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Microbial Exopolysaccharides, Their Structures, Formation Mechanisms, and Effects on Human Health: Food-Related Microorganisms

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ABSTRACT

A biofilm is a complex matrix formed by microorganisms that includes exopolysaccharides, proteins, extracellular DNA, various enzymes, and the microorganisms themselves. Biofilm cell is a more stable form of microorganism than planktonic cell. Microbial cells attach themselves to the surface after certain signals or changes, colonize to create a more favorable environment for their growth and viability, and secrete exopolysaccharide. This component is the basic matrix of biofilm. The diversity of exopolysaccharides within biofilms varies significantly depending on their specific composition, contributing uniquely to the characteristics of biofilms. This diversity in biofilms underscores the need for targeted control strategies. Biofilms can be beneficial or harmful depending on the situation and where they develop. Accordingly, microbial biofilms have dual effects on health. Biofilms can have both harmful effects on health, such as contributing to antibiotic resistance and persistent infections, while biofilms formed by beneficial microorganisms play a crucial role in enhancing food functionality. Moreover, the formation of biofilm in certain foods can contribute to the enhancement of the product matrix, particularly by improving its texture. In this review, the structures of these biofilms, their basic components, their possible safety concerns, and health benefits are discussed. Moreover, this review deals with biofilm producing bacteria in foods and assesses the prevention strategies for biofilm formation within the food industry.

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Introduction

Microorganisms generally found in planktonic cells or individual cells which is more sensitive to environmental conditions than biofilm producing cells (Malheiro & Simões, 2017). They can develop by adhering to surfaces and forming structures called biofilms on the surfaces they adhere to (López et al., 2010). Anthonie van Leeuwenhoek was the first to mention the existence of biofilms, and this process dates back to the 17th century (Percival et al., 2011). Dental plaque is the most well-known and typical example of biofilm formation (Høiby, 2017). One of the well-known microorganisms that play a role in biofilm formation on implants belongs to *Staphylococcus* spp. (Hall-Stoodley et al., 2004).

The theory and subsequent studies conducted by Costerton et al. (1999) explained by which microorganisms come together and hold on to living and non-living materials. Bacteria are generally free-floating in nature. However, they can also live as a colony and form biofilm (Valen & Scheie, 2018). A biofilm that can be single or multilayered is a collection of microorganisms that are comprised of an extracellular polymeric substance. Biofilm formation basically follows the steps of planktonic bacteria: (i) to adhere to the surface, (ii) to form colonies on the surface, and (iii) to develop there (Satpathy et al., 2016). The target of a self-generated biofilm matrix or exopolysaccharide (EPS), composed of proteins, polysaccharides, and extracellular DNA, is to guard cells from environmental factors, including competitive microorganisms, antibiotics, and the immune system of the host, which will adversely affect microbial activity (Schilcher & Horswill, 2020).

The biggest concern about biofilm is that it makes microorganisms more resistant to certain antibiotics or pH changes (Sharma et al., 2019). Accordingly, if a microorganism is pathogenic, biofilm enables cells to escape from the host immune system. Depending on the conditions, several Gram-positive and Gram-negative planktonic bacteria can secrete biofilms (Chen et al., 2013). Biofilms, which are good survival strategies for microorganisms, would also effects in food sector, such as spoiling foodstuffs, catalyzing reactions that cause metal corrosion, and causing equipment failure and economic loss (Alvarez-Ordóñez et al., 2019). This review presents the formation, structure, characteristics and functional properties of biofilms, addressing safety concerns, microorganisms involved in their production, technological aspects, and future perspectives.

Classifications of Biofilms and Their Structure

Biofilms often appear as non-uniform structures (Flemming & Wingender, 2010) and the main matrix components, EPSs, protein, and DNA are distributed between cells as shown in Figure 1. EPS, which is generally hydrophobic, is composed of polysaccharides. Microbial EPS can be divided into two categories including cell surface-associated capsular ones and EPSs secreted as free polymers. For microorganisms, the roles of EPS (Figure 1) include adhesion, cohesion, mechanical stability, adaptation, and protection against environmental stresses (Rather et al., 2021). Hernández-Jiménez et al. (2013) have concluded that human macrophages recognize and phagocytize planktonic cells more rapidly and efficiently than biofilm cells. To understand the transformation from planktonic cells to cell aggregation in biofilm, it is necessary to examine the biofilm formation process.

EPS can be extracted and purified by applying physical and chemical processes (Di Martino, 2018). Microbial growth conditions and growth stages affect the composition and characterization of EPS structure (Yoshida et al., 2015). One of the exciting facts about EPS is that some of the EPS contains rare sugars (Roca et al., 2015). These sugars in EPS are classified into categories containing fucose or rhamnose. The structure of polysaccharides determines biofilm formation (Di Martino, 2018). Polysaccharides with high molecular weights of about 10 to 1000 kDa are classified as homopolymers and heteropolymers (Nwodo et al., 2012). Homopolysaccharides contain only one type of monosaccharide while heteropolysaccharides are made up of different repeating units with varying sizes. Although homopolysaccharides are synthesized by extracellular enzymes, heteropolysaccharides are synthesized by a complex sequence of interactions involving intracellular enzymes (Abedfar & Hosseininezhad, 2016). Considering the EPS produced by *Cupriavidus pauculus* KPS 201, a rhamnose homopolymer containing protein, uronic acid, and nucleic acid was observed in its composition (Pal & Paul, 2013). Homopolysaccharides of lactic acid bacteria (LAB) consist of repeating units of one type of monosaccharide such as D-glucose or D-fructose (Saadat et al., 2019). On the other hand, heteropolysaccharides are also produced by the mesophilic LAB which are *Lactococcus lactis, Lactococcus lactis* subsp. *cremoris*, *Lactobacillus casei, Lactobacillus sake*, and *Lactobacillus rhamnosus* and by the thermophilic lactic acid bacteria which are *Lactobacillus helveticus Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, and *Streptococcus thermophilus* (Saadat et al., 2019).

As mentioned before, EPS contributes to the biofilm matrix. *Pseudomonas aeruginosa* produces EPS called alginate in its biofilm (Valentine et al., 2020). Alginate protects *Pseudomonas aeruginosa* cells from phagocytosis. *Escherichia coli* produces phosphoethanolamine cellulose and it provides intercellular connections (Flemming and Wingender, 2010; Moradali and Rehm, 2020). Different major polysaccharides can also be found in the biofilm structure of different microorganisms. For instance, *Staphylococcus epidermis* produces polysaccharide intercellular adhesin (PIA), which is a homoglycan composed of β-1,6-linked 2-deoxy-2-amino-d-glucopyranosyl residues (Gowrishankar & Pandian, 2017). *Pseudomonas aeruginosa* produces Pel and Psl polysaccharides in their biofilms (Gowrishankar & Pandian, 2017; Cherny & Sauer, 2020). The diversity of bacterial EPSs classified to date is summarized in Table 1. Although some studies are provided in Table 1 to show the diversity of EPS, there are studies on the production and characterization of similar EPSs for a wider variety of bacterial species and even strains in the literature. Additionally, Figure 2 represents the microorganisms identified as producers of these EPSs. Heteropolysaccharides, including gellan from *Sphingomonas elodea* ATCC 31461 and xanthan from *Xanthomonas campestris*, have distinct monosaccharide compositions, with gellan comprising β-D-glucose, L-rhamnose, and D-glucuronic acid units, and xanthan containing D-glucose, D-glucuronic acid, and L-rhamnose units (Wang et al., 2015; West, 2021).

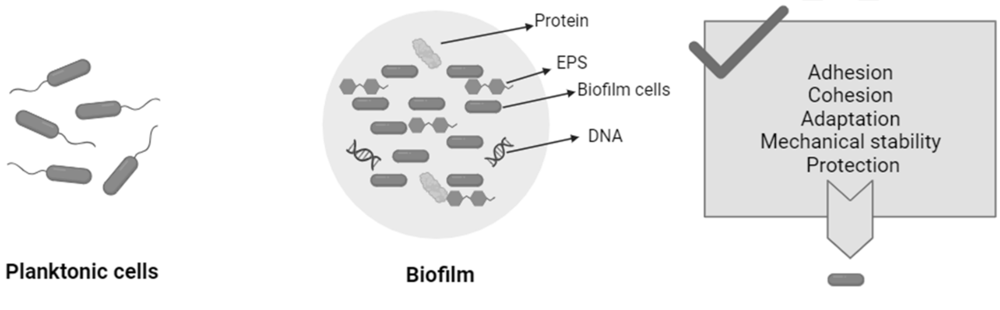

Figure 1. Biofilm structure and function of EPS component (created with BioRender version 2023).

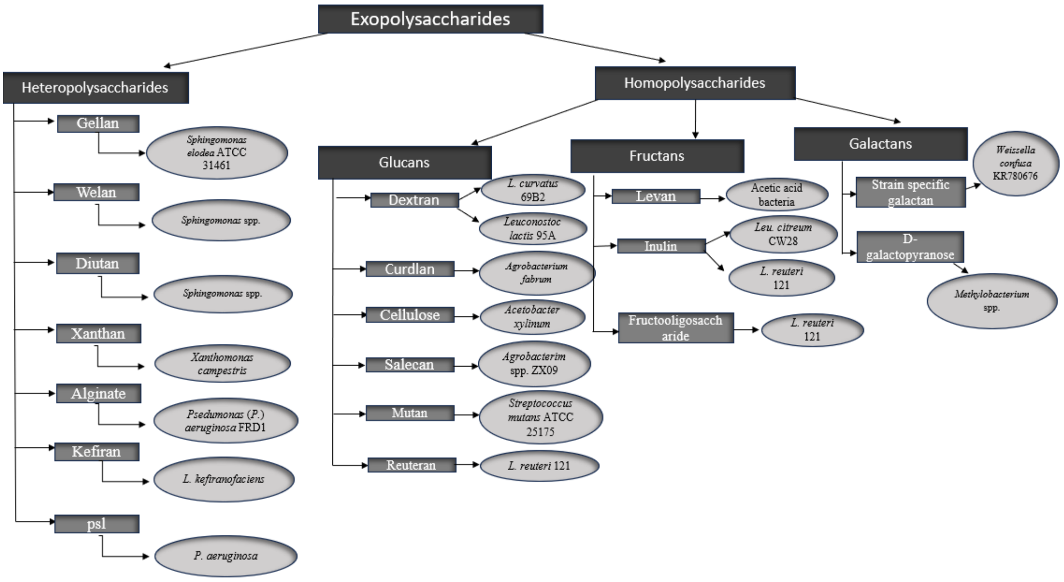

Figure 2. Bacterial sources of hetero- and homopolysaccharides.

Table 1. Exopolysachharides defined in bacterial biofilms

Microbial exopolysaccharides			
Homopolysaccharides		*Heteropolysaccharides*	
Dextran	(Palomba et al., 2012)	Gellan	(West, 2021)
Curdlan	(Laxmi et al., 2018)	Welan	(Zhao et al., 2021)
Cellulose	(Ghozali et al., 2021)	Diutan	(Coleman et al., 2008)
Salecan	(Xiu et al., 2011)	Xanthan	(Nejadmansouri et al., 2021)
Mutan	(Banas et al., 2007)	Alginate	(Bustamante-Torres et al., 2022)
Reuteran	(Meng et al., 2016)	Kefiran	(ZajŠEk et al., 2011)
Levan	(Anguluri et al., 2022)	psl	(Cherny and Sauer, 2020)
Inulin	(Van Hijum et al., 2002; Ortiz-Soto et al., 2009)	Gellan	(West, 2021)
Fructooligosaccharide	(van Hijum et al., 2002)	Welan	(Zhao et al., 2021)
Strain specific galactan	(Kavitake et al., 2016)		
D-galactopyranose	(Verhoef et al., 2003)		

Homopolysaccharides are further categorized into glucans, fructans, and galactans. Glucans such as dextran, produced by *Leuconostoc* lactis 95A (Palomba et al., 2012), and cellulose, synthesized by *Acetobacter xylinum* (Ghozali et al., 2021), consist of glucose monomers. Fructans composed of fructose units like inulin and levan are synthesized by bacteria such as acetic acid bacteria and lactic acid bacteria, respectively (Van Hijum et al., 2002; Ortiz-Soto et al., 2009; Anguluri et al., 2022). Lastly, galactans include D-galactopyranose, synthesized by *Methylobacterium* spp., composed mainly of galactose units (Verhoef et al., 2003). This classification is crucial for understanding the biosynthesis, application, and control of these biopolymers in various industries.

Biofilm Formation by Microorganisms

Bacteria exist in nature in two forms: (i) planktonic (individual) cells and (ii) microbial aggregates (biofilm cells) (Figure 1). Biofilm formation is seen when planktonic cells come together and attach to the surface where they are located. The formation of these microcolonies is followed by the maturation of the biofilms to form large colonies (Ghanbari et al., 2016; Sauer et al., 2022).

Biofilm formation begins with the contact of planktonic cells with the surface. In the first stage, planktonic cells are free in the environment. After that, cells are aggregated, and biofilm formation occurs by increasing the cell density (Flemming & Wingender, 2010). Microorganisms attach to the surface with weak interactions like Van der Waals interactions and establish colonies there. Colonization is achieved by polysaccharides found in or secreted by microorganisms (Sauer et al., 2022). Flagella structures also facilitate the colonization. Adhesion provides long-term binding to the surface. At the same time, the aggregation of cells allows them to recognize each other and promotes the total cell density. After increasing cell density, a maturation process and a stable structure are observed. They then separate into clusters and establish colonies elsewhere (Flemming and Wingender, 2010; Yin et al., 2019; Sauer et al., 2022). Microbial biofilm formation occurs due to factors such as nutrient competition, oxygen depletion, pH, and fluctuating temperatures (Rumbaugh & Sauer, 2020). The biopolymers known as EPS are responsible for adhesion to surfaces and cohesion in the biofilm (Flemming & Wingender, 2010). EPS improves the resistance of biofilms to antimicrobial agents (Yüksel et al., 2018).

Biofilms act as a protective barrier for microorganisms. They form biofilm structures to adapt to stress conditions, to perform intercellular communication, and to establish colonization. Cell-to-cell communication, known as quorum sensing, is one of the metabolic processes leading to biofilm formation and mainly mediated by small diffusible molecules called autoinducers (Banerjee et al., 2019). Once the concentration of autoinducers reaches a threshold, cells detect these signals, initiate local colonization, and begin forming a biofilm. This process is highly conserved evolutionarily, however, different autoinducers are used for Gram-negative and Gram-positive bacteria (Fuqua et al., 1994).

Concerning Microorganisms in Foods

Foodborne disease is an issue that affects public health all around the world. About 800 foodborne outbreaks have been reported annually in the United States (Qiu et al., 2021). In 2006-2016, a great majority of these outbreaks were caused by *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes*. While pathogenic or spoilage microorganisms can pose risks to food safety, food quality, and human health, starter cultures and probiotic microorganisms can be beneficially used in food production, applications, and the development of functional foods. Fermentation, which is a process step used in the production of food products, such as yogurt, wine, and pickles, is a good example of this situation. Food microorganisms are summarized in Figure 3.

Pathogenic and Spoilage Microorganisms

Food spoilage can be defined as a deterioration or change in the sensory properties of food that are unacceptable to consumers. This process is caused by different spoilage microorganisms. Microorganisms involved in food spoilage belong to bacteria and fungi (Schmeisser et al., 2007). *Zygosaccharomyces* spp., *Saccharomyces* spp., *Debaryomyces hansenii* and *Candida* yeasts generally cause spoilage in foods, changing color, odor, and texture. Lorenzo et al. (2018) have stated that the most common spoilage bacteria in foods consist of *Lactococcus* spp., *Leuconostoc* spp., *Lactobacillus* spp., *Pediococcus* spp., *Streptococcus* spp., *Carnobacterium* spp., *Brochothrix thermosphacta*, *Kurthi zapfilai,* and *Weissella* spp. Among these microorganisms, species such as *Lactococcus*, *Leuconostoc*, *Lactobacillus*, and *Streptococcus* spp. are particularly used as starter cultures in food production. However, biofilm formation can vary depending on the species and even the strain (Wallis et al., 2018). Additionally, LAB, known as Generally Recognized as Safe (GRAS), are used in food products. Not all industrial cultures produce biofilms, but cultures that produce EPS, the main component of biofilms, can be preferred to use for enhancing the textural properties of the final product (Berthold-Pluta et al., 2019). Regarding this, detailed information is provided under the section on starter cultures.

Food pathogens are microorganisms threatening public health and food safety. The most common food pathogens encountered in many parts of the world have been identified as *Clostridium botulinum*, *Campylobacter*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella*, and some toxin producer molds (Bintsis, 2017). Canned food produced in unfavorable conditions contains *Clostridium botulinum*. This bacterium causes botulism by producing neurotoxins that affect the human nervous system (Parkinson et al., 2017). *Escherichia coli* O157:H7, which is mostly encountered in undercooked meat, meat products and unpasteurized milk and dairy products, is also a foodborne pathogen (Bedasa et al., 2018). *Listeria monocytogene*s, which appears in foods, such as raw and processed milk and dairy products, meat and meat products, and seafoods, causes listeriosis (Shamloo et al., 2019). *Campylobacter enteritis* is the most common cause of enteric infections. *Campylobacter jejuni* infection occurs after consuming or contacting contaminated poultry, meat, milk, or water (Facciolà et al., 2017; Hansson et al., 2018). *Salmonella* spp., which is one of the main pathogens occasioned by food infections, occurs in unpasteurized products, cocoa, and cake mixes. In this group, the two main *Salmonella* serotypes transmitted from animals to humans are *Salmonella* Enteritidis and *Salmonella* Typhimurium (Demirbilek, 2018; Sharma et al., 2019; Olaimat et al., 2020).

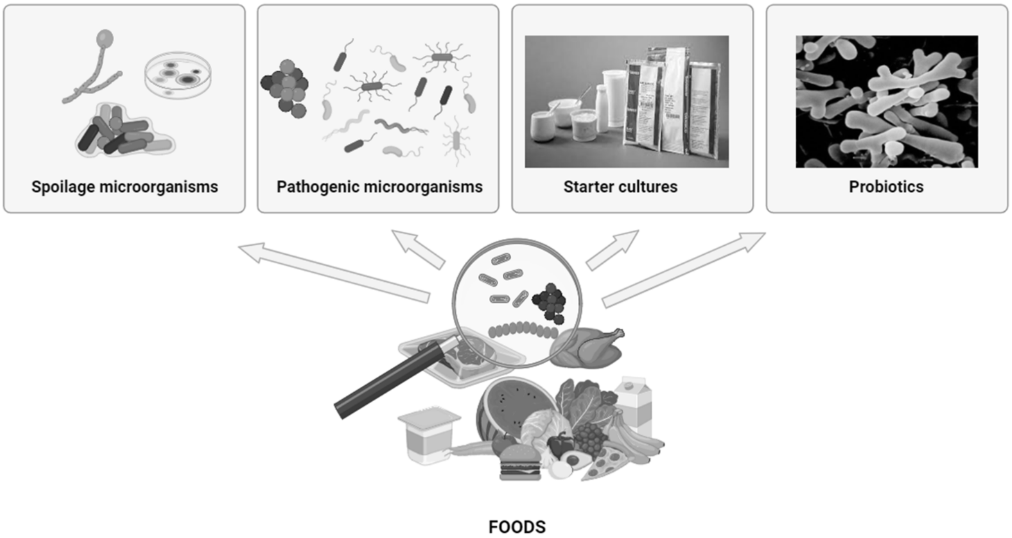

Figure 3. Microorganisms found in food products (created with BioRender version 2023).

Foodborne pathogens and their biofilms are the main causes of foodborne diseases. It has been reported that *Listeria monocytogenes* forms biofilms in food processing environments (Wang et al., 2022). *Campylobacter jejuni*, which causes gastrointestinal disorders, is frequently observed in poultry products. Pokhrel et al. (2022) have reported that this pathogen forms a biofilm when exposed to oxidative stress conditions. Essentially, biofilm formation occurs through various stress responses or quorum sensing mechanisms (Zhao et al., 2023). *Salmonella* biofilms are responsible for various foodborne diseases and contamination of the food processing environment (Seo & Kang, 2020). In this context, the use of some probiotic bacteria and lactic acid bacteria as antibiofilm agents in the food industry has been suggested (Vuotto et al., 2014).

Starter Cultures

Starter cultures can be defined as living microorganisms that contain a single type or mixture of microbial cells and are added to foods to produce final characteristic product (García-Díez & Saraiva, 2021). Starter cultures are responsible for executing the fermentation process in foods like dairy products and meat products. Besides the role of initiating fermentation, starter cultures are known to inhibit the growth of unwanted microbiota in food product (Laranjo et al., 2019). LAB, which is the most common example for starter cultures in the production of food products such as cheese and yogurt. LAB produce lactic acid from lactose, accelerating the acidification in foods. Thus, it enhances food safety through its antimicrobial effects while also improving the sensory properties of the food (Altieri et al., 2017).

Some strains of *Lactobacillus*, such as *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Lactobacillus fructivorans,* one of the most well-known starter cultures, appear to form biofilms (Kubota et al., 2008). Biofilm formation is not always harmful. With the formation of biofilm, microorganisms, such as *Acetobacter* and *Gluconobacter* spp., which are used in vinegar production, grow more on wooden chips and this ensures efficient production (Giudici et al., 2017). In another study on starter culture for fermented milk production, biofilms of *Lactobacillus plantarum* culture had a positive effect on planktonic probiotic *Lactobacillus plantarum* cells (Hu et al., 2019). Moreover, EPS in biofilm produced by LAB has been reported to improve the texture and viscosity of foods (De Souza et al., 2023).

Probiotics

The term 'probiotic' is derived from the Greek word meaning 'for life.' Probiotics are living microorganisms that have a positive influence on the host when consumed in adequate quantities (Özkan et al., 2021). Probiotics have positive effects such as balancing the intestinal microbiota, alleviating the effects of lactose intolerance, protecting against cancer, and supporting the immune system (Binda et al., 2020). Commonly used probiotics are *Lactobacillus*, *Bifidobacterium, Bacillus*, and *Streptococcus* species. The action mechanisms of probiotics are stimulation and support of the immune system and inhibition of pathogenic microorganisms by producing antimicrobial substances, blocking their attachment sites, and competing for nutrients (Bermudez-Brito et al., 2012). The biofilm-forming probiotics as *Bifidobacterium longum* subsp. *infantis* and *Lactobacillus reuteri* have demonstrated an ability to effectively delay microbial spoilage (Speranza & Corbo, 2017). Probiotics are able to secrete products, such as hydrogen peroxide, bacteriocins, organic acids, and lactic acids, to inhibit the pathogens. Interestingly, probiotics not only promote gut health but also exhibit effective biofilm inhibition. This inhibition occurs through various mechanisms, such as competing with pathogens for adhesion sites, producing antimicrobial substances, and disrupting the communication signals essential for biofilm formation (Barzegari et al., 2020).

Effects of Biofilms on Human Health and Safety Concerns

Biofilms may cause several infectious diseases. Biofilm-forming organisms can cause human infections, such as keratitis on contact lenses, chronic sinusitis, and wound infection (Del Pozo & Patel, 2007). In biofilm studies, it has been observed that bacteria within biofilms can exhibit antibiotic resistance due to several factors, including the limited penetration of antibiotics into the biofilm, the chemical composition and thickness of biofilm matrix, and the inherent resistance mechanisms of the microorganisms themselves (Singh et al., 2010; Sharma et al., 2019). The increased antibiotic resistance of biofilm cells compared to planktonic cultures, combined with endotoxin production by Gram-negative bacteria, plays a significant role in the progression of diseases (Saxena et al., 2019). These factors contribute to the persistence and severity of infections, making them more challenging to treat effectively. Another factor contributing to the resistance of biofilms against antibiotics is the accumulation of enzymes, such as β-lactamases, that degrade antibacterials within the biofilm matrix (Dincer et al., 2020).

Moreover, biofilm formed microorganisms can be evaded from immune systems by hiding themselves or staying dormant. This escape mechanism might lead to an increase in local tissue damage. Also, in the literature, there is evidence for the interaction between biofilm formation and cancer (Ivanenko, 2021). Cancer cells can be defined as cells that can uncontrolledly cell division. The aggregation of cancer cells leads to tumor formation. Cancer cells and biofilms are similar in a way that both try surviving in extreme conditions (Ivanenko, 2021). Biofilms can be involved in tumor formation, promotion, progression (Choi et al., 2023). Evidence shows that biofilm formation can lead to changes in the surrounding tissue environment that to the aid of cancer cells (Upadhyay et al., 2023).

Technological Strategies for Preventing Biofilm Formation in the Food Industry

Biofilm formation is an important problem that threatens food safety in the food industry (Lindsay & von Holy, 2006). Biofilm formation is a dynamic process, not a static one, with biofilm structures changing based on the type of microorganisms, the surface they attach to, and the environmental components (Tang et al., 2011). The first step in controlling and preventing biofilm formation is to take necessary precautions before biofilm formation (Meyer, 2003). Effective and appropriate cleaning should be done at regular intervals in the food plant. In addition, angular and blunt equipment should not be used, as corner points generally cause accumulation, and round structures are easier to clean. Silver-plated surfaces show inhibitory properties of biofilm formation and polymer coatings show antibiofilm properties (Knetsch & Koole, 2011; Vera-González & Shukla, 2020).

Different biofilms have different spectral properties and the structure of the relevant biofilm can be studied using image processing methods (Grichkin et al., 2017; Achinas et al., 2020). In the most food factories, mechanical processes are applied to the equipment surface to remove the biofilm during cleaning (Meyer, 2003). Automatic brushing or high-pressure cleaning is more effective than gel cleaners or low-pressure cleaning. There are problems in sanitation and disinfection due to the resistant structures of biofilms, affecting both food safety and food quality (Abebe, 2020). In addition to traditional biofilm control mechanisms, ultrasonication, electric fields, enzymes, or hurdle technologies such as using hydrogen peroxide and ultraviolet light together, can be used to break down the EPS (Simões et al., 2010; Chemat et al., 2011; Vankerckhoven et al., 2011). However, the effectiveness of each technique may differ according to the applied surface, the type of bacteria that form biofilm, and the application methods (Meyer, 2003).

Since the use of chemicals for biofilm removal does not give effective results in all cases, different strategies have been developed instead of using traditional cleaning and disinfection methods. Innovative prevention strategies against biofilms are summarized in Table 2. Modern approaches such as nanotechnology-based applications, quorum sensing inhibitors (QSIs), enzymatic biofilm disruptors, phage therapy, biologically based strategies, and photodynamic therapy (PDT) hold promise in preventing and eradicating biofilms (Zhu et al., 2022; Ribeiro et al., 2022). The mode of action of these methods may affect the early stages of biofilm formation or other phases of the biofilm development process. One of the strategies to remove biofilms involves the use of enzymes to degrade the EPS of biofilms (Johansen et al., 1997). The different structures of EPS (Table 1) require the use of different types of enzymes. For this purpose, enzymes such as DNases (human DNase I), proteases (Savinase, Everlase, Polarzyme), amylases (BAN-alpha-amylase, AMG-glucoamylase), cellulases, and glycoside hydrolases (Dispersin B, alginate lyase) are used in the disruption of biofilms (Mayton et al., 2021; Molobela et al., 2010). Another novel method is related to bacteriophage application to prevent the formation of biofilms by microorganisms (Squires, 2018). Bacteriophages are viruses that specifically infect bacteria, allowing for targeted action against biofilm-forming pathogens without harming beneficial microorganisms, unlike broad-spectrum antibiotics (Tian et al., 2021). Moreover, bacteriophages produce enzymes such as depolymerases, which degrade the EPS within biofilms, weakening the biofilm structure and enhancing susceptibility to antimicrobial agents (Topka-Bielecka et al., 2021). However, there are some difficulties with the use of phage theory. One of these challenges is that bacteriophages reach target bacteria and bind to specific receptors found on bacterial cells. In planktonic cells, this connection is much easier than biofilm cells. The microbial cells in the biofilm are enclosed by a matrix, which prevents bacteriophages from easily binding to their receptors (Pires et al., 2017).

Table 2. Novel and traditional elimination methods for biofilm

Novel Methods	Description	Advantages	Limitations	References
Enzymatic Degradation	Targeting destruction of EPS	Prolong resistance development	Heterogeneity structure of EPS, accordingly single enzyme might not effective	(Ramakrishnan et al., 2022; Wang et al., 2023)
Quorum Sensing Inhibitors	Blockage on cell-to-cell communication	Usage of natural product as an inhibitor	Targeting and specificity problems	(Machado et al., 2020)
Photodynamic Therapy	Usage of light, molecular oxygen, and photosensitizer against microorganisms	Eliminate microorganisms by not using harsh chemicals	Low penetration of PS with biofilm Short-life time of ROS	(Hu et al., 2022)
Nanotechnology based methods	Development of new surfaces as an antibacterial surface	Prevention of adhesion ability of microorganisms	Potential of leaching	(Li et al., 2021; Birkenhauer, & Neethirajan 2015
Bacteriophage	Usage of “virus” to eliminate biofilm formation.	Alternative approaches to antibiotic usage	Limited host-range	(Topka-Bielecka et al., 2021)
Traditional Methods	Description	Advantages	Limitations	References
Cleaning in place	Cleaning of equipment via circulating fluid flow	Less time consuming	Not easy to remove accumulated biofilms	(Loeffler, 2006)
Ultrasound cleaning	Usage of mechanical energy	No usage of chemical	Low intensity does not affect bacterial biofilm	(Elafify et al., 2024)
Antibiotics	Disruption of microorganisms’ cellular integrity	Easy to use Adaptable for combined therapy	Development of antibiotic resistance	(Sullivan et al., 2020)

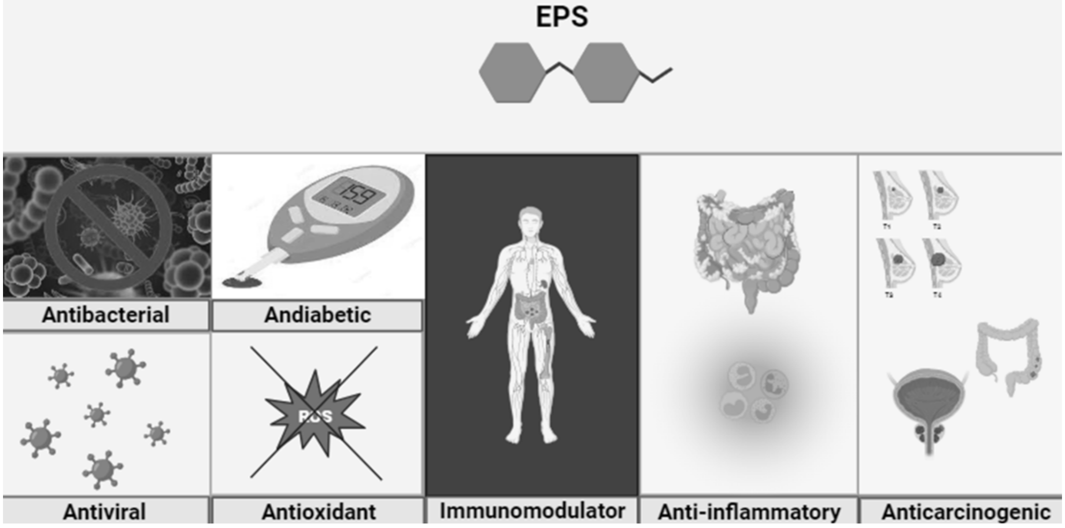

Figure 4. Functional properties of EPSs secreted by microorganisms (created with BioRender version 2023).

PDT is an emerging strategy with diverse applications, particularly noted for its potent antimicrobial effects (Songca & Adjei, 2022). PDT relies on chemical reactions triggered by the interaction of molecular oxygen, a photosensitizer, and light (Niculescu & Grumezescu, 2021). When these three components come together, they produce toxic compounds known as reactive oxygen species (ROS) or singlet oxygen. These compounds degrade the matrix of biofilms, breaking down polysaccharides and protein. This weakening of the matrix leads to the disruption of the biofilm. Silver nanoparticles are widely recognized for their strong antimicrobial properties (Liao et al., 2019). They can penetrate biofilm matrices and disrupt bacterial cells by generating ROS, damaging cell membranes, and interfering with bacterial DNA. Other metallic nanoparticles, such as gold, zinc oxide, and titanium dioxide, are also effective in inhibiting biofilm formation and promoting the degradation of existing biofilm (Pourmehdiabadi et al., 2024). Another approach involves using QSIs to effectively disrupt biofilm formation and inhibit the production of virulence factors (Wang et al., 2024). Quorum sensing relies on a variety of molecules in its process, and QSIs can interfere with this system, thereby reducing the formation of biofilms and the pathogenicity of bacteria. To enhance the efficacy of QSIs, they can be used in combination with antibiotics. QSIs work through three primary mechanisms: directly inhibiting the production of the signal molecule, promoting the breakdown of the signal molecule, or inhibiting the interaction between the signal molecule and its receptor (Carradori et al., 2020). Developing new technologies and methodologies will give people the power to control unwanted microbial cell growth.

Functional Properties of Secreted Biofilms

EPS, which differs in terms of physical and chemical properties, is secreted by microorganisms out of the cell (Karygianni et al., 2020). Although it is a matter of great concern that pathogenic microorganisms produce EPS, these biopolymers produced by beneficial bacteria can have positive effects on human health. It has been observed that EPSs have beneficial effects, such as antioxidant, immunomodulator, anticarcinogenic, anti-inflammatory, and antiviral, in several studies (Angelin & Kavitha, 2020; Wu et al., 2021; Zayed et al., 2022). Ayyash et al. (2020) have reported that EPSs produced by *Lactobacillus plantarum* isolates among LAB have antioxidant, antidiabetic, and antitumor activities. Jeong et al. (2017) revealed that the EPS synthesized by *Lactobacillus. kefiranofaciens* DN1 showed both bacteriostatic and bactericidal activity against *Listeria monocytogenes* and *Salmonella* Enteritidis. The functional properties of EPS are summarized in Figure 4. Secreted EPSs can be used in many industries, such as food, pharmacology, textiles, and cosmetics. The physicochemical and functional properties of some EPS species make them basic microbial synthesis products with various biotechnological applications (Osemwegie et al., 2020; Zayed et al., 2022).

Tarannum et al. (2023) conducted a study on the antioxidant activity of EPS produced by new bacterial strains isolated from bovine milk. Among the 63 isolates, 4 strains exhibited higher EPS production and significant antioxidant activity, along with high antibiofilm activity against *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 25922. Another study demonstrated that *Lactobacillus plantarum* 12 exhibited effects against *Shigella flexneri*, revealing that the EPS produced by *Lactobacillus plantarum* 12 not only inhibited the biofilm formation but also reduced the antibiotic resistance of *Shigella flexneri* (Song et al., 2020).

Due to the significant diversity of the gut microbiota, maintaining a balance among the various microorganisms is of paramount importance. To prevent and address imbalances within the gut microbiota, several strategies have been developed. Among these strategies, the use of probiotics, prebiotics, and postbiotics has emerged as a promising solution. During the journey of probiotics in the body, they are faced with different conditions and their effectiveness might be decreased until they reach their target (Han et al.,2021). To overcome these challenges, embedding probiotics in biofilms may present an effective solution. Biofilms are known for their resistance to environmental changes, including variations in pH, temperature, nutrient derivation, and exposure to antibiotics, thereby offering enhanced protection and viability for the probiotics (Gao et al., 2022).

Concluding Remarks and Future Perspectives

This review has provided a comprehensive overview of microbial biofilms, their structures, formation mechanisms, and their implications for human health, particularly in the context of food-related microorganisms. Biofilms are specialized structures that enable microorganisms to survive and proliferate in challenging environmental conditions. Although the general mechanism for biofilm formation is the same for most microorganisms, species- or even strain-specific behaviors are unique to each microorganism. The formation of biofilms by pathogenic and spoilage microorganisms presents serious challenges for food safety and public health, as these biofilms are often at the center of persistent infections and can accelerate the degradation of food products, leading to both safety risks and economic losses in the food industry. Conversely, biofilms produced by beneficial microorganisms, particularly those rich in EPS, offer valuable technological and functional benefits. These EPS can enhance food quality by improving texture, stability, and extending shelf life. However, the dual nature of biofilms, as both beneficial and harmful, underscores the need for ongoing research. Future studies should aim to better understand the specific roles of EPS in promoting health and food quality, as well as to develop innovative and sustainable strategies to prevent the formation of harmful biofilms. Advancing our knowledge in these areas is crucial for maximizing the benefits of microbial biofilms while minimizing their risks.

Declarations

The authors have declared that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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