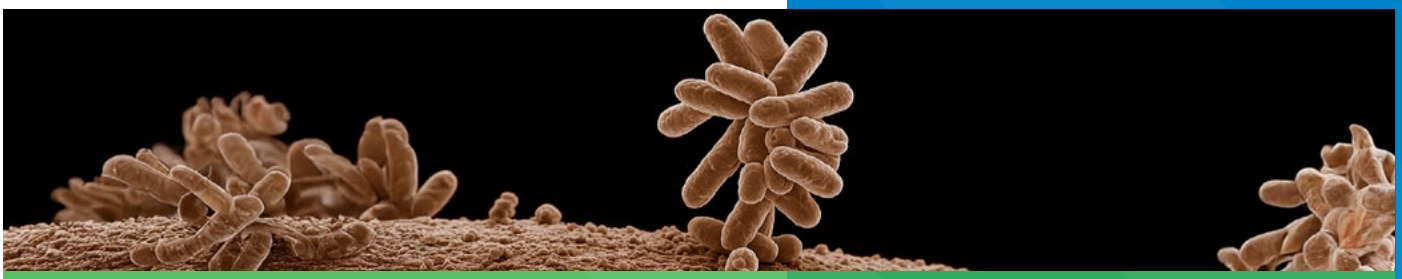

Biofilm Attachment

Surface-Dependent Mechanisms of Biofilm Attachment in Food and Dairy Environments

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Surface-Dependent Mechanisms of Biofilm Attachment in Food and Dairy Environments

Implications for Predicting Hotspots, Enhancing Sanitation, and Targeting Surveillance

Biofilms are persistent and costly challenges in food processing, contributing to recurring contamination, reduced shelf life, and significant food safety risks. Despite their microbiological complexity, biofilms exhibit predictable behaviors in processing environments, providing valuable insights to allow quality assurance (QA) managers to better anticipate and address biofilm-related risks.

This paper focuses on the attachment phase of biofilm formation, where the choice of surface material significantly influences bacterial colonization. We examine how surface properties, such as porosity, hydrophilicity, hydrophobicity, and surface free energy, determine bacterial preferences. Specifically, we discuss why Gram-positive organisms tend to favor hydrophilic, porous substrates, while Gram-negative bacteria prefer moderately hydrophobic materials. The role of surface appendages (pili, flagella) and biochemical factors, including the production of extracellular polymeric substances (EPS), is also explored in depth, linking molecular and cellular mechanisms to biofilm development and persistence.

We also examine how environmental factors such as ionic strength, pH, and the presence of conditioning films further modulate attachment dynamics, bacterial survival, and the progression to more resilient biofilms. We emphasize that biofilm hotspots in food plants are often predictable based on material properties and process conditions, enabling targeted monitoring and sanitation that improve operational efficiency and reduce the costs of contamination and noncompliance.

Introduction

Biofilms remain one of the most persistent and costly challenges for food and dairy processors. When bacteria irreversibly attach to equipment surfaces and become embedded within a matrix of extracellular polymeric substances (EPS), they acquire heightened resistance to cleaning-in-place (CIP) systems, sanitizers, and physical disruption, making them nearly impossible to dislodge.[1] Their presence on food processing equipment threatens product quality and creates food safety risks, conditions that in turn lead to spoilage, economic loss, and tainted brand reputation.[2]

Our previous white papers, *Biofilms in Dairy Processing*[3] and *Quorum Sensing and Biofilms*[4], provided broad overviews of biofilm ecology and communication. The first paper described how biofilms persist in dairy facilities, why they resist conventional sanitation, and the implications for product quality and safety. The second explored how quorum sensing and EPS production enable bacterial communities to coordinate their activity, reinforce attachment, and adapt to environmental stresses. These papers focused on explaining biofilm resilience and practical industry challenges.

This paper takes a different approach. Here, we dive more deeply into the science of biofilm attachment at the molecular, cellular, and physicochemical levels. Rather than simply describing why biofilms persist, we analyze how bacteria interact with specific materials such as stainless steel, polytetrafluoroethylene (PTFE) commonly used as nonstick coatings or gasket materials in food and dairy equipment, ethylene-propylene-diene monomer (EPDM) rubber, and glass. We focus on mechanistic pathways, such as hydrophobic and electrostatic forces, wall teichoic acids, lipopolysaccharides, pili, curli, and extracellular polysaccharides, that determine whether Gram-positive or Gram-negative organisms are more likely to colonize particular surfaces.

The goal of this work is to provide a more rigorous scientific explanation of attachment and to contemplate a predictive capability that may derive from that understanding. By linking biochemical mechanisms to material properties, we can anticipate hotspots where Gram-positive and Gram-negative organisms differ in their propensity to form biofilms in food and dairy processing environments. Understanding this predictive approach requires a deeper scientific engagement, bridging fundamental microbiology with applied sanitation, providing both mechanistic clarity and practical guidance for the industry.

Overview: Mechanisms of Bacterial Attachment

Bacterial adhesion is the first crucial step in biofilm formation. It allows microorganisms to establish a presence on surfaces in both natural and industrial settings, including biotic and abiotic environments. This initial contact results from a complex interplay of physical, chemical, and biological forces that dictates how microbial cells approach, interact with, and ultimately adhere to a surface. The early stages of bacterial attachment are usually divided into reversible or irreversible phases. Each phase involves different molecular interactions and environmental factors that decide whether adhesion becomes permanent. In food processing environments, understanding how these mechanisms function is crucial for preventing and controlling biofilms, which can persist despite thorough cleaning efforts.

Reversible Attachment and the Role of Surface Forces

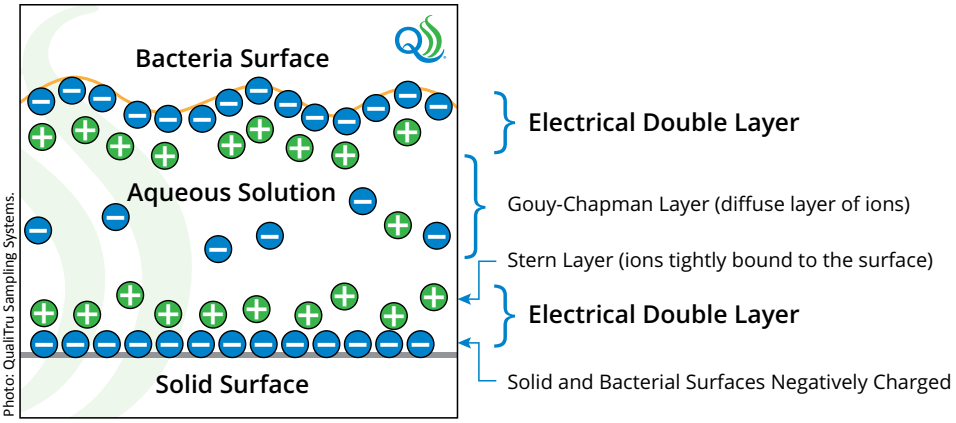
Long-range, non-specific interactions primarily influence the reversible phase of bacterial adhesion. Interactions, including van der Waals attractions, electrostatic forces, and hydrophobic effects (Figure 1), shape the physicochemical environment at the cell-substrate interface.[5] Operating over distances of tens to hundreds of nanometers, these forces change how bacteria respond to nearby surfaces without requiring direct contact.

As explained by van Loosdrecht et al.,[6] van der Waals forces result from temporary dipole-induced dipole interactions between atoms and molecules. While each force is individually weak, they become important when added together across the large surface area of a bacterial cell and the substratum. The strength of the van der Waals attraction depends on the properties of the medium between the surfaces, which is usually water in food processing environments, and the shape of the interacting surfaces. This means that a bacterium moving toward a flat stainless steel surface would likely experience more van der Waals attraction compared to one heading for a curved or irregular polymer surface. This is due to the larger, more continuous contact area, which enhances overall molecular interactions. Van der Waals attractions are always attractive and act between all materials, providing a general baseline of attractive potential that must be balanced by repulsive forces.

Electrostatic interactions, however, are more selective and rely heavily on the charge profiles of both the microbial cell envelope and the substrate surface. Most bacterial cells carry a negative charge due to the presence of ionizable groups, such as carboxyl and phosphate, on their outer membranes.[7,8] Industrial surfaces, such as stainless steel, glass, and certain plastics, also have a net negative charge when hydrated, mainly due to the dissociation of surface hydroxyl groups. This creates a natural electrostatic repulsion that prevents bacteria from approaching the surface closely.

The strength and range of this electrostatic barrier are not constant, however, and depend on the ionic

Figure 1: Interface of Bacterial and Solid Surfaces in Aqueous Solution



This illustration shows the formation of the electrical double layer (EDL) at the interface between a solid surface and a bacterial surface in an aqueous solution. The Stern layer (directly adjacent to the surface) contains tightly bound ions (positive near the negatively charged surface), while the Gouy-Chapman layer (further from the surface) contains more dispersed ions that help balance the charge. The positive ions (e.g., hydrogen, sodium, potassium) are attracted to the negative charges on both the bacterial and solid surfaces, while negative ions (e.g., chloride, hydroxide) remain more spread out in the aqueous phase, leading to an overall charge balance. This structure governs electrostatic interactions and plays a crucial role in bacterial adhesion and surface interactions.

makeup and conductivity of the surrounding medium. For example, in solutions with higher ionic strength, such as those containing divalent cations like calcium or magnesium (e.g., milk or orange juice), the electrical double layers around both the cell and the surface become compressed. This reduces the distance over which repulsion occurs, allowing for a closer approach and enabling van der Waals and hydrophobic forces to take precedence.[9] Thus, changes in the conductivity or salinity of the environment can shift the balance from repulsion to attraction, playing a crucial role in supporting initial adhesion under specific conditions.

Some bacteria have ways to reduce electrostatic repulsion. For instance, *Staphylococcus aureus* and *Listeria monocytogenes* modify their cell walls through surface proteins that alter local charge densities. Similarly, exopolysaccharides produced by bacteria such as *Pseudomonas aeruginosa* or *Escherichia coli* can mask negative charges and create “docking zones” where repulsion is lower. Other organisms, including *Pseudomonas fluorescens* and *Salmonella enterica*, utilize ionic bridging, in which divalent cations such as calcium or magnesium form electrostatic bonds between the negatively charged bacterial surface and stainless steel or glass, thereby enhancing attachment and biofilm stability.[10-12] In dairy environments, this mechanism is particularly relevant for *P. fluorescens*, a frequent milk spoilage organism whose adhesion to stainless steel is strengthened by divalent cations present in milk.

This complex balance of attractive and repulsive forces is often described using Derjaguin–Landau–Verwey–Overbeek (DLVO) theory, developed in the 1940s by Boris Derjaguin, Lev Landau, Evert Verwey, and Theo Overbeek to explain colloid stability in aqueous solutions.[13,14] In this framework, bacteria approaching a surface experience both van der Waals attractions and electrostatic repulsions, which together create an interaction energy landscape. Two types of potential energy drops can form. The secondary minimum is a shallow reduction in potential energy that occurs at a greater distance from the surface, where adhesion is weak and reversible. As cells move closer to the surface, van der Waals attractions increase sharply and begin to outweigh electrostatic repulsion. This state is more thermodynamically stable, resulting in a significantly deeper drop in potential energy. This primary minimum favors strong and essentially irreversible attachment. Cells that reach this primary minimum are much more likely to remain fixed to the surface and progress toward biofilm development.[6,8]

While DLVO theory provides useful predictions of general adhesion trends, it oversimplifies biological complexity by treating bacteria as inert particles and assuming perfectly clean, ideal surfaces. For example, it does not account for structures such as pili or flagella, nor

Overcoming Electrostatic Repulsion in Initial Bacterial Attachment

Even when both the bacterial cell and surface carry net negative charges, bacteria can still attach to surfaces through various mechanisms. While van der Waals forces (weak, transient dipole interactions) play a minor role, the main contributors to attachment are hydrophobic interactions, ion bridging (via divalent cations like Ca^{2+} or Mg^{2+}), and adhesin-mediated binding. These mechanisms help bacteria overcome the initial repulsion caused by electrostatic forces.

For Gram-negative bacteria, hydrophobic interactions are crucial when bacterial surfaces contain hydrophobic regions that interact with hydrophobic sites on surfaces like PTFE or rubber. Additionally, pili and LPS (lipopolysaccharide) help facilitate attachment. For Gram-positive bacteria, surface proteins, including teichoic acids, interact with hydrophilic surfaces, while hydrophobic adhesins facilitate attachment to hydrophobic sites.

Key mechanisms include:

1. **Van der Waals Forces:** Weak, transient dipole-induced dipole interactions can help bacteria approach surfaces, allowing other forces to take over.
2. **Hydrophobic Interactions:** Hydrophobic regions on bacterial surfaces can interact with hydrophobic sites on materials, overcoming electrostatic repulsion and promoting adhesion.
3. **Ion Bridging (Divalent Cations):** Divalent cations like Ca^{2+} or Mg^{2+} can bridge the negative charges on the bacterial cell and surface, neutralizing repulsion and aiding attachment.
4. **Steric Interactions and Adhesin-Mediated Binding:** Bacterial surface proteins such as pili or adhesins can bind to specific receptors or ligands, allowing stronger, more targeted attachment that isn't based solely on electrostatic forces.

While van der Waals forces contribute to the initial attachment, hydrophobic interactions, ion bridging, and adhesin-mediated binding are crucial in overcoming electrostatic repulsion and enabling bacterial adhesion to surfaces.

for the ways bacteria actively alter their surface chemistry and gene expression in response to contact. DLVO theory further ignores the influence of conditioning films, bacterial strain-level variations, and hydrodynamic shear forces common in food and dairy processing systems.[12] These limitations led to the development of extended DLVO models (XDLVO), which include additional interactions such as hydrophobic forces and steric hindrance. Although still approximations, these models provide a more realistic framework for microbial adhesion.

Despite their limitations, DLVO-based models remain valuable because they describe the initial energy landscape that determines whether adhesion is thermodynamically favorable. They help identify conditions where attachment is likely or unlikely, and they allow comparisons of how changes in ionic strength, surface potential, or material type affect adhesion probability. When combined with empirical observations, DLVO and XDLVO models provide a conceptual foundation for strategies aimed at reducing bacterial attachment in food and dairy environments.

Among the additional forces emphasized in extended models, hydrophobic interactions are especially important. The degree of hydrophobicity in both the bacterial cell envelope and the material surface has a strong influence on adhesion. Bacterial hydrophobicity arises from lipids, surface proteins, and extracellular polymers, and it can change in response to growth phase or environmental stress.[15] For example, nutrient limitation often increases hydrophobicity as a survival strategy. Hydrophobic bacteria tend to adhere readily to nonpolar surfaces such as PTFE, polypropylene, and rubber. In contrast, hydrophilic surfaces such as glass or oxidized stainless steel develop hydration layers that act as barriers to hydrophobic contact.

At the molecular level, these hydrophobic interactions are driven by entropy. When two nonpolar surfaces approach each other in water, the ordered water molecules surrounding them are released into the bulk solution, thereby increasing the system's entropy and lowering its free energy. Bos and colleagues[5] point out that this thermodynamic gain promotes closer contact between bacterial cell surfaces and hydrophobic materials. Consequently, cells with greater surface hydrophobicity experience stronger attractive forces on nonpolar substrates. This makes hydrophobicity a key factor in adhesion strength and stability during the early stages of biofilm formation.

Irreversible Attachment: Molecular Anchoring and EPS Secretion

The shift from reversible to irreversible attachment occurs when bacterial surface molecules detect contact and activate cellular processes that anchor the cell securely to a surface (Figure 2). This transition is aided by specialized surface structures that help overcome repulsive forces and establish stable adhesion. Among the most important are fimbriae, pili, flagella, and a wide range of molecules collectively known as adhesins, which include fimbrial and non-fimbrial surface proteins, polysaccharides, and other macromolecules that promote binding.[16-18]

Fimbriae and pili are thread-like protein extensions that project outward from the bacterial cell, enabling close contact and recognition of ligands on abiotic surfaces and host tissues. In Gram-negative bacteria, type I and type IV pili play central roles in attachment and early microcolony formation. Type I pili mediate binding through mannose-specific adhesins. Type IV pili contribute to both adhesion and twitching motility, which allow bacteria to move along surfaces and colonize favorable sites.[19,20] This motility-driven exploration enables the population to spread across surfaces, enhancing the likelihood of successful and stable attachment.

Lacking an outer membrane, Gram-positive bacteria rely more heavily on cell wall-anchored adhesins for irreversible attachment. These adhesins are large surface proteins that selectively bind to environmental ligands, including carbohydrates and proteins associated with contact surfaces. In dairy processing facilities, such ligands may include casein and whey proteins that are left behind due to incomplete cleaning. In meat processing, proteins such as fibronectin

Figure 2: Mechanisms of Type IV Pilus-Mediated Surface Attachment

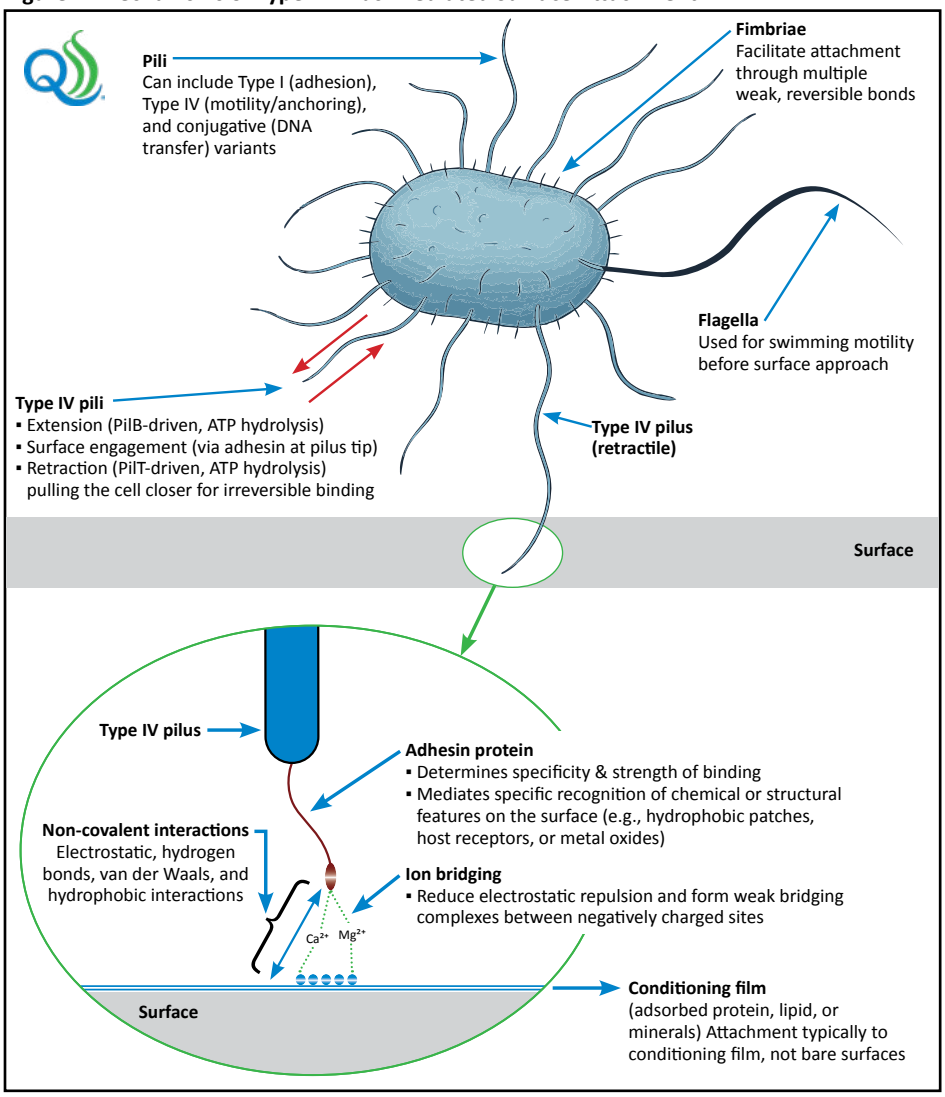


Photo: QualiTru Sampling Systems.

In many Gram-negative food spoilage and pathogenic bacteria, including *Pseudomonas spp.*, *Acinetobacter spp.*, *Aeromonas spp.*, and enteric genera such as *Escherichia coli*, *Salmonella enterica*, *Klebsiella pneumoniae*, and *Enterobacter spp.*, Type IV pili are retractile filaments that extend from the cell surface to engage nearby substrates through a tip-associated adhesin. Both pilus extension and retraction are ATP-dependent processes driven by motor proteins: PilB, an ATPase that powers pilus extension, and PilT, an ATPase that drives retraction to pull the cell into close contact for irreversible binding. Attachment occurs primarily to a conditioning film of adsorbed proteins, lipids, or minerals rather than to bare surfaces. Non-covalent forces, such as electrostatic, hydrogen-bonding, hydrophobic interactions, van der Waals forces, and Ca^{2+}/Mg^{2+} ion bridging, reduce charge repulsion and stabilize contact between the pilus tip and the surface. Fimbriae contribute to early, weak, reversible attachment, while Type I pili promote stable adhesion that reinforces irreversible attachment, and conjugative pili mediate DNA transfer between cells. Together, these surface structures coordinate the transition from reversible to irreversible adhesion that precedes biofilm formation and subsequent maturation.

In contrast, Gram-positive bacteria such as *Listeria monocytogenes* lack pili entirely but employ surface-anchored adhesins, including the Internalin family proteins (InlA, InlB, and related members), which are covalently attached to the cell wall by sortase enzymes. These adhesins mediate direct contact with food-contact surfaces, contributing to their ability to colonize equipment, persist in processing environments, and initiate biofilm formation through non-pilus mechanisms.

or collagen can adhere to equipment surfaces, creating binding sites for bacteria to colonize.[21]

The specificity of adhesin–ligand interactions influences the range of surfaces a bacterium can colonize and contributes to the architecture of early biofilm formation. Importantly, these surface structures are not merely passive binding agents but additionally act as mechanosensors. When fimbriae, pili, or adhesins detect contact with a surface, they trigger regulatory cascades that alter gene expression, including the activation of pathways for EPS production. This initial mechanosensory response is later reinforced by quorum sensing, which synchronizes EPS secretion and other biofilm-related behaviors across the population.[16] By integrating local mechanosensory cues with population-level signaling, bacteria ensure that biofilm formation is both timely and coordinated.

Flagella also play a dual role in surface attachment, functioning as both motility organelles and mechanosensors. Their first role is to propel bacteria toward nutrient-rich or otherwise favorable environments. Once cells encounter a surface, however, the mechanical resistance on the flagellar motor signals the cell that contact has been made, and it is time to upregulate adhesion and early biofilm genes.[22] In several food-related species, including *L. monocytogenes*, *P. fluorescens*, and *Bacillus cereus*, flagellar filaments mediate temporary adhesion interactions with surfaces. These short-lived contacts stabilize cells long enough to establish irreversible attachment.[19,20,23,24] In this way, flagella provide a bridge between transient motility and stable surface colonization.

Following this recognition and anchoring phase, bacterial gene expression shifts decisively toward the production of EPS. Extracellular polymeric substance is a water-rich matrix of polysaccharides, proteins, nucleic acids, and lipids that performs multiple roles, including strengthening adhesion, providing a scaffold for biofilm development, protecting cells from environmental stress, and trapping nutrients to support growth.[25,26] While quorum sensing is not the primary trigger of this transition, it becomes increasingly important as the biofilm matures, coordinating EPS secretion and regulating group behaviors across the community.[4]

The precise composition of EPS differs by species and environmental conditions, but it consistently fulfills its protective and anchoring roles. For example, Colvin et al.[27] note that in *S. aureus*, polysaccharide intercellular adhesin (PIA) is a significant contributor to cell-to-cell adhesion and biofilm cohesion. At the same time, in *P. aeruginosa*, the exopolysaccharides Pel and Psl play critical roles in surface attachment and maintaining structural integrity. Beyond providing bulk structure, EPS also contributes to surface interactions at the molecular level. Components of the matrix can engage in ionic and hydrogen bonding with substrata, reinforcing attachment. Similarly, Gammudi et al.[28] note that EPS molecules may form ionic complexes with divalent metal ions, which can strengthen

surface adhesion or alter local surface chemistry to favor continued colonization. They go on to point out that, in this context, irreversible attachment does not typically involve covalent bonding to the surface but arises from the cumulative effect of many noncovalent interactions—such as ionic, hydrogen, hydrophobic, and van der Waals forces—that together create a highly stable linkage reinforced by the EPS matrix. These chemical interactions highlight the dual role of EPS as both a structural scaffold and a dynamic interface between bacterial cells and their environment.

Environmental Modulation of Attachment Dynamics

Environmental factors such as temperature, pH, ionic strength, and nutrient availability have a strong influence on both reversible and irreversible attachment processes. For example, acidic pH levels can increase the adhesion of certain bacteria by protonating carboxyl groups on the cell surface. This reduces the repulsion between negatively charged surfaces, facilitating a closer approach and allowing attractive forces to establish a more stable attachment. Similarly, high ionic strength compresses the electrical double layer around the cell and the surface, which also permits a closer approach and strengthens adhesion.[9]

Temperature affects bacterial adhesion in several ways, including altering cell membrane fluidity, regulating the expression of adhesion factors, and changing the viscosity of EPS. In dairy and beverage plants, Hareld and Zottola[29] showed that these effects are intensified by CIP cycles, where rapid heating and cooling impose repeated stresses on attached cells. While CIP effectively removes most planktonic cells, sessile bacteria embedded within biofilms or protected in hard-to-clean niches can survive these treatments. Once normal production resumes, these survivors may disperse progeny, and new cells introduced with raw materials or the environment can colonize available surfaces. Together, these processes contribute to persistent contamination despite routine cleaning.[30]

Nutrient availability exerts a complex influence on bacterial attachment. Under nutrient-poor conditions, many species enhance the expression of adhesion proteins and increase EPS production as a survival strategy, ensuring close association with surfaces where scarce nutrients may accumulate.[31,32] Yet once a biofilm matures, prolonged nutrient limitation can have the opposite effect by activating dispersal mechanisms that release cells to search for more favorable environments. In this way, nutrient signals regulate not only the initial decision to attach but also the timing of detachment, highlighting how environmental cues continually shape attachment behavior throughout the biofilm lifecycle.[33]

Surface Conditioning and the Role of Organic Films

By default, this discussion evaluates bacterial adhesion to residue-free native surfaces, since inherent properties such as hydrophilicity, hydrophobicity, and porosity strongly influence biofilm risk. However, when films of proteins, fats, or minerals persist after cleaning, they diminish these differences and make most surfaces colonizable. These conditioning films reduce the relative tendencies exhibited by native surfaces, underscoring the importance of validated cleaning procedures and vigilant surveillance.

Van Houdt and Michiels[34] determined that these conditioning layers, which range in size from nanometers to micrometers, significantly alter the underlying material by modifying surface energy, masking charge, and altering hydrophobicity. In practice, these changes may enhance bacterial adhesion by reducing electrostatic repulsion and altering the local chemical environment. For example, in dairy and food processing environments, residues left on equipment surfaces can alter surface free energy and wettability, creating more hydrophobic conditions that favor bacterial attachment. Sinde and Carballo[35] demonstrated that such changes promote *L. monocytogenes* adhesion by allowing closer contact and localized retention on stainless steel, rubber, and PTFE.

The presence of conditioning films also lowers the accuracy of thermodynamic or surface energy models, which usually assume clean, uncoated materials. Stainless steel is preferred for food manufacturing due to its inertness and smooth finish; however, it becomes more vulnerable to colonization as surface residues accumulate. These films not only improve bacterial retention but may also induce early EPS production and biofilm development by changing microbial gene expression or providing nutrient sources.[15,23,30] This highlights the need for well-designed in-process sampling and environmental monitoring protocols that can detect early microbial attachment before a stable and resistant biofilm community can form.

Surface Chemistry and Surface-Specific Attachment Mechanisms

The surface properties of food contact materials have a profound influence on microbial adhesion and the subsequent development of biofilms. Although bacterial physiology and genetic regulation are critical determinants of biofilm behavior, the interaction between the physicochemical features of the substratum and its surrounding environment often determines whether initial contact results in stable attachment. Surfaces differ in surface energy, hydrophobicity, charge distribution, and topography. These properties determine the balance of attractive and repulsive forces that cells experience when they approach each other.[36-38] Understanding these interactions is particularly important in the context of food and beverage processing environments, where stainless steel, polymers, glass, and elastomeric materials are commonly used and are frequently exposed to fluctuating thermal and chemical conditions.

Biofilm formation, then, is not uniform across materials, but reflects the unique interaction of microbial communities with the physicochemical environment presented by each surface type.

Stainless Steel

Among all materials, stainless steel is of particular importance because of its prevalence in dairy, beverage, and food processing facilities. It has long been

Figure 3. Comparison of hydrophobic and hydrophilic surface properties

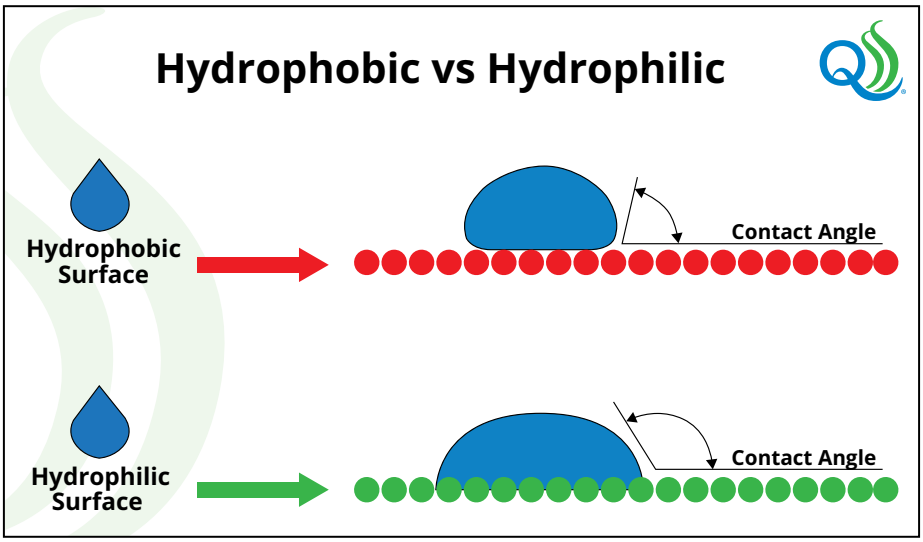


Photo: QualiTru Sampling Systems.

Hydrophobic surfaces repel water, resulting in a high contact angle and a rounded droplet. Hydrophilic surfaces attract water, lowering the contact angle and allowing the droplet to spread. These surface energy differences influence microbial attachment: hydrophobic materials (e.g., PTFE, EPDM rubber) tend to favor adhesion by Gram-positive bacteria, whose cell walls contain nonpolar teichoic acids and surface proteins that interact favorably with other hydrophobic surfaces by displacing interfacial water. In contrast, hydrophilic materials (e.g., stainless steel, glass) are more readily colonized by Gram-negative species, whose outer membranes expose polar, charged lipopolysaccharides that form electrostatic and hydrogen-bonding interactions with high-energy, hydrophilic surfaces.

favored for its corrosion resistance, smoothness, and apparent cleanability. Nevertheless, numerous studies have demonstrated that stainless steel is far from immune to microbial colonization.[23,28]

A balance of surface energy, electrostatic interactions, and microtopography governs the adhesion of bacteria to stainless steel. Early experimental studies by Fletcher[37] demonstrated that despite its reputation as a hygienic surface, stainless steel can readily support microbial attachment when exposed to aqueous environments containing organic matter. This apparent paradox reflects the reality that stainless steel surfaces present complex oxide layers and surface charges that interact with bacterial cell envelopes in ways that favor colonization under certain conditions.

Atomic force microscopy (AFM) studies have provided detailed quantification of the adhesion forces between bacteria and stainless steel. Using this method, Sheng and colleagues[38] demonstrated that electrostatic interactions between negatively charged bacterial surfaces and the positively charged oxide layer of stainless steel play a significant role in the initial attachment process. Ionic strength was observed to enhance adhesion forces, as increased concentrations of ions in solution help neutralize the electrostatic repulsion between the cell and surface, thereby facilitating closer contact and making attachment more likely. Conversely, the presence of organic nutrients initially reduced bacterial adhesion by reducing surface hydrophobicity and altering surface charge characteristics. These conditioning films rapidly become favorable for bacterial attachment, however, as proteinaceous residues and other organic compounds on the surface provide ligands for bacterial adhesion.[39]

Microscopic observations corroborate the role of surface conditioning and oxide chemistry in attachment. Gammudi et al.[28] used epifluorescence and scanning electron microscopy to show that biofilms on AISI 304 and 316 stainless steels are not uniformly distributed but tend to concentrate at imperfections and surface heterogeneities. Extracellular polymeric substances were typically observed at the cell–substratum periphery, underscoring their role in mediating contact with the steel surface. The same study highlighted the ability of sulfate-reducing bacteria and *Pseudomonas spp.* to colonize stainless steel despite its polished finish, suggesting that smoothness alone does not prevent colonization if other conditions favor attachment.

The distribution of bacterial strains also influences the outcomes of adhesion. Mafu et al.[23] demonstrated that *L. monocytogenes* adheres readily to stainless steel, with adhesion patterns influenced by environmental conditions and cell physiology. Similarly, Sinde and Carballo[35] showed that both *Salmonella spp.* and *L. monocytogenes* can adhere strongly to stainless steel, although *Listeria* generally exhibited higher levels of attachment across tested conditions. These findings highlight the significance of bacterial surface properties, including hydrophobicity and surface free energy, in determining adhesion to stainless steel surfaces.

Hydrophobicity plays a dual role (Figure 3). Stainless steel is typically considered less hydrophobic than polymers such as PTFE or rubber; however, contact angle measurements vary depending on the alloy composition, finish, and cleaning treatments. Sinde and Carballo[35] found, for example, that commercial sanitizers increased the surface free energy of stainless steel and reduced bacterial attachment, demonstrating that chemical modification of surface wettability can directly affect colonization potential. In contrast, untreated stainless steel supported significant bacterial attachment, as other factors, including surface charge, conditioning films, and microtopography, facilitated colonization. This apparent dichotomy reflects the multifactorial nature of adhesion, which, while hydrophobicity contributes, also involves electrostatics, conditioning layers, and EPS secretion, all playing critical roles.

The microstructure and topography of stainless steel contribute to bacterial adhesion. Even finely polished surfaces contain microgrooves and imperfections that can provide shelter for cells against shear forces and cleaning treatments. Harimawan et al.[40] emphasized the role of oxide composition and surface roughness in modulating adhesion of different bacterial species. Their findings support the idea that adhesion strength is not simply a function of hydrophobicity or surface free energy, but also of local topographical factors that present niches for colonization.

An additional dimension is provided by the role of EPS–metal interactions. Studies have shown that EPS can form ionic complexes with divalent metal

ions, such as calcium and iron, which accumulate on stainless steel surfaces.[28] These complexes strengthen adhesion and may even contribute to microbially influenced corrosion (MIC), a process by which biofilms accelerate deterioration of stainless steel surfaces. Extracellular polymeric substances, therefore, not only secure cells to the surface but can alter the surface chemistry itself in ways that reinforce biofilm persistence.

More recently, attention has turned to engineered stainless steel surfaces designed to resist colonization. Hage and co-workers[41] reviewed advances in antimicrobial stainless steel surfaces, noting that alloying with elements such as copper or applying nanostructured coatings can reduce bacterial adhesion and biofilm development. Similarly, Friedlander et al.[42] demonstrated that peptide-coated surfaces can disrupt bacterial colonization, suggesting that surface modification may represent a promising strategy for reducing contamination risk in food plants. However, these technologies remain emerging, and practical deployment in industrial settings is still limited.

Taken together, research on stainless steel illustrates the complexity of bacterial adhesion. Surface charge, oxide chemistry, microtopography, conditioning films, and microbial physiology all converge to determine attachment outcomes. While stainless steel remains the industry standard for hygienic design, it is not inherently resistant to biofilm formation. Instead, its performance depends heavily on cleaning protocols, surface modifications, and the broader ecological conditions present in food and beverage processing environments.

Polymers

Polymers are widely used in food processing environments in various forms, including gaskets, seals, conveyor belts, tubing, and more. Their chemical composition and surface energy characteristics differ substantially from stainless steel, which alters bacterial attachment dynamics. Generally, polymers tend to be more hydrophobic, and this property significantly influences bacterial colonization. Among polymers, PTFE is notable for its extremely low surface free energy, making it highly hydrophobic and resistant to wetting by aqueous solutions.

Bacterial adhesion to PTFE is consistently higher than to stainless steel when measured under comparable conditions. When studying the adhesion of *Salmonella spp.* and *L. monocytogenes* to various surfaces, Sinde and Carballo[35] observed that both adhered more strongly to PTFE than to stainless steel, reflecting the preference of bacterial cells for hydrophobic surfaces. The same study showed that rubber surfaces also supported higher bacterial loads than stainless steel, with PTFE ranking as the most adherent material. These findings align with thermodynamic predictions, which suggest that surfaces with lower free energy present more favorable conditions for reducing surface tension between cells and surfaces.[36]

The high affinity of bacteria for hydrophobic polymers is a function of surface chemistry, surface roughness, and microtopography. Fletcher[37] demonstrated that even subtle variations in the smoothness of polymer surfaces can alter adhesion patterns. The ability of hydrophobic materials to adsorb organic residues further complicates their behavior. In food processing environments, PTFE and other polymers may become coated with proteins and lipids from food products, forming residual conditioning layers that mask intrinsic surface properties and create new interfaces that can enhance bacterial attachment.[49]

Despite their susceptibility to microbial attachment, materials like PTFE can be more easily sanitized than stainless steel under certain conditions. Sinde and Carballo[35] reported that PTFE showed the most significant reduction in bacterial attachment after exposure to commercial sanitizers compared to rubber or stainless steel. This paradox may be because conditioning films do not adhere as strongly to PTFE, allowing sanitizers to remove attached cells more effectively. Nevertheless, the initial attachment potential of PTFE and similar polymers underscores the risk associated with their widespread use in food handling systems.

Glass

The physicochemical properties of glass differ from those of both stainless steel and polymers. Glass is generally hydrophilic, with a relatively high surface free energy, and exhibits a smooth, non-porous topography when clean.

Early studies provided insight into the adhesion behavior of bacteria on glass surfaces. Fletcher[37] showed that bacterial attachment to glass varied according to cell taxonomy and environmental conditions, with hydrophobic organisms attaching more poorly to glass than to polymers or hydrophobic surfaces. The hydrophilic character of glass creates a less favorable environment for hydrophobic interactions, which are often a dominant force in bacterial adhesion. Nevertheless, hydrophilic interactions and electrostatic attraction can still allow colonization under certain pH or ionic strength conditions.

Microscopic observations further demonstrate that bacterial adhesion to glass is not uniform. Fletcher[37] further noted that roughness and scratches on glass surfaces could act as preferential attachment sites, despite the material's overall smoothness. Conditioning films also play a role here. For example, Barnes et al.[39] demonstrated that milk proteins can readily adsorb to hydrophilic materials, such as glass, thereby altering their surface energy and providing ligands for bacterial adhesins. Once such films are established, glass may support bacterial colonization at levels comparable to other food contact materials.

Although glass surfaces are less commonly used in modern dairy and beverage plants, their study has been essential for establishing baseline principles of microbial adhesion. Comparing bacterial behavior on glass, polymers, and stainless steel highlights the significance of hydrophobicity and the chemical modifications introduced by conditioning layers in influencing adhesion outcomes.

Rubber

Elastomeric materials, such as ethylene-propylene-diene monomer (EPDM) rubber, are widely used in food processing equipment, particularly in

Table 1. Biofilm Attachment Quick Reference — Materials in Food and Dairy Plants

Material	Surface character	Primary adhesion drivers	Relative adhesion	Conditioning film effect	Sanitizer response	Hotspots / notes	QA/monitoring actions
Stainless steel (AISI 304/316)	Hydrophilic oxide layer; charge varies w/ pH/cleaners; smooth but microgrooved	Electrostatics (strength ↑ w/ double-layer compression); conditioning films; microdefects; EPS–metal ion complexes	Baseline (↓ PTFE/rubber; ↑ when residues present)	Protein/fat films quickly mask surface and ↑ ligands for adhesion	Some sanitizers ↑ surface free energy and ↓ attachment; effectiveness depends on residues	Imperfections, welds, scratches; EPS at periphery; micro induced corrosion risk via Ca2+/Fe2+ complexes	Prioritize sampling at welds/defects/Δ flow; verify residue removal
PTFE and similar polymers	Highly hydrophobic; very low surface free energy; smooth	Hydrophobic interactions; rapid protein /lipid film formation	Highest among listed materials (often > SS, glass, rubber)	Readily adsorbs proteins/lipids; films drive attachment despite native hydrophobicity	Larger sanitizer effect vs. Rubber/ SS; films detach more readily	Gaskets, valves, tubing, belts; initial attachment high even when clean	↑ surveillance; validate sanitizer contact time; inspect for film removal and re-formation
Rubber (e.g., EPDM)	Moderately–strongly hydrophobic; rougher; microcrevices	Hydrophobic interactions; rapid protein film formation; microdefects	Intermediate (PTFE > rubber > stainless ≈ glass when clean)	Rapid, persistent protein films (casein/whey) facilitate attachment	Less effect vs. PTFE; porosity and elasticity can limit efficacy	Gaskets, seals; niches resist CIP; reservoir for recolonization	Short inspection & replacement intervals; aggressive CIP and validate; aggressive monitoring
Glass	Hydrophilic; high surface free energy; smooth but scratches possible	Electrostatics at certain pH/ionic strength; conditioning films overcome resistance	Lower when clean → rises with films of scratches	Milk proteins readily adsorb → ↑ attachment to levels comparable to other materials	Effective when films minimal; scratches can harbor cells	Scratched zones; legacy equipment; less common as food-contact today	Check for scratches; ensure film removal; decommission damaged pieces

tubing and gaskets, where flexibility is crucial. Rubber is generally more hydrophobic than stainless steel and less hydrophobic than PTFE. This characteristic influences bacterial colonization. Sinde and Carballo[35] demonstrated that *L. monocytogenes* and *Salmonella spp.* adhered more readily to rubber than to stainless steel, placing rubber between stainless steel and PTFE in terms of adhesion potential.

The hydrophobicity of rubber promotes the adsorption of organic matter, leading to the rapid formation of conditioning layers in processing environments. Barnes et al.[39] observed that milk proteins, including caseins and whey proteins, readily coat rubber surfaces, facilitating bacterial attachment. This makes rubber particularly problematic in dairy processing, where residual protein films are common.

Surface roughness also plays a role. Finishes on rubber components are often less uniform, providing micro-crevices where bacteria can evade shear forces and cleaning agents. Mafu et al.[23] reported that *L. monocytogenes* adhered firmly to food contact surfaces with microdefects, a finding directly applicable to rubber seals and tubing. Such imperfections can shelter sessile cells during CIP procedures, allowing them to survive and recolonize surfaces once processing resumes.

Sanitizing rubber surfaces presents unique challenges. Although some sanitizers reduce bacterial adhesion by altering surface energy, the porosity and elasticity of rubber can limit their effectiveness. Sinde and Carballo[35] found that reductions in bacterial adhesion on rubber were less pronounced than on PTFE following sanitizer treatment, suggesting that cleaning protocols may need to be more aggressive for rubber components. The persistence of bacterial cells within the microstructure of rubber highlights its vulnerability as a contamination source in processing lines.

When considered together, polymers, glass, and rubber demonstrate the critical role of surface hydrophobicity and free energy in bacterial adhesion. Polymers such as PTFE, with very low surface free energy, favor hydrophobic interactions and show the highest bacterial attachment. Glass, with its hydrophilic properties, generally resists hydrophobic adhesion but can still be colonized once conditioning films form. Rubber surfaces are intermediate. In their native state, rubber surfaces support higher levels of colonization than stainless steel or glass, but lower than PTFE (Table 1).

Conditioning layers, particularly protein films, are a unifying factor that diminishes intrinsic material differences by providing new interfaces that bacteria can exploit. These films are formed as proteins, lipids, and other organic molecules from the product or environment adsorb onto surfaces, creating new physicochemical conditions that alter surface charge, hydrophobicity, and availability of ligands for bacterial adhesins, thereby promoting stable bacterial attachment. Unlike transient layers that adsorb during processing and are swept away during cleaning, conditioning films persist even after cleaning and can become nearly impossible to remove once dried. These persistent films provide bacterial binding sites, supporting biofilm formation, which makes subsequent cleaning more challenging and necessitates careful monitoring.[23]

Thermodynamics, Free Energy, and Electrostatic Considerations

The adhesion of bacteria to surfaces is often described in terms of surface free energy and thermodynamic favorability. Absolom et al.[36] developed a thermodynamic model of bacterial adhesion based on changes in surface free energy. According to this model, bacterial adhesion is favored when contact with a surface results in a reduction of the system's free energy. Hydrophobic materials, which have relatively low initial surface free energy, experience a greater relative decrease in free energy when bacteria attach. This means that, although the total drop in free energy may not be larger,

the relative reduction in free energy is greater for hydrophobic surfaces than for hydrophilic ones, making adhesion to hydrophobic surfaces more thermodynamically favorable.

In practical terms, the surfaces encountered in food processing environments vary widely in this regard. Glass and stainless steel are generally hydrophilic, with relatively high surface free energies that make them less favorable for hydrophobic interactions. On the other hand, PTFE and EPDM are strongly hydrophobic, characterized by low surface free energies and high-water contact angles. This difference helps explain why polymers like PTFE and EPDM tend to support higher bacterial attachment than glass or stainless steel.

Electrostatic interactions further complicate the thermodynamic picture. Sheng et al.[38] demonstrated that ionic strength, pH, and nutrient conditions significantly influence the adhesion forces between bacterial cells and stainless steel surfaces. At higher ionic strengths, the electrical layer surrounding both cells and surfaces is compressed. This reduces repulsive forces, allowing for a closer approach. At pH values near the bacterial isoelectric point, adhesion is enhanced because electrostatic repulsion is minimized. In practical terms, this means that environments rich in salts or minerals, such as cheese brines, whey residues, or certain cleaning solutions, can increase ionic strength, reduce electrostatic barriers, and promote bacterial adhesion to equipment surfaces. These findings underscore that adhesion is best understood as the outcome of multiple overlapping hydrophobic, electrostatic, and steric forces operating in a given environment.

Implications for Food and Beverage Processing

The persistence of bacterial attachment across a wide range of surfaces has significant implications for food safety and quality. The ability of bacteria to colonize stainless steel, polymers, rubber, and glass underscores that no material is inherently "safe" from biofilm formation. Instead, contamination risk is managed through design, cleaning, ongoing surveillance, and operational practices.

Hygienic design principles emphasize minimizing crevices, using materials with smooth finishes, and selecting surfaces that are more easily cleaned. Nevertheless, studies repeatedly show that sessile bacteria can survive CIP treatments, particularly when protected within microdefects or conditioning films.[43,44] Survivors act as reservoirs for colonization once processing resumes.

The comparative behavior of materials also informs equipment choices. Stainless steel, while not immune to colonization, supports lower bacterial adhesion than rubber or PTFE and is therefore preferred when possible. However, where polymers or elastomers are unavoidable, more aggressive surveillance, cleaning, and preventive maintenance protocols may be necessary.

Ultimately, the challenge of biofilm control lies not in selecting a single material that resists adhesion, but in understanding the interplay between surface properties, environmental conditions, and microbial physiology. Thermodynamic models provide a helpful framework, but the decisive factors are often biological and include the ability of bacteria to adapt, secrete EPS, and exploit conditioning films. Emerging engineered surfaces may offer new tools, but for now, comprehensive sanitation strategies remain the most reliable defense against persistent biofilms in food processing facilities.

Table 2. Physiological drivers of surface attachment by Gram class and actionable QA implications

Attachment Driver	Gram-Positive	Gram-Negative	QA/Monitoring Implications
Cell Envelope	Thick peptidoglycan (PG) with WTAs/LTAs; highly polar, net negative; sortase-anchored adhesins.	Thin PG in periplasm; outer membrane (OM) + LPS (Lipid A, core, O-antigen); embedded OM proteins.	Different contact chemistries: expect different material preferences and responses.
Primary attachment forces	Electrostatic interactions with surface; hydrogen bonding via teichoic acids; specific sortase-anchored adhesins.	Hydrophobic interactions with plastics/elastomers; OM proteins/adhesins; electrostatics modulated by LPS and ions.	Match sampling sites to likely forces: polar/oxide sites for Gram+; hydrophobic/low-energy for Gram–.
Dominant appendages	Flagella; pili in select taxa; sortase-anchored adhesins confer specificity.	Type I/IV pili, fimbriae, flagella; autotransporters, two-partner systems, chaperone-usher assembly.	Under flow, expect pili-mediated "grappling"; flagella act as mechanosensors that kick off EPS production.
Surface preference (clean)	Polar/hydrophilic: stainless steel (SS), glass, oxide-coated metals.	Hydrophobic: PTFE, PP, PE, EPDM/rubber; can also adhere to SS when LPS exposes adhesins.	Prioritize sampling on polymers/elastomers for Gram–; welds/oxidized SS/glass for Gram+.
Conditioning film effect	Protein/fat films ↑ adhesion (ligands + cation bridging).	Films ↓ barriers & expose/anchor adhesins; rough LPS variants ↑ adhesion.	Validate removal of protein/fat films; include post-CIP swabs on high-risk parts.
Adaptive strategies	Hydrophobic adhesins; EPS to mask chemistry; WTA D-alanylation/glycosylation tune charge.	LPS smooth↔rough switching (O-antigen length); curli, Pel/Psl EPS systems; OM protein regulation.	Expect strain/state variability— "now you see it, now you don't." Build trend-based monitoring, not one-offs.
Ionic strength & pH	Ca^{2+}/Mg^{2+} bridge WTAs to surfaces; ↓ pH reduces repulsion.	Ca^{2+}/Mg^{2+} stabilize LPS, compress double layers → ↑ adhesion.	Track hardness/salinity (brines, whey, cleaners). Adjust CIP setpoints and sanitizer selection accordingly.
Sanitizer response (typical)	Effective if clean of films; rough elastomers remain problematic.	PTFE often largest ↓ post-sanitizer vs. rubber/SS; biofilm matrix resists.	Pre-rinse to strip films, follow with sanitizer with verified contact time; rotate chemistries.
Common plant hotspots	Welds, scratches, SS valves, deadlegs, gaskets near SS; cold rooms for Listeria.	Gaskets, PTFE valves, tubing, belts, polymer housings, stagnant low-flow areas; drains.	Map by material + flow + residue; increase frequency where those overlap.
Representative taxa	*Listeria, Staphylococcus, Enterococcus, Corynebacterium.*	*Pseudomonas, Acinetobacter, Salmonella, E. coli.*	Tune testing accordingly.

Physiological Factors Influencing Bacterial Attachment

Bacterial attachment to surfaces is not a uniform process but one shaped by the fundamental structural and biochemical differences between major taxonomic groups. The transition from reversible contact to irreversible adhesion reflects the combined influence of cell envelope architecture, extracellular appendages, and secreted polymers. These features determine not only whether a bacterium can adhere at all, but also the types of surfaces it preferentially colonizes and the stability of the resulting biofilm. In food and beverage processing environments, where surfaces range from stainless steel and glass to plastics, elastomers, tiles, or concrete, these differences have direct implications for contamination control. Understanding the distinct adhesion strategies of Gram-positive and Gram-negative organisms provides a framework for predicting where persistent biofilms are most likely to develop.

Although all bacteria must solve the same basic challenge of overcoming repulsive forces at the cell–surface interface, they do so with tools that reflect their specific cell wall organization. Gram-positive organisms rely on their thick peptidoglycan layers and surface polymers, such as teichoic acids, to generate strong electrostatic interactions that are reinforced by sortase-anchored adhesins, which confer biochemical specificity. Gram-negative organisms, by contrast, operate with a thinner peptidoglycan layer shielded by an asymmetric outer membrane rich in lipopolysaccharides and embedded proteins. This arrangement favors interactions with hydrophobic substrates and provides multiple pathways for adhesin secretion and appendage assembly. By examining these contrasting physiological strategies in detail, the following discussion highlights how structural biology at the cellular level translates into persistent biofilm challenges at the industrial scale.

For practical purposes, the distinction between Gram-positive and Gram-negative organisms is not about which group adheres "better" to surfaces overall, but instead that each group shows characteristic preferences and patterns of attachment. These patterns reflect differences in their cell surface structures and adhesion strategies, which translate into consistent tendencies to colonize specific types of niches (Table 2). The guidance for QA managers is therefore parallel rather than comparative. The goal is not to rank one group against the other, but to recognize where each is more likely to be found when contamination arises and where to focus attention to prevent contamination from occurring.

Gram-Positive Bacterial Attachment Mechanisms

The Cell Envelope and Surface Chemistry

The Gram-positive cell envelope (Figure 4) consists of a thick peptidoglycan layer that provides structural integrity and protection to the bacterial cell. This peptidoglycan is typically 30-40 layers deep and is heavily cross-linked, making it rigid and capable of withstanding environmental stresses. Embedded within the peptidoglycan matrix are wall teichoic acids (WTAs), which are covalently attached to the peptidoglycan, and lipoteichoic acids (LTAs), which are anchored in the cytoplasmic membrane beneath the peptidoglycan and extend outward through it.[11,45-47] Both WTAs and

Spore Attachment — A Passive but Persistent Strategy

While much of the discussion of attachment focuses on vegetative cells, spore-forming genera such as *Bacillus*, *Geobacillus*, *Alicyclobacillus*, and *Clostridium* introduce a different persistence strategy.

Spores are not active colonizers. Their protective coats lack adhesins and appendages, and their largely passive interaction with surfaces is governed by hydrophobic, electrostatic, and van der Waals forces. However, this passive adhesion, combined with extreme resistance to cleaning, heat, and sanitizing agents, allows spores to remain embedded in microscopic niches, crevices, or polymeric fittings long after vegetative cells are eliminated. Once favorable conditions return, spores germinate into vegetative cells, which then engage in true adhesion and biofilm development.

This survival-first strategy explains why spore-forming organisms are among the most persistent contaminants in food and beverage processing environments, even when biofilm control measures appear effective.

Figure 4: Gram-Positive Cell Envelope

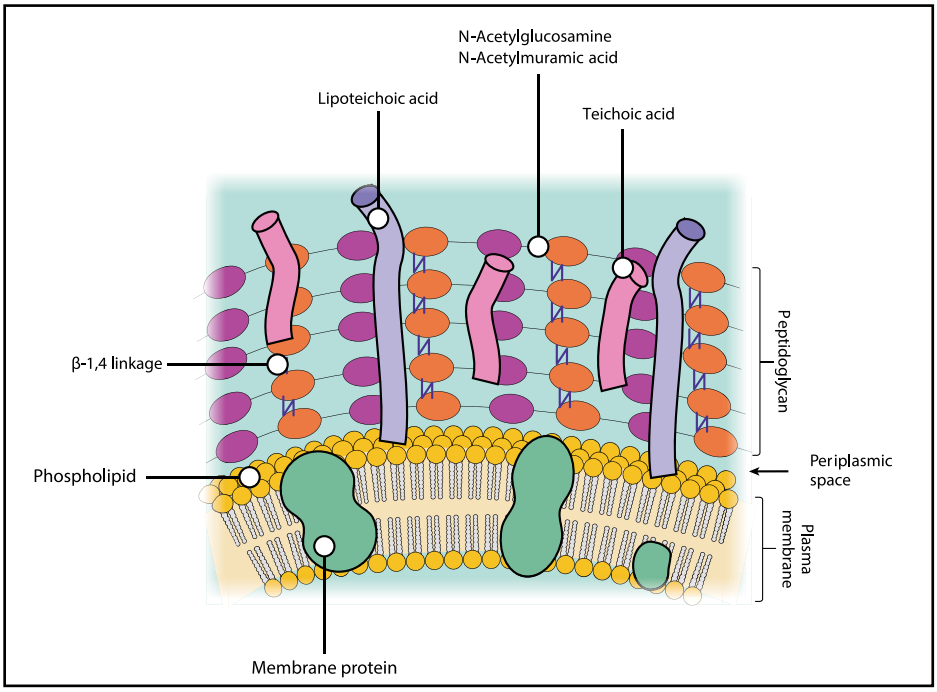


Photo: Bass stock/Shutterstock

Diagram of Gram positive bacteria envelope, including teichoic and lipoteichoic acids, membrane and proteins.

LTAs are polymers typically consisting of repeating glycerol or ribitol phosphate units. Their phosphate-rich backbones impart a net negative surface charge and strong hydrophilicity, which underpin the cell's tendency to interact with polar, hydroxylated surfaces such as glass, stainless steel, or other oxide-coated metals.

These interactions with abiotic surfaces are not purely passive. Divalent cations, such as calcium and magnesium, can act as bridges between phosphate groups in the teichoic acids and hydroxyl groups on surfaces, thereby strengthening adhesion and stabilizing otherwise weak electrostatic attractions. This bridging is particularly relevant in dairy and beverage plants, where hard water and dairy residues provide a steady supply of divalent ions that can facilitate attachment.

Furthermore, WTAs are not static polymers. Biochemical modifications, particularly D-alanylation (the addition of D-alanine to teichoic acids), can fine-tune surface charge, influencing both the rate and strength of adhesion. D-alanine esters reduce the net negative charge of teichoic acids, minimizing repulsion between the bacterial surface and negatively charged materials.[11,48] Additionally, glycosylation (the attachment of glucose molecules) of teichoic acids alters the spatial arrangement of surface molecules, either shielding adhesins or exposing them, which affects their ability to bind to other molecules.[49] These modifications act as "surface switches," allowing Gram-positive bacteria to regulate adhesion in response to environmental conditions.

Sortase-Anchored Adhesins

While teichoic acids create a general electrostatic environment that facilitates initial contact between bacteria and surfaces, sortase-anchored proteins add biochemical specificity to adhesion. Sortases are a family of membrane-associated transpeptidases responsible for covalently attaching surface proteins to the peptidoglycan cell wall, thereby stabilizing them in an orientation favorable for substrate binding.[21] These enzymes recognize a conserved LPXTG motif (Leucine–Proline–any amino acid–Threonine–Glycine) at the C-terminus of target proteins. Upon recognition, the sortase cleaves the peptide bond between threonine and glycine, forming an acyl-enzyme intermediate. Through a series of reactions, the resulting protein is incorporated into the peptidoglycan layer. As a result, the processed proteins are permanently displayed on the bacterial surface as adhesins. By covalently linking adhesins to the cell wall, sortase enzymes ensure both stability and accessibility, enabling strong and specific interactions with abiotic molecules such as stainless steel-bound proteins, mineral residues, or other conditioning film components. This molecular anchoring mechanism is therefore indispensable for the transition from reversible attachment to stable biofilm formation.[50,51]

Environmental cues can tightly regulate adhesin expression. In *L. monocytogenes*, for example, factors such as temperature, osmolarity, nutrient availability, and ionic strength all influence the transcription of surface proteins. In separate reports, Janež et al.[49] and Bierne and Cossart[52] demonstrated the ecological flexibility of *L. monocytogenes* by noting that adhesins involved in virulence are often downregulated at body temperature but upregulated under cooler ambient conditions, such as those commonly found in food processing environments. Additionally, the glycosylation of WTAs can influence adhesin accessibility, linking modifications in the cell wall polymers to changes in protein presentation. These factors help explain why the same bacterial strain may exhibit different attachment behaviors depending on the environmental context.

Motility Structures: Flagella and Pili

Although less common in Gram-positive bacteria than in their Gram-negative counterparts, flagella and pili provide valuable contributions to attachment. In *L. monocytogenes*, flagella are predominantly expressed at temperatures below 30 °C, precisely those encountered in most food-processing environments.[53] These structures facilitate not only motility but also the first stage of biofilm development, when reversible surface contact is essential. Mutations in flagellin genes have been shown to alter biofilm initiation and architecture, underscoring their dual role in both propulsion and adhesion.[54]

Pili (fimbriae) are unevenly distributed among Gram-positive taxa. When present, they extend the reach of bacterial cells, allowing for weak yet strategic initial interactions with surfaces. Bacteria such as *Enterococcus spp.* and *Corynebacterium spp.* express pili that play a key role in these early contacts.[55] While these interactions are initially weak, pili facilitate the transition to stronger adhesion by enabling the bacterial cells to anchor more effectively as they express additional adhesins. Over time, this initial weak attachment can be reinforced through the action of surface proteins or exopolysaccharides, contributing to the formation of a stable biofilm. In this way, pili are crucial for initiating colonization, providing the foundation for stronger adhesin-mediated attachment that follows.

Flagella and pili also function as mechanosensors, detecting contact with a surface and transmitting signals that activate biofilm-related gene expression. Upon surface contact, flagellar rotation may stall, generating intracellular signals that increase the production of adhesins, EPS, and stress-response factors.[19] This sensory function is complemented by interactions with quorum sensing systems, allowing bacterial populations to coordinate their transition from planktonic to sessile growth.[4,56,57] Thus, these appendages bridge the gap between physical attachment and biochemical adaptation.

Adaptation to Hydrophobic Surfaces

Although Gram-positive organisms preferentially colonize polar, hydrophilic surfaces, they can adapt to the hydrophobic polymers and elastomers common in food processing environments. Colonization of these low-surface-energy materials requires additional effort. To do so, bacteria must synthesize adhesins with hydrophobic domains or secrete exopolysaccharides that mask surface chemistry. These strategies incur a significant energetic cost, but theyenable survival on substrates that would otherwise resist Gram-positive adhesion. When present, conditioning films of fats or proteins further reduce the barrier. This adaptive flexibility explains why Gram-positive biofilms persist across

diverse environments and why controlling Gram-positive bacterial contamination in food and beverage plants remains challenging despite cleaning and sanitation efforts.[8,35,58]

Gram-Negative Bacterial Attachment Mechanisms

The Cell Envelope as a Dynamic Surface

Gram-negative bacteria are structurally distinct from Gram-positive organisms in ways that profoundly influence their adhesion strategies. The Gram-negative cell envelope (Figure 5) is a multilayered system comprising the inner cytoplasmic membrane, a thin yet resilient peptidoglycan layer, and an asymmetric outer membrane that contains LPS. This architecture provides both a physical barrier and a chemically diverse surface for interactions with abiotic materials.

The outer LPS layer is central to the way Gram-negative bacteria interact with surfaces. Each LPS molecule has three parts. (1) Lipid A is a hydrophobic anchor that embeds the molecule into the outer membrane. (2) The core oligosaccharide, composed of short chains of sugars such as heptose and mannose derivatives (e.g., 3-deoxy-D-manno-octulosonic acid, or KDO), provides structural stability and links lipid A to the outermost region of the cell envelope. (3) The O-antigen is a repeating chain of sugars that can vary dramatically in length and composition. These sugars may include glucose, galactose, rhamnose, or unusual deoxy- or amino-sugars, and their diversity explains why O-antigen structure varies so widely between species and even among strains of the same species. This variation in O-antigen structure strongly influences surface charge, hydrophobicity, and the extent to which the O-antigen extends from the cell surface.[59,60]

The same bacterial species can exist in either a smooth or rough form, reflecting phenotypic variation in LPS that influences how cells interact with their environment. When bacteria produce smooth LPS with long O-antigen chains, the extended sugars form a hydrated, negatively charged shield. This barrier masks adhesins and hydrophobic membrane regions, reducing the ability of cells to make direct contact with either hydrophilic surfaces, such as stainless steel, or hydrophobic materials like plastics.

The net effect is a cell surface that is less adhesive but more protective, helping cells resist environmental stress or immune attack. By contrast, in bacteria with rough LPS, which lack these extended sugar chains, the underlying hydrophobic domains and surface proteins are exposed. This enhances adhesion, particularly to hydrophobic substrates such as plastics or rubber, which are common in food processing environments, and can facilitate stronger interactions with polar surfaces when adhesins are unmasked. The smooth–rough distinction, therefore, reflects a biological tradeoff. Smooth LPS protects at the expense of adhesion, whereas rough LPS promotes colonization but leaves the cell more vulnerable.

This dual capacity has important implications for food safety. Smooth LPS variants of pathogens such as *Salmonella* or *E. coli* are less adhesive but more resistant to environmental stress, allowing them to survive sanitation cycles more effectively. Rough variants, by contrast, adhere readily to hydrophobic equipment surfaces and initiate biofilm formation more quickly and efficiently. Although rough cells are more vulnerable as free (planktonic) cells or during the earliest stages of attachment, once incorporated into a biofilm, they are equally protected and difficult to eradicate. The practical consequence is that pathogens are unpredictable; the same strain may pass through one day without adhering strongly, yet on the next produce variants that colonize aggressively—the classic "now you see it, now you don't."

Spoilage organisms, such as *Pseudomonas* and *Acinetobacter*, also possess both smooth and rough LPS forms. In food plant environments, however, they typically behave in a more consistently adhesion-oriented manner, largely because their O-antigens are shortened, leaving adhesins and hydrophobic regions exposed. Abundant EPS and surface-active polymers further reinforce these more chemically active regions. As a result, spoilage organisms more reliably create quality problems, while pathogens are more dangerous precisely because they can behave in multiple ways, making them harder to predict and control. For processors, the safest assumption is that pathogenic Gram-negative bacteria are always capable of persisting, and sanitation

Figure 5: Gram-Negative Cell (*E. coli*) Envelope

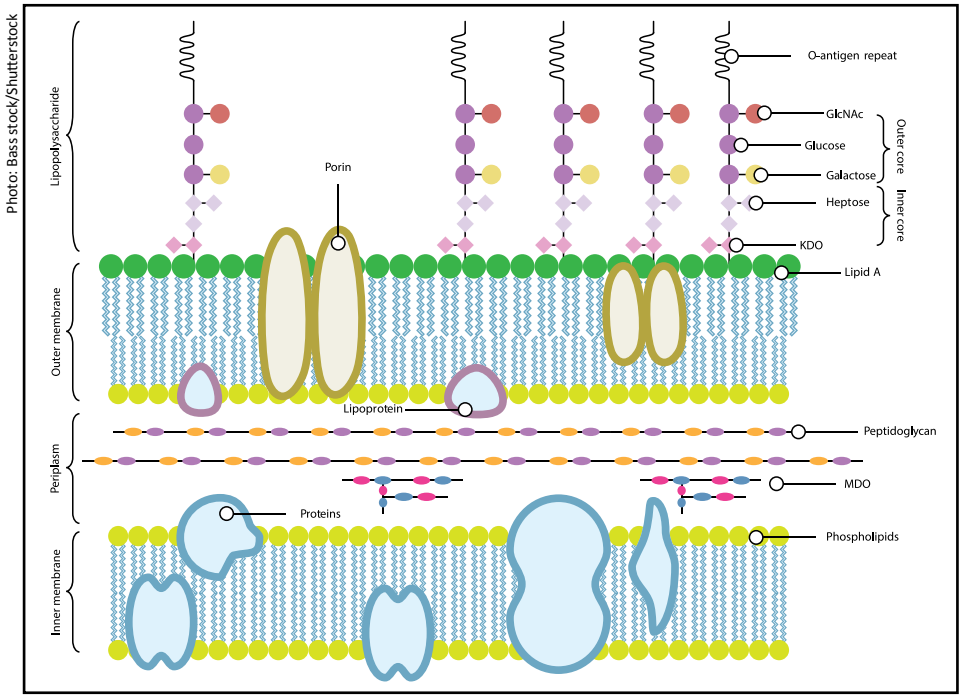


Diagram of Gram-negative bacterium (*E. coli*) cell envelope - inner, outer membrane, LPS, peptidoglycan.

and environmental monitoring programs must be designed with this unpredictability in mind.[27,35,60]

This capacity to vary surface chemistry provides Gram-negative bacteria with remarkable flexibility. Even small genetic or regulatory changes in LPS biosynthesis can alter surface affinity, giving populations an adaptive advantage when encountering new materials in food processing environments.

Electrostatics, Ionic Strength, and Adhesion

Electrostatic interactions are fundamental in Gram-negative attachment, but ionic conditions strongly modulate their effect. Both bacterial surfaces and common food-contact materials carry net negative charges, creating an initial electrostatic barrier to adhesion. High concentrations of divalent cations such as Ca^{2+} or Mg^{2+} reduce this repulsion by neutralizing surface charges. These ions can also stabilize LPS structure and act as molecular bridges between negatively charged groups on cells and surfaces.[35]

This explains why Gram-negative adhesion patterns can differ between low- and high-ionic-strength environments (Table 3). In dilute aqueous conditions, electrostatic repulsion dominates and inhibits attachment, while in nutrient-rich or hard-water environments, adhesion efficiency increases. Such context dependence underscores the importance of considering not only material properties but also process water and food chemistry when assessing contamination risks.

Outer Membrane Proteins and Secretion Systems

Whereas Gram-positive bacteria rely heavily on sortase-anchored proteins, Gram-negatives deploy adhesins through a variety of secretion systems, including autotransporters, two-partner secretion systems, and the chaperone-usher pathway.[56] Each mechanism delivers adhesins across the outer membrane and displays them at specific sites along the cell surface where they are best positioned to initiate attachment.

Autotransporters are single-protein systems with two functional parts. At the C-terminal end of the protein, a β-barrel forms a hollow cylinder that embeds in the outer membrane. This creates a pore through which other parts of the protein can pass. The second functional protein segment, known as the passenger domain, traverses the hollow center of the barrel and emerges on the outside of the cell. Once exposed, the passenger domain folds into its active shape and functions as the adhesin.

Two-partner secretion systems introduce an additional level of complexity. In these systems, one protein forms a translocation pore in the outer membrane while a separate partner protein passes through and is displayed externally as the adhesin. This division of labor enables the secretion of larger or more specialized adhesins that could not be produced by an autotransporter alone.

The chaperone-usher pathway, in contrast, assembles long, filamentous structures such as pili and fimbriae. In this system, periplasmic "chaperones" guide individual pilin protein subunits to a separate usher protein in the outer membrane. The usher protein then polymerizes these subunits into a fiber that extends outward from the cell. These pili not only increase the physical reach of bacteria but also provide multiple binding sites, making them especially effective for initiating contact with both biotic and abiotic surfaces.

The activity of these secretion pathways is tightly regulated by environmental signals. In *Escherichia coli*, for instance, curli fibers—extracellular ropelike protein structures (amyloid structures)—are produced only under specific conditions of nutrient limitation and lower temperature. Curli enhance adhesion to hydrophobic surfaces and strengthen cohesion within biofilms, making them especially important for persistence in the cool, nutrient-limited environments often found in food processing facilities.[56]

Cellular Appendages

Fimbriae and pili are arguably the most characteristic attachment structures of Gram-negative bacteria. Functioning as long, filamentous appendages that reach beyond the cell body, these organelles

Table 3. Effect of pH and Ionic Strength on Bacterial Adhesion of Pseudomonas fluorescens

Condition	Adhesion Tendency	Notes
Low pH (pH 5.5)	High	Increased hydrophobicity and reduced electrostatic repulsion promote bacterial adhesion.
Neutral pH (pH 7.0)	Moderate	Electrostatic forces balance; moderate adhesion occurs due to both repulsion and attraction.
High pH (pH 8.5)	Low	Increased electrostatic repulsion weakens adhesion, limiting bacterial attachment.
Low Ionic Strength (0.001 M KNO_3)	Low	Dominant DLVO repulsion prevents bacterial approach, limiting adhesion.
Moderate Ionic Strength (0.01 M KNO_3)	High	Charge screening reduces repulsion, allowing optimal bacterial adhesion through electrostatic and hydrophobic interactions.
High Ionic Strength (0.1 M KNO_3)	Slightly Reduced	Further compression of the electrical double layer and possible steric hindrance reduce adhesion, despite decreased repulsion.

Adhesion is maximized under moderately acidic conditions and intermediate ionic strength (0.01 M KNO_3), where electrostatic repulsion is minimized and hydrophobic interactions are enhanced. These findings illustrate how subtle shifts in environmental parameters can strongly influence initial surface attachment.

Adapted from: Rochex A, Godon J-J , Bernet N, Escudie R. Role of shear stress on composition, diversity and dynamics of biofilm bacterial communities. *Water Research*, 2008;42:4915-4922.

facilitate initial contact with abiotic surfaces. Under flow conditions, such as those found in piping systems or tanks, they play vital roles in helping bacteria withstand hydrodynamic shear.

As noted earlier, Type I pili attach to surfaces by binding to mannose residues anchored to the surface.[19] This attachment can be blocked, however, when free mannose is present in the environment because it competes for the same cellular binding sites. In food processing facilities, where sugar residues are commonly found in conditioning films, this competitive binding can determine whether bacteria adhere strongly or remain loosely attached. Type IV pili are more versatile. In addition to their role in twitching motility, surface exploration, and biofilm initiation, these pili have the unique ability to extend, attach, and retract. This allows cells to "grapple" surfaces and then pull themselves into closer proximity, increasing the likelihood of stable adhesion.[27]

The mechanical flexibility of pili is vital in food processing environments, where surfaces are rarely pristine. Pili can establish weak but persistent interactions with conditioned materials, allowing cells to survive CIP cycles or flowing liquid until stronger adhesins or extracellular polymers can consolidate attachment.

Flagella are primarily known for their role in motility, but in Gram-negative bacteria, they also serve as adhesive and sensory structures. The rotation of flagella brings bacterial cells into transient contact with surfaces. When this rotation is hindered by surface collision, it triggers signaling pathways that can alter gene expression. This mechanosensory function has been shown to stimulate the production of adhesins, EPS, and stress-response factors, thereby accelerating the transition from planktonic to surface-associated growth.[19]

Flagella serve not only as propellers but also as adhesive and sensory organelles. In species such as *Salmonella* and *Pseudomonas*, flagellin proteins interact directly with abiotic surfaces, providing a mechanical foothold during the early stages of biofilm formation. Through their combined functions in movement, surface detection, and attachment, flagella equip cells with a versatile system for establishing biofilms in complex environments.

Exopolysaccharides and Cell Signaling

The maturation of Gram-negative biofilms depends heavily on exopolysaccharide production. Among the best-characterized systems are the Pel and Psl polymers of *P. aeruginosa*. Pel is a cationic, glucose-rich polymer that binds strongly to anionic extracellular DNA. Divalent cations, such as Ca^{2+} and Mg^{2+}, further stabilize this interaction by bridging the negatively charged sites on multiple DNA strands to each other and to the abiotic surface. This binding of Pel to DNA and subsequent cross-linking of DNA helps reinforce the biofilm matrix, making it more stable and resistant to environmental stresses.

Psl, on the other hand, is a neutral, mannose-rich polymer that assembles into fibrous scaffolds on surfaces and between cells, reinforcing both initial adhesion and intercellular cohesion. Together, Pel and Psl act like complementary building materials. Pel provides the 'cement' that locks cells together, while Psl creates the 'scaffolding' that anchors the community to surfaces. This synergistic function, achieved through the interaction of chemically distinct polymers, provides *P. aeruginosa* biofilms with both stability and flexibility as they mature. This helps explain why *P. aeruginosa* biofilms are notoriously resistant to cleaning and disinfection in industrial settings.[27,57,59]

Attachment in Gram-negative bacteria is not only structural but also regulatory, with many appendages and surface components acting as signaling platforms that integrate both mechanical and chemical information from the environment. For instance, the retraction of Type IV pili activates cyclic-di-GMP signaling pathways, which are central regulators in the transition to biofilm growth.[27] Elevated cyclic-di-GMP levels lead to increased adhesin expression and EPS production, anchoring cells in a sessile state. Other surface structures, such as flagella and fimbriae, similarly contribute to initial attachment and the subsequent regulation of biofilm formation.

Quorum sensing further coordinates these behaviors at the population level. In *Pseudomonas aeruginosa*, quorum signals regulate the expression of virulence factors, surface appendages, and matrix components, ensuring synchronized attachment and biofilm maturation across the community. These complex signaling networks tightly link surface contact, gene regulation, and multicellular behavior, orchestrating the biofilm lifecycle.[4]

It is important to distinguish between the mechanisms of initial adhesion and those that govern biofilm persistence. Initial attachment depends primarily on surface chemistry and adhesins, while persistence involves processes such as exopolysaccharide elaboration, quorum sensing, and resistance to shear or sanitizers. Once initial contact is secured, these persistence traits determine whether contamination remains transient or develops into a mature, resilient biofilm.

Strain-Level Variability

Just as surface chemistry varies among Gram-positive species due to differences in their surfactome composition, Gram-negative bacteria exhibit considerable variability at the strain level, particularly in their outer membrane proteins, LPS, and pili. For example, strains of *P. aeruginosa* differ in the relative production of Pel, Psl, and alginate, altering both adhesion strength and resistance to cleaning agents.[27,57] Similarly, *E. coli* strains vary in curli expression, influencing their capacity to persist on hydrophobic plastics or stainless steel under nutrient-limited conditions.[56] Such differences, however, usually do not change the general surface preferences dictated by Gram classification. Gram-negatives still favor hydrophobic substrates. These differences, however, influence whether a given strain is capable of forming a biofilm under specific process conditions.

Gram-negative bacteria employ an integrated strategy for surface attachment that reflects their complex envelope structure and regulatory networks. LPS composition establishes

a dynamic surface chemistry, while secretion systems precisely deliver adhesins. Pili, fimbriae, and flagella initiate both reversible and irreversible contact, acting as mechanical grappling hooks and as sensory organs. Exopolysaccharides such as Pel and Psl consolidate attachment into robust biofilm matrices, while signaling pathways ensure coordinated community behavior.

This multi-layered approach allows Gram-negative organisms to colonize a broad spectrum of materials, with a notable preference for hydrophobic surfaces. In food processing environments, their adaptability poses a persistent challenge, as local variations in surface conditioning, water chemistry, and strain-specific traits can dramatically affect biofilm formation. Recognizing these mechanistic underpinnings is essential for predicting contamination risks and designing effective cleaning, surveillance, and control strategies.

Conclusion

The mechanisms by which Gram-positive and Gram-negative bacteria attach to surfaces reflect their fundamental structural differences yet converge on a common outcome: the capacity to persist in food processing environments despite cleaning and sanitation efforts. Gram-positive organisms rely heavily on their thick peptidoglycan walls, teichoic acids, and sortase-anchored adhesins to establish strong, specific bonds with polar surfaces such as stainless steel and glass. Gram-negative organisms, on the other hand, utilize their asymmetric outer membrane, LPS, and secretion systems to favor interactions with hydrophobic substrates, such as polyethylene, PTFE, or conditioned plastics.

These general taxonomic patterns are further shaped by motility structures, extracellular polymers, and regulatory systems that fine-tune adhesion according to environmental conditions. Flagella and pili act not only as physical tools for attachment but also as sensors that activate biofilm-related gene networks. Exopolysaccharides consolidate these early interactions into structured communities, while modifications to wall polymers or LPS adjust surface chemistry on demand. Strain-level variation adds another layer of complexity, determining not only how strongly a given isolate adheres but also whether it forms a biofilm at all.

For food processors, the practical significance lies in recognizing that the risk of surface contamination is not uniform. Polar, hydrophilic materials may preferentially harbor Gram-positive organisms such as *L. monocytogenes* or *S. aureus*, whereas hydrophobic plastics and elastomers may favor Gram-negative species like *P. aeruginosa* or *Salmonella*. Local conditions, ranging from ionic strength and organic residues to temperature and nutrient availability, can alter these patterns.

Understanding the biochemical and physical foundations of attachment, therefore, provides more than academic insight. It should guide the development of cleaning, monitoring, and prevention strategies tailored to the taxa most likely to persist on particular surfaces. By anticipating where Gram-positive and Gram-negative organisms are most prone to establish biofilms, processors can better allocate resources, refine sanitation protocols, sharpen monitoring strategies, and target interventions that disrupt adhesion at its earliest stages.

The Hierarchy of Chemical Bonds in Biofilm Attachment

When bacteria first encounter a surface, the strength of the chemical forces involved determines whether adhesion remains reversible or progresses to stable, mature biofilm formation. Each bond type corresponds to a stage in biofilm development:

- **Van der Waals forces (weakest):** Weak attractions caused by brief shifts in electron distribution around atoms; these fleeting forces let bacteria make the first reversible "touch" with a surface.
  - *Analogy*: Like static cling between clothes fresh from the dryer.
  - *Biofilm stage*: Reversible attachment — cells can still be easily dislodged.
- **Hydrogen bonds:** Moderate-strength attractions that form when a hydrogen atom already linked to oxygen or nitrogen is drawn toward another nearby electronegative atom. These stabilize interactions between bacterial polymers and hydroxyl-rich surface films.
  - *Analogy*: Like hook-and-loop fasteners holding fabric together — stronger than static, but still easy to peel apart.
  - *Biofilm stage*: Early stabilization — stronger than van der Waals alone, but detachment is still possible.
- **Ionic bonds:** Stronger electrostatic attractions between oppositely charged groups; these help anchor negatively charged bacterial cell walls to positively charged sites on metals or proteins on the surface.
  - *Analogy*: Like two magnets snapping together — attachments are strong, but the pull weakens when pH or temperature changes alter the environment.
  - *Biofilm stage*: Irreversible attachment — cells are firmly bound and begin secreting extracellular polymers.
- **Covalent bonds (strongest):** Very strong bonds created when atoms share electrons; while rare in direct adhesion, they provide permanence in bacterial cell walls and extracellular polymers that cement biofilms.
  - *Analogy*: Like welding metal pieces together — essentially permanent under normal conditions; requires enzymes or strong chemical reactions to disrupt covalent bonds.
  - *Biofilm stage*: Maturation and persistence — covalent linkages in EPS and cell walls stabilize the biofilm; enzymatic cleavage later enables dispersion

Takeaway: Biofilm formation follows this progression—from weak, reversible forces that initiate contact to strong, covalent-linked polymers that make biofilms persistent and resistant.

Strength ladder (weakest → strongest):

Van der Waals → Hydrogen → Ionic → Covalent

Glossary of Biofilm Terms for Food and Dairy Processors

Acyl-Homoserine Lactones (AHLs): Signaling molecules used by Gram-negative bacteria in quorum sensing. AHLs bind to receptors on bacterial cells, initiating gene expression related to biofilm formation, virulence, and other collective behaviors.

Adhesins: Surface proteins or structures (fimbrial or non-fimbrial) that allow bacteria to attach to surfaces or other cells. They act like molecular 'glue.'

Autoinducer-2 (AI-2): A signaling molecule used by both Gram-negative and Gram-positive bacteria for interspecies communication. AI-2 is involved in regulating quorum sensing and coordinating behaviors like biofilm formation and virulence.

Biofilm formation phases: The stages in the development of a biofilm, including reversible attachment, irreversible attachment, production of extracellular polymeric substances (EPS), biofilm maturation, and dispersal or detachment of cells.

Biofilm dispersal: The active release of bacterial cells from a mature biofilm, often triggered by nutrient limitation or environmental stress, enabling colonization of new surfaces.

Casein and whey proteins: Major dairy proteins that can form residues on processing surfaces, acting as conditioning films and providing ligands for bacterial attachment.

Cleaning-in-place (CIP): An automated cleaning system used in food and beverage plants to sanitize equipment without disassembly. Biofilms often resist CIP treatments.

Conditioning films (or conditioning layers): Thin layers of proteins, fats, minerals, or other organic materials that remain on surfaces after cleaning. These films can mask the surface's natural properties, creating favorable conditions for bacterial attachment and biofilm formation.

Conjugative pili (sex pili): Specialized pili in Gram-negative bacteria that mediate direct cell-to-cell contact. They form a physical bridge through which plasmids and other genetic material can be transferred, enabling horizontal gene transfer. Traits passed this way may include tolerance to sanitizers, resistance to heavy metals, enhanced metabolic pathways, or virulence factors — all of which can strengthen biofilm persistence in food plant environments.

Covalent bonds: Strong chemical bonds formed when atoms share electrons. In biofilm attachment, covalent bonds can be involved in the anchoring of adhesins to bacterial surfaces, especially in Gram-positive bacteria where sortase enzymes mediate the covalent attachment of surface proteins to the cell wall, strengthening bacterial adhesion to surfaces. Strongest chemical bond.

Cyclic-di-GMP: A bacterial signaling molecule that regulates the switch from free-floating to biofilm lifestyles. High levels promote surface adhesion and EPS production.

Curli: Amyloid fibers produced by some Enterobacteriaceae that function as adhesins, reinforcing attachment and biofilm cohesion.

DLVO theory: Derjaguin–Landau–Verwey–Overbeek theory, a model explaining how electrostatic repulsion and van der Waals attraction influence bacterial adhesion.

Electrostatic interactions: Attractive or repulsive forces between charged bacterial surfaces and charged substrata. Important in determining whether bacteria approach or are repelled by a surface.

Electrical double layer: A layer of charged ions surrounding both bacteria and surfaces, creating electrostatic repulsion. Its thickness depends on ionic strength of the solution.

E**PS-degrading enzymes:** Enzymes that break down extracellular polymeric substances (EPS) in biofilms. These enzymes can facilitate the removal of biofilms by degrading the matrix, making bacteria more susceptible to sanitizers and physical disruption.

Exopolysaccharides: Long-chain carbohydrate polymers secreted by bacteria that form a crucial component of the biofilm matrix. These polysaccharides typically consist of repeating sugar units such as glucose, galactose, mannose, rhamnose, and acetylated sugars. Exopolysaccharides promote bacterial attachment to surfaces, contribute to biofilm cohesion, and protect bacteria from environmental stresses.

Extracellular Polymeric Substances (EPS or EPS Matrix): A complex mixture of biopolymers secreted by microorganisms, primarily bacteria, that form the structural matrix of biofilms. EPS typically includes polysaccharides, proteins, lipids, and extracellular DNA (eDNA). The EPS Matrix serves several functions, including providing physical stability to the biofilm, facilitating bacterial attachment to surfaces, protecting embedded bacteria from environmental stresses (e.g., antimicrobial agents, desiccation, UV radiation), and allowing communication within the biofilm community through mechanisms like quorum sensing.

Fimbriae: Thin, hair-like surface appendages on bacteria that enable attachment to surfaces or host tissues. Often interchangeable with pili.

Flagella: Whip-like appendages used for motility, which can also act as mechanosensors signaling surface contact and aiding initial adhesion.

Gram-positive / Gram-negative: Two categories of bacteria based on cell wall structure. Gram-positives have thick peptidoglycan layers and teichoic acids. Gram-negatives have thinner walls plus an outer membrane with lipopolysaccharides.

Hydrogen bonds: Weak electrostatic (charge-based) attraction between a partially positively charged hydrogen atom (that is covalently bonded to a highly electronegative atom like oxygen or nitrogen) and a nearby lone pair of electrons on another electronegative atom. In biofilm attachment, hydrogen bonds can form between bacterial surface molecules (such as proteins or polysaccharides) and the surface of materials, contributing to bacterial adhesion. Stronger than van der Waals forces; weaker than ionic bonds.

Hydrophobic interactions: Attractions that occur when nonpolar bacterial surfaces interact with nonpolar materials, driven by the release of ordered water molecules.

Hydrophilicity / Hydrophobicity: Surface characteristics influencing water interactions. Hydrophilic surfaces attract water, while hydrophobic surfaces repel water.

Ionic bonds: Electrostatic interactions between positively and negatively charged ions. In biofilm attachment, ionic bonds can form between charged bacterial surface components (like teichoic acids in Gram-positive bacteria) and the charged surfaces of materials, helping to stabilize attachment, especially when divalent cations (like Ca2+ or Mg2+) are present. Stronger than hydrogen bonds; weaker than covalent bonds.

Lipopolysaccharides (LPS): Molecules in the outer membrane of Gram-negative bacteria. Smooth LPS contain O-antigen chains; rough LPS lack them, influencing adhesion behavior.

Mechanosensing: The ability of bacterial structures such as pili or flagella to detect physical contact with a surface and trigger biofilm-related gene expression.

Microbially induced corrosion (MIC): Deterioration of metals, such as stainless steel, accelerated by microbial activity, often due to EPS–metal ion interactions in biofilms.

Motility structures: Appendages like flagella and pili that allow bacteria to move toward surfaces and facilitate initial attachment. These structures also help bacteria "sense" the surface and trigger the transition from reversible to irreversible attachment.

Nutrient limitation: Scarcity of nutrients that can trigger bacteria to enhance adhesion and EPS production, or in mature biofilms, to disperse cells.

Pel and Psl polysaccharides: Exopolysaccharides of Pseudomonas aeruginosa. Pel acts as a 'cement' locking cells together; Psl forms 'scaffolding' that anchors biofilms to surfaces.

Polysaccharide intercellular adhesin (PIA): A major EPS component in Staphylococcus aureus biofilms, contributing to cohesion and cell-to-cell adhesion.

Quorum sensing: Bacterial communication using chemical signals to coordinate behaviors like EPS production, adhesion, and biofilm maturation.

Quorum sensing inhibitors (QSIs): Chemical compounds that interfere with bacterial communication systems, specifically quorum sensing, which regulates biofilm formation and other collective behaviors. QSIs can disrupt biofilm development and reduce bacterial virulence.

Reversible vs. irreversible attachment: Early adhesion can be weak and reversible, but once bacteria activate anchoring mechanisms and EPS secretion, attachment becomes stable and irreversible.

Sortase-anchored adhesins: Surface proteins in Gram-positive bacteria that are covalently attached to the cell wall by sortase enzymes. These adhesins play a key role in bacterial attachment to surfaces, particularly in the formation of biofilms.

Sortase enzymes: Gram-positive enzymes that covalently anchor adhesins to the cell wall, making them available for stable contact with surfaces.

Steric hindrance: Physical blocking caused by bulky molecules or polymers that prevent close contact between bacterial cells and surfaces.

Surface free energy: A measure of a material's tendency to interact with liquids or other substances. High surface free energy tends to favor hydrophilic interactions, while low surface free energy favors hydrophobic interactions, influencing bacterial adhesion.

Teichoic acids (wall and lipoteichoic acids): Phosphate-rich polymers in Gram-positive bacteria that extend outward from the cell wall, contributing to surface charge and adhesion properties.

Type I pili: Fimbrial appendages in Gram-negative bacteria that bind specifically to mannose residues on surfaces or host tissues.

Type IV pili: Fimbrial appendages in Gram-negative bacteria that mediate adhesion and twitching motility, aiding surface colonization.

Van der Waals forces: Weak attractions between molecules caused by temporary dipoles. Individually weak but collectively significant for bacterial adhesion.

XDLVO theory: Extended DLVO models that include hydrophobic and steric interactions in addition to electrostatics and van der Waals forces, providing a more realistic framework.

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Clarence Johnson (retired) spent the most recent 25 years of his career founding and managing three biotechnology/medical technology companies that developed and marketed proprietary technology to enhance cardiovascular, respiratory, and skeletal muscle health. In his early career, Mr. Johnson spent 10 years working with dairy companies to solve quality control mysteries and teach food microbiology to dairy plant operators in the U.S. and abroad. He was an early adopter of Hazard Analysis and Critical Control Point quality management and carried the principles into dairy production. Mr. Johnson has advanced degrees in microbiology and biochemistry from the University of Minnesota, holds 12 patents, and has published extensively in scientific, medical, and lay publications.

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