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Mechanisms and Therapeutic Potential of Probiotics as Biofilm Disruptors

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Abstract
Biofilms, which are structured populations of bacteria encased in a self-produced extracellular polymeric substance (EPS), provide a serious challenge in clinical medicine, industry, and the environment due to their inherent resilience to antimicrobials and host defenses. As the search for effective, non-antibiotic methods to combat biofilms has intensified, there has been considerable interest in probiotics, which are bacteria that offer health benefits when administered in sufficient amounts. This review aims to evaluate the mechanisms, efficacy, and therapeutic potential of probiotics as biofilm-disrupting agents, with an emphasis on their ability to prevent, weaken, or eradicate pathogenic microbial biofilms across clinical and environmental settings. The objectives of this study were to assess the efficacy of different probiotic species and strains in disrupting established biofilms of clinically relevant pathogens such as *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans*, and others. To evaluate synergistic interactions between probiotics and conventional antimicrobial agents, examining whether combined therapy enhances biofilm eradication or reduces antimicrobial resistance. This study carefully examines the novel role of specific probiotic strains as potent disruptors of biofilms. Among the various mechanisms that probiotics employ is competitive exclusion, the production of antimicrobial substances (bacteriocins, organic acids, hydrogen peroxide), the release of enzymes that degrade biofilms, disruption of quorum sensing (QS), and modification of host immune responses. Probiotics' ability to combat biofilms created by common pathogens such as *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans*, and oral infections is rigorously assessed. Current and prospective uses in industrial biofouling management, agriculture, and medical domains (wound healing, urinary tract infections, dental caries/periodontitis, gastrointestinal infections) are investigated. Probiotics are a viable, environmentally friendly method of controlling biofilms, and in the age of growing antibiotic resistance, they may be used as an adjuvant or substitute treatment.

Keywords: Probiotics, Biofilm, Biofilm disruption, Antimicrobial resistance, Quorum sensing

Introduction

The most common way that microorganisms develop in industrial, clinical, and ecological settings is through biofilms. These intricate, surface-associated communities are surrounded by a self-generated extracellular polymeric substance (EPS) matrix that is mostly made up of lipids, proteins, polysaccharides, and nucleic acids (Flemming & Wingender, 2010). According to Stewart and Costerton (2001) and Davies (2003), the resident microorganisms benefit greatly from this structure. These benefits include improved nutrient capture, protection from environmental stresses like desiccation and UV light, and, most importantly, a significantly higher tolerance to antimicrobial agents and host immune defenses (up to 1000-fold compared to planktonic cells). Biofilm-associated infections are therefore notoriously hard to eliminate, and they play a major role in treatment failures, chronicity, and the worldwide antimicrobial resistance (AMR) epidemic (Jamal et al., 2018; Adeola et al., 2022). Conventional antimicrobial treatments frequently fall short against biofilms, requiring aggressive strategies such as combination treatments, high-dose/long-duration antibiotics, or surgical debridement/device removal, all of which are expensive and risky (Hoiby et al., 2010). This has sparked a lot of study on complementary or

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alternative approaches to disrupting and preventing biofilms. Probiotics have been a popular and environmentally friendly choice among these. "Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" is how the World Health Organization (WHO) defines probiotics (FAO/WHO, 2002). Historically linked to gut health (e.g., Lactobacillus, Bifidobacterium), their acknowledged advantages have broadened to encompass immune system regulation, barrier function augmentation, and direct antagonistic action against pathogens (Hill et al., 2014). Importantly, an increasing amount of data indicates that certain probiotic strains have strong potential to prevent the formation of biofilms and to break up existing biofilms via various methods (Borges et al., 2014; Rather et al., 2021; Igere et al., 2024). By examining their modes of action, effectiveness against important infections, and present and future uses, this study seeks to present a thorough examination of probiotics functioning as biofilm disruptors.

Biofilm formation and challenges

Biofilm Lifecycle: The process of biofilm formation is dynamic and involves several stages:

1. Initial Reversible Attachment: Free-floating (planktonic) cells temporarily stick to the surface through electrostatic and van der Waals weak forces (Garrett et al., 2008).
2. Irreversible Attachment: Cells start generating EPS and increase attachment by utilizing adhesins (such as fimbriae and pili) (Donlan, 2002).
3. Microcolony Formation and EPS Production: As cells multiply, they create microcolonies that are encased in a growing EPS matrix. The biofilm's distinctive structural and protective element is this matrix (Flemming and Wingender, 2010).
4. Maturation: water channels for the passage of waste and nutrients are among the intricate three-dimensional structures that the biofilm forms. Microbial diversity may rise.

To colonize new surfaces, cells disperse (either actively or passively) from the biofilm and return to a planktonic condition.

Biofilm Resistance Mechanisms: There are several reasons why biofilms are resistant:

Physical Barrier: According to Høiby et al. (2010), the thick EPS matrix physically prevents immune cells and antimicrobial chemicals from penetrating.

Metabolic Heterogeneity: Zones of slow or non-growing cells (persisters) are produced by gradients of nutrients, oxygen, and waste products. These zones are naturally less vulnerable to antimicrobials that target active metabolism (Lewis, 2007).

Modified Microenvironment: Some antibiotics may become inactive in the biofilm due to localized acidic or anaerobic conditions (Anderl et al., 2000).

Enhanced Horizontal Gene Transfer: Antimicrobial resistance genes can spread more easily when cells are near one another (Molin & Tolker-Nielsen, 2003).

Stress Response Activation: According to Mah & O'Toole (2001), the formation of biofilms triggers general stress responses that improve resistance.

Persister Cells: A tiny subset goes into a highly tolerant, inactive state.

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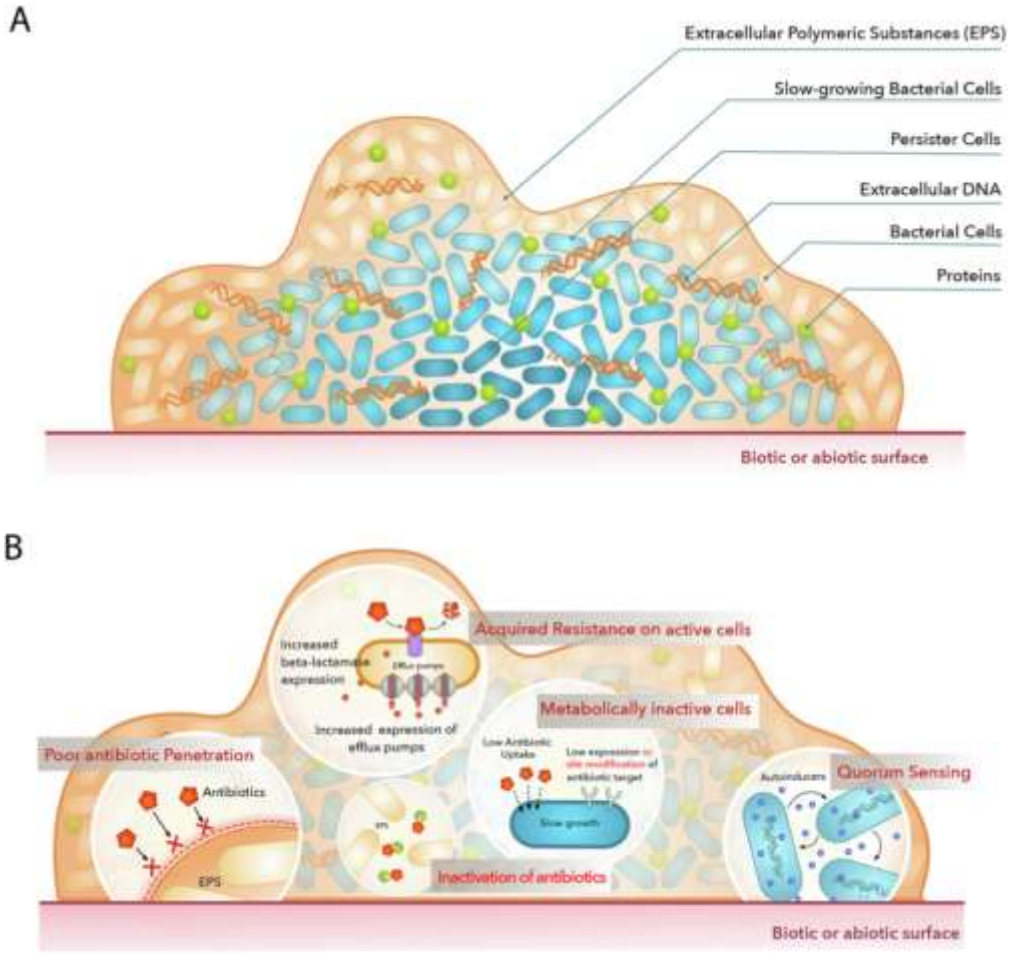

Figures 1a & b: Schematic diagram of biofilms and their resistance mechanisms (Souto et al., 2020)

Probiotics' mechanisms of biofilm disruption

1. Surface colonization and competitive exclusion
2. Acidification and byproducts of metabolism
3. Enzymes that break down EPS and biosurfactants
4. Interference with quorum sensing (QS inhibition)
5. Clearance mediated by the host and immunomodulation

Probiotics fight biofilms using a complex set of mechanisms, frequently working in concert (Borges et al., 2014; Rather et al., 2021).

1. Competitive Exclusion:

Receptor Blockade: Probiotics compete with pathogens for adhesion sites on host tissues or abiotic surfaces. They can bind to host receptors (e.g., mucins, epithelial cells), physically blocking pathogen access. For example, *Lactobacillus rhamnosus* GG and *L. acidophilus* compete with uropathogenic *E. coli* (UPEC) for binding sites on uroepithelial cells (Karlsson et al., 2012).

Nutrient Competition: Probiotics actively consume essential nutrients (e.g., iron, carbon sources) in the microenvironment, limiting their availability for pathogen growth and biofilm formation (Valenti et al., 2018). Lactobacillus spp. is known for its efficient nutrient utilization.

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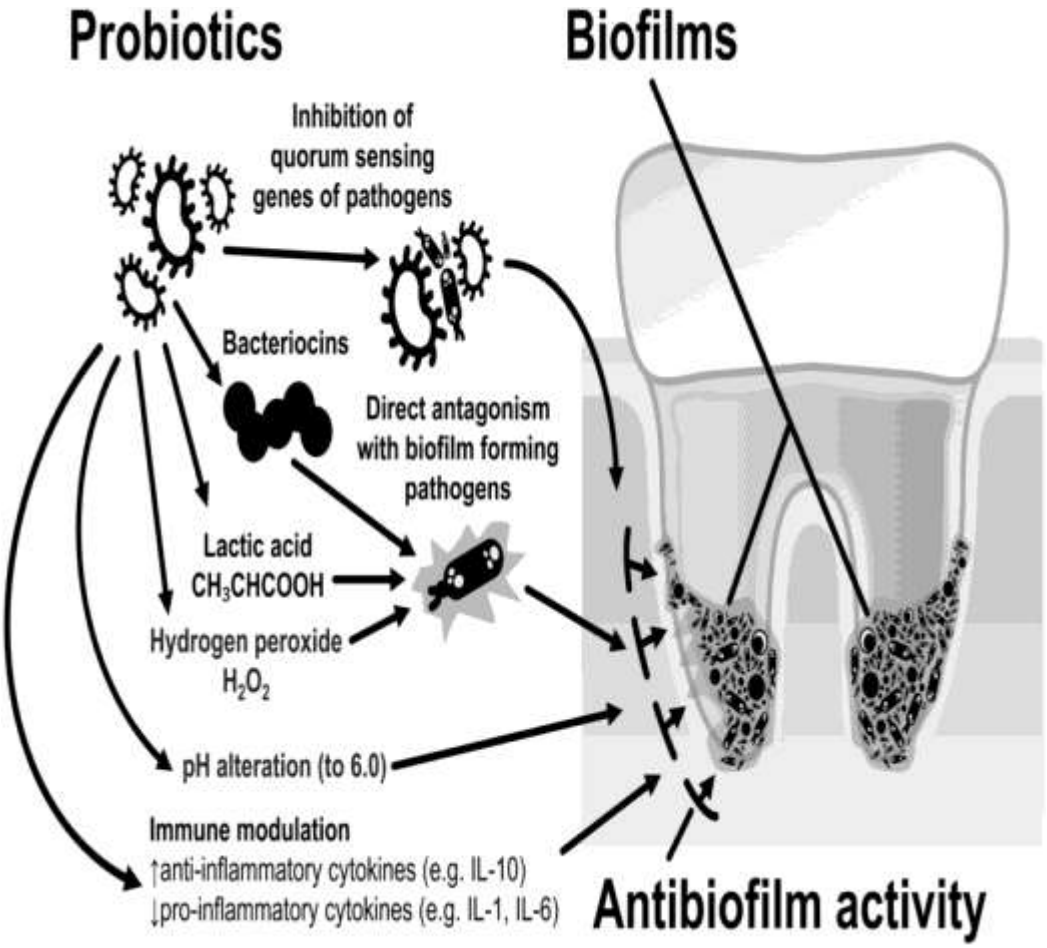

Figure 2: Schematic representation of different mechanisms of probiotic antibiofilm activity (Souto et al., 2020)

Antimicrobial Substance Production: Lactic acid, acetic acid, and other organic acids: significant metabolic byproducts of bifidobacteria and lactobacilli. They make the environment less pH-friendly, which makes it harder for many pathogens to thrive. Furthermore, internal pH homeostasis and metabolic processes can be disrupted by undissociated acid molecules penetrating bacterial membranes (Alakomi et al., 2000). Additionally, the EPS matrix becomes unstable in acidic environments (Walencka et al., 2008). The bactericidal agents are Heat-stable antimicrobial peptides or proteins that are ribosomally synthesized and have a comparatively limited or wide range of activity. Among them are reuterin (*Lactococcus reuteri*), plantaricin (*Lactococcus plantarum*), and nisin (*Lactococcus lactis*). Crucially, some bacteriocins have actions against biofilm cells, and they can either kill or inhibit closely related bacteria (Dobson et al., 2012; Mathur et al., 2018). They frequently target membrane integrity or the synthesis of cell walls.

Several lactobacilli, including *L. crispatus* and *L. jensenii*, produce hydrogen peroxide (H_2O_2). Microbial membranes, proteins, and DNA are all harmed by the strong oxidizing agent H_2O_2. It has been demonstrated to prevent the growth of biofilms and the survival of pathogens in the oral and vaginal niches, such as *Staphylococcus aureus* and *Gardnerella vaginalis* (O'Hanlon et al., 2011; Valenti et al., 2018). Surface-active substances known as biosurfactants lower surface tension. By interfering with cell-surface and cell-cell interactions, probiotic biosurfactants (such as those derived from Lactobacillus species) can disrupt pre-formed biofilms, decrease initial pathogen adherence, and improve the penetration of other antimicrobials (Gudina et al., 2010). Diacetyl, ethanol, or CO_2 are produced by certain probiotics and may have antibacterial properties.

Biofilm-Degrading Enzyme Secretion: Enzymes that can hydrolyze important elements of the biofilm EPS matrix are produced by probiotics: DNases: Degrade extracellular DNA (eDNA), which is an essential adhesive and structural element of many bacterial biofilms (e.g., *P. aeruginosa, S. aureus*). DNases are produced in large quantities by Bacillus species (Kaplan, 2011).

Proteinaceous EPS components are broken down by proteases and dispersin B-like enzymes, which can also interfere with adhesins. Proteases produced by *Bacillus subtilis* and *Lactobacillus fermentum* are effective against *P. aeruginosa* and *S. aureus* biofilms (Rather et al., 2021). Within the EPS matrix, glycosidases and hydronidases

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break down polysaccharides, such as glycosaminoglycans (Mu et al., 2023). Both *L. acidophilus* and *L. casei* have shown hyaluronidase activity that is pertinent to the dissolution of biofilms.

Interference with Quorum Sensing (QS): Bacteria employ this cell-density-dependent communication system to coordinate gene expression, including the formation of biofilms and virulence factors. The production of quorum quenching (QQ) enzymes is one way that probiotics can interfere with QS. Some lactobacilli (like *Lactobacillus acidophilus*) and Bacillus species (including *Bacillus cereus* and *B. thuringiensis*) generate enzymes called lactonases, which hydrolyze the acyl-homoserine lactones (AHLs) required by Gram-negative bacteria, and oxidoreductases, which alter signaling molecules. QS signal molecules (autoinducer-2 (AI-2) and AHLs) are broken down or rendered inactive by these enzymes (Chu et al., 2011).
ii. Manufacturing QS Inhibitors (QSIs): Tiny compounds that prevent signal molecules from attaching to receptors or obstruct the creation of signals? Probiotic-produced metabolites, including furans and peptides, can function as QSIs.
iii. Probiotics may create signal mimics that compete with pathogen signals for receptor binding, hence causing competition for QS receptors. According to Borges et al. (2014), QS interference results in decreased production of virulence factors (such as toxins and proteases), impaired biofilm development, and increased vulnerability to immune clearance and antimicrobials.

Modulation of Host Immunological Responses: Probiotics affect the local immunological environment by interacting with immune cells (such as dendritic cells and macrophages) and host epithelial cells: Improving Barrier Function: Reduce pathogen penetration and biofilm initiation on mucosal surfaces by strengthening tight junctions between epithelial cells and encouraging the synthesis of mucin. By boosting anti-inflammatory cytokines like IL-10 and decreasing excessive pro-inflammatory cytokine production like TNF-α and IL-8, it can mitigate tissue damage brought on by inflammation linked to biofilms (Sicard et al., 2017; Mu et al., 2023). Boosting Innate Immunity: Increase neutrophil and macrophage phagocytic activity against biofilm cells (Valenti et al., 2018). The removal of biofilms and infection resolution is indirectly facilitated by this immunological regulation.

Efficacy against specific pathogens and biofilms
Research demonstrates probiotic efficacy against biofilms formed by diverse pathogens:
Gram-Positive Pathogens
MRSA and other *Staphylococcus aureus* strains are inhibited by Lactobacillus species (e.g., *Lactobacillus rhamnosus, Lactobacillus fermentum*, and *Lactobacillus casei*) through the use of organic acids, bacteriocins, and biosurfactants. The production of strong lipopeptides (like surfactin) and enzymes (like DNase and proteases) by Bacillus species (like *B. subtilis* and B. amyloliquefaciens) is efficient against *S. aureus* biofilms (Rather et al., 2021). Strong anti-biofilm activity is demonstrated by the nisin produced by *Lactococcus lactis* (Mathur et al., 2018).
Enterococcus faecalis: Probiotics such as *Lactobacillus acidophilus, Lactobacillus plantarum*, and *Lactobacillus casei* prevent biofilm formation on urinary catheters and in root canals by producing bacteriocins and acid (Souto et al., 2020).

Gram-Negative Pathogens
Pseudomonas aeruginosa: A significant biofilm-forming agent in wounds, medical equipment, and lungs with cystic fibrosis. Its biofilm formation is inhibited by probiotics (*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus plantarum*, and others) through organic acids, biosurfactants, and especially QQ enzymes (lactonases) that target AHLs, lowering pyocyanin, elastase synthesis, and biofilm architecture (Kim et al., 2018).
Escherichia coli (including UPEC): In addition to producing acids and bacteriocins (such as colicins from *E. coli*), probiotics (*L. rhamnosus* GR-1, *L. reuteri* RC-14, *L. crispatus*, and Bacillus spp.) also compete for adhesion sites in the gut and urinary tract and break up biofilms.
Salmonella species: Through competition, acid production, and maybe QQ, Lactobacillus species and Bifidobacterium species prevent the formation of biofilms on surfaces that come into contact with food and the intestinal epithelium (Giaouris et al., 2015).
Candida albicans is a fungus that causes a lot of mucosal and device-associated biofilm infections. Through acids, H_2O_2, biosurfactants, and possibly QQ of fungal farnesol signaling, probiotics, especially Lactobacillus species (*L. rhamnosus, L. acidophilus, L. reuteri*, and *L. crispatus*), prevent Candida adhesion, hyphal formation (a crucial virulence factor), and biofilm formation (Rossoni et al., 2018; Mu et al., 2023). Competition is essential for adhesion sites on the vaginal epithelium.
Dental Caries (*Streptococcus mutans* biofilms): Oral Biofilms Probiotics such as Bifidobacterium DN-173 010,

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L. rhamnosus GG, *L. reuteri*, and *L. casei* Shirota compete with cariogenic bacteria, generate acids that inhibit *S. mutans*, and may produce enzymes or bacteriocins that target plaque biofilm (Llena et al., 2019).
Probiotics *(L. reuteri, L. brevis, L. casei,* and Bacillus spp.) can lower the load of pathogenic biofilms (such as Porphyromonas gingivalis and Aggregatibacter actinomycete comitans), alter the host immune response, and prevent the production of inflammatory cytokines in cases of periodontal disease (Polymicrobial biofilms) (Teughels et al., 2011; Invernici et al., 2018; Mu et al., 2023).

Application of probiotic biofilm disruptors

Medical Applications

i. Catheter-associated UTIs (CAUTIs) and urinary tract infections (UTIs): By competing with uropathogens (such as *E. coli* and Enterococcus) for bladder/vaginal adhesion and breaking down biofilms, probiotic strains (like *L. rhamnosus* GR-1 and *L. reuteri* RC-14) given orally or intravaginally help prevent recurrent UTIs (Reid, 2017; Schwenger et al., 2015). Research is being done on coating catheters with probiotics or their byproducts (Trautner et al., 2003).

ii. Healing Wounds (Burns, Chronic Wounds): In animal models and certain clinical studies, topical administration of probiotics (*L. plantarum, L. casei*, Bacillus spp.) or their cell-free supernatants (CFS) containing antimicrobials/QQ enzymes can lower the burden of biofilms (*S. aureus, P. aeruginosa*), reduce inflammation, and promote healing (Mu et al., 2023). Probiotic-impregnated dressings are being developed.

iii. Gastrointestinal Infections & Disorders: Probiotics treat and prevent GI infections caused by biofilms (such as Salmonella, pathogenic *E. coli*, and *Clostridium difficile*) by immune regulation, competitive exclusion, and antimicrobial generation. According to Sicard et al. (2017) and Derrien & van Hylckama, (2015), they might also interfere with biofilms that are linked to the pathophysiology of inflammatory bowel disease (IBD).

iv. Vaginal Health: By reestablishing the protective lactobacilli-dominated microbiota, generating lactic acid/H_2O_2/bacteriocins, preventing the formation of pathogen (Gardnerella, Candida) biofilms, and modifying local immunity, probiotics, mostly Lactobacillus strains, are used to treat and prevent bacterial vaginosis (BV) and vulvovaginal candidiasis (VVC) (Borges et al., 2014).

V. Oral Health: To prevent dental caries (by targeting the *S. mutans* biofilm) and periodontal disease (by targeting the pathogenic subgingival biofilms), probiotic lozenges, gums, mouthwashes, and toothpastes are used in conjunction with mechanical hygiene (Twetman & Keller, 2012; Invernici et al., 2018).

vi. Otitis Media: By preventing the production of nasopharyngeal biofilms by bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae*, nasal/oral probiotics may help prevent recurrent otitis media (Marchisio et al., 2015).

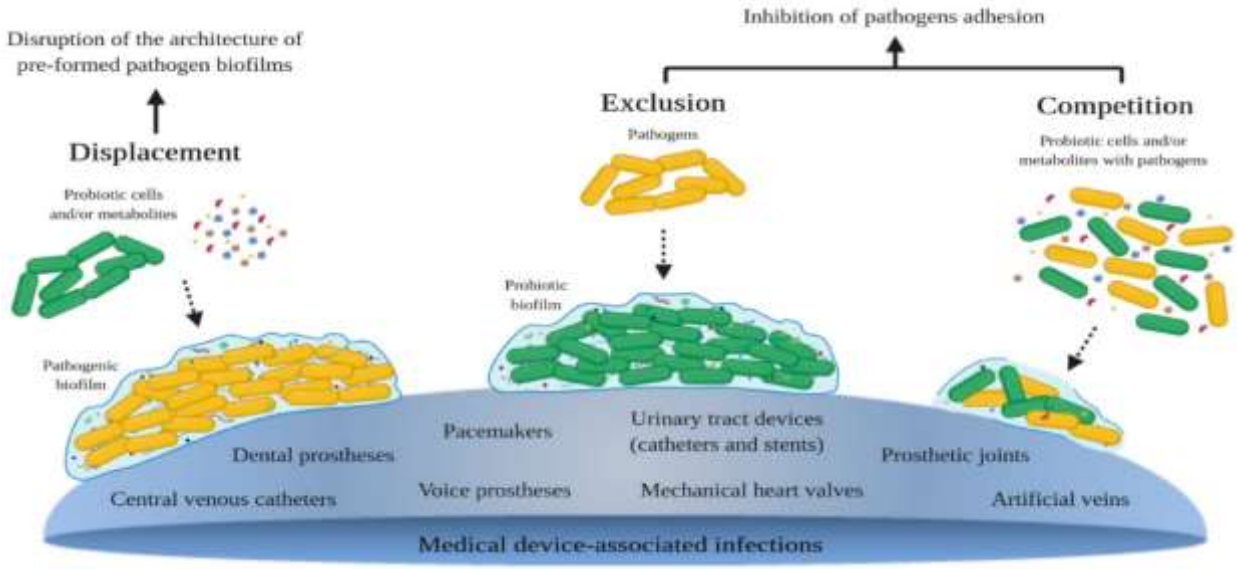

Figure 3: The use of Probiotics to fight Biofilms in Medical device-associated infections **(**Giordani et al., 2021)

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Industrial Applications

i. Food Industry: To stop spoilage and harmful bacteria (like *Listeria monocytogenes*) from forming biofilms on food surfaces and processing equipment, probiotics and their bacteriocins (like nisin) are utilized as natural preservatives (Giaouris et al., 2015).

ii. Water Systems and Industrial Biofouling: As an eco-friendly substitute for biocides, QQ probiotics, particularly Bacillus spp., are being investigated for managing biofilms in cooling towers, pipelines, and membrane filtration systems (Kim et al., 2018; Mitra et al., 2021).

Agricultural Applications

i. Plant Protection: By acting as biocontrol agents, probiotic bacteria (such as Bacillus and *Pseudomonas fluorescens*) can stop phytopathogen biofilms from forming on plant surfaces or inside plant tissues, hence lowering illness (Ongena & Jacques, 2008).

ii. Animal husbandry: According to Callaway et al. (2008), probiotics in feed or water might lessen pathogen biofilms in the animal's gut, enhancing health and lowering the need for antibiotics.

Conclusion

One of the biggest challenges in the fight against chronic infections and biofouling is biofilms. Equipped with a wide range of mechanisms, such as immune modulation, quorum quenching, the production of antimicrobials and biofilm-degrading enzymes, and competitive exclusion, probiotics present a promising, multifaceted, and frequently environmentally friendly approach to biofilm disruption and prevention. Their effectiveness against biofilms produced by important bacterial and fungal pathogens in industrial, medicinal, and agricultural settings is supported by a substantial body of preclinical data. Probiotics are a useful weapon in the continuous fight against problems linked to biofilms, especially in the crucial area of antimicrobial stewardship.

Authors' Contribution

All the authors contributed to this research paper, ranging from the research work to the writing and proofreading of the manuscript.

Conflict of Interest

There is no conflict of interest on the part of the authors with regard to the publishing of this article.

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