to interact with the surface.

Davies [25] reported that active transport, mediated by bacterial flagella activity and chemotaxis, has been considered an important mechanism enabling bacteria to interact with a surface. However, using *E. coli* and insertion mutagenesis to disrupt flagella operons and the *che* operon, responsible for chemotaxis, Pratt and Kolter [86] demonstrated that motility is important for initial interaction with an abiotic surface, but chemotaxis played no part in bacterial attachment and was described by the authors as dispensable in initial biofilm formation in *E. coli*. Conversely, Klausen et al. [57] reported that the flagella of *Pseudomonas aeruginosa* were not a necessary factor in the initial attachment of this organism to a solid surface, but played a role in biofilm development. Of interest also is a report by O'Toole and Kolter [75] detailing that mutant non-flagellated cells of *Pseudomonas fluorescens* were defective in attachment to several inert surfaces, compared

to non-mutant flagella producing cells. However, a change in the medium, with citrate instead of glucose as a carbon source, reinstated the cells attachment ability, suggesting the environmental conditions in which the cells exist may have an effect on attachment. Recent research has tended to focus on characterization of important factors involved in all aspects of biofilm formation from initial attachment to biofilm maturation. A common theme reported in biofilm deficient mutants is the disruption of the genes involved in flagella synthesis [69, 75, 86, 96]. However, the methods employed to isolate biofilm deficient mutants are often not very suitable for the isolation of mutants carrying mutations solely in the initial attachment phase. The involvement of flagella in initial attachment of bacteria to abiotic surfaces is still not well understood, but this may be a reflection on different laboratory conditions, different bacterial strains and surfaces employed.

Attachment

There are two generally accepted theories on the attachment of bacteria to solid surfaces. The first of these theories has a two-step process [59, 65]. The first step involves the bacteria being transported close enough to allow initial attachment to take place, with the forces involved in this initial attachment being van der Waals forces, electrostatic forces and hydrophobic interactions [16, 36, 115]. During this initial contact bacteria still show Brownian motion and can be easily removed by fluid shear forces, e.g. rinsing [65]. The next crucial step in the attachment process is the irreversible attachment of cells to the surface, described by Dunne [30] as bacteria locking on to the surface by the production of exo-polysaccharides and or specific ligands, such as pili or fimbriae that may complex with the surface. At the end of this stage much stronger physical or chemical forces are required to remove the bacteria from the surface, e.g. scraping, scrubbing or chemical cleaners.

In the transition from reversible attachment to irreversible attachment, various short range forces are involved, including covalent and hydrogen bonding as well as hydrophobic interactions [59]. Poortinga et al. [84] expanded on the idea of covalent bonding in bacterial attachment and suggested that bacteria either donated electrons to, or accepted electrons from the substratum. Whatever the electron transfer, these results suggest that electron transfer between cell surface and the substratum plays an important role in bacterial attachment to inorganic and presumably to organic surfaces too.

The three-step model proposed by Busscher and Weerkamp [14] involves Lifshitz–van der Waals forces operating over several hundred nanometers (nm) as a first step. The second operates over distances of about 20 nm involving Lifshitz–van der Waals forces and electrostatic interactions. The third step occurs at distances of around 5 nm, where specific adhesion receptors may facilitate strong adhesion.

The transition time from reversible to irreversible attachment has been an area of comparatively little work in the past. However, Meinder et al. [66] demonstrated that over a time as short as ten minutes, attachment of thermophilic *Streptococcus* became 100 times less reversible. Flint et al. [32] found that cultures of *Streptococcus thermophilus* and *Bacillus cereus* attached to stainless steel in less than 60 s and subsequent washing with distilled water proved ineffective in removing attached cells. Schwab et al. [95] noted that numbers of *L. monocytogenes* attached to stainless steel were essentially the same either after 5 min or 24 h incubation. Butler et al. [15] reported similar findings with meat surfaces, noting considerable attachment of bacterial cells taking place in the first minute of contact, though small increases were also seen over time. Vadillo-Rodríguez et al. [108] used atomic force microscopy (AFM) to measure the forces required to move away the AFM tip from the surface of *Streptococcus thermophilus* after the AFM tip had been in contact with the cell surface from anywhere between 1 and 200 s. An increasing amount of force was required to remove the AFM tip from the cell surface over time, which corroborates earlier work mentioned above, suggesting the bond strength increases between cell surface and substratum over a relatively short period of time.

Surface charge

Bacterial cells generally have a net negative charge on their cell wall at neutral pH [92]. However, the magnitude of the charge varies from species to species and is probably influenced by cultural conditions [36, 56] age of the culture [122] ionic strength [24] and pH [47]. The charge on the cell surface is often determined as its zeta-potential, which is calculated from the mobility of the bacterial cell in the presence of an electrical field under defined salt concentration and pH. Most bacteria have a negative zeta potential at physiological pH (pH 7) [36, 61, 68], however, Jucker et al. [53] isolated a bacterial strain of *Stenotrophomonas* (*Xanthomonas*) *maltophilia*, with a positive zeta potential at physiological pH. This was compared with a strain of *Pseudomonas putida* with a negative zeta potential at physiological pH. The *S. maltophilia* demonstrated high attachment efficiency to glass and Teflon, both of which have a negative surface charge. But as the ionic strength of the suspending medium was increased, a drop in attachment efficiency of *S. maltophilia* was noted as well as a change to a negative zeta potential, suggesting the importance of surface charge in attachment of *S. maltophilia* to glass and Teflon. Conversely, at high ionic strength the negatively charged *P. putida* demonstrated higher attachment efficiency and a decreasing (move towards zero) zeta

potential, suggesting high ionic strength suppresses or overwhelms the natural surface charge of bacteria. Mafu et al. [64] also concluded that high ionic strengths suppressed electrostatic interactions between *L. monocytogenes* and various inert surfaces. Furthermore, Giaouris et al. [35] reported that higher sodium chloride concentrations (10.5%) inhibited the adherence of *Salmonella enterica* to stainless steel coupons. One explanation for the above observations mentioned by Jucker et al. [53] and Van der Wal et al. [113] is that the bacterial cell surface charge originates from the dissociation of acidic groups such as carboxyl, phosphate and amino groups as well as basic groups found on the cell surface. Consequently, the zeta potential of the bacterial cell strongly depends upon the ionic strength of the suspending medium and the higher the ionic strength the more ions are available to shield and thus neutralise the charge of the cell surface. Electrostatic interaction chromatography (ESIC) has been used by several groups in the past to measure the overall surface charge of bacteria [26, 32, 52, 71, 83, 107]. The relative surface charge of cells is assessed by the affinity of cells for either the anionic or cationic resins. The ESIC method usually involves passing a culture through an anionic or cationic column and comparing the relative retention to elution ratio of bacterial cells. The ESIC method assesses most bacteria as possessing a net negative charge, in line with most zeta potential measurements. Jones et al. [52] noted that some isolates of *S. epidermidis* exhibited a high interaction with both anionic and cationic exchange resins. They suggested there were localised regions of positively charged (cationic) molecules on the bacterial cells surface, but over the whole cell surface, negatively charged (anionic) molecules outnumbered the cationic molecules, resulting in the cell having net negative charge.

Surface charge can also be influenced by the pH of the suspending medium as reported by Husmark and Ronner [47]. They demonstrated a maximum level of attachment of *Bacillus cereus* spores to surfaces when the pH of the suspending medium was equal to the isoelectric point of the Bacillus spores, in this case pH 3. In the pH range above the isoelectric point (above pH 4) the observed decrease in spore attachment was thought to result from electrostatic repulsion between the spore surface and the substratum, because both surfaces had a negative charge.

Other groups to have reported positive correlations between cell surface charge and attachment include, Ukuku and Fett [107], Dickson and Koohmaraie [26] and Van Loosdrecht et al. [115]. On the other hand, Flint et al. [32] compared 12 strains of thermophilic *Streptococci* and their attachment to stainless steel with respect to their surface charge, measured by separation through anionic and cationic exchange resins. They were unable to divine any relationship between numbers of cells attaching to stainless steel and cell surface charge. As commented by Flint et al. [32], at pH 7 all the thermophilic *Streptococci* cells displayed a negative surface charge and this is likely to repel the bacterial cell from surfaces such as stainless steel, owing to the inherent negative surface charge of stainless steels. Gilbert et al. [36] noted that increasing negative charge on the surface of *E. coli* resulted in reduced attachment, but no such correlation could be drawn for *S. aureus*, demonstrating that attachment cannot solely be explained by surface charge, but may be one of the contributing factors to bacterial attachment. Narendran [72] also reported that bacterial attachment to meat surfaces could not be explained by the bacterial surface charge alone and suggested that bacterial attachment is very complex with many bacterial surface characteristics involved. The surface of the meat is also more complex than that of relatively inert surfaces, such as stainless steel.

The surface charge of inert surfaces to which bacteria can attach is also highly likely to play an important role in bacterial attachment. Fukuzaki et al. [34] reported that the zeta potential of stainless steel particles at pH 7 was weakly negative, with the stainless steel having an isoelectric point between pH 4.0 and 4.5. Bren et al. [9] proposed that hydroxyl groups of surface oxides can interact with H^+ and OH^- groups according to the following reaction,

$$MeOH_2^+ \overset{H^+}{\leftrightarrow} MeOH \overset{OH^-}{\leftrightarrow} MeO^- + H^+ \quad (Me = \text{metal})$$

The ratio of metals that are protonated (positively charged groups), neutral or dissociated (negatively charged groups) is obviously very dependent upon the pH of the overlying medium. Thus in low pH medium the dominant group would be $MeOH_2^+$ but at neutral or higher pH values MeOH or MeO^- groups may dominate. Different metals may also have slightly different pKa and pKb values, so thus different ratios of metal oxides at the surface may produce metal surfaces with varying surface charges at the same pH value. A possible example of this may have been reported by Takehara and Fukuzaki [101], when they observed that stainless steel treated with HNO_3, ozone and 300°C heat treatment contained different ratios of Chromium and Iron oxides at the surface. The different treated stainless steel surfaces also demonstrated different relative adsorption curves for H^+ and OH^- titrations, suggesting that the surface treatment may also play an important role in the surface charge of stainless steel and in turn have a role to play in the attachment of bacteria.

Hydrophobicity

Hydrophobic interactions have widely been suggested as being responsible for much of the adherence of cells to

surfaces, Hood and Zottola [46]. Hydrophobicity is an interfacial phenomenon. It is very difficult to evaluate the results of most adhesion tests solely on the basis of hydrophobicity, since so many parameters are involved in interfacial systems of interest [28]. Although the hydrophobic effect has been known for some time, it has been difficult to assign to it a satisfying definition [29]. Put simply, a hydrophobic molecule would rather exist in another hydrophobic environment than in a hydrophilic environment, such as water. Husmark and Ronner [48] demonstrated that bacterial spores generally attach at a higher rate than vegetative cells to surfaces. They attributed this observation to the higher hydrophobicity of spores and the hair like structures covering the surface of the spores. Zita and Hermansson [126] correlated cell surface hydrophobicity of *E. coli* strains to the attachment to activated sludge flocs found in the treatment of waste water, suggesting that cell surface hydrophobicity may play an important role in the attachment of *E. coli*. Hydrophobicity of a cell surface is not only the domain of bacteria, but has also been attributed to the adhesion of *Cryptosporidium parvum* and *Giardia lamblia* to solid surfaces [23] and in the attachment of yeast to stainless steel surfaces in the apple processing industry [11]. Davies [25] concluded that differences in surface hydrophobicity of different bacterial cells result from the properties conferred upon the cell surface by molecules such as proteins and lipids. Evidence that hydrophobicity of cells may be related to protein structures on the cell surface does exist. Paul and Jeffrey [80] noted that treatment of cells with proteolytic enzymes decreased the hydrophobicity of *Vibrio proteolytica* and this in turn reduced the adherence to hydrophobic surfaces such as polystyrene. Oakley et al. [74] reported that *Streptococcus sanguis* cells treated with trypsin demonstrated reduced adhesion to hexadecane (a highly hydrophobic organic liquid), presumably because hydrophobic proteins were removed from the cell surface. X-ray photoelectron spectroscopy (XPS) analyses of bacterial surfaces by Reid et al. [89] and Millsap et al. [68] found that bacterial strains with high hydrophobicity ratings also tended to have a higher nitrogen/carbon ratio. Conversely cells with a higher hydrophilic rating tended to have higher oxygen/carbon ratio. These results tend to indicate that the presence of proteinaceous material at the cell surface increases the hydrophobicity of the cell surface. Walker et al. [122] also suggested differences that they reported in the surface hydrophobicities of 3 and 18 h cultures of *E. coli* are related to the surface proteins, specifically a decrease in hydrophilic (acidic) proteins present on the cell surface. The role of lipopolysaccharide (LPS) in attachment to solid surfaces remains confused [12, 87, 88] but evidence is mounting that the presence of LPS on a cells surface tends to make a bacterial cell more hydrophilic in nature and that the loss of LPS from a cell surface results in the cell surface becoming more hydrophobic in nature [2, 70, 76] Interestingly, Kannenberg and Carlson [55] reported that a reduction in oxygen levels of the medium induced structural modifications in the LPS of the bacterium Rhizobium, resulting in an increase in surface hydrophobicity of the cell. This tends to indicate that the bacterial cell is quite capable of sensing changes in its external environment and in turn changing a major cell surface characteristic such as surface hydrophobicity.

Much debate has existed as to which is the best method to measure bacterial surface hydrophobicity. The three most popular methods include Bacterial Adherence To Hydrocarbons, commonly called the BATH test, as described by Rosenberg et al. [93], which is now generally called the MATH test (microbial adherence to hydrocarbons). The others are hydrophobic interaction chromatography (HIC) [97] and Water Contact Angle measurements [110]. In the MATH test, evidence exists that hydrophobicity is not the only interaction taking place between microbial cell and organic solvent (hydrophobic compound) such as hexadecane. [Indeed, both hexadecane and xylene have been found to disrupt cell walls of *S. thermophilus* and *Anoxybacillus* spp. respectively (Flint, personal communication)]. Ahimou et al. [1], Busscher et al. [13] and Van der Mei et al. [111] have all reported that the MATH test can be influenced by electrostatic interactions, with Busscher et al. [13] reporting that hexadecane, the most commonly used hydrocarbon to measure hydrophobicity, is negatively charged in water, with a zeta potential of between –50 and –80 mV. Van der Mei et al. [112] concluded that the MATH test should be measured at pH values where the zeta-potential of the test organism and/or hydrocarbon are near zero to reduce the potential interference of electrostatic interactions. Doyle [29] suggested the MATH test should be performed under either high ionic strength or at the isoelectric point of the bacterial cells to minimise any possible electrostatic interactions, making any measurement of attachment to any hydrophobic hydrocarbon such as hexadecane valid.

Hydrophobic interaction chromatography (HIC) involves the interaction of hydrophobic cells with a hydrophobic column, such as Phenyl-Sepharose, with cells demonstrating high hydrophobicity being retained in the column and cells with low hydrophobicity being eluted. As early as 1978, Smyth et al. [97] noted that increasing ionic concentration, in this case NaCl, affected cell attachment to a HIC column such as Phenyl-Sepharose. Wiencek et al. [124] also reported that a high ionic strength was necessary to overcome electrostatic repulsion between bacterial spores and a hydrophobic column containing Phenyl-Sepharose. Wiencek et al. [124] used both BATH and HIC methods to measure relative cell hydrophobicity on

bacterial spores and noted that the relative hydrophobicites as determined by BATH and HIC generally agreed, even though a high ionic strength of NaCl was used to mask electrostatic repulsion between bacterial spores and Phenyl-Sepharose.

Evidence that hydrophobicity is a strong predictor of cell attachment to surfaces varies from group to group, with Peng et al. [83], Gilbert et al. [36], Iwabuchi et al. [49], Liu et al. [62] and Van Loosdrecht et al. [116] suggesting a strong correlation between hydrophobicity and cell attachment to surfaces. Van Loosdrecht et al. [116] went so far as to suggest that surface hydrophobicity is the key factor in determining bacterial attachment to solid surfaces and that surface charge can only become important when surface hydrophobicity is minimal. However, it must be noted that Van Loosdrecht et al. [116] used polystyrene discs, which are very hydrophobic, to measure cell adhesion, thus possibly favouring hydrophobic interactions. On the other hand Sorongan et al. [98], Parment et al. [79], Parkar et al. [78] and Flint et al. [32] concluded that hydrophobicity had little to no relationship in determining bacterial cell attachment. Nevertheless, hydrophobicity may be one of the many factors involved in initial attachment of micro-organisms to surfaces.

DLVO theory

The DLVO (Derjaguin, Landau, Verwey, and Overbeek) theory of colloid stability has been used by several groups to try to explain attachment of micro-organisms to surfaces [42, 90]. According to the DLVO theory, particle adhesion is driven by the sum of the Lifshitz–van der Waals interactions, usually described as attractive, and also electrostatic interactions, which may be repulsive or attractive, depending upon the charge of the two surfaces interacting. Rijnaarts et al. [91, 92] concluded that electrostatic interaction is as a general rule repulsive between inert surfaces and bacterial surfaces at neutral pH, as most inert surfaces and bacterial surfaces are negatively charged at neutral pH. Bacterial cell surface charge originates from the presence of carboxyl, phosphate and amino groups in either dissociated or protonated form and the surface charge consequently depends upon the pH [85] and/or ionic concentration [113] of the suspending medium. Poortinga et al. [85] suggested that at physiological pH values, i.e. between pH 5 and 7 bacterial cells are generally negatively charged due to the excess of carboxyl and phosphate groups over amino groups.

One critical aspect to the DLVO theory is ionic strength of the solution. Rijnaarts et al. [90, 92] described how at low ionic strength solution, for example <0.001 M, long range electrostatic repulsion dominates bacterial attachment, but at high ionic strength (>0.1 M) other factors such as steric interactions (hydrophobicity) dominate. Many workers have now realised that the DLVO theory does not take into account that the bacterial cell surface is not a model colloid particle but a highly dynamic surface that responds to changes in ionic strength, pH, the presence of macromolecules and even the presence of other surfaces [85]. These environmental changes may induce conformation changes to surface structures such as flagella and fimbriae that may have an important role in cell attachment. Pembrey et al. [82] reported that the methods used to prepare cells for cell surface analysis can have an influence on the values of cell surface parameters, especially the use of high salt buffers vs. low salt buffers. Even centrifugation at 15,000*g* was suggested by Pembrey et al. [82] to cause enough modification to cell wall structures to bring about differences in electrophoretic mobility and hydrophobicity compared with cells of the culture that were not centrifuged at 15,000*g*. Work presented by Pembrey et al. [82] and Castellanos et al. [17] tends to imply that the bacterial cell surface is not just an inert rigid structural component of the cell, but a delicate and complex array of proteins, carbohydrates and other components that the cell uses to sense its immediate environment, which can be easily damaged or altered by chemical and/or physical stress.

Methods for analysing electrostatic interaction such as zeta-potential and electrophoretic mobility measurements tend to give results in terms of the cell surface overall charge, or the net surface charge at the macroscopic level. Dan [24] suggested that the DLVO approach to bacterial adhesion tended to treat bacterial cells as traditional colloidal particles, characterised by having an even surface and a evenly distributed surface charge. The problem remains that cells contain many complicated surface structures such as flagella, pili, fimbriae, glycoproteins, carbohydrates, teichoic acids and other biological materials composed of proteins in *Bacillus* species up to 9% of total cell proteins are associated with the cell wall [104]. These complicated surface structures may exert their own localised cell surface charge at a microscopic cell surface level that could possibility mediate attachment through local electrostatic attraction despite the cell's having an overall electrostatic repulsion. Interestingly, Jones et al. [52], measuring the cell surface charge of *S. epidermidis* strains, noted that some strains revealed a marked interaction with the cationic-exchange resin (negatively charged resin), though all strains exhibited as expected a highly negative cell surface, suggesting that different regions on the surface on the cell display different surface charges, even though the overall cell charge may be negative.

Surface roughness and micro-topography

Stainless steel is the most common food contact material used in the food industry today, as it is easy to fabricate, durable, chemically and physiologically inert at a variety of food processing temperatures and pressures, generally corrosion resistant and usually easy to clean [45]. However, the micro-topography of stainless steel examined under SEM [127] and more recently AFM [3] reveals cracks and crevices, which could provide a greater area for cell attachment and possible protection from cleaning chemicals and fluid forces. Verran and Whitehead [121] concluded that surfaces with scratches and pits of similar size to microbial cells retained higher numbers of cells than surfaces with surface features much larger than microbial cells. Various groups have observed greater cell attachment on surfaces with high surface roughness and thus concluded surface roughness is an important factor in bacterial attachment to inert surfaces [60, 81]. On the other hand, Mafu et al. [64], Vanhaecke et al. [114] and Flint et al. [33] have reported no correlation between surface roughness and bacterial attachment to inert surfaces. Arnold and Bailey [3] reported that electro-polished stainless steel showed significantly fewer bacterial cells attaching and that biofilm formation on the electro-polished stainless steel was slower than untreated surfaces. Parkar et al. [77] commented that electro-polished stainless steel produced only a small reduction in initial attachment of thermophilic bacilli, but biofilm formation on electro-polished stainless steel was patchy and less dense than on normal 316 stainless steel. Arnold and Bailey [3] described electro-polishing as removing metal from an object's surface through an electrochemical process similar to, but the reverse of electroplating. Removal of metal ions, they suggested, reduces the chemical reactivity of the surface, changing the electrostatic interactions between metal and the surface of the microorganism and thus rendering the surface less susceptible to bacterial attachment. Besides surface roughness, surface topography may also play a part in cell attachment to surfaces [59]. Several groups have observed with the use of SEM that bacteria are able to attach within the surface cavities of the steel surface [119, 127]. Verran et al. [119] and Jullien et al. [54] suggested that stainless steel topography had little effect on the total numbers of bacterial cells attaching, and that surface topography may affect biofilm development by protecting cells from removal and thus allowing biofilm re-growth to occur more rapidly. Flint et al. [33] also commented that surface topography around the critical size close to the diameter of the bacterial cells may entrap bacteria on the stainless steel surface, thus providing cells with some degree of protection from cleaning agents.

Staphylococcus species

Key aspects of *Staphylococcus* initial attachment to solid surfaces are thought to include surface hydrophobicity [43, 44] surface proteins [22, 27, 40, 58, 103, 118] and teichoic acid structure [38]. Several surface proteins have been implicated in the ability of *Staphylococcus* strains to attach to inert surfaces. Cucarella et al. [22] identified two mutants of *Staphylococcus aureus* with the use of the transposon Tn917, with a significant decrease in attachment to inert surfaces. Both mutants had the Tn917 transposon inserted at the same locus on the chromosome of the bacteria. This locus encoded a cell wall-associated protein of 2,276 amino acids with a molecular weight of 254 kDa [4] called BAP (Biofilm Associated Protein) for short. All isolates of *S. aureus* harbouring the BAP gene were highly adherent to inert surfaces and were strong biofilm producers. Tormo et al. [105] reported that other strong biofilm producers from *S. epidermidis*, *S. chromogenes*, *S. xylosus*, *S. simulans* and *S. hyicus* all produced a BAP-like protein with an amino acid sequence similarity of greater than 80% suggesting that the BAP surface protein is an important protein involved in attachment of *Staphylococcus* to surfaces.

Other groups have also described the isolation of mutants unable to attach to solid surfaces or unable to form a biofilm due to the loss of a surface protein. Heilmann et al. [39] isolated a transposon-insertion mutant of *S. epidermidis* unable to attach to polystyrene. In comparison with the wild type, the mutant lacks five cell surface associated proteins with masses of 120, 60, 52, 45 and 38 kDa. Restoration of the 60 kDa band by complementation studies demonstrated that only the 60 kDa band is required for initial attachment to polystyrene. Also noted was a decrease in the hydrophobicity of the mutant compared with the wild type strain and the more pronounced ability of the mutant to attach to a hydrophilic surface, in this case glass. Heilmann et al. [39] suggested that the observed increase in attachment to glass by the mutant compared with the wild type may be a result of the mutant's lacking the five surface proteins, allowing hydrophilic surface structures to become unmasked, thus making the cell surface more hydrophilic. This in turn increases the likelihood of hydrophilic/hydrophilic interaction between mutant bacterial cell surface and the glass surface, compared with hydrophobic/hydrophilic interaction between the wild type bacterial cell surface and the glass surface. Further analysis by Heilmann et al. [40] showed that the 60 kDa adhesion protein appeared to be a protein fragment of a much larger protein that bears sequence homology to an autolysin (AtlE) found in *S. aureus*. Heilmann et al. [40] proposed that the 60 and 52 kDa bands are the product of the cleaved 120 kDa band, similar to the AtlE found in *S. aureus*, which is composed

of two lytic active domains of 60 and 52 kDa in size. The ability of the 60 kDa to bind to both polystyrene surfaces and plasma protein coated surfaces suggests that it is a multifunctional surface protein allowing cells to attach to solid surfaces and host cell surfaces. Veenstra et al. [118] identified a 280 kDa surface protein, subsequently named SSP1 (*Staphylococcus* Surface Protein) from *S. epidermidis* and with the use of immunogold labelling followed by electron microscopy suggested that SSP1 is located on fimbriae-like structures on the cell surface. Proteolytic cleavage of SSP1 by trypsin resulted in SSP2 of 250 kDa as demonstrated by SDS-PAGE. The proteolytic cleavage of cells with SSP1 on the surface coincided with the loss of adhesive function and increased concentration of SSP2, suggesting the conversion of SSP1 to SSP2. Veenstra et al. [118] suggested that the bacterial cell might be able to control its own phenotype between high adherent and low adherent by the proteolytic cleavage of SSP1 to SSP2 until a more favourable environment is reached and adhesiveness can be restored.

No one has reported on the epidemiological distribution among isolates of the Genus *Staphylococcus* of the surface proteins associated with attachment, namely 250 kDa reported by Veenstra et al. [118], 60 kDa reported by Heilmann et al. [40] and 254 kDa reported by Cucarella et al. [22]. The question still remains open on the distribution of the above mentioned surface proteins among *Staphylococcus* isolates: do some *Staphylococcus* isolates possess all three surface proteins or do some isolates only have one or even none? If some *Staphylococcus* isolates do possess all three surface proteins associated with attachment, then does each protein have a specific affinity with a particular surface, e.g. hydrophobic or hydrophilic surfaces, or are they all generic in terms of overall surface affinity? Do other bacteria associated with attachment to solid surfaces also possess multiple surface proteins as *Staphylococcus* appears to be able to express, or do other bacteria have a smaller or possibly larger repertoire of surface proteins that can be called upon to help in initial attachment of cells?

Listeria species

Listeria monocytogenes is an important pathogenic bacterium most commonly transmitted by food. Numerous studies have shown *L. monocytogenes* to be capable of attaching to stainless steel [7, 10, 73]; Glass [18, 19] and plastics [100]. The ability of *L. monocytogenes* to attach to and colonise surfaces during food processing and storage is thought to be important in the contamination of food products prior to consumption [102]. Several groups [8, 63, 73] have demonstrated that dominant or persistent strains of *L. monocytogenes* isolated from food processing plants have a higher attachment rate to surfaces than sporadic or non-persistent strains. Amongst the large number of strains examined, significant variation was noted in the ability of some isolates to attach to stainless steel [63, 73]. Borucki et al. [8] concluded that persistent strains of *L. monocytogenes*, as well as demonstrating higher initial attachment rates, also show increased biofilm formation. The higher attachment and biofilm formation rates of persistent *L. monocytogenes* strains probably demonstrate the natural selection within food processing plants of strains of *L. monocytogenes* with increased ability to attach to solid surfaces, resist sanitizers and form extensive biofilms.

The question remains "What aspects of the cell surface of *L. monocytogenes* allow it to attach to solid surfaces and thus cause contamination of food products?" Chae et al. [19] concluded that hydrophobicity played no part in the ability of *L. monocytogenes* to attach to glass surfaces, but cell surface hydrophobicity was correlated with attachment to polystyrene (a strongly hydrophobic surface), suggesting the possible importance of hydrophobic interactions in cell attachment. Chae et al. [19] also reported a positive correlation between total carbohydrate production and biofilm formation over 24 h. This observation does not relate total carbohydrate production with cell attachment, but merely suggests higher carbohydrate production helps in biofilm development and growth. Vatanyoopaisarn et al. [117] showed the importance of flagella on the initial attachment of *L. monocytogenes* to stainless steel over the first 10 h by comparing the attachment rates of a flagella producing strain and a non-flagella producing mutant. However, after 24 h, the two strains showed similar levels of attachment. This tends to suggest that structural and/or chemical changes are occurring at the cell surface during ageing of the culture, allowing greater interaction between cells and surface that predominate over the effect flagella have on attachment. Chavant et al. [20] reported that cell surface hydrophobicity of *L. monocytogenes*, which was generally hydrophilic in nature, varied with the age of the culture and also with the temperature of incubation, with stationary phase cultures and higher incubation temperatures generally increasing cell surface hydrophobicity. Results obtained by Chavant et al. [20] support the hypothesis of Vatanyoopaisarn et al. [117] that the cell surface is constantly changing over the life of a culture and is responding to different environmental stimuli. Both Briandet et al. [10] and Giovannacci et al. [37] reported that *L. monocytogenes* incubated at lower temperatures, i.e. <8°C demonstrated a weaker negative charge compared with cells incubated at either 15, 20 or 37°C at neutral pH. Briandet et al. [10] also noted a rapid reduction in the electrophoretic mobility at around pH 4, of cells incubated at 20 or 37°C. They related this with protein or peptidoglycan-associated $COOH/COO^-$ ($4 < pKa < 5$) carboxyl groups present on the cell surface becoming protonated and thus reducing the overall nega-

tive charge of the cell. However, cells grown at 8°C demonstrated no such rapid reduction in electrophoretic mobility and thus they suggested that cells cultured at 8°C might contain fewer carboxyl groups than cells grown at 20 or 37°C. The surface of *L. monocytogenes* is negatively charged as with most bacteria at neutral pH [92], but several authors have reported the lack of an isoelectric point over the pH range 2–7 in *L. monocytogenes*, suggestive of the presence of compounds on the cell surface with very low pKa values [10, 37, 67]. Generally speaking, most bacteria have an isoelectric point around pH 2 or 3.5 [72].The absence of an isoelectric point within the pH range 2–7 was hypothesized by Rijnaarts et al. [91] to be linked to the presence of phosphate groups with a very low pKa (pKa < 2.1) in the phosphodiester bridges of cell wall teichoic acids. These results reinforce the view stated earlier that the cell surface of *L. monocytogenes* is a dynamic, highly changing cell organelle, continually sensing and changing in response to changes in the environment and no one factor may be responsible for overall attachment of *L. monocytogenes* to inert surfaces.

Conclusion

The interaction between surface and bacterial cell surface appears to be mediated by a complex array of chemical and physical interactions, with each affected by the chemical and physical environment to which the bacterial cell and the surface are currently or recently exposed. The multiple factors involved in cell attachment, such as surface conditioning, mass transport, surface charge, hydrophobicity, surface roughness, growth medium and surface microtopography can make it difficult to characterise the role and the overall importance each factor has in attachment. The understanding of bacterial attachment to solid surfaces such as stainless steel may help in the future development of surfaces with no or reduced attachment, or in developing an effective sanitation programme and thus reducing the potential contamination of processed products by spoilage or pathogenic bacteria.

References

1. Ahimou F, Poquot M, Thonart P, Rouxhet PG (2001) Influence of electrical properties on the evaluation of the surface hydrophobicity of *Bacillus subtilus*. J Microbiol Methods 45:119–126
2. Al-Tahhan RA, Sandrin TR, Bodour AA, Maier RM (2000) Rhamnolipid-induced removal of lipopolysaccharide from *Pseudomonas* aeruginosa: effect on cell surface properties and interaction with hydrophobic substrates. Appl Environ Microbiol 66:3262–3268
3. Arnold JW, Bailey GW (2000) Surface finishes on stainless steel reduce bacterial attachment and early biofilm formation: scanning electron and atomic force microscopy study. Poult Sci 79:1839–1845
4. Arrizubieta MJ, Toledo-Arana A, Amorena B, Penadés JR, Lasa I (2004) Calcium inhibits BAP-dependent multicellular behavior in *Staphylococcus aureus*. J Bacteriol 186:7490–7498
5. Barnes L-M, Lo MF, Adams MR, Chamberlain HHL (1999) Effect of milk proteins on adhesion of bacteria to stainless steel surfaces. Appl Environ Microbiol 65:4543–4548
6. Beech IB, Sunner J (2004) Biocorrosion: towards understanding interactions between biofilms and metals. Curr Opin Biotechnol 15:181–186
7. Beresford MR, Andrew PW, Shama G (2001) *Listeria monocytogenes* adheres to many materials found in food-processing environments. J Appl Microbiol 90:1000–1005
8. Boruck MK, Peppin JD, White D, Loge F, Call DR (2003) Variation in biofilm formation among strains of *Listeria monocytogenes*. Appl Environ Microbiol 69:7336–7342
9. Bren L, English L, Fogarty J, Policoro R, Zsidi A, Vance J, Drelich J, Istphanous N, Rohly K (2004) Hydrophilic/electron-acceptor surface properties of metallic biomaterials and their effect on osteoblast activity. J Sci Technol 18:1711–1722
10. Briandet R, Meylheuc T, Maher C, Bellon-Fontaine MN (1999) *Listeria* monocytogenes Scott A: cell surface charge, hydrophobicity, and electron donor and acceptor characteristics under different environmental growth conditions. Appl Environ Microbiol 65:5328–5333
11. Brugnoni LI, Lozano JE, Cubitto MA (2007) Potential of yeast isolated from apple juice to adhere to stainless steel in the apple juice industry. Food Res Int 40:332–340
12. Burks GA, Velegol SB, Paramonova E, Lindenmuth BE, Feick JD, Logan BE (2003) Macroscopic and nanoscale measurements of the adhesion of bacteria with varying outer layer surface composition. Langmuir 19:2366–2371
13. Busscher HJ, van de Belt-Gritter B, van der Mei HC (1995) Implications of microbial adhesion to hydrocarbons for evaluating cell surface hydrophobicity 1. Zeta potentials of hydrocarbon droplets. Colloids Surf B Biointerfaces 5:111–116
14. Busscher HJ, Weerkamp AH (1987) Specific and non-specific interactions in bacterial adhesion to solid substrata. FEMS Microbiol Rev 46:165–173
15. Butler JL, Stewart JC, Vanderzant C, Carpenter ZL, Smith GC (1979) Attachment of microorganisms to pork skin and surfaces of beef and lamb carcasses. J Food Prot 42:401–406
16. Carpentier B, Cerf O (1993) Biofilms and their consequences with particular reference to hygiene in the food industry. J Appl Bacteriol 75:499–511
17. Castellanos T, Ascencio F, Bashan Y (1997) Cell-surface hydrophobicity and cell-surface charge of *Azospirillum* spp. FEMS Microbiol Ecol 24:259–172
18. Chae MS, Schraft H (2000) Comparative evaluation of adhesion and biofilm formation of different *Listeria monocytogenes* strains. Int J Food Microbiol 62:103–111
19. Chae MS, Schraft H, Hansen LT, Mackereth R (2006) Effects of physicochemical surface characteristics of *Listeria monocytogenes* strains on attachment to glass. Food Microbiol 23:250–259
20. Chavant P, Martinie B, Meylheuc T, Bellon-Fontaine MN, Hebraud M (2002) *Listeria monocytogenes* LO28: surface physicochemical properties and ability to form biofilms at different temperatures and growth phases. Appl Environ Microbiol 68:728–737
21. Corpe WA (1974) Periphytic marine bacteria on the formation of microbial film on solid surfaces. In: Colwell R, Morita R (eds) Effect of the ocean environment on microbial activity. University Park Press, Baltimore, pp 397–417

22. Cucarella C, Solano C, Valle J, Amorena B, Lasa I, Penades JR (2001) BAP a *Staphylococcus aureus* surface protein involved in biofilm formation. J Bacteriol 183:2888–2896
23. Dai X, Boll J, Hayes ME, Aston DE (2004) Adhesion of *Cytosporidium parvum* and *Giardia lamblia* to solid surfaces: the role of surface charge and hydrophobicity. Colloids Surf B Biointerfaces 34:259–263
24. Dan N (2003) The effect of charge regulation on cell adhesion to substrates: salt-induced repulsion. J Colloid Interface Sci 27:41–47
25. Davies DG (2000) Physiological events in biofilm formation. In: Allison D, Gilbert P, Lappin-Scott M, Wilson M (eds) Community structure and co-operation in biofilms, pp 37–51
26. Dickson JS, Koohmarare M (1989) Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. Appl Environ Microbiol 55:832–836
27. Donlan RM (2001) Biofilm formation: a clinically relevant microbiological process. Clin Infect Dis 33:1387–1392
28. Doyle RJ, Rosenberg M, Drake D (1990) Hydrophobicity of oral bacteria. In: Doyle RJ, Rosenberg M (eds) Microbial cell surface hydrophobicity. American Society of Microbiology, Washington DC, pp 387–419
29. Doyle RJ (2000) Contribution of the hydrophobic effect to microbial infection. Microbes Infect 2:391–400
30. Dunne MW (2002) Bacterial adhesion—seen any good biofilms lately? Clin Microbiol Rev 15:155–166
31. Fletcher M (1976) The effect of proteins on bacterial attachment to polystyrene. J Gen Microbial 94:400–404
32. Flint SH, Brooks JD, Bremer PJ (1997) The influence of cell surface properties of thermophilic *Streptococci* on attachment to stainless steel. J Appl Microbiol 83:508–517
33. Flint SH, Brooks JD, Bremer PJ (2000) Properties of the stainless steel substrate influencing the adhesion of thermoresistant *Streptococci*. J Food Eng 43:235–242
34. Fukuzaki S, Urano H, Hagata K (1995) Adsorption of pectin onto stainless steel surfaces: role of electrostatic interactions. J Jpn Soc Food Sci Technol-Nippon Shokuhin Kagaku Kogaku Kaishi 12:700–708
35. Giaouris E, Chorianopoulos N, Nychas G-JE (2005) Effect of temperature, pH, and water activity on biofilm formation by *Salmonella enterica* Enteritidis PT4 on stainless steel surfaces as indicated by the bead vortexing method and conductance measurements. J Food Prot 68:2149–2154
36. Gilbert P, Evans DJ, Evans E, Duguid IG, Brown MRW (1991) Surface characteristics and adhesion of *E. coli* and *Staphylococcus epidermidis*. J Appl Bacteriol 71:72–77
37. Giovannacci I, Ermel G, Salvat G, Vendeuvre JL, Bellon-Fontaine MN (2000) Physicochemical surface properties of five *Listeria monocytogenes* strains from a pork-processing environment in relation to serotypes, genotypes and growth temperature. J Appl Microbiol 88:992–1000
38. Gross M, Cramton SE, Götz F, Peschel A (2001) Key role of teichoic acid net charge in *Staphylococcus aureus* colonization of artificial surfaces. Infect Immun 69:3423–3421
39. Heilmann C, Gerke C, Perdreau-Remington F, Gotz F (1996) Characterization of Tn917 insertion mutants of *Staphylococcus epidermidis* affected in biofilm formation. Infect Immun 64:277–282
40. Heilmann C, Hussan M, Peters G, Gotz F (1997) Evidence for autolysin mediated primary attachment of *Staphylococcus epidermidis* to a polystyrene surface. Mol Microbiol 24:1013–1024
41. Helke DM, Somers EB, Wong ACL (1993) Attachment of *Listeria monocytogenes* and *Salmonella typhimurium* to stainless steel and buna-N in the presence of milk and milk components. J Food Prot 56:479–484
42. Hermansson M (1999) The DLVO theory in microbial adhesion. J Colloid Interface Sci 14:105–119
43. Hogt AH, Dankart J, Hulstaert CE, Feijen J (1986) Cell surface characterists of coagulase negative *Staphylococcus* and their adherence to fluorinated poly(ethylenepropylene). Infect Immun 51:294–301
44. Hogt AH, Dakart J, Vries JA, Feijen J (1983) Adhesion of coagulase negative *Staphylococcus* to biomaterials. J Gen Micro 129:2959–2968
45. Holah J, Gibson H (2000) Food industry biofilms. In: Allison D, Gilbert JP, Lappin-Scott H, Wilson M (eds) Community Structure and co-operation in biofilms. pp 211–235
46. Hood SK, Zottola EA (1995) Biofilms in food processing. Food Control 6:9–18
47. Husmark U, Rönner U (1990) Forces involved in adhesion of *Bacillus* spores to solid surfaces under different environmental conditions. J Appl Bacteriol 69:557–562
48. Husmark U, Rönner U (1992) The influence of hydrophobic electrostatic and morphologic properties on the adhesion of *Bacillus* spores. Biofouling 5:335–344
49. Iwabuchi N, Sunairi M, Anzai H, Morisaki H, Nakajima M (2003) Relationships among colony morphotypes, cell surface properties and bacterial adhesion to substrata in *Rodococcus*. Colloids Surf B Biointerfaces 30:51–60
50. Jeong DK, Frank JF (1994) Growth of *Listeria monocytogenes* at 21°C in biofilms with microorganisms isolated from meat and diary environments. Lebensm-Wiss Technol 27:415–424
51. Johl S (1988) Bacterial adhesion to processing surfaces in the meat industry. PhD Thesis, University of Surrey, UK
52. Jones D.S, Adair CG, Mawhinney MW, Gorman SP (1996) Standardization and comparison of the methods employed for microbial cell surface hydrophobicity and charge determination. Int J Pharm 131:8489
53. Jucker BA, Harms H, Zehnder AJB (1996) Adhesion of the positively charged bacterium *Stenotrophomanas (Xanthomonas) maltophilia* 70401 to glass and teflon. J Bacteriol 178:5472–5479
54. Jullien C, Benezech T, Carpentier B, Lebret V, Faille C (2002) Identification of surface characteristics relevant to the hygienic status of stainless steel for the food industry. J Food Eng 56:77–87
55. Kannenberg EL, Carlson RW (2001) Lipid A and *O*-chain *M*odifications cause *Rhizobium* lipopolysaccharides to become hydrophobic during bacteroid development. Mol Microbiol 39:379–391
56. Kim KY, Frank JF (1994) Effect of nutrients on biofilm formation by *Listeria monocytogenes* on stainless steel. J Food Prot 58:246–251
57. Klausen M, Heydorn A, Ragas P, Lambertsen L, Aaes-Jørgensen A, Molin S, Tolker-Nielsen T (2003) Biofilm formation by *Pseudomonas aeruginosa* wild type, flagella and type IV Pili mutants. Mol Microbiol 48:1511–1524
58. Knobloch JKM, Bartscht K, Sabottke A, Rohde H, Feucht H, Mack D (2001) Biofilm formation by *Staphylococcus epidermidis* depends on functional RsbU. An activator of the zyB operon: differential activation mechanisms due to ethanol and salt stress. J Bacteriol 183:2624–2633
59. Kumar CG, Anand SK (1998) Significance of microbial biofilms in food industry: a review. Int J Food Microbiol 42:9–27
60. Lecleroq-Perlat M-N, Lalande M (1994) Cleanability in relation to surface chemical composition and surface finishing of some materials commonly used in food industries. J Food Eng 23:501–517
61. Lerebour G, Cupferman S, Bellon-Fontaine MN (2004) Adhesion of *Staphylococcus aureus* and *Staphylococcus epidermidis*

to Episkin® reconstructed epidermis model and to an inert 304 stainless steel substrate. J App Micro 97:7–16
62. Liu Y, Yang S, Li Y, Xu H, Qin L, Tay J (2004) The influence of cell and substratum suface hydrophobicities on microbial attachment. J Bacteriol 110:251–256
63. Lundén JM, Miettinen MK, Autio TJ, Korkeala HJ (2000) Persistent *Listeria monocytogenes* strains show enhanced adherence to food contact surfaces after short contact times. J Food Prot 63:1204–1207
64. Mafu AA, Roy D, Foulet J, Magny P (1990) Attachment of *Listeria monocytogenes* to stainless steel, glass, polypropylene and rubber surfaces after short contact times. J Food Prot 53:742–746
65. Marshall KC, Stout R, Mitchell R (1971) Mechanisms of the initial events in the absorption of marine bacteria to surfaces. J Gen Micro 68:337–348
66. Meinders JM, Van de Mei HC, Busscher JH (1995) Deposition efficiency and reversibility of bacterial adhesion under flow. J Colloid Interface Sci 176: 329–341
67. Meylheuc T, Giovannacci I, Briandet R, Bellon-Fontaine MN (2002) Comparison of the cell surface properties and growth characteristics of *Listeria monocytogenes* and *Listeria innocua*. J Food Prot 65:786–793
68. Millsap KW, Reid G, van der Mei HC, Bussher H (1997) Cluster analysis of genotypically characterized *Lactobacillus* species based on physicochemical cell surface properties and their relationship with adhesion to hexadacane. Can J Microbiol 43:284–291
69. Montie TC, Doyle-Huntzinger D, Craven RC, Holder IA (1982) Loss of virulence associated with absence of flagellum in an isogenic mutant of *Pseudomonas aeruginosa* in the burned-mouse model. Infect Immun 38:1296–1298
70. Norman RS, Frontera-Suau R, Morris PJ (2002) Variability in *Pseudomonas aeruginosa* lipopolysaccharide expression during crude oil degradation. Appl Environ Microbiol 68:5096–5103
71. Mozes N, Rouxhet PG (1987) Methods for measuring hydrophobicity of micro-organisms. J Microbiol Methods 6:99–112
72. Narendran V (2003) Bacterial attachment to meat surfaces. PhD Thesis, Massey University
73. Norwood DE, Gilmour A (1999) Adherence of *Listeria monocytogenes* strains to stainless steel coupons. J Appl Microbiol 86:576–582
74. Oakley J.D, Taylor KG, Doyle RJ (1985) Trypsin-susceptible cell surface characteristics of *Streptococcus sanguis*. Can J Micro 31:1103–1107
75. O'Toole GA, Kolter R (1998) The initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signaling pathways: a genetic analysis. Mol Microbiol 28:449–461
76. Park KY, So JS (2000) Altered cell surface hydrophobicity of lipopolysaccharide-deficient mutant of *Bradyrhizobium japonicum*. J Microbiol Methods 41:219–226
77. Parker SG, Flint SH, Brooks JD (2003) Physiology of biofilms of Thermophilic *Bacilli*—potential consequences for cleaning. J Ind Microbiol Biotechnol 30:553–560
78. Parker SG, Flint SH, Palmer JS, Brooks JD (2001) Factors influencing attachment of Thermophilic *Bacilli* to stainless steel. J App Microbiol 90:901–908
79. Parment PA, Svanborg-Eden C, Chaknis MJ, Sawant AD, Hagber GL, Wilson LA, Adhearn DG (1992) Hemagglutination (Fimbriae) and hydrophobicity in adherence of *Serratia marcescens* to urinary tract epithelium and contact lenses. Curr Microbiol 25:113–118
80. Paul JH, Jeffrey WH (1985) Evidence for separate adhesion mechanisms for hydrophilic and hydrophobic surfaces in *Vibrio proteolytica*. Appl Environ Microbiol 50:431–437
81. Pedersen K (1990) Biofilm development on stainless steel and PVC surfaces in drinking water. Water Res 24:239–243
82. Pembrey RS, Marshall KC, Schneider RP (1999) Cell surface analysis techniques: what do cell preparation protocols do to cell surface properties? Appl Environ Microbiol 65:2877–2894
83. Peng JS, Tsai WC, Chou CC (2001) Surface characteristics of *Bacillus cereus* and its adhesion to stainless steel. Int J Food Microbiol 65:105–111
84. Poortinga AT, Bos R, Busscher HJ (2001) Charge transfer during *Staphylococcal* adhesion to TiNOX® coating with different specific resistivity. Biophys Chem 91: 273–279
85. Poortinga AT, Bos R, Norde W, Busscher HJ (2002) Electric double layer interactions in bacterial adhesion to surfaces. Surf Sci Rep 47:1–32
86. Pratt LA, Kolter R (1998) Genetic analysis of *E. coli* biofilm formation: roles of flagella, motility, chemotaxis and type I Pili. Mol Microbiol 30:285–293
87. Razatos A, Ong YL, Boulay F, Elbert DL, Hubbell JA, Sharma MM, Georgiou G (2000) Force measurements between bacteria and poly(ethylene glycol)-coated surfaces. Langmuir 16:9155–9158
88. Razatos A, Ong YL, Sharma MM, Georgiou G (1998) Molecular determinants of bacterial adhesion monitored by atomic force microscopy. Proc Natl Acad Sci 95:11059–11064
89. Reid G, Bialkowska-Hobrzanska H, van der Mei HC, Bussher HJ (1999) Correlation between genetic, physico-chemical surface characteristics and adhesion of four strains of *Lactobacillus*. Colloids Surf B Biointerfaces 13:75–81
90. Rijnaarts HHM, Norde W, Bouwer EJ, Lyklema J, Zehnder AJB (1995) Reversibility and mechanism of bacterial adhesion. J Colloid Interface Sci 4:5–22
91. Rijnaarts HHM, Norde W, Lyklema J, Zehnder AJB (1995) The isoelectric point of bacteria as an indicator for the presence of cell surface polymers that inhibit adhesion. Colloids Surf B Biointerfaces 4:191–197
92. Rijnaarts HHM, Norde W, Lyklema J, Zehnder AJB (1999) DLVO and steric contributions to bacterial deposition in media of different ionic strengths. J Colloid Interface Sci 14:179–195
93. Rosenberg M, Gutnick D, Rosenberg E (1980) Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. FEMS Microbiol Lett 9:29–33
94. Sasahara KC, Zottaloa EH (1993) Biofilm formation by *Listeria monocytogenes* utilizes a primary colonizing microorganisms in flowing systems. J Food Prot 56:1022–1028
95. Schwab U, Hu Y, Wiedmann M, Boor K J (2005) Alternative sigma factor σ^B is not essential for *Listeria monocytogenes* surface attachment. J Food Prot 68:311–317
96. Smit G, Kijne JW, Lugtenberg BJJ (1989) Roles of flagella, lipopolysaccharide, and a Caþ2-dependent cell surface protein in attachment of *Rhizobium leguminosarum biovar viciae* to Pea root hair tips. J Bacteriol 171:569–572
97. Smyth CJ, Jonsson P, Olsson E, Soderland O, Rosengren J, Hjerten A, Adstrom T (1978) Differences in hydrophobic surface characteristics of porcine enteropathogenic *E. coli* with or without K88 antigen as revealed by HIC. Infect Immun 22:462–472
98. Sorongon ML, Bloodgood RA, Burchard RP (1991) Hydrophobicity adhesion and surface exposed proteins of gliding bacteria. Appl Environ Microbiol 57:3193–3199
99. Speers JGS, Gilmour A (1985) The influence of milk and milk components on the attachment of bacteria to farm dairy equipment surfaces. J Appl Bacteriol 59:325–332
100. Stepanović S, Ćirković I, Ranin L, Švabić-Vlahović M (2004) Biofilm formation by *Salmonella* spp. and *Listeria monocytogenes* on plastic surface. Lett Appl Microbiol 38:428–432

101. Takehara A, Fukuzaki S (2002) Effect of the surface charge of stainless steel on adsorption behaviour of pectin. Biocontrol Sci 7:9–15
102. Taylor CM, Beresford M, Epton HSA, Sigee DC, Shama G, Andrew PW, Roberts IS (2002) *Listeria monocytogenes rel*A and *hpt* mutants are impaired in surface-attached growth and virulence. J Bacteriol 184:621–628
103. Timmerman CP, Fleer A, Besnier L, DeGraff L, Cremers F, Verhoef J (1991) Characterisation of a proteinaceous adhesion of *Staphylococcus epidermidis* which mediates attachment to polystyrene. Infect Immun 59:4187–4192
104. Tjalsma H, Bolhuis A, Jongbloed JDH, Bron S, van Dijl JM (2000) Signal peptide-dependent protein transport in *Bacillus subtilis*: a genome-based survey of the secretome. Microbiol Mol Biol Rev 64:515–547
105. Tormo MA, Knecht E, Götz F, Lasa N, Penadés JR, (2005) Bap-dependent biofilm formation by pathogenic species of *Staphylococcus*: evidence of horizontal gene transfer? Microbiology 151:2465–2475
106. Trachoo N, Brooks JD (2005) Attachment and heat resistance of *Campylobacter jejuni* on *Enterococcus faecium* biofilm. Pak J Biol Sci 8:599–605
107. Ukukul DO, Fett WF (2002) Relationship of cell surface change and hydrophobicity to strength of attachment of bacterial to cantaloupe rind. J Food Prot 65:1093–1099
108. Vadillo-Rodríguez V, Busscher HJ, Norde W, de Vries J, van der Mei HC (2004) Atomic force microscopic corroboration of bond aging for adhesion of *Streptococcus thermophilus* to solid substrata. J Colloid Interface Sci 278:251–254
109. Vadillo-Rodrìguez V, Busscher HJ, van der Mei HC, de Vries J, Norde W (2005) Role of *Lactobacillus* cell surface hydrophobicity as probed by AFM in adhesion to surfaces at low and high ionic strength. Colloids Surf B Biointerfaces 41:33–41
110. Van de Mei HC, Bos R, Busscher HJ (1998) A reference guide to microbial cell surface hydrophobicity based on contact angles. Colloids Surf B Biointerfaces 11:213–221
111. Van der Mei HC, De Vries J, Buscher HJ (1993) Hydrophobic and electrostatic cell surface properties of thermophilic dairy *Streptococci*. Appl Environ Microbiol 59:4305–4312
112. Van der Mei HC, van de Belt-Gritter B, Busscher HJ (1995) Implications of microbial adhesion to hydrocarbons for evaluating cell surface hydrophobicity 2. Adhesion mechanisms. Colloids Surf B Biointerfaces 5:117–126
113. Van der Wal A, Norde W, Zehnder AJB, Lyklema J (1997) Determination of the total charge in the cell walls of gram-positive bacteria. Colloids Surf B Biointerfaces 9:81–100
114. Vanhaecke E, Remon J-P Mears M, Roes F, Rudder DD, van-Peteghem A (1990) Kinetics of *Pseudomonas aeruginosa* adhesion to 304 and 316-L stainless steel role of cell surface hydrophobicity. Appl Environ Microbiol 56:788–795
115. Van Loosdrecht MCM, Lyklema J, Norde W, Schroa G, Zehnder AJB (1987) Electrophoretic mobility and hydrophobicity as a measure to predict the initial steps of bacterial adhesion. Appl Environ Microbiol 53:1898–1901
116. Van Lossdrecht MCM, Lyklema J, Norde W, Schroa G, Zehnder AJB (1987) The role of bacterial cell wall hydrophobicity in adhesion. Appl Environ Microbiol 53:1893–1897
117. Vatanyoopaisarn S, Nazli A, Dodd CE, Rees CED, Waites WM (2000) Effect of flagella on initial attachment of *Listeria monocytogenes* to stainless steel. Appl Environ Microbiol 66:860–863
118. Veenstra GC, Cremers FFM, van Dijk H, Fleer H (1996) Ultrastructural organization and regulation of biomaterial adhesion of *Staphylococcus epidermidis*. J Bacteriol 178:537–541
119. Verran J, Rowe DL, Boyd RD (2001) The effect of nanometer dimension topographical features on the hygienic status of stainless steel. J Food Prot 64:1183–1187
120. Verran J, Whitehead KA (2006) The effect of surface topography on the retention of microorganisms. Food Bioprod Proc 84(C4):253–259
121. Verran J, Whitehead KA (2006) Assessment of organic materials and microbial components on hygiene surfaces. Food & Bioprod Proc 84(C4):260–264
122. Walker SL, Hill JE, Redman JA, Elimelech M (2005) Influence of the growth phase on adhesion kinetics of *Escherichia coli* D12g. Appl Environ Microbiol 71:3093–3099
123. Whittaker LJ, Klier CM (1996) Mechanisms of adhesian by oral bacteria. Annu Rev Microbial 50:513–552
124. Wiencek MK, Klapes AN, Foegeding PM (1990) Hydrophobicity of *Bacillus* and *Clostridium* spores. Appl Environ Microbiol 56:2600–2605
125. Yang J, Bos R, Belder GF, Engel J, Busscher HJ (1999) Deposition of oral bacteria and polystyrene particules to quartz and dental enamel in a parallel plate and stagnation point flow chamber. J Colloid Interface Sci 220:410–418
126. Zita A, Hermansson M (1997) Effects of bacterial cell surface structures and hydrophobicity on attachment to activated sludge flocs. Appl Environ Microbiol 63:1168–1170
127. Zoltai PT, Zoltola EA, McKay L (1981) Scanning electron microscopy of microbial attachment to milk contact surface. J Food Prot 44:204–208