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Biofilm: The dark side of bacteria

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Abstract

Nowadays, microbial contaminations are the main challenge for food industries having consistent sanitary implications on public health and economic aspects. At this purpose, the biofilm, defined as bacterial structured communities, represents real environmental resistance forms both in nature and at the industrial level. It involves both pathogenic and commensal bacteria that produce extracellular polymeric substances (EPS) named as matrix which is a physical barrier to the external world (e.g., protection from biocides' exposure, antimicrobials, etc.). The biofilm formation can be influenced by many variables e.g., surface characteristics, pH, temperature, and organic residues. When biofilm gains the irreversible step, its structural complexity is enforced by a solid communication performed through the *quorum sensing*. Therefore, this review wants to provide an overview about the main mechanisms and the potential impacts of biofilm productions among industrial realities also empathizing innovative and sustainable approaches for its control and prevention.

Key words Bacteria, Biofilm, Food industries, Food safety, Public health

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Introduction

Among food industries, the microbiological contaminations and their managements represent crucial and current issues. The wide differences and complexity of the modern food supply chains provide ideal environmental conditions (e.g., substrates, abiotic or biotic surfaces, etc.) to the bacterial growths starting from the primary productions to the different multiple processing stages. Indeed, the biofilm-associated bacteria have demonstrated resistance against many external stressors such as the classical cleaning agents, and biocides used as solo or in combined forms (1).

Biofilm Formation and Structure. In the scientific literature, the biofilm has been defined as structured microbial communities which are irreversibly attached to abiotic or biotic surfaces included within a matrix, composed by extracellular polymeric substances (EPS), which is produced by the same involved bacterial genera. More in detail, the matrix also contains other organic molecules e.g., polysaccharides, proteins, lipids, and extracellular DNA that also contribute to the structural stability and protective functions (1,2). The biofilm formation can be considered dynamic and characterized by multiple stages starting from the planktonic one to the *mature* biofilm (3).

The first step is represented by the weak electrostatic interactions between bacteria and surfaces, and it can be also facilitated by any microbial structures such as flagella, pili, and fimbriae which confer mobility and making easy the colonization process. Once attached, bacteria start to produce the EPS enforcing their anchoring to the surfaces. At this point, the biofilm formation can be considered reversable.

The bacterial growth, coupled to a consistent EPS synthesis, permits the construction of a three-dimensional structure that is characterized by microcolonies, developed as multiple microcolonies, and channels that facilitate nutrient and waste transport (4). This complexity is supported by *quorum sensing*, that is a cell-to-cell communication mechanism. It is mediated by the synthesis of molecular signals e.g., N-acyl homoserine lactones (AHLs) in Gram-negative bacteria and autoinducing peptides in Gram-positive bacteria. These chemical signals are responsible for the genetic expression of specific bacterial genomic regions which empathize the EPS production (5). Indeed, the potential disruption of *quorum sensing* pathways has been considered as possible and promising method to control the biofilm formation.

At this step, the biofilm is considered *mature* and irreversible; it is ready for the final stage that is represented by the environmental dispersion of cells and by the colonization of other surfaces (4). From a general perspective, the high heterogeneity, of biofilm forming strains, has a crucial role; indeed, it implies the coexistence of different metabolisms which require different nutrients, oxygen, and metabolic byproducts within the biofilm. This condition can lead to growth of different bacterial subpopulations with specific metabolic and stress-response profiles (5).

Factors affecting biofilm development

Both intrinsic and extrinsic factors are fundamental for the biofilm formation among the food processing environments. Moreover, specific cell interactions modulates the bacterial genetic expressions responsible for the phenotypic activation of the biofilm synthesis e.g., *Listeria monocytogenes* and *Pseudomonas* spp. (3,6). Among the extrinsic factors, it is mandatory to consider surface's physio-chemical characteristics (e.g., presence of conditioning film, pH, hydrophobicity, etc.), environmental ones (temperature and humidity), and the detection of organic substrates useful for the bacterial metabolisms. It is important to consider that multi-species biofilms represent the real situation in food industries; indeed, the mono-species ones can be marginally observed (4). Finally, these complex communities also enforce the horizontal dialogues of mobile genetic forms, between different bacteria, also involved in the antimicrobial resistance phenomenon (3,4).

Impact of food safety and public health

Among biofilm producer pathogens, *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Staphylococcus aureus* have demonstrated to be resistant to the common cleaning and disinfection industrial procedures (3). In addition to the sanitary repercussions, the biofilm formation has clear economic damages for food industries; it implies many costs related to the products recalls, epidemiological investigations, corrective actions to be applied, etc. (4). From a public health perspective, the biofilm persistence, in the food processing plants, increases the possibilities of cross-contamination and diffusion of foodborne pathogens in the environment and finally involving the consumers (1,6).

Strategies for biofilm control in the food industry

The biofilm control and eradication, in food industries, can be realizable through the combination of

three variables: well-structured preventive measures, effective cleaning and disinfection protocols, and the development of innovative strategies of eradication.

Preventive measures

The preventive measures are focused on the minimization of the biofilm formation risk, and it is based on the principles mentioned among the so-called *good manufacturing practices, good hygiene practices*, and on the rigorous application of the HACCP system. Furthermore, the industrial productive flows should be designed and constructed in order to minimize the presence of environmental niches that ca facilitate the biofilm formation (3).

Well-structured monitoring plans are high recommended trying to reduce the impact of possible biofilm detections; recently, innovative molecular diagnostic tools, based on quantitative PCR coupled with next generation sequencing, have consistently improved the sensitivity and specificity of this method (1).

Cleaning and disinfection

Cleaning and disinfection protocols (e.g., scrubbing, high-pressure washing) preserve their efficacy only at the reversable stage of biofilm formation. Indeed, the constant and growing EPS synthesis contributes to protect bacterial cells from biocides (3). At *mature* stage, most of biocides, used as solo or in the combined form with other molecules, have low chances to damage the biofilm structures (4).

Innovative eradication strategies

In the recent decades, researchers have focused their attention on effective innovative strategies to eradicate biofilm from food industries. Among them, enzymatic cleaners (such as proteases, DNases, and polysaccharide-degrading enzymes) can disrupt the EPS matrix determining the exposure of the bacterial cells to the biocides (3). Another promising biotechnology is represented by the bacteriophage that can eliminate biofilm-forming bacteria though the synthesis of endolysins released in the matrix (1). The antimicrobial peptides have attracted more attention due to their ability to disrupt microbial membranes determining the inhibition of biofilm formation and maturation (4). Finally, the *quorum sensing* inhibitors have been largely used because they specifically interfere in the bacterial communication preventing the biofilm maturation; in this way, bacteria become susceptible to the normal used biocides (5).

Conclusions

Although, the biofilm issue has been widely studied among different food producing industries, it still requires further scientific studies that elucidate the bio-molecular mechanisms underlying biofilm formation, development, and persistence (1).

The adoption of innovative and sustainable eradication protocols, in collaborations with food industries, academic and research institutions, will be fundamental to apply scientific discovers into practical solutions for the food industry. Integrated and multi-disciplinary are necessary to develop sustainable, cost-effective strategies for biofilm management in complex food processing environments.

Conflicts of Interest. The Author declares no conflict of interest

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