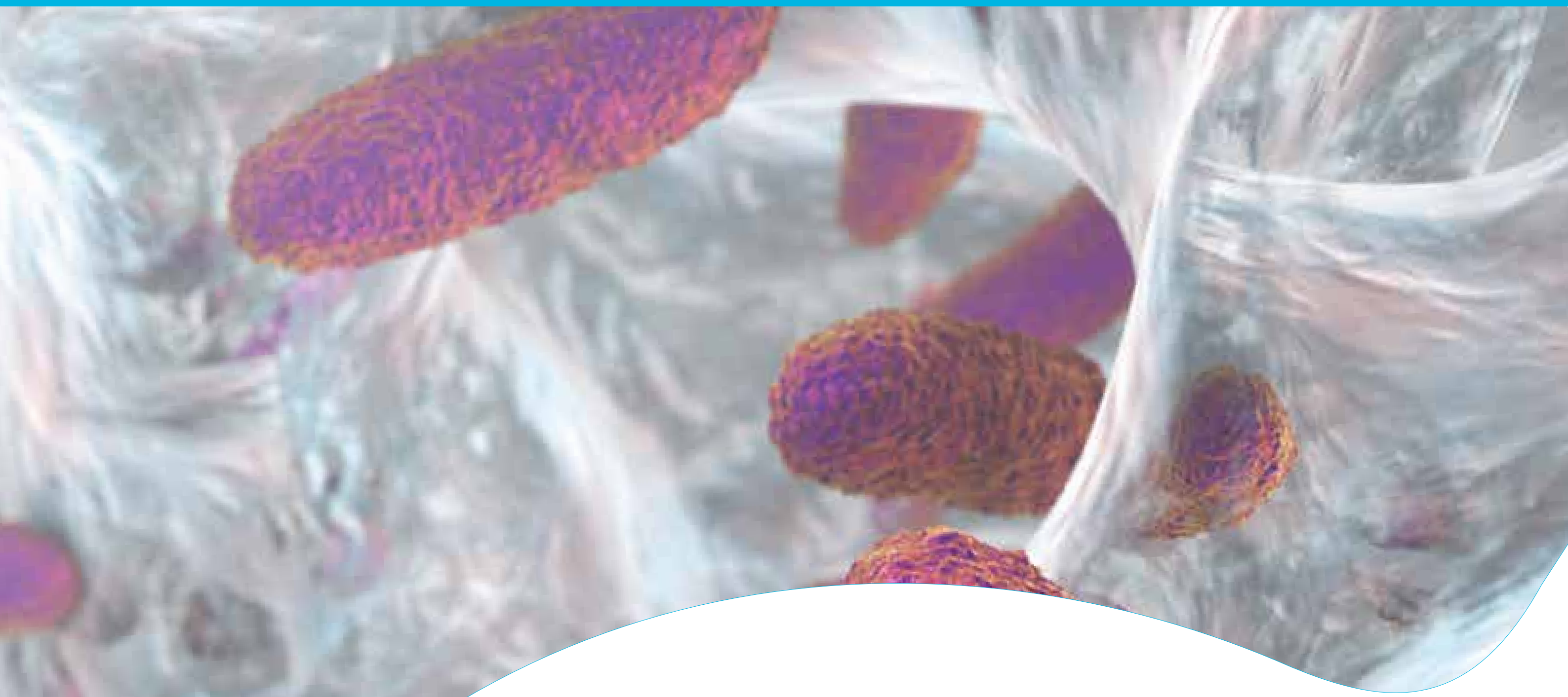

COMPONENTS & FUNCTIONS OF BACTERIAL BIOFILMS

One of the major concerns in the field of hygiene in the food industry is the formation of bacterial biofilms. Biofilms are groups of micro-organisms that accumulate in a solid-liquid interface and are surrounded by a mucilaginous matrix.[1] The biofilm matrix is the extracellular material, produced by micro-organisms themselves, in which are embedded the cells of the biofilm. It consists of a conglomeration of different types of biopolymers - known as Extracellular Polymeric Substances (EPS) - which form the support for the three-dimensional structure of the biofilm. The formation of biofilms allows cells a life form completely different from the planktonic state, protecting them from adverse environments and facilitating their survival.

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The formation of biofilms on surfaces follows a sequential process shown in Figure 1, which starts with the adhesion of cells to the surface. This gives rise to the formation of microcolonies, which induces phenotypic changes in the grouped cells, thus helping these cells to adapt to the new environment and begin to produce EPS to form a biofilm matrix and make it grow. The mature biofilm has a structure that extends perpendicularly to the surface, often mushroom-shaped, with channels through which water can circulate.

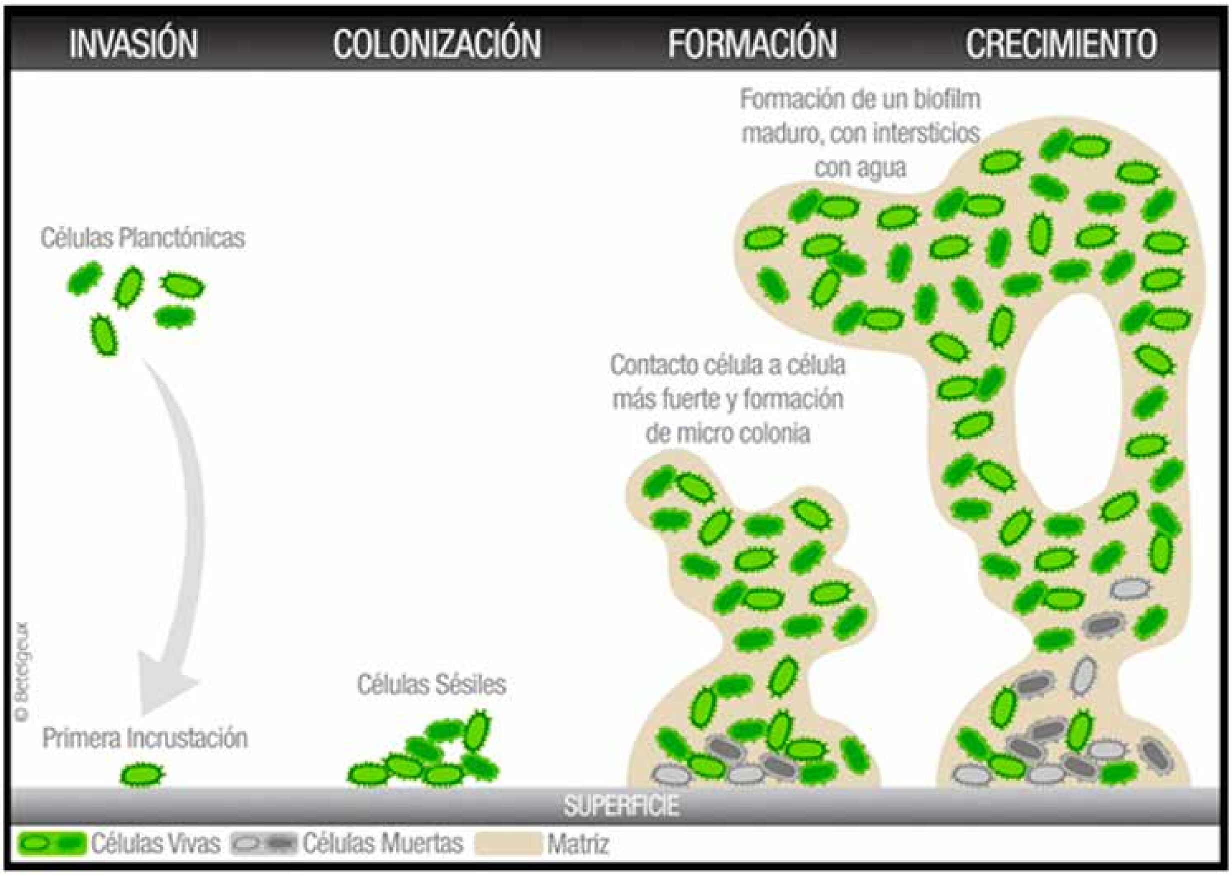

Figure 1. Stages of a mature biofilm formation on a surface.

The biofilm matrix retains the cells and keeps them close to each other, allowing a high degree of interaction, including intercellular communication and the formation of synergistic microconsortiums. The matrix also protects the organisms against drying, oxidants, biocides, some antibiotics and metallic cations, ultraviolet radiation and immune defences. However, the cells of the biofilm are not completely immobilized, but can move inside the biofilm and detach from it.

There is a great variety of EPS that can form the matrix of biofilms, depending on the micro-organisms present, the shear forces experienced during their formation, the temperature and the availability of nutrients. In general, the major components of the biofilm matrix are water, polysaccharides, proteins, nucleic acids, lipids and other biopolymers. Figure 2 represents schematically the variety of EPS that make up the biofilm matrix and how these are distributed among its cells.

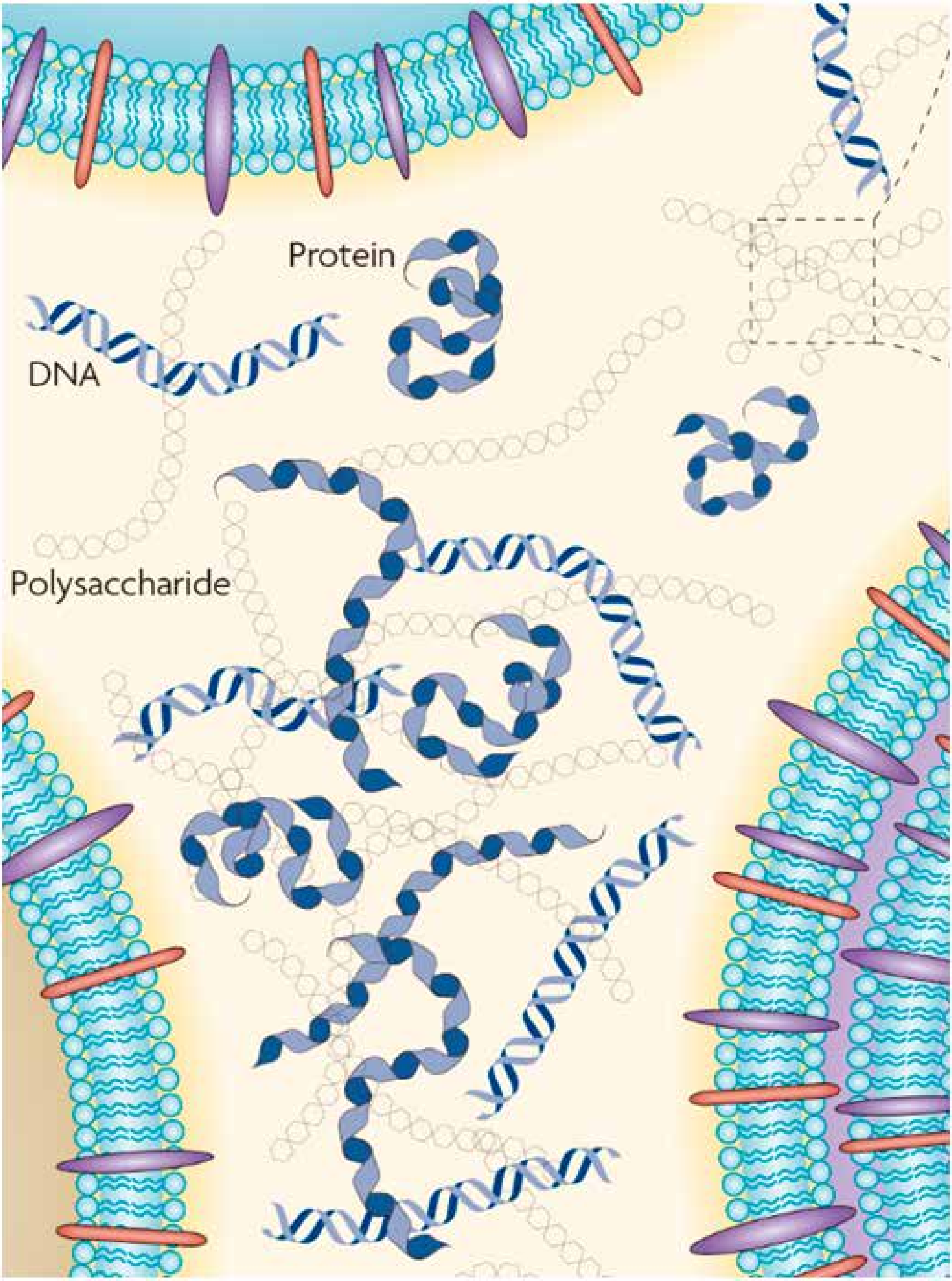

Figure 2. Representation of the distribution of the major components of the biofilm matrix (polysaccharides, proteins and DNA) among the cells that inhabit the biofilm.[2]

The structure of the biofilm is influenced by the EPS that make up its matrix, as well as many other factors such as hydrodynamic conditions, nutrient concentration, bacterial mobility, intercellular communication, and metal ions that may exist in the environment.

Below are some of the main components of the biofilm matrix:

Exopolysaccharides

Polysaccharides constitute a major fraction of the extracellular matrix, and they appear ubiquitously in biofilms formed in different environments: salt water, fresh water, soils, infections in humans, laboratory cultures, etc. Most are long, linear or branched molecules, with molecular mass around 106 D[3] and consist of a mixture of neutral and charged sugars, so they are heteropolysaccharides. They can also contain organic or inorganic substituents that significantly affect their physical and biological properties. For example, one of the most common exopolysaccharides is alginate, constituted by D-manuric and L-guluronic acids that is produced in Pseudomonas aeruginosa biofilms, ,one of the most studied biofilm models.[4]

Exopolysaccharides fulfil various essential functions for the formation of biofilms, generally associated with their adhesion to surfaces and the maintenance of structural integrity. Their chemical nature may vary depending on the microorganisms that produce them, although the exopolysaccharides found in the biofilm matrix do not necessarily reflect the microbial distribution of the biofilm, because cells capable of producing exopolysaccharides can be accommodated in mixed species biofilms [5] .

Extracellular proteins

The proteins present in the extracellular matrix have functions that allow the growth of the biofilm and the survival of the cells housed through access to nutrients or the regulation of the integrity and stability of the biofilm.

The biofilm matrix contains several extracellular enzymes, generally associated with exopolysaccharides, many of which are involved in the degradation of biopolymers. The presence of enzymes that degrade components of the extracellular matrix converts this matrix into an external digestive system that degrades biopolymers to products of low molecular mass that can be assimilated and used as sources of energy and carbon. For example, the degradation of exopolysaccharides is mainly due to hydrolases and lyases.

These enzymes can act on EPS produced by the same bacterium that produces the enzyme or on EPS from other species. In addition, the degradation of structural EPS plays an important role in biofilm development, as it allows the dispersal of sessile cells from biofilms, which allows the formation of new biofilms. This dispersion occurs in response to environmental changes such as scarcity of nutrients or sudden availability of nutrients.

In addition, other enzymes can even degrade the surfaces that host the biofilm, as in the case of redox enzymes that contribute to microbial corrosion.[6]

Non-enzymatic matrix proteins, such as those associated with cell walls or lectins (carbohydrate-binding proteins), are involved in the formation and stabilization of the matrix polysaccharide network and constitute a bond between the bacterial surface and extracellular EPS. These proteins promote the formation of biofilms in various bacterial species and play key roles such as adherence to inanimate surfaces and host cells.[7]

Finally, protein appendages, such as pili, fimbriae and flagella, can also act as structural elements through their interaction with other EPS of the biofilm matrix.

Extracellular DNA

The extracellular DNA (eDNA) constitutes an integral part of the biofilm matrix and its way of life.[8] For example, it has been shown that eDNA is a major component in the biofilms matrix of P. aeruginosa, where it acts as intercellular connector, and in fact the presence of DNAase inhibits the formation of these biofilms.[9] In biofilms of other species, eDNA acts as an adhesive or even as antimicrobial, causing cell lysis by chelating cations that stabilize lipopolysaccharides and the bacterial outer membrane.

Surfactants and lipids

Unlike polysaccharides, proteins and DNA, which are hydrophilic molecules, other EPS with hydrophobic properties exist in the biofilm matrix. This hydrophobic nature has been associated with methyl and acetyl substituents in polysaccharides or lipids, which are crucial for the adhesion of bacteria to hydrophobic surfaces.

In addition, other EPS with surfactant capacity, such as surfactin, viscosin and emulsan, can disperse hydrophobic substances and facilitate their availability.

Biosurfactants have been identified as factors that promote the initial formation of microcolonies, thus facilitating the migration of associated bacteria to the surface and the formation of mushroom-shaped structures, preventing the colonization of channels and contributing to the dispersion of the biofilm.

Water

The majority component of the biofilm matrix is water. The exopolymer matrix provides a highly hydrated environment that loses water more slowly than its environment and therefore protects the biofilm cells against fluctuations in water potential. The bacteria respond actively to desiccation by producing EPS.[10] In addition, the exopolymer matrix can act as a molecular filter, retaining cations, anions, apolar compounds and particles in the aqueous phase. EPS contain apolar regions, groups with potential for the formation of hydrogen bonds, anionic groups (in uronic acids and proteins) and cationic groups (in amino sugars).

EPS and the mechanical properties of the biofilm

In general, biofilms show viscoelastic properties. They can experience both reversible elastic responses and irreversible deformations, depending on the forces acting on the exopolymer matrix. This fact suggests that there are fluctuating binding points between EPS components that remain joined by weak physical chemical interactions such as hydrogen bonds, van der Waals forces and electrostatic interactions (see Figure 2). The cross-linking of biopolymers contributes to the stability of the matrix.[11] Also, the interaction of multivalent inorganic ions with EPS can significantly influence the mechanical properties of biofilms. For example, the presence of Ca2+ increases the mechanical stability of biofilms of Pseudomonas aeruginosa, due to the crosslinking of alginate polianionic molecules.[9]

In conclusion, the matrix of the bacterial biofilm is an extremely complex environment that allows the hosted cells a way of life completely different from that of the planktonic state. Exopolymer substances (EPS) are essential for the formation of biofilms and their variety provides various functions that enable the survival of cells in the biofilm against external aggressions and their expansion. The complexity of the biofilm matrix and the level of protection provided by this form of life to micro-organisms mean that its formation poses a significant risk to food safety and that the control of biofilms requires the application of specific hygiene procedures and the use of specialized tools.

About the Author

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