Review Article

Controlling Biofilm Formation of Foodborne Pathogens Utilizing Probiotics in the Food Industry

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Abstract

The formation of biofilms by microorganisms on food-contact surfaces poses a significant challenge in the agro-food industry. These biofilms act as protective shelters for harmful bacteria, allowing them to survive harsh food preparation conditions and resist antimicrobial agents, including conventional sanitizers and cleaning agents. Addressing this issue is critical for ensuring food safety and mitigating contamination risks. Probiotics, beneficial microorganisms widely used in food production, have emerged as a promising solution for controlling biofilm formation. Through mechanisms such as displacement, exclusion, and competition, probiotics inhibit the adhesion and subsequent development of biofilms by foodborne pathogens. Recent studies highlight the potential of specific probiotics and their byproducts to disrupt existing biofilms, reducing bacterial resistance and contamination risks. This review synthesizes current research on the application of probiotics in biofilm management, focusing on their mechanisms of action, effectiveness across various food systems, and practical implications for the agro-food sector. The use of probiotics represents a sustainable and innovative strategy to control biofilm formation and enhance food safety. By leveraging their unique properties, the agro-food industry can address challenges associated with biofilm-associated contamination, ensuring safer food production processes.

Keywords: Biofilm, Biofilm formation, Food industry, Probiotics, Inhibition of biofilm, Quorum sensing

Introduction

Foodborne illnesses continue to pose a serious global threat to public health, contributing significantly to morbidity, mortality, and economic losses across the world [1]. A major challenge in controlling these infections is the way foodborne pathogens build biofilms. In food processing settings, biofilms—structured colonies of microbial cells encased in a self-made polymeric matrix—stick to surfaces like rubber, plastic, and stainless steel [2]. Once formed, these biofilms give bacteria a safe haven that increases their resilience to environmental stressors, disinfectants, and antimicrobial treatments. This raises the possibility of ongoing contamination in systems used to produce food [3, 4].

The contamination of food products can occur at various stages along the food supply chain—including production, processing, packaging, transportation, and final preparation—making biofilm control a critical component of food safety management [5]. Not only do biofilms act as reservoirs for pathogens, but they also lead to sensory and quality degradation in food, affecting taste, smell, texture, and shelf life [6].

Common biofilm-forming foodborne pathogens include *Salmonella spp.*, *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perfringens*, and *Vibrio spp.*, all of which have been linked to significant outbreaks and food recalls globally [7]. According to the European Food Safety Authority (EFSA), *Salmonella* and *Campylobacter* were the most frequently reported causative agents of zoonotic foodborne diseases in 2020, followed by *Listeria* and *Yersinia* [8].

The food industry has used a variety of techniques, including mechanical scrubbing, thermal processing, chemical sanitisers, and surface coatings, to reduce the production of biofilms. However, because of the tenacity of bacteria buried in biofilms and the possible hazards to the environment and

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human health posed by chemical treatments, these methods frequently fail [9, 10]. Consequently, the search for safer, sustainable, and more effective alternatives has gained momentum.

One promising approach is the use of probiotics—live microorganisms that confer health benefits when administered in adequate amounts [11]. Beyond their traditional role in gut health, probiotics have demonstrated potential in inhibiting biofilm formation by foodborne pathogens through various mechanisms, including competitive exclusion, production of bacteriocins and organic acids, co-aggregation with pathogens, and disruption of quorum sensing (QS) pathways [12-14]. Recent studies have shown that strains such as *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus*, *Lactobacillus acidophilus*, and *Pediococcus acidilactici* exhibit strong anti-biofilm properties in in vitro and situ models [15, 16].

Moreover, the concept of probiotic cocktails, combining multiple strains, has emerged as a more robust strategy for biofilm disruption, offering synergistic effects and broader antimicrobial spectra [17]. Despite the promising findings, there remains a need to explore the practical application of probiotics in food processing environments, the stability of their anti-biofilm activity under industrial conditions, and their regulatory acceptance as part of food safety interventions.

The purpose of this narrative review is to examine the current understanding of foodborne pathogen biofilm formation and the growing role of probiotics as an all-natural and successful biofilm management method in the food business.

Materials and Methods

Literature Selection Criteria

A comprehensive search of the available literature was conducted using several electronic databases, including PubMed, Scopus, and Web of Science. Studies published between 2000 and 2024 were considered for inclusion. The selection process involved the following inclusion criteria:

- Studies focused on foodborne pathogens and probiotic interventions in the food industry.
- Peer-reviewed articles, clinical trials, experimental studies, and systematic reviews.
- Articles available in English.

Exclusion criteria included:

- Studies not related to probiotics or foodborne pathogens.
- Non-peer-reviewed sources (e.g., conference abstracts, editorials).
- Studies focusing on non-food-related applications of probiotics.

The final selection was based on the relevance and methodological quality of the studies, ensuring a comprehensive representation of the current state of research.

Results and Discussion

• *Biofilm Formation*

Biofilms are cohesive structures composed of microorganisms that adhere to biotic and abiotic surfaces. Microbes wrap themselves in an extracellular matrix through the secretion of extracellular polymers. Essentially, the outer layers of microbes connect with the surfaces of intricate groups of microbes. Bacteria generate a polymer matrix composed primarily of biomolecules. This polymer matrix creates a well-aerated and moisturized combination which plays a crucial role in maintaining the biofilms and their 3-D (dimensional) configurations. Significantly, the features of biofilms offer microorganisms safeguard from natural elements along with the increase of the defense against antibacterial treatments, hence helping with the endurance and harmfulness of microbes. Therefore, the production of bacterial biofilms is a crucial component of the bacterium's mechanism for survival [10]. The morphological composition and susceptibility to ecological influences along with living attributes of microbes in biofilms differ significantly from the microbes in plankton. Additionally, the 3-D arrangement that biofilms have served as an inherent defense along with a shielding coating against microbes [11].

The development and growth processes of biofilms are continuing, ever-changing, and intricate procedures that work based on factors such as the matrix, culture media, essential cell properties, messenger molecules, cellular biomolecule processes, and hereditary regulation. The process of biofilm development comprises five consecutive stages and these stages are shown in **Figure 1**:

This figure illustrates the sequential stages of biofilm development: initial attachment, irreversible attachment, maturation I and II, and dispersion. It highlights how free-floating (planktonic) bacteria adhere to surfaces, form microcolonies, develop complex extracellular matrices, and eventually disperse to colonize new sites. Understanding these stages is critical for identifying points of intervention to disrupt biofilm formation [12].

In order to create an appropriate surface layer, bacterial biofilms begin with the absorption of either organic (like proteins, lipids, polysaccharides, fatty acids, etc.) or inorganic (like inorganic salt, water, etc.) molecules. Following that, this layer is integrated into a variety of Extracellular Polymeric Substances (EPS) in either single or mixed communities [13]. After bacteria have adhered to biotic and abiotic outer layer, they engage in intercellular communication using an extracellular signaling mechanism known as QS. By encouraging particular genetic material in microbes to produce extracellular matrix, including EPS and

proteins, QS regulates the entire biofilm growth process, leading to the gradual development of a fully developed and mature biofilm structure. QS's ability to communicate between cells is crucial to the formation of biofilms. Bacterial cells use a process called QS to control the production, release, and buildup of signal molecules outside of their cells through chemical communication [14].

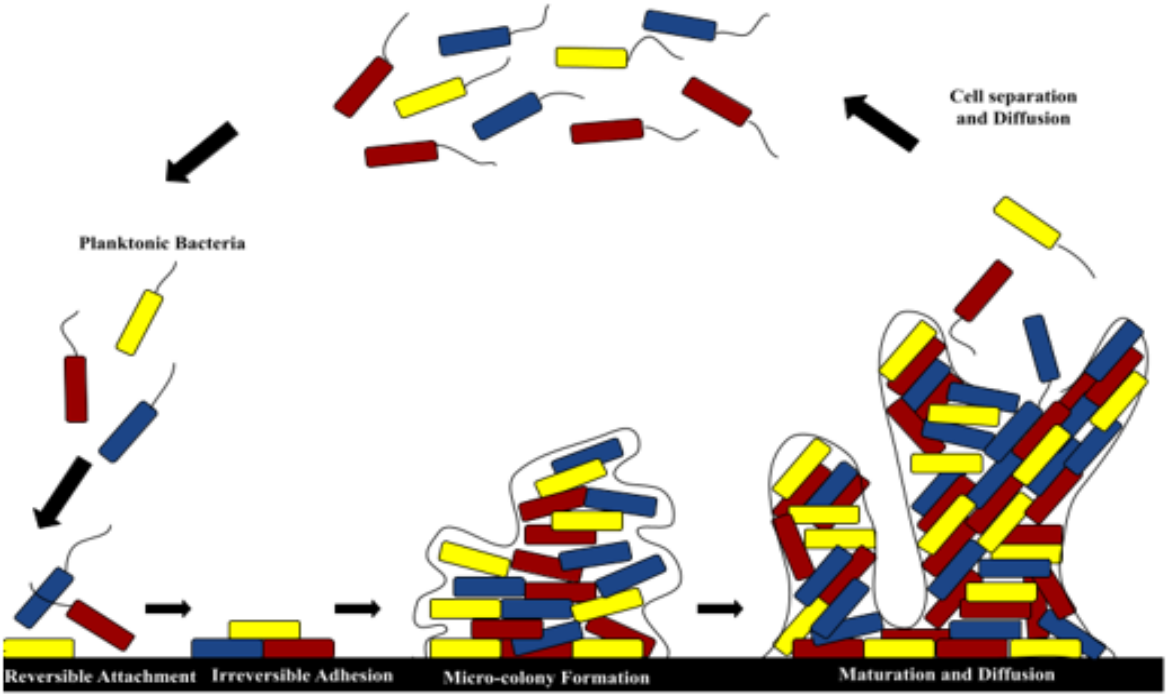

Figure 1. Stages of Biofilm Formation

Role of Biofilm in Food Industry

Inter-mixing of pollutants in food may occur at any point in the food supply progression, including manufacturing, procedure of processing, preservation, dissemination, and preparation of food by customers [3]. Food contamination can result in alterations in flavor, fragrance, surface quality, and visual aspects that are regarded as unsatisfactory and unwanted [4]. Raw, uncooked, little processed food, primarily derived from animals but also including fruits and vegetables, is very susceptible to bacterial contamination [13]. Bacteria tend to attach themselves to surfaces that come into touch with food and create biofilms, rather than remaining in a free-floating state in the water. Biofilms present a significant hygiene threat as they serve as reservoirs for foodborne pathogens. These microbes modify the sensory food qualities by releasing lipases and proteases [15]. The presence of nutrients along with dampness on the outer layers facilitates the growth of biofilms that occur on many hard materials such as fruits, meats, bones, and food industry equipment made of stainless steel, plastic, polystyrene, and glass [16].

Listeria monocytogenes is a major foodborne pathogen that forms biofilm when in contact with hard surfaces like stainless steel, plastics, etc. Food is contaminated when comes into contact with such hard surfaces [17].

It has been discovered that slaughter areas, such as equipment used in chicken processing, can produce isolates of *Salmonella*. Because the environment is typically damp, biofilm development is highly favorable. Though the prevalence of *Salmonella* biofilms in food processing environments is poorly understood, research has demonstrated and confirmed *Salmonella's* ability to attach to and form biofilms on surfaces such as those found in food processing plants' stainless steel, cement, and plastic [17].

Foodborne diseases are caused by using items like milk and other dairy products and are caused by *Staphylococcus aureus*, a significant foodborne pathogen with the ability to form biofilms. In the dairy sector, biofilm formation often happens on almost every surface of technological systems [17].

- ### Pathogenic and Foodborne Microbes

In 2020, *Salmonella* spp. was responsible for the most reported cases of foodborne illnesses, followed by *Listeria monocytogenes*. Other important bacteria that cause

foodborne diseases include *Vibrio* spp., *Clostridium* spp., *Staphylococcus* spp., and *Pseudomonas aeruginosa* [5]. Symptoms of *Salmonella* include fever, diarrhoea, and gastrointestinal distress. Acute gastroenteritis and more serious conditions including meningitis and abortion can be brought on by *L. monocytogenes*. Acute gastroenteritis can be brought on by *S. aureus* [18].

The pathogenic microbes that cause foodborne diseases are given in **Table 1** with their source of infection and symptoms caused by them.

Table 1. Pathogenic and Foodborne Microbes with their source of infection and symptoms

Microorganism	Disease	Source of Infection	Symptoms	References
Staphylococcus aureus	Staphylococcal food poisoning	Contamination through improper food handling practices Cross-contamination inadequate cleaning.	Nausea Vomiting Retching Diarrhea	[19, 20]
***Salmonella* sp.**	Salmonellosis	Contamination of raw foods of animal origin like meat, poultry, eggs, and unpasteurized milk cross-contamination in kitchens.	Diarrhea Abdominal cramps Nausea Fever	[21-23]
Campylobacter jejuni	Campylobacteriosis	Contaminated water unpasteurized milk raw or undercooked poultry meats and seafood.	Diarrhea abdominal cramps nausea fever	[24, 25]
Escherichia coli	Hemolytic Uremic Syndrome	Consumption of undercooked meat raw shellfish contaminated food	Bloody diarrhea abdominal cramps	[26, 27]
Listeria monocytogenes	Listeriosis	contaminate food products during harvesting, processing, preparation, packing, transportation, or storage Contamination can occur through raw materials, water, soil, incoming air, and even pets spreading the bacteria in the home environment if they consume contaminated food.	Fever Muscle Aches Nausea Vomiting Diarrhea Headache stiff neck Confusion	[28, 29]
Clostridium botulinum	Botulism	spores are found on the surfaces of fruits, vegetables, and seafood. The toxin is most commonly formed when food is improperly processed (canned) at home, especially low-acid foods like vegetables and meats that are not processed under pressure to kill the spores. Improperly handled commercial food products	Vertigo double or blurred vision loss or light reflex difficulty in swallowing dry mouth Weaknesses respiratory paralysis	[30]
Vibrio cholerae	Cholera	Primarily non-saline fresh waterborne Can also be associated with foods of terrestrial origin	Abdominal cramps Diarrhea Vomiting Fever	[30]
Pseudomonas aeruginosa	Gastroenteritis	Ingestion of contaminated water or food Improper storage Poor food hygiene	Diarrhea Fever Vomiting Nausea	[31, 32]

- ## *Methodology to Manage and Inhibit Biofilm Development*

The best way to get rid of biofilms in the food business is to actively prevent them from growing and, as important, prevent bacteria from entering food processing facilities. Implementing an efficient hygiene procedure and ensuring the design of the plant and equipment is essential to restrict the entry of microbes into industries and prevent their interaction with food [15, 33]. To reduce areas where microorganisms can take shelter and proliferate, it is advisable to avoid gaps and cracks [15]. The selection of exterior layer materials and coverings is crucial in preventing the growth of biofilms [33]. Furthermore, the implementation of a Hazard Analysis and Critical Control Point system (HACCP) is crucial to maintaining the well-being and standard of food [34]. The methods to regulate and avoid biofilm formation are shown in **Figure 2** in a flow diagram.

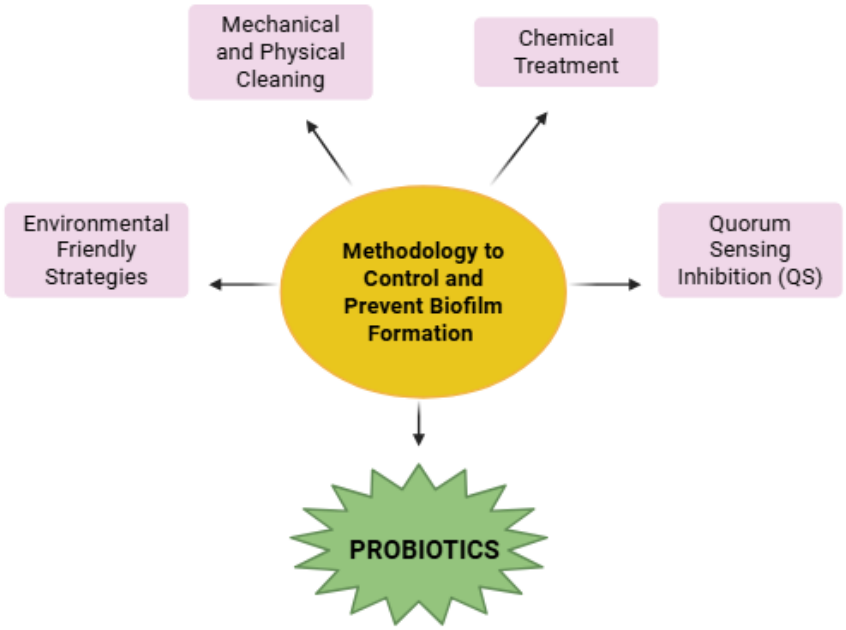

Figure 2. Flow Diagram of Methodology to Regulate and Avoid Biofilm Formation

There are a few methods that help to regulate and avoid biofilm development, and these are described in this paragraph. After biofilms have developed in food contact areas, the initial methods used to remove them are Mechanical and Physical Cleaning techniques [33]. These techniques include high-pressure cleaning and the injection of extremely hot steam [16]. The biofilm is destroyed as a result of these activities interfering with the extracellular matrix [8]. One method for managing biofilm is chemical treatment with detergents, sanitizers, and decontaminants [35, 36]. It has been demonstrated that chemical treatments such sodium hydroxide, hydrogen peroxide, peracetic acid, and sodium hypochlorite can decrease biofilms [6]. Environmentally friendly methods for controlling biofilm include employing enzymes, bacteriophages, natural substances like concentrated plant oils, and chemicals produced by bacteria, for instance, bacteriocins and biosurfactants [36]. Enzymes, such as proteases, lipases, and polysaccharides, are large as well as biodegradable biologically active molecules that can prevent biofilm formation. Bacteriophages are viruses that specifically infect prokaryotic cells. Essential oils are composed of a combination of secondary metabolites derived from plants, such as phenol, thymol, and carvacrol [8]. QS inhibition is seen as an alternative method to control biofilm formation, however, the exact connection between the two is not yet completely comprehended [6]. Microorganisms produce quorum-quenching chemicals as a means of competing with nearby cells [13].

All these methods discussed above have some disadvantages which make them unable to be used in certain cases. The properties of the surface being cleaned, the type of biofilm, the strength of the cleaning agents, and the length of time and temperature of the CIP (Clean-in-Place) process all affect how effective the cleaning process is [37]. In the case of Chemical treatment, the dosage and the timing concerning these chemical products are typically adjusted to eradicate aquatic plankton bacteria, making them potentially ineffective against biofilms [34]. In addition, biofilms exhibit greater resistance to certain biocidal agents, such as chlorine-containing and quaternary ammonium sanitizers [6]. While dealing with Environmentally friendly methods the bacteriophages have shortcomings in effectively directing against microbes within biofilms because of the presence of the extracellular matrix. Some of the essential oils may irritate the dermis and internal human system [6]. QS inhibition can occur via a number of methods. Autoinducers (AI) are QS signaling molecules that inhibitors can bind to competitively, quorum-quenching enzymes can degrade AI signals, small regulatory RNAs (sRNAs) can post-transcriptionally modulate QS genes, and AI can be directly blocked. QS genes can be inhibited and the QS mechanism suppressed by interfering with just one element of the QS pathway [38]. All conventional methods to control biofilm formation have certain limitations. Probiotics have therefore become a viable substitute tactic to prevent biofilm formation in the food sector, lowering the possibility of antibiotic resistance linked to foodborne infections [5].

When taken in sufficient quantities, probiotics—live microorganisms commonly known as "good bacteria"—produce health advantages. They are frequently present in cultured milk products and fermented foods. Probiotics can be found in abundance in these fermented foods.

Although probiotics can be derived from a range of microorganisms including bacteria, yeasts, and molds, the most widely used types belong to the bacterial genera *Lactobacillus*, *Lactococcus*, *Streptococcus*, and *Bifidobacterium*. Probiotics are known to help mitigate adverse health effects and support overall well-being [39].

Several studies have shown that some probiotics, especially lactic acid bacteria (LAB), can control the production of biofilms by different pathogens and prevent microbial cell adhesion. **Figure 3** provides an illustration of this method [40, 41].

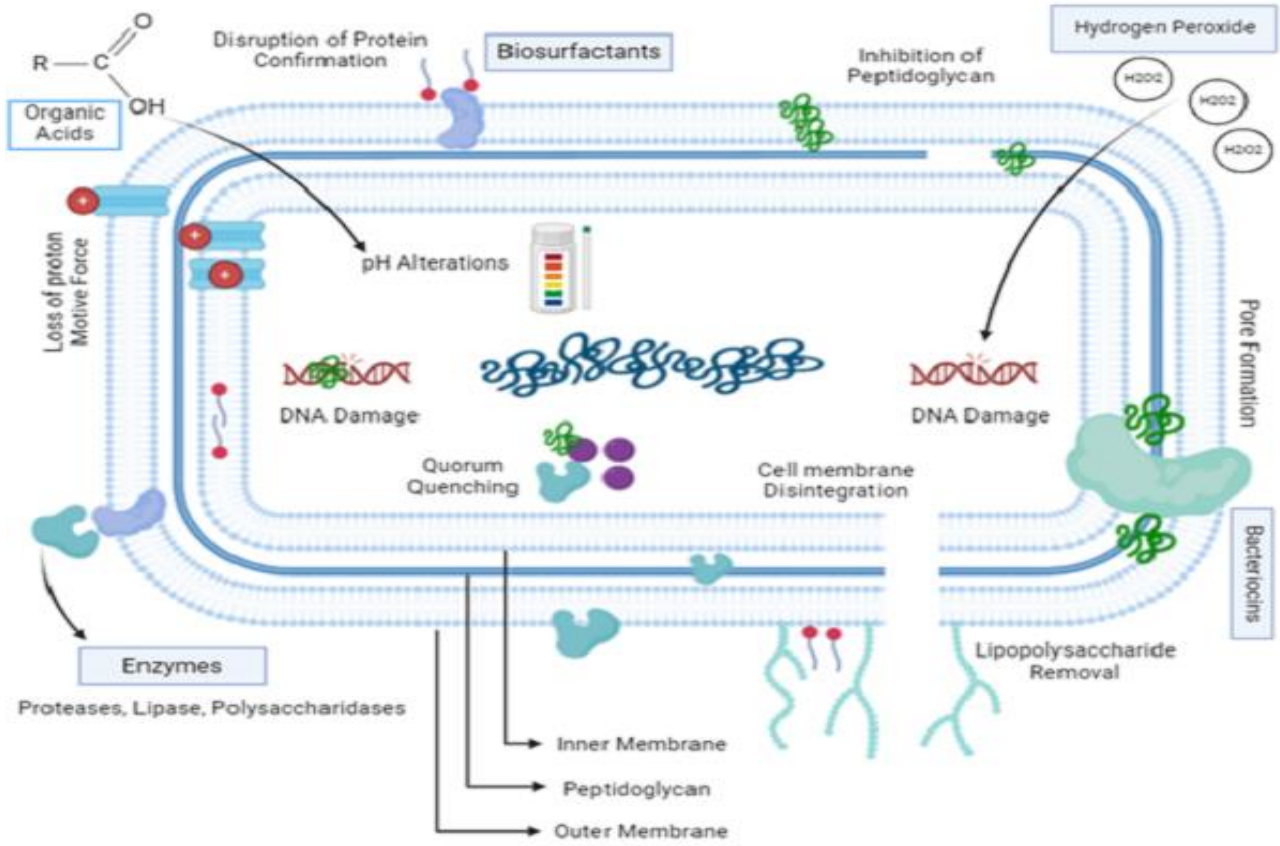

Figure 3. Mechanism of Probiotics to Inhibit Biofilm Development

The competition for resources and binding sites, as well as the release of antimicrobial compounds produced from microorganisms, such as bacteriocins, biosurfactants, organic acids, hydrogen peroxide, and restricted exopolysaccharides, may all contribute to the antagonistic effect [42]. Furthermore, earlier studies have shown that probiotics improve food safety by preventing QS activity [38]. Probiotics work by using Bacteriocins to undermine the integrity of bacterial cells. This is accomplished by releasing the proton motive force and either preventing the formation of peptidoglycans or by creating pores in bacterial membranes. Lactic acids and other organic acids lower pH, which may prevent microorganisms from growing while having no effect on probiotics because they can tolerate low pH levels [42]. Foodborne viruses can enhance their existence in the gastrointestinal tract by creating biofilms after they enter the human body. The production of these biofilms is controlled by a process called QS. Therefore, probiotics, which are consumed through fermentation-based foods, have a twofold impact on both the well-being and standard of food, as well as on gut health. This is potentially achieved by interfering with the QS activity of harmful bacteria [38].

Methods Using Probiotics to Inhibit Biofilm Development

Probiotics can suppress the proliferation of microorganisms and prevent biofilm development using certain methods which are displacement, exclusion, or competition, and are displayed in **Figure 4** in a Flow Diagram [9].

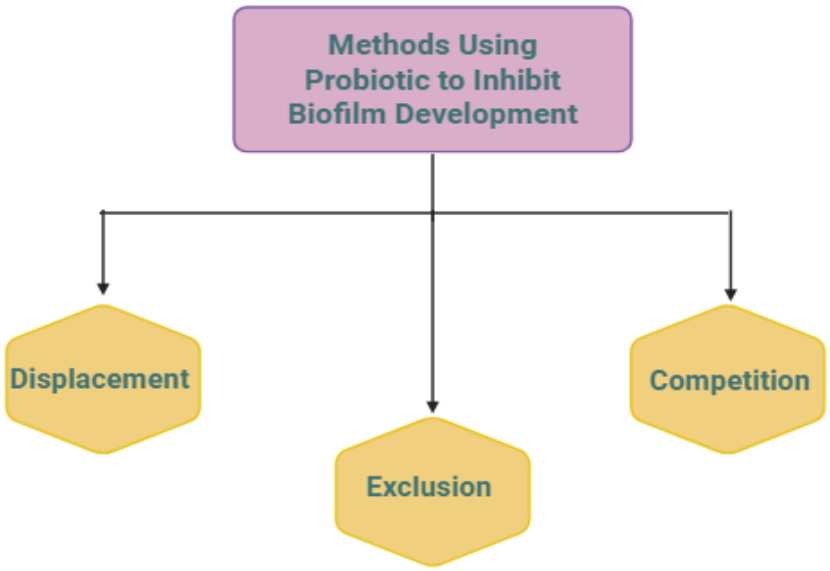

Figure 4. Flow Diagram of Methods Using Probiotics to Inhibit Biofilm Development

This flow diagram categorizes the primary probiotic strategies for inhibiting biofilm formation into three core mechanisms: displacement, exclusion, and competition.

Displacement

Displacement involves the introduction of probiotics and/or their metabolites to disturb pre-existing biofilms [9].

Probiotics such as *Lactobacillus* and *Bifidobacterium* species can compete with pathogenic bacteria for attachment sites on surfaces. This competition results in the displacement of pathogenic bacteria and the prevention of their colonization [43].

Exclusion

Exclusion involves applying probiotic biofilms and/or their metabolites onto food contact surfaces to hinder the attachment of pathogenic bacteria [44].

Exclusion refers to the targeted prevention of the formation of harmful biofilms by probiotic strains through mechanisms such as the generation of antimicrobial compounds and competition for resources. Probiotics can generate bacteriocins, organic acids, and other antimicrobial substances that hinder the growth and formation of biofilms by harmful bacteria such as *Salmonella* and *Listeria* [43].

Competition

The competition includes the first-hand contact between probiotics and/or their metabolites and microbes present in food that can cause illness [9].

Probiotics can disturb the microbial balance, prevent pathogenic colonization, and reduce biofilm growth in different situations by actively competing with pathogens for resources necessary for biofilm formation [43].

Outcomes Obtained by Performing the Displacement Method

Research regarding utilizing probiotics to manage and block the production of biofilms within the food sector has been growing. Different probiotic genera are commonly used. The prevailing techniques employed for biofilm evaluation include crystal violet (CV) staining and colony forming units (CFU) counting. Multiple materials have been investigated for the production of biofilms, with glass and polystyrene being the most commonly used followed by stainless steel. Wood, rubber, silicone, polyvinyl chloride, and polytetrafluoroethylene were also examined. Microtiter plates served as the main platform for biofilm formation in the bulk of the studies. Of the anti-biofilm compounds that were analysed, probiotic cells and cell-free supernatant (CFS) were the most often investigated, making up 34% and 31% of the research, respectively. To a lesser degree, EPS, biosurfactants, crude extracts, and bacteriocins were also investigated. **Table 2** illustrates how probiotics can use the displacement approach to regulate the biofilms that form on meals. The following probiotics are used to regulate the production of biofilms:

Table 2. The capability of probiotics to control the biofilms produced by foodborne microbes on the food by utilizing the displacement method

Probiotics	Foodborne microbes	Outcomes obtained (Biofilm Inhibition)	References
***Lactiplantibacillus* spp.**	*Bacillus cereus* *Staphylococcus aureus* *Escherichia coli* *Pseudomonas aeruginosa* *Salmonella typhimurium* *Listeria monocytogenes*	No inhibition 100% inhibition 93.7% inhibition 99.9% inhibition 99.6% inhibition 100% inhibition	[45-48]
***Lacticaseibacillus* spp.**	*Listeria monocytogenes* *Escherichia coli* *Acinetobacter baumannii* *Cronobacter sakazakii* *Pseudomonas aeruginosa*	16–52% inhibition 58–84% inhibition 28–63% inhibition 10–51% inhibition 48-76% inhibition	[40] [49] [50] [51] [52]
***Lactobacillus* spp.**	*Staphylococcus aureus* *Pseudomonas aeruginosa* *Listeria monocytogenes*	18–87% inhibition 77% inhibition 48% inhibition	[53] [54] [50] [51]
***Limosilactobacillus* spp.**	*Escherichia coli* *A. baumannii* *Chromobacterium violaceum*	58–84% inhibition 28–63% inhibition 3-40% inhibition	[45] [55] [40] [56]
***Ligilactobacillus* spp.**	*Listeria monocytogenes*	63% inhibition	[50]
***Lactilactobacillus* spp.**	*Listeria monocytogenes*	100% inhibition	[57] [58]
***Pediococcus* spp.**	*S. Typhimurium* *Escherichia coli* *Staphylococcus aureus* *E. faecalis* *P. aeruginosa* *C. violaceum*	33% inhibition 52% inhibition 75% inhibition 50% inhibition 32% inhibition 40% inhibition	[58] [56] [59]
***Leuconostoc* spp.**	*Staphylococcus aureus* *Escherichia coli* *E. faecalis*	77% inhibition 62% inhibition 53% inhibition	[59]
***Lactococcus* spp.**	*Listeria monocytogenes*	inhibition	[60] [61]
***Enterococcus* spp.**	*Listeria monocytogenes*	inhibition	[60]
Probiotic Cocktails	*Listeria monocytogenes* *Salmonella heidelberg* *Salmonella gallinarum* *Staphylococcus aureus* *Campylobacter jejuni*	98% inhibition 99.99% inhibition 99.99% inhibition 99.99% inhibition 14.9% inhibition	[62] [63]

Lactiplantibacillus spp.

B.cereus, *S. aureus, E. coli, P. aeruginosa,* and *S.typhimurium* were tested for the antibacterial activity of *Lactiplantibacillus* cells [45, 46]. There was no *B. cereus* inhibition. However, the eradication ratio of well-defined biofilms produced by *P. aeruginosa* and *E. coli* was above 99.9% and 94%, respectively, while the presence of biofilms dropped by a maximum of 100%. *S. aureus*

Pre-existing *L. monocytogenes* biofilms were effectively broken up by different concentrations of bacterial inhibitors produced by *Lactiplantibacillus plantarum* strains [47, 48]. After being treated with bacteriocins, most cell membranes were damaged, which caused intracellular contents to flow out. The byproducts generated by probiotics are released into the surrounding environment and can be gathered in the CFS. The dissemination of *P. aeruginosa* as well as *L. monocytogenes* biofilms was induced to different extents by CFS derived from *Lactiplantibacillus* strains [64, 65].

While *Lactiplantibacillus* spp. showed 99.9% inhibition of *P. aeruginosa* biofilms [45, 46], these results were derived from idealized lab conditions (e.g., polystyrene surfaces, nutrient-rich media). Industrial applications may face challenges such as variable temperatures or nutrient competition, which were not addressed in these studies.

Lacticaseibacillus spp.

The reduction of biofilm was less significant for *S. typhimurium* as compared to *L. monocytogenes* when using *Lacticaseibacillus* [43]. Probiotic varieties within this genus exhibited significant biofilm-resistant effectiveness counter to many diseases. The biological mass and biochemical activity of *Vibrio parahaemolyticus* biofilms were decreased by 20% and 41%, respectively [66]. The mature biofilms of *S. aureus*, *E. coli,* and *Acinetobacter baumannii* achieved reductions of 65–77%, 58–84%, and 28–63%, correspondingly [40]. The inhibition of biofilm formation in *S. aureus* might be linked with the synthesis of lactic acid, without the participation of bacteriocins [53]. The application of CFS destroyed biofilms formed by *Cronobacter sakazakii* (10-51%) and *Listeria monocytogenes* (16-52%) [49, 50].

Biosurfactants derived from *Lacticaseibacillus* cultures effectively disrupted the pre-existing biofilms of *S. aureus*, *E. coli*, *Bacillus subtilis,* and *P. aeruginosa* [51]. These metabolites disrupt the cellular membranes, causing seepage and subsequent cellular apoptosis [52].

Biosurfactants from Lacticaseibacillus disrupt cellular membranes [52], but their industrial use may be limited by stability issues during high-temperature processing, a gap not yet explored in current research.

Lactobacillus spp.

The CFS derived from *Lactobacillus* caused the disintegration of biofilms, resulting in a decrease in the biomass density of *S. aureus*, *P. aeruginosa,* and *L. monocytogenes* biofilms by 18% to 87%. The inhibition of S. aureus was not linked to bacteriocin synthesis, as it was demonstrated that the LAB strain under investigation creates lactacin B in the case of the bacteria being grown in mixed cultures [53]. Biosurfactants obtained through *Lactobacillus* effectively disrupted already-formed biofilms of *S. aureus* at concentrations ranging from 45% to 63% [51].

Limosilactobacillus spp.

The *Limosilactobacillus* strains completely eradicated *S. aureus* biofilms, while after treating *E. coli* and *P. aeruginosa* with probiotics, no culturable cell was found [45]. Additionally, when *P. aeruginosa* biofilms came into contact with the CFS of *Limosilactobacillus*, they completely broke down [55]. The production of lactic, acetic, and formic acids as well as bacterial inhibitors that function in acidic conditions may be responsible for the reported inhibitory effect. Fully established biofilms of *A. baumannii* (with a range of 28–63%) and *E. coli* (with a range of 58–84%) were successfully dispersed by CFS [40]. The crude extract from *Limosilactobacillus* decreased *P. aeruginosa* by approximately 32% and *Chromobacterium violaceum* by approximately 40%. The extract's EPS and other metabolites, which have inhibitory effects on QS, are primarily responsible for this reduction [56].

Ligilactobacillus spp.

Although they have been tested against fewer diseases, a variety of lactobacilli have also been investigated for their capacity to prevent the production of biofilms. Ligilactobacillus CFS was used to diminish the biofilm of L. monocytogenes by 63% [50].

Lactilactobacillus spp.

Lactilactobacillus can decrease the fully developed biofilm of *L. monocytogenes*. Additionally, its bacteriocin extract has demonstrated a more pronounced anti-biofilm impact [57].

Pediococcus spp. and Leuconostoc spp.

Additionally, *Pediococcus* and *Leuconostoc*, among other, LAB, indicated remarkable anti-biofilm effectiveness. The *Pediococcus* cultures showed a substantial inhibitory effect regarding the development of *S. typhimurium*, *L. monocytogenes*, *E. coli,* and *S. aureus*. They could reduce already existing biofilms made of polyvinyl chloride, stainless steel, or glass [18]. Furthermore, the formation of *S. typhimurium* biofilm decreased by 33% when exposed to the CFS derived from *Pediococcus*. This reduction was attributed to the rupture of the extracellular matrix caused due to the bacteriocin made by *Pediococcus* [58]. Crude extracts of *Pediococcus* reduced the dispersion of *C. violaceum* and *P. aeruginosa* biofilms by 40% and 32% respectively [56].

In contrast to both Positive and Negative Gramme microorganisms, EPS produced from Pediococcus and Leuconostoc showed antibacterial qualities [59]. Mature biofilms of S. aureus, E. coli, and Enterococcus faecalis were successfully dispersed by them, leading to a 33%–80% decrease in biomass.

Lactococcus spp. and Enterococcus spp.

The use of LAB (*Lactococcus* and *Enterococcus* genera) led to a notable decrease in the count of culturable biofilm cells of *L. monocytogenes* in poly-species biofilms. The reduction was observed when the treatment was carried out at temperatures of 4 °C or 8 °C, respectively. These temperatures are known to slow down or suppress the growth of probiotics [60]. A separate investigation showed that the presence of *L. monocytogenes* biofilms dropped by 2.7 logarithmic units upon immersion in a solution containing bacteriocin derived from *Lactococcus* [61].

Probiotic Cocktails

After 24 hours of exposure, a mixture of *Lactobacillus animalis, Lactobacillus amylovorus*, and *Pediococcus acidilacti* cells efficiently reduces the growth of *L. monocytogene*s biofilm by 98%. Additionally, this mixture eliminated surface-adhered *L. monocytogenes* cells even after 72 hours [62]. *Salmonella Heidelberg, Salmonella gallinarum, S. aureus*, and *Campylobacter jejuni* have been shown to be susceptible to the anti-biofilm effects of a mixture of *Bacillus* and *Pediococcus* species [63]. The reductions in the presence of mature biofilms ranged from

14.9% for *C. jejuni* to 99.99% for *S. Heidelberg, S. gallinarum,* and *S. aureus*. The extent of reduction varied based on the type of substrate (Ground dirt, timber, and styrene foam) and the duration of Interaction. *Lactobacillus* spp. and *Pediococcus* spp. Demonstrated broader-spectrum biofilm inhibition compared to *Leuconostoc* spp., likely due to higher bacteriocin production [43, 58]. However, *Pediococcus* efficacy varied significantly with substrate type (e.g., 99.99% reduction on stainless steel vs. 14.9% on styrene foam [63]), suggesting surface material critically impacts outcomes

Potential Applications of Probiotics in the Food Industry

While numerous in vitro studies support the anti-biofilm potential of probiotics, translating these findings into practical, industrial applications is essential for real-world impact. The following are promising avenues through which probiotics can be integrated into food safety strategies:

- ***Probiotic Surface Sprays***

It is possible to directly apply probiotic-based sprays to surfaces that come into touch with food, like cutting boards, conveyor belts, and stainless-steel equipment. By forming a protective biofilm of advantageous microorganisms, these sprays prevent harmful germs from colonizing. For example, *Lactobacillus plantarum* has been tested in surface treatments to reduce *Listeria monocytogenes* biofilms on food processing surfaces [67].

- ***Active Packaging Materials***

Incorporating probiotic cultures into biodegradable packaging materials enables the continuous release of antimicrobial compounds. Studies have explored the embedding of *Lactobacillus rhamnosus* in edible films and coatings to inhibit surface contamination on ready-to-eat foods like sliced meats and cheese [68].

- ***Food-Safe Coatings***

Edible coatings enriched with probiotics can be applied to perishable products such as fruits, vegetables, and dairy to prolong shelf life and prevent pathogen attachment. These coatings combine barrier properties with probiotic-driven antagonism against spoilage organisms [67].

- ***Cleaning-In-Place (CIP) Systems***

Probiotic-infused cleaning agents used in CIP protocols for tanks and pipelines can offer an eco-friendly alternative to harsh chemical sanitizers. Some pilot-scale studies have shown that probiotic solutions can reduce biofilm load in dairy and beverage production lines more sustainably [68].

- ***Bioaugmentation in Drainage Systems***

Probiotics may also be used in industrial drainage and wastewater systems to degrade organic residues and suppress biofilm-forming pathogens at source points often missed during routine cleaning [67, 68].

Real-world examples of such applications include trials conducted in dairy plants using *Lactobacillus casei* for surface hygiene management, and integration of probiotic coatings in meat packaging to reduce spoilage and pathogen survival.

These emerging approaches reflect the shift toward natural, sustainable food safety interventions, meeting both regulatory standards and consumer demand for chemical-free processing environments.

No studies have assessed probiotic sprays under high-pressure cleaning in meat plants, raising concerns about their practicality. Future work should test probiotic cocktails in pilot-scale facilities to validate scalability.

Conclusion

Biofilm formation by foodborne pathogens poses a significant challenge in the food industry, contributing to contamination, food spoilage, and public health risks. Traditional methods such as mechanical cleaning, chemical treatments, and antimicrobial agents have limitations, including resistance development and environmental concerns. Probiotics offer a promising, sustainable alternative by inhibiting biofilm formation through competitive exclusion, displacement, and production of antimicrobial metabolites like bacteriocins, organic acids, and biosurfactants. Studies demonstrate the efficacy of probiotic strains, particularly LAB, in disrupting biofilms formed by pathogens such as *Listeria monocytogenes*, *Salmonella spp.*, and *Staphylococcus aureus*. By disrupting QS, probiotics not only improve food safety but also support gut health. Although further research is required to maximize the use of probiotics, incorporating them into food safety plans has the potential to significantly lower the dangers associated with biofilms in the food sector.

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