

Bacterial biofilms of interest to the food industry: review

Tales Henrique Vertuan Pereira¹, Emanoelli Aparecida Rodrigues dos Santos¹, Evelyn Cristine da Silva¹, Isabela de Lima Rocha^{2*}, Camila Lampugnani², Vinicius Cunha Barcellos², Julia Arantes Galvão³, Juliano Gonçalves Pereira¹, Luciano dos Santos Bersot², Leonardo Ereno Tadielo^{3*}

¹São Paulo State University (UNESP), Botucatu campus, School of Veterinary Medicine and Animal Science, Distrito de Rubião Júnior, SN, 18618-970, Botucatu, São Paulo, Brazil.

²Federal University of Paraná (UFPR), Palotina campus, Department of Veterinary Sciences, Rua Pioneiro, 2153, Jardim Dallas, 85950-000, Palotina, Paraná, Brazil.

³Department of Veterinary Medicine, Federal University of Paraná, Rua dos Funcionários, 1540, 80035-050, Curitiba, Paraná, Brazil

*Corresponding author, e-mail: isabeladelimarocha@gmail.com

Abstract. Biofilms are communities of microorganisms fixed on biotic or abiotic surfaces, and their formation causes an important impact on the food industries, leading to cross-contamination, in addition to increased costs for companies due to the deterioration of structure and equipment, and the increased use of sanitizers and disinfectants. Therefore, it is essential to act mainly to prevent biofilm formation. In relation to bacterial biofilms, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella*, *Escherichia coli*, *Bacillus cereus*, and *Pseudomonas aeruginosa* have been cited as the main biofilm formers, in addition to their importance as pathogens and disease-causing agents, impacting human health and deteriorating food and equipment. Several studies aim to seek measures to prevent and control the formation of biofilms, including the use of sanitizers alone, or in combination with each other, the use of essential oils, nanoparticles, electrochemically activated water, and biofilms formed by non-pathogenic and non-deteriorating microorganisms that act as competition. Thus, it is concluded that reducing the impact of these microorganisms in the food industries is possible.

Keywords: Biofilms, contamination, food industry, food quality

Biofilmes bacterianos de interesse para a indústria alimentícia: revisão

Resumo. Os biofilmes são comunidades de microrganismos aderidos a superfícies bióticas ou abióticas, cuja formação gera impactos significativos na indústria alimentícia. Esses impactos incluem a contaminação cruzada e o aumento dos custos operacionais, decorrentes da deterioração de equipamentos e da necessidade de maior uso de sanitizantes e desinfetantes. Por isso, a prevenção da formação de biofilmes é de extrema importância. Entre os principais formadores de biofilmes bacterianos estão *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli*, *Bacillus cereus* e *Pseudomonas aeruginosa*. Esses microrganismos destacam-se não apenas por sua capacidade de formar biofilmes, mas também por sua relevância como patógenos causadores de doenças, afetando a saúde humana, além de atuarem como agentes de deterioração de alimentos e equipamentos. Diversos estudos têm buscado alternativas para prevenir e controlar a formação desses biofilmes, como o uso de sanitizantes isolados ou combinados, aplicação de óleos essenciais, nanopartículas, água eletroquimicamente ativada e a utilização de biofilmes formados por microrganismos não patogênicos e não deteriorantes, que atuam por competição. Dessa forma, é possível mitigar os impactos negativos desses microrganismos na indústria de alimentos.

Palavras-chave: Biofilmes, contaminação, indústria de alimentos, qualidade dos alimentos

Biopelículas bacterianas de interés para la industria alimentaria: Revisión

Resumen. Las biopelículas son comunidades de microorganismos adheridas a superficies bióticas o abióticas, cuya formación genera impactos significativos en la industria alimentaria. Estos impactos incluyen la contaminación cruzada y el aumento de los costos operativos, debido a la degradación de los equipos y a la necesidad de un mayor uso de desinfectantes y sanitizantes. Por ello, la prevención de la formación de biopelículas es de suma importancia. Entre los principales formadores de biopelículas bacterianas se encuentran *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli*, *Bacillus cereus* y *Pseudomonas aeruginosa*. Estos microorganismos se destacan no solo por su capacidad de formar biopelículas, sino también por su relevancia como patógenos causantes de enfermedades que afectan la salud humana, además de actuar como agentes de deterioro de alimentos y equipos. Diversos estudios han buscado alternativas para prevenir y controlar la formación de estas biopelículas, tales como el uso de sanitizantes aislados o combinados, la aplicación de aceites esenciales, nanopartículas, agua activada electroquímicamente y el uso de biopelículas formadas por microorganismos no patógenos y no deteriorantes que actúan por competencia. De esta manera, es posible mitigar los impactos negativos de estos microorganismos en la industria alimentaria.

Palabras clave: Biopelículas, contaminación, industria alimentaria, calidad de los alimentos

Introduction

Biofilms are defined as sessile communities, aggregates of microorganisms irreversibly attached to biotic or abiotic surfaces, encased within a matrix composed of polymeric substances, proteins, and DNA. In response to environmental changes and as a survival mechanism under stress, bacteria embedded in biofilms secrete certain enzymes that facilitate a communication mechanism known as quorum sensing ([Natress, 2023](#); [Rumbaugh & Sauer, 2020](#)).

In recent years, various anti-biofilm strategies have been investigated. It is well established that controlling an already established biofilm is significantly more challenging than preventing its formation ([Borges et al., 2012](#); [Landini et al., 2010](#)). To support the development of effective prevention and control strategies, many studies have aimed to deepen our understanding of the cellular processes involved in biofilm formation, including initial adhesion, bacterial motility, EPS (extracellular polymeric substance) production, and quorum-sensing mechanisms ([Landini et al., 2010](#)).

In addition to contributing to food cross-contamination and the subsequent transmission of foodborne illnesses, biofilms increase operational costs through higher energy and disinfectant consumption. Furthermore, they can cause significant damage to equipment and infrastructure in food processing environments, due to both microbial-induced deterioration and corrosion, as well as the improper use of biocidal agents ([Carrascosa et al., 2021](#); [Rahman et al., 2022](#); [Shi & Zhu, 2009](#)). As a result, it is essential to understand the factors that promote biofilm development and the strategies available to prevent and inhibit their formation—an approach considered the first and most effective step in biofilm control within the food industry ([Rossi et al., 2022](#)).

The objective of this study was to describe the main species of biofilm-forming microorganisms found in food processing environments, as well as to explore key aspects related to their formation, prevention, and control.

Methodology

To conduct this review, searches were carried out in databases containing academic and scientific publications using the keywords “Biofilms in industries,” “Biofilms in meatpacking plants,” and “Biofilms in food,” which returned 409, 594, and 15,900 results, respectively, in the following databases: Capes Journals, PubMed, and Google Scholar, covering the period from 2010 to 2023.

Based on a brief analysis of abstracts, results, and conclusions, 30 articles were selected that focus specifically on methods for biofilm prevention and control. These articles, available in full online in English and Portuguese, were used as the main references. Additionally, books and epidemiological data were consulted to complement the content presented in this review.

Bacterial biofilms

Microorganisms can form biofilms through cell multiplication, surface adhesion, and the production of extracellular polymeric substances (EPS) ([Flemming et al., 2016](#)). Biofilms can develop on nearly all biotic and abiotic surfaces, posing serious challenges across various sectors, including the food and medical industries. They are commonly found on food contact surfaces and equipment, medical devices (e.g., implants), hospital instruments, water distribution pipelines, and even in natural aquatic systems.

The multilayered structure of cells and EPS creates a compact and complex biofilm architecture that hinders the penetration of antimicrobial agents. This limits their ability to reach inner layers, drastically reducing their efficacy ([Borges, 2020](#); [Percival et al., 2011](#)). Consequently, when microorganisms form biofilms, their resistance to conventional antimicrobial agents increases significantly, making these biofilms difficult to eliminate ([Borges, 2020](#)).

Biofilms are composed of over 97% water, with the remaining content made up of a wide array of structural and functional components that form the biofilm matrix ([Flemming et al., 2016](#)). Microorganisms themselves constitute only 2 to 5% of this matrix, although they secrete the polymeric substances that dominate its organic content. The EPS matrix plays a critical role in defining the morphology, structural integrity, cohesion, and overall functionality of the biofilm, comprising approximately 50 to 90% of its total organic matter ([Borges, 2020](#); [Oliveira et al., 2010](#)).

A solid understanding of microbial biofilms, including their structure, composition, and formation processes, is essential for developing effective prevention and control measures, especially in the food industry, where their impact can be widespread and costly ([Oliveira et al., 2010](#)).

The formation of a biofilm involves a series of physical, biological, and chemical processes that occur in sequential stages, involving one or more species of microorganisms associated with each other. Initially, the presence of a substrate is necessary, which is essential for the production of a matrix that will be formed after the deposition of planktonic microorganisms. In the aqueous phase, these microorganisms absorb nutrients from the external environment and produce EPS components that will initiate the binding and stabilization of the matrix ([Flemming et al., 2016](#); [Simões, 2012](#)). This is followed by the initial development of a conditioning film, the attachment of microorganisms to the latter through adhesion mechanisms, which is mediated by structures such as flagella, pili, or adhesins, the formation of microcolonies, the synthesis of EPS, and the development of quorum sensing ([Landini et al., 2010](#); [Rastegari et al., 2020](#); [Simões, 2012](#); [Singh, 2019](#)).

Quorum sensing is defined as a mechanism that allows microbial communication based on the constant production, release, and detection of signaling molecules into the environment, called autoinducer molecules ([Paluch et al., 2020](#); [Whiteley et al., 2017](#)). Thus, its main role is to regulate processes vital to the cell, such as the production of virulence factors or biofilm formation ([Paluch et al., 2020](#)).

After being attracted to the substrate formed, microorganisms begin to grow and multiply if they are not removed through appropriate cleaning and sanitization techniques ([Natress, 2023](#)). They then bind reversibly to the substrate, promoting adhesion. This binding depends heavily on the hydrophilic and hydrophobic properties of the adhesion surface ([Singh, 2019](#)).

Biofilm consolidation is achieved through the deposition of EPS by microorganisms, allowing them to adhere to surfaces and to each other, as well as protecting them from adverse conditions ([Natress, 2023](#)). This class of EPS includes some extracellular polysaccharides, such as alginate-like exopolysaccharides, cellulose, capsular polysaccharides, and lipopolysaccharides, among others. In addition to these, proteins such as exoenzymes, adhesion proteins (such as Bap – biofilm-associated protein), lipoproteins, glycoproteins, and nucleic acids such as eDNA (extracellular DNA) and other compounds ([Borges, 2020](#); [Seviour et al., 2019](#)).

Thus, the primary structure consolidates and provides mechanical and chemical stability to the cells as they proceed to establish an irreversible bond and form a three-dimensional sessile community. Once attached, the bacteria create a microenvironment to continue their development and multiplication, becoming resistant to adverse or disruptive environmental conditions ([Coughlan et al., 2016](#); [Paluch et al., 2020](#)).

Biofilm acts as a sponge system that absorbs various molecules from the environment, providing nutrient acquisition and matrix stabilization ([Flemming et al., 2016](#); [Oliveira et al., 2010](#)). Similarly, their physicochemical properties allow biofilms to retain and stabilize extracellular digestive enzymes produced by biofilm cells, transforming the matrix into an external digestive system. Thus, biofilms can digest biodegradable components of the substrate, which are exposed to enzymes in the matrix, and maintain their development ([Flemming et al., 2016](#)).

Cross-contamination of food can occur through the detachment of bacterial cells from the surface of a biofilm. This detachment, which can happen at any time during the biofilm formation process, occurs when the bonds between cells are weakened, largely due to the increase in repulsive forces at the expense of the attractive forces imposed on biofilm bacteria, and its occurrence can initiate a new adhesion process ([Santana, 2023](#); [Singh, 2019](#)). Given the importance of food contamination by biofilms, the main biofilm-forming pathogens of interest to the food processing industry are listed below.

Main biofilm-forming microorganisms

Listeria monocytogenes

Bacteria of the genus *Listeria* are small, Gram-positive coccobacilli, facultative anaerobes, catalase-positive, and oxidase-negative. The genus comprises 30 known species, among which *Listeria monocytogenes* is recognized as a significant foodborne pathogen. It has been identified as the etiological agent of various diseases in both humans and animals worldwide, including septicemia, abortion, and meningitis. The populations most at risk include adults, pregnant women, and immunocompromised individuals. In humans, contamination primarily occurs through the ingestion of contaminated foods such as meat, dairy products, vegetables, seafood, and ready-to-eat products ([Colagiorgi et al., 2017](#); [Quinn et al., 2018](#); [Sereno et al., 2019](#)).

From a pathogenic perspective, *L. monocytogenes* is capable of invading both phagocytic and non-phagocytic cells, surviving and replicating intracellularly, and spreading from cell to cell without exposure to humoral immune defenses ([Quinn et al., 2018](#)). This microorganism also demonstrates a high capacity to form biofilms and adhere to various surfaces commonly found in food processing environments, including stainless steel, polypropylene, polystyrene, PVC, rubber, and glass, the latter showing the highest adhesion capacity. Although optimal biofilm production occurs at 37° C, *L. monocytogenes* is also capable of forming biofilms at refrigeration temperatures ranging from 4° C to 12° C, demonstrating significant adaptability to cold storage conditions, particularly in slaughterhouses and refrigerated facilities. It may also be found in mixed biofilms alongside other pathogenic species ([Colagiorgi et al., 2017](#); [Tadielo et al., 2022](#)).

The presence of pili, flagella, and membrane proteins enables this pathogen to adhere to surfaces in food processing environments. Additionally, it can survive in acidic conditions for extended periods and form biofilms even under anaerobic conditions ([Carrascosa et al., 2021](#)). [Guilbaud et al. \(2015\)](#) described a characteristic honeycomb-like structure in biofilms formed by *L. monocytogenes*, consisting of multiple cohesive cell layers distributed heterogeneously, with void spaces and pockets containing dead cells and extracellular DNA (eDNA).

[Tadielo et al. \(2022\)](#) demonstrated the in vitro formation of mixed-species biofilms involving *L. monocytogenes* and *Salmonella Typhimurium* on polypropylene surfaces. These biofilms exhibited complex and robust architectures, potentially hindering their removal during cleaning procedures. The study also highlighted the involvement of specific genes associated with biofilm formation, multidrug resistance, and sanitizer tolerance.

Due to its pathogenic potential and increasing antimicrobial resistance, *L. monocytogenes* is subject to regulatory control. In Brazil, Normative Instruction No. 09 (April 8, 2009) established procedures for

controlling *L. monocytogenes* in ready-to-eat animal-origin products ([BRAZIL, 2009](#)). According to [Oliveira et al. \(2018\)](#), resistance has been reported, albeit at a low frequency, to antimicrobials such as penicillin, clindamycin, erythromycin, tetracyclines, gentamicin, ciprofloxacin, and ampicillin. Notably, [Sereno et al. \(2019\)](#) reported ampicillin-resistant isolates obtained from various surfaces, tools, and processing stages in a pig slaughterhouse in Brazil.

Recognizing its public health significance, the Brazilian Health Regulatory Agency ([ANVISA, 2025](#)) included *L. monocytogenes* in Normative Instruction No. 161 (July 1, 2022), which mandates microbiological limits for this pathogen in ready-to-eat foods. For general products, the maximum acceptable count is 10^2 CFU/g or mL, while for foods intended for infants or for special dietary purposes, *L. monocytogenes* must be absent in 25 g or mL ([BRAZIL, 2022](#)).

Exemptions from routine testing are allowed only for foods that meet at least one of the following conditions: shelf life shorter than five days; $\text{pH} \leq 4.4$; water activity (a_w) ≤ 0.92 ; or $\text{pH} \leq 5.0$ combined with $a_w \leq 0.94$. These parameters are based on the established understanding that *L. monocytogenes* cannot proliferate under such restrictive environmental conditions, despite its adaptability to a wide range of pH and a_w values ([Colagiorgi et al., 2017](#)).

In conclusion, the severity of illnesses caused by *L. monocytogenes* and its potential for resistance to multiple antimicrobials underscore the need for robust prevention strategies and continuous monitoring throughout the food production chain—including farms, slaughterhouses, and processing facilities.

Escherichia coli

Escherichia coli (*E. coli*), a member of the Enterobacteriaceae family, is a Gram-negative, oxidase-negative, generally motile bacillus characterized by peritrichous flagella and fimbriae ([Pitout, 2012](#)). It is considered one of the most important pathogens in both food safety and public health due to its ubiquitous presence, ability to form biofilms, the diseases associated with contaminated food, and its increasing multidrug resistance ([Oliveira et al., 2016](#); [Pitout, 2012](#); [Quinn et al., 2018](#)).

While many strains of *E. coli* are harmless and function as commensal organisms in the intestinal microbiota of humans and animals, contributing to host physiological health, some strains are opportunistic pathogens, especially when they colonize extraintestinal sites such as the mammary glands or urinary tract, particularly in immunocompromised individuals. Pathogenic *E. coli* strains possess various virulence factors that enable them to colonize mucosal surfaces and cause systemic infections, including meningitis, pneumonia, and septicemia. Exploring the diversity of *Listeria monocytogenes* biofilm architecture by high-throughput confocal laser scanning microscopy and the predominance of the honeycomb-like morphotype.

The pathogenic versatility of *E. coli* is largely due to the acquisition or deletion of specific genes that confer virulence properties to certain strains. Numerous studies have documented the biofilm-forming ability of *E. coli*, as well as the presence of resistance and virulence genes, which are often associated with multidrug resistance ([Rivas et al., 2010](#)).

Pathogenic strains of *E. coli* are classified based on their virulence factors, such as fimbrial and afimbrial adhesins, toxins, and invasins, into distinct pathotypes, including: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC) e enterohemorrhagic *E. coli* (EHEC). These pathotypes are primarily associated with diarrheal syndromes, often accompanied by vomiting, fever, and dehydration ([BRAZIL, 2022](#)). Among these: ETEC and EPEC are more commonly linked to watery diarrhea of short duration. Notably, EPEC ranked among the top 10 pathogens identified in Waterborne and Foodborne Disease (WBFD) outbreaks reported between 2012 and 2021 ([BRAZIL, 2022](#)), EIEC causes dysentery, characterized by diarrhea with mucus and blood, resembling infections caused by *Shigella* spp. and EHEC, particularly serogroup O157:H7, is of significant concern due to its production of Shiga toxins (Stx1 and Stx2), the same toxins produced by *Shigella dysenteriae* type I. These toxins exert a cytotoxic effect on intestinal epithelial cells, potentially leading to necrosis, perforation, and in severe cases, hemolytic-uremic syndrome (HUS), which is associated with high hospitalization and mortality rates ([Quinn et al., 2018](#); [Rivas et al., 2010](#)).

Given the public health risks posed by *E. coli*, particularly its biofilm-forming ability, multidrug resistance, and the severity of associated illnesses, ongoing research into its biofilm formation, as well as prevention and control strategies, is critical to reducing food contamination and protecting consumer health.

Salmonella spp.

Salmonella is a genus of Gram-negative bacilli within the Enterobacteriaceae family ([Santos et al., 2020](#)). It comprises two species capable of causing disease in humans: *Salmonella enterica* and *Salmonella bongori* ([Al-Rifai et al., 2020](#); [Foti et al., 2009](#)). *S. enterica* is further divided into six subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica*, with *S. enterica* subsp. *enterica* being the most relevant to public health due to its zoonotic potential and the existence of over 2,600 serovars ([Ibrahim & Morin, 2018](#); [Quinn et al., 2018](#)).

Among these, *S. Typhimurium* and *S. Enteritidis* are the primary serovars associated with foodborne transmission. *S. Enteritidis*, along with *S. Heidelberg*, is commonly linked to the consumption of poultry products and is responsible for paratyphoid fever. Both pose significant public health concerns due to their prevalence and ability to contaminate foods of animal origin ([BRAZIL, 2020](#); [Quinn et al., 2002](#)).

Salmonella spp. is motile due to the presence of peritrichous flagella, with exceptions such as *S. Gallinarum* and *S. Pullorum*, which are non-motile and pathogenic to poultry. These bacteria are facultative anaerobes, mesophilic (optimum growth between 7° C and 45° C), and capable of surviving freezing conditions in organic matter for extended periods. They tolerate a pH range of 4.5 to 9.0, are catalase-positive, oxidase-negative, and typically ferment sugars such as maltose, mannitol, mannose, sorbitol, and trehalose, though they do not ferment lactose ([BRAZIL, 2020](#); [Quinn et al., 2002](#)).

Between 2012 and 2021, *Salmonella* spp. ranked as the third most frequently reported etiological agent in outbreaks of foodborne and waterborne diseases ([BRAZIL, 2022](#)). Pathogenicity begins in the mucosa of the small intestine and colon, where the bacteria penetrate the epithelial barrier, inducing phagocytosis and triggering an inflammatory response characterized by increased activity of the reticuloendothelial system and prostaglandin release. These responses result in watery diarrhea, which may or may not contain blood, in addition to nausea and vomiting ([Carmo et al., 2005](#); [Oliveira et al., 2010](#)).

In Brazil, Normative Instruction No. 20 (October 21, 2016) regulates the control and monitoring of *Salmonella* spp. in poultry farms and slaughterhouses under the Federal Inspection Service (SIF). The goal is to reduce pathogen prevalence and ensure a sufficient level of consumer protection ([BRAZIL, 2016](#)). Despite stringent regulations from farm to processing plant, *Salmonella* spp. remains a major challenge for the food industry.

A particular concern is the biofilm-forming capacity of *Salmonella* spp. in both farms and food processing environments. For example, *S. Enteritidis* is known to form biofilms on various surfaces, particularly hydrophobic materials such as plastics, stainless steel, rubber, glass, and cement g ([Carmo et al., 2005](#); [Oliveira et al., 2010](#); [Quinn et al., 2018](#)).

Biofilm formation varies among serovars and is influenced by surface type and temperature, affecting bacterial persistence in processing environments ([Obe et al., 2022](#)). Within a single serovar, significant differences in biofilm-forming capacity may exist, likely due to genetic differences, including the presence of biofilm-associated genes ([Borges, 2020](#)). For instance: *S. Schwarzengrund* has been linked to the formation of strong and resilient biofilms and *S. Heidelberg* and *S. Newport* tend to produce weaker biofilms, which are easier to remove through sanitation. However, the rise of multidrug-resistant strains, especially within the *Heidelberg* serovar, presents a growing threat ([Bassini, 2017](#); [Obe et al., 2022](#)). Understanding the biofilm-producing capabilities and resistance patterns of different *Salmonella* serovars is critical for implementing targeted prevention and control strategies.

Bacillus cereus

The genus *Bacillus* comprises large, Gram-positive, rod-shaped bacteria, many of which are aerobic or facultatively anaerobic and capable of producing endospores. While most species are non-pathogenic

and commonly found in the environment, two species are of particular concern: *Bacillus anthracis*, the causative agent of anthrax, a severe zoonosis and *Bacillus cereus*, a major cause of foodborne disease outbreaks ([Quinn et al., 2018](#))

B. cereus is a motile, spore-forming organism commonly associated with gastrointestinal illnesses, though it can also cause invasive infections, particularly in immunocompromised individuals. These include bacteremia, pneumonia, meningitis, brain abscesses, endophthalmitis, ocular keratitis, necrotizing skin infections, osteomyelitis, pyelonephritis, and endocarditis ([Batista, 2018](#); [Dewey-Mattia et al., 2018](#)).

This microorganism grows optimally at temperatures between 28° C and 35° C, tolerates pH values between 4.9 and 9.3, and can survive in environments with up to 7.5% salt and water activity (aw) $\geq$ 0.95. *B. cereus* produces two types of enterotoxins responsible for distinct gastrointestinal syndromes: diarrheal syndrome, caused by thermolabile toxins produced during vegetative growth in the small intestine. Symptoms include abdominal pain and profuse watery diarrhea, typically appearing 4 to 16 hours post-ingestion and lasting up to 24 hours and emetic syndrome, caused by thermostable toxins produced in food prior to ingestion. Symptoms such as nausea and vomiting occur rapidly, between one to cinq hours after eating contaminated food ([Batista, 2018](#); [Dewey-Mattia et al., 2018](#)).

B. cereus is responsible for an estimated 2–5% of foodborne illness cases globally. Foods most commonly involved include rice, meat, eggs, dairy, baked goods, stews, vegetables, creams, fruits, and even contaminated water. It has also been isolated from hospital equipment, catheters, disinfectants, and bedding, indicating its ability to survive in clinical settings ([Batista, 2018](#); [Dewey-Mattia et al., 2018](#); [Nunes et al., 2016](#)).

In Brazil, ([ANVISA, 2025](#)) regulations set maximum permissible limits for *B. cereus* in various food categories to ensure consumer safety ([BRAZIL, 2022](#)). The widespread presence of this bacterium is largely due to its environmental origin (soil) and failures in hygiene practices throughout the production and supply chain ([Rahnama et al., 2023](#)).

Between 2012 and 2021, *B. cereus* was the fourth most commonly reported pathogen in foodborne outbreaks in Brazil ([BRAZIL, 2022](#)). Its ability to form biofilms contributes to cross-contamination, food spoilage, and equipment deterioration, further complicating sanitation efforts ([Batista, 2018](#)). Given its public health significance, rigorous preventive practices and hygiene controls are essential to reduce the risk of contamination and ensure food safety.

Staphylococcus aureus

Another highly relevant pathogen, *Staphylococcus aureus*, belongs to the genus *Staphylococcus*, whose species are Gram-positive cocci. These bacteria are classified based on characteristics such as colony morphology, type of hemolysis, biochemical profile, and ribosomal RNA restriction enzyme patterns ([Alves et al., 2020](#)). *S. aureus* is widely distributed in nature and exists as a commensal organism on the skin of animals and humans, as well as in the mucous membranes of the upper respiratory and lower urogenital tracts. It may also be transiently present in the gastrointestinal tract and is relatively environmentally stable ([Galié et al., 2018](#); [Quinn et al., 2018](#)).

S. aureus is a facultative anaerobe, non-spore-forming, non-motile, and catalase-negative bacterium. It is considered extremely opportunistic, capable of causing a variety of infections in both animals and humans ([Galié et al., 2018](#); [Quinn et al., 2018](#)).

Importantly, *S. aureus* is a notorious biofilm-forming microorganism ([Paluch et al., 2020](#)) and one of the leading causes of foodborne illnesses, in addition to being a major pathogen in dairy mastitis. Outbreaks of staphylococcal food poisoning are frequently associated with poultry, meat products, eggs, milk, and dairy derivatives due to their susceptibility to contamination by the bacterium's wide variety of exotoxins and other virulence factors ([Galié et al., 2018](#); [Innes et al., 2021](#); [Membré et al., 2011](#)). These enterotoxins are heat-stable and are secreted during bacterial growth in food matrices, typically introduced through poor handling practices or contamination from animal sources. Even foods with low water activity, such as those high in salt or sugar, can support the development of this pathogen ([Galié et al., 2018](#)).

S. aureus is a mesophilic microorganism, capable of growing at temperatures between 7° C and 47° C. Although the bacterium itself may be inactivated during high-temperature food processing, its enterotoxins remain active due to their thermostability. The optimal pH for bacterial growth is between 7.0 and 7.5, although there are reports of its multiplication in foods with a pH range of 4.2 to 9.3 ([Akbar & Anal, 2013](#); [de Boer et al., 2009](#); [Moreira et al., 2016](#)).

From 2012 to 2021, *S. aureus* was the second most frequently isolated microorganism in reported foodborne and waterborne disease outbreaks (DTHA) in Brazil ([BRAZIL, 2022](#)). Foods requiring intensive manual handling are commonly associated with contamination, likely due to inadequate hygiene practices and biofilm-mediated cross-contamination. Among the most frequently implicated foods are desserts, cheeses, pasta dishes, lasagna, and pancakes ([Akbar & Anal, 2013](#); [de Boer et al., 2009](#); [Moreira et al., 2016](#)).

Biofilm formation by *S. aureus* is a critical virulence factor. These biofilms have been detected in cow mammary glands, milking equipment, and various medical devices, including orthopedic implants, pacemakers, vascular catheters, and heart valves. Clinically, this can lead to infections such as osteomyelitis, endocarditis, and skin infections. The bacterium demonstrates strong adhesion to a variety of materials, including glass, metals (copper, chromium, titanium), and plastics (polyester, silicone, polyethylene) ([Bhattacharya et al., 2015](#); [Parastan et al., 2020](#)).

The structural matrix of *S. aureus* biofilms is composed of polysaccharides, proteins, and extracellular DNA (eDNA), which contribute to antimicrobial resistance. This makes biofilm-associated cells significantly more resistant to disinfectants and antibiotic treatments. The emergence and spread of multidrug-resistant (MDR) strains of *S. aureus* is a growing concern, posing significant challenges to both public health and food safety systems ([Parastan et al., 2020](#)).

Pseudomonas aeruginosa

Microorganisms of the genus *Pseudomonas* spp. are among the most common psychrotrophic spoilage organisms, especially in stored foods with exposure to air, high moisture content, and neutral pH, such as meat and dairy products ([Xu et al., 2019](#)). The most significant species from both economic and health perspectives is *Pseudomonas aeruginosa*, recognized as an opportunistic pathogen, a major food spoiler, and a cause of various diseases ([Antunes & Reis, 2021](#)).

Pseudomonas aeruginosa is a Gram-negative, obligate aerobic, motile bacillus that occurs worldwide. It is primarily found in water, soil, and plants, but also colonizes the skin, mucous membranes, and feces of animals and humans ([Quinn et al., 2018](#)). This bacterium shows a particular preference for moist tissues, such as the upper respiratory tract, kidneys, urinary tract, and gastrointestinal tract, and is known for its high resistance to various antimicrobials. Notably, patients with cystic fibrosis often suffer lung infections caused by *P. aeruginosa* biofilms, which exhibit significant resistance to antibiotic treatments ([Simões, 2012](#)).

Several studies have documented its resistance to carbapenems—including meropenem and imipenem, which are crucial antimicrobials for treating highly resistant bacteria ([Quinn et al., 2018](#); [Raposo et al., 2017](#)). Furthermore, the formation of biofilms by *P. aeruginosa* can occur faster than with other microorganisms, and the bacterium produces defense mechanisms that enhance its persistence and multi-drug resistance ([Carrascosa et al., 2021](#); [Nunes et al., 2016](#); [Sahade, 2019](#)). Pathogenic strains produce various toxins and enzymes that facilitate invasion and tissue damage. Attachment to host cells is mediated by fimbriae, while toxins such as exotoxin A, phospholipase C, and proteases contribute to tissue injury ([Quinn et al., 1962](#)).

In food processing environments, *P. aeruginosa* exhibits psychrotrophic behavior, colonizing various surfaces including floors and drains. It is a significant contributor to the deterioration of food and equipment. The bacterium's high production of extracellular polymeric substances (EPS) enhances its ability to attach to surfaces and promotes biofilm formation, particularly on stainless steel materials ([Carrascosa et al., 2021](#); [Raposo et al., 2017](#)).

A study by [Costa et al. \(2022\)](#) comparing biofilm formation on stainless steel and polypropylene surfaces identified *P. aeruginosa* alongside *S. Typhimurium* as the microorganisms responsible for the most challenging biofilm problems, highlighting the threat these pathogens pose to the food industry.

General strategies for biofilm prevention and control

Numerous studies have described and evaluated various methods to prevent and eradicate biofilm formation ([Borges et al., 2012](#)). Among these, the use of chemicals with antimicrobial properties, such as antibiotics, biocides, and disinfectants, is the most common approach for controlling harmful biofilms. However, no single method has proven completely effective on its own, often necessitating the combination of two or more antibiofilm agents to achieve better results ([Simões, 2012](#)).

Sessile biofilm communities exhibit significant tolerance to antimicrobial agents, which often requires the use of high concentrations of chemicals. This approach, however, can lead to the emergence of resistant or persistent microbial strains, especially in industrial environments ([Borges et al., 2012](#); [Serenó et al., 2019](#)).

In the food industry, routine biofilm control primarily relies on sanitization processes using disinfectants. These disinfectants vary in toxicity and efficacy, and their effectiveness can decline over time due to microbial resistance development ([Colagiorgi et al., 2017](#); [Oliveira et al., 2014](#)). Commonly used disinfectants include sodium hypochlorite, quaternary ammonium compounds (such as benzalkonium chloride), and peracetic acid. Selection is often based on factors like ease of application and cost. However, practical limitations exist; inappropriate use, prolonged exposure, and suboptimal concentrations of disinfectants can promote the natural selection of resistant strains within microbial populations. Therefore, periodic evaluations of disinfectant efficacy are essential to ensure continued effectiveness ([Colagiorgi et al., 2017](#); [Menegaro et al., 2016](#)).

Additionally, the presence of organic matter on surfaces—such as food residues—can significantly reduce the effectiveness of sanitizers like sodium hypochlorite. This necessitates prior mechanical cleaning to remove debris and lower the organic load on equipment and surfaces before chemical treatment.

Studies have demonstrated that the use of sublethal doses of biocidal agents can diminish or negate sanitizer effectiveness, highlighting the importance of using appropriate concentrations. To prevent the development of microbial resistance, it is recommended to rotate disinfectants with different active ingredients regularly ([Colagiorgi et al., 2017](#); [Hua et al., 2019](#)).

Studies show that several factors directly influence the effectiveness of sanitizers, including the amounts and concentrations used, contact time, type of surface, and the microorganisms that compose the biofilms ([Colagiorgi et al., 2017](#)). Understanding these factors is necessary to achieve maximum efficacy.

Furthermore, the use of essential oils, enzymes, nanoparticles, electrochemically activated water, and bacteriophages are alternative methods for controlling bacterial biofilms in animal production facilities and the food industry. However, some of these approaches are still underexplored ([Borges, 2020](#); [Galié et al., 2018](#); [Simões, 2012](#)).

For example, bacteriophages can serve as a viable option for biofilm control ([Simões, 2012](#)). Bacteriophages are viruses that infect bacteria, making them natural predators of these microorganisms. They are known to penetrate biofilms by invading the intact matrix and producing enzymes that degrade extracellular polymeric substances (EPS) ([Chegini et al., 2020](#); [Harper et al., 2014](#); [Simões, 2012](#)). It should be noted that bacteriophages have advantages over antibiotics in inhibiting infections caused by bacterial biofilms. Specifically, bacteriophages can penetrate the inner layers of biofilms, unlike antibiotics, which mainly affect bacterial cells on the surface ([Chegini et al., 2020](#)).

Important characteristics of bacteriophages include ease of isolation, high specificity, absence of toxicity, low production cost, harmlessness to humans, and a great capacity to evolve to overcome bacterial resistance ([Duarte et al., 2016](#); [Flemming et al., 2016](#); [Rahman et al., 2022](#)). Some studies have demonstrated the effectiveness of bacteriophages against biofilms formed by pathogenic bacteria such as *Salmonella* spp., *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, and *Pseudomonas*

aeruginosa. However, further studies on their application in the food industry are needed to ensure their complete safety ([Gdoura-Ben Amor et al., 2022](#); [Chegini et al., 2020](#); [Garcia et al., 2017](#); [Gray et al., 2018](#)).

Meanwhile, other studies are focusing on the applicability of enzymes in biofilm control. Despite their high cost and the need to combine more than one type of enzyme, enzymes can be combined with sanitizers to achieve better results ([Stiefel et al., 2016](#)). Some enzymes, such as endolysins, can be isolated from bacteriophages for use in degrading biofilms, such as those of *L. monocytogenes*, and can also be combined with other enzymes ([Schmelcher & Loessner, 2016](#)). In their work, [Stiefel et al. \(2016\)](#) evaluated the effectiveness of combining enzymes and cleaning products to remove biofilms in vitro and demonstrated that *Staphylococcus aureus* biofilms were efficiently removed using an active protease. However, for *Pseudomonas aeruginosa*, a combination of proteases, polysaccharidases, lipases, and DNases was necessary to successfully remove biofilms.

Electrochemically activated water (a biocidal agent produced from water, salt, and electricity) may also be a promising alternative for biofilm control, given its low cost and biodegradable nature. This method is reported to have broad-spectrum bactericidal efficacy due to the synergistic effects of available chlorine concentrations, pH, and oxidation-reduction potential. Several studies have demonstrated its efficacy against microorganisms important to the food industry ([Zhao et al., 2021](#)). For example, [Wilsman \(2018\)](#) tested its use to control *Salmonella heidelberg*, achieving good results in reducing microbial load in the planktonic phase, although the agent was not completely eliminated. Other studies report positive results with its application in controlling biofilms of *L. monocytogenes*, *E. coli*, *Staphylococcus aureus*, and *P. aeruginosa* ([Gómez-López et al., 2013](#); [Yan et al., 2022](#)). However, further research is needed to confirm the absence of harmful residues and to ensure food safety when using this method.

Other methods, such as the use of silver nanoparticles, magnesium, chitosan, lipids, and magnetic nanoparticles, have been investigated in several studies for controlling biofilms ([Borges, 2020](#)). Among these, copper is an alternative method that has been employed in the food industry, water distribution systems, and medical facilities ([Gomes et al., 2020](#)). The antimicrobial properties of copper surfaces have been tested in hospital environments, showing a significant reduction in bacterial load ([Schmidt et al., 2016](#)). Similar studies in the food industry have corroborated these findings. Some have demonstrated the effectiveness of combining copper with sanitizers such as sodium hypochlorite and quaternary ammonium compounds for mitigating biofilms formed by important pathogens, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp. ([Pontin, 2020](#); [Steenackers et al., 2012](#)).

[Pontin \(2020\)](#) found that surfaces made of electrolytic copper and coated with brass showed enhanced benefits in reducing microorganisms without corrosion when used with benzalkonium chloride (a quaternary ammonium compound) and sodium hypochlorite. Based on these results, the recommendation was made to adopt surfaces based on these metals in industries to prevent *Salmonella* Enteritidis biofilms. [Parra et al. \(2018\)](#) also demonstrated the bactericidal effect of copper against microorganisms isolated from poultry, with significant reductions in *Salmonella enterica* and *Listeria monocytogenes*. In addition to these pathogens, evidence suggests that copper may also be effective in controlling biofilms formed by *E. coli*, *P. aeruginosa*, and *S. aureus* ([Gomes et al., 2020](#); [Santo et al., 2011](#)).

According to [ANVISA \(2005\)](#), the recommended daily intake of copper is 0.9 to 1.6 mg, with a maximum limit of 10 mg for adults aged 19 to 50 ([BRAZIL, 2005](#)). Although there is no legislative ban on the use of copper, some studies indicate the potential risk of metal residues in food, which may limit the feasibility of this method. Therefore, further studies are required to confirm the safety of copper-based approaches ([Gomes et al., 2020](#); [Pontin, 2020](#)).

Silver nanoparticles, which are also used in food packaging, have been studied for controlling biofilms of various pathogens, with promising results in studies involving *L. monocytogenes*, *Salmonella* spp., *E. coli*, *Bacillus cereus*, *P. aeruginosa*, and *S. aureus* ([Deshmukh et al., 2019](#)).

Another promising approach is the use of plant-derived essential oils as alternatives to chemicals and microbial derivatives such as bacteriophages and enzymes. Essential oils play a key role in protecting

plants against bacteria, fungi, viruses, insects, and animals. Several herbs and spices are well-known for their antimicrobial properties and have been traditionally used worldwide as flavorings, food preservatives, or medicinal agents ([Perricone et al., 2015](#)). Many studies have explored the application of essential oils for biofilm control ([Rossi et al., 2022](#)). Chemical classes of aldehydes and phenols present in these oils—such as cinnamaldehyde, carvacrol, eugenol, and thymol—have shown effectiveness against biofilms ([Gray et al., 2018](#); [Perricone et al., 2015](#); [Rossi et al., 2022](#)).

[Rossi et al. \(2022\)](#) raised concerns regarding the potential emergence of microbial resistance to essential oils and toxicological issues, particularly relating to their safety in food manufacturing, allergenic potential, and possible mutagenic effects on microorganisms. Further studies are needed to clarify these aspects.

Several plant species have been associated with antibiofilm activity through their essential oils. For instance, [Rossi et al. \(2022\)](#) reported that *Dodartia orientalis* essential oil reduced the microbial load of *S. aureus*, *E. coli*, and *S. Enteritidis* biofilms by decreasing bacterial adhesion. Similarly, [Galié et al. \(2018\)](#) described the antibiofilm effects of *Salvia officinalis* and *Cinnamomum cassia* essential oils, while [Carvalho \(2021\)](#) reported successful control of *L. monocytogenes* biofilms using lemon balm (*Melissa officinalis*) essential oil, also noting its preservative potential in the food industry. [Santos et al. \(2023\)](#) demonstrated the effectiveness of black pepper (*Piper nigrum*) essential oil—which contains the active ingredient piperine—in controlling biofilms of *L. monocytogenes*, *S. Typhimurium*, and *P. aeruginosa* due to its antimicrobial, antifungal, and antioxidant properties.

Other essential oils frequently cited include citronella (*Cymbopogon nardus*), lemongrass (*Cymbopogon citratus*) ([Gray et al., 2018](#)), and rosemary (*Rosmarinus officinalis*) ([Santos et al., 2023](#)). Rosemary oil, rich in phenolic compounds, was even more effective than peracetic acid at inhibiting *L. monocytogenes* adhesion. These studies indicate that essential oils are promising alternatives; however, further research is needed to establish standardized application methods on different surfaces, compare their efficacy alone or combined with other sanitizers, and evaluate their impact on the sensory properties of foods ([Santos et al., 2023](#)).

Recently, the use of non-pathogenic and non-spoilage microorganisms, such as *Bacillus* species and lactic acid bacteria (LAB), has gained attention for biofilm control ([Mgomi et al., 2023](#); [Notário, 2019](#)). LAB are Gram-positive, catalase-negative, non-spore-forming rods or cocci that primarily produce lactic acid through carbohydrate fermentation. Their ability to perform anaerobic fermentation, along with their survival, physiological, and probiotic properties, and their capacity to form biofilms and antagonize pathogens, makes them viable candidates for controlling pathogenic biofilms in the food industry ([Mgomi et al., 2023](#)). These non-pathogenic microorganisms can inhibit pathogen adhesion and biofilm formation by competing for substrates and colonization sites, and by producing antimicrobial substances such as hydrogen peroxide, organic acids, and bacteriocins ([Seo & Kang, 2020](#)).

For example, [Notário \(2019\)](#) tested biofilms formed by *Bacillus* spp. and LAB such as *Pediococcus acidilactici* and found they controlled *S. Heidelberg* growth within the first 24 hours of contact, though they were less effective against *Campylobacter jejuni*, another foodborne pathogen. Further studies exploring other species are recommended. [Jara et al. \(2020\)](#) demonstrated that two *Lactobacillus* spp. strains prevented *L. monocytogenes* adhesion on glass surfaces but called for more research to assess whether this could control pathogen spread and affect virulence.

In dairy products, [Aman et al. \(2021\)](#) reported that *Pediococcus pentosaceus* and *Lactobacillus fermentum*, isolated from dairy derivatives, effectively inhibited biofilms formed by *P. aeruginosa*, a spoilage organism isolated from rancid butter.

Other studies showed good results using biofilms of *Streptomyces* spp. to inhibit *L. monocytogenes* on stainless steel surfaces ([Kim et al., 2019](#)). Additionally, *Paenibacillus peoriae*, *Streptomyces cirratus*, and *Pseudomonas extremorientalis* were effective against *Salmonella enterica* biofilms on stainless steel ([Kim et al., 2019](#)). [Seo & Kang. \(2020\)](#) also demonstrated the use of bacteriocins from *Pediococcus acidilactici* against *S. Typhimurium* biofilms on stainless steel. Further research is recommended to confirm the efficacy of these biocontrol methods against relevant food industry pathogens and spoilage organisms.

Given the range of available methods, it is essential to emphasize the importance of preventing biofilm formation through rigorous and well-structured quality control systems. This includes implementing self-control programs (SCPs) recommended by the Ministry of Agriculture, Livestock, and Food Supply (MAPA) and ANVISA, such as Good Manufacturing Practices (GMP), Standard Sanitation Operating Procedures (SSOP), and Hazard Analysis and Critical Control Points (HACCP), among others. Compliance with relevant food safety legislation is also crucial ([BRAZIL, 2017](#); [BRAZIL, 2022](#)). These hygiene and sanitary measures are fundamental for industries aiming to produce safe food, as they reduce microbial load and prevent contamination of surfaces and equipment during food processing.

Final considerations

Biofilms pose significant challenges to the food industry and public health by facilitating cross-contamination and the spread of foodborne pathogens. Additionally, they contribute to food spoilage and surface corrosion, leading to increased use of disinfectants, sanitizers, and energy within industrial processes. Therefore, preventing biofilm formation and implementing effective mitigation strategies are essential. Among the most commonly used disinfectants, sodium hypochlorite, peracetic acid, and quaternary ammonium compounds remain widely recommended for industrial sanitation due to their proven ability to eliminate nearly all identified pathogens, despite variability in their effectiveness.

Alternative approaches, including essential oils, bacteriophages, electrochemically activated water, nanoparticles, and biofilms of non-pathogenic and non-spoiling microorganisms, show promise as viable options. However, further research is needed to better understand their practical applications, potential residual effects on food products, and any associated risks to consumer health.

Finally, it is critical that food industries implement comprehensive self-monitoring programs to maintain hygiene standards and ensure food safety throughout processing.

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