

The Battle of Probiotics and Their Derivatives Against Biofilms

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Abstract: Biofilm-related infections have been a major clinical problem and include chronic infections, device-related infections and malfunction of medical devices. Since biofilms are not fully available for the human immune system and antibiotics, they are difficult to eradicate and control; therefore, imposing a global threat to human health. There have been avenues to tackle biofilms largely based on the disruption of their adhesion and maturation. Nowadays, the use of probiotics and their derivatives has gained a growing interest in battling against pathogenic biofilms. In the present review, we have a close look at probiotics with the ultimate objective of inhibiting biofilm formation and maturation. Overall, insights into the mechanisms by which probiotics and their derivatives can be used in the management of biofilm infections would be warranted.

Keywords: antibiotic resistance, biofilm, probiotics, lactobacillus, sepsis, infection

Introduction

Biofilms are the aggregates of micro-organisms that are embedded in a self-produced polymeric matrix in a sessile state.¹ In the history of microbiology, biofilms have been detected earlier; however, only recently has their clinical burden been fully recognized. According to the National Institutes of Health (NIH), biofilms are involved in approximately 65% and 80% of all microbial and chronic infections, respectively. In the clinic, microbial biofilms through colonization on implants (prosthetic heart valves, catheters and joint replacement) and medical devices, account for hospital-acquired infections that make the patients easily infected by certain pathogens. Moreover, biofilm infections lead to different disorders, for instance, diabetes mellitus, dental caries, medical implants and wound infections that significantly affect the quality of life, cancer development, and subsequently, increase the global morbidity rate.¹

Hardly are biofilms detectable with routine diagnostic tests; therefore, the management of their infections are challenging in the clinic.² Methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus mutans*, *Pseudomonas aeruginosa*, *S. epidermidis* and *Gardnerella vaginalis* are the most common biofilm formers in the clinic.³ Different strategies like new generations of antibiotics and the inhibition of biofilm formation by quorum sensing (QS) inhibitors have been developed. Due to the challenges of these therapeutic agents in the clinic, there is a demand for developing new strategies. Recent evidence indicates that one of the strongest options for fighting pathogenic biofilms would be probiotics.

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Probiotics are living bacteria that confer a health-related profit to the host when administered in acceptable doses. This action of probiotics is mediated by interacting with host gut microbiota. High-throughput approaches including transcriptomics, metabolomics, proteomics and metagenomics have revealed that probiotics present beneficial for the host and they can modify host mucosal and systemic immune responses and protect the host against pathogens.⁴ *Lactobacillus* (lactic Acid Bacteria, LAB) and *Bifidobacterium* are the most important microbial genera that are generally used in the preparations of probiotics. These strains support a balanced immune function, healthy gut microbiome and improved nutrient absorption and lead to a healthy host.⁵ They are also capable to potentially modulate the microbial ecology of biofilms by pathogens' growth inhibition, adhesion and co-aggregation. Furthermore, probiotics exert antimicrobial activities against the gastrointestinal (GI) tract pathogens via declining luminal pH, competing for adhesion sites and nutrients and producing antimicrobial agents such as bacteriocins, hydrogen peroxide and organic acids (Tables 1 and 2). Based on these properties, probiotics present effectiveness in managing biofilms.

To date, some articles have been published on the beneficial effects of probiotics on the pathogenic biofilms formation in the wound as well as oral and infectious diseases. In a clinical trial, the use of *Bifidobacterium animalis* subsp. *lactis* HN019 twice a day for 30 days could promote benefits in the treatment of patients with chronic periodontitis.⁶

In this review, first, we have an overview on the mechanisms of biofilms formation and approaches for combating biofilms. Then, we highlight the novel probiotic-based progressive strategy to manage the pathogenic biofilms with emphasizing on probiotics' molecular mechanisms of actions.

Biofilm Formation

A biofilm is an agglomeration of micro-organisms on biotic and abiotic substances.⁷ The formation of biofilm is not accidentally, it is programmed with a complex mechanisms, whereby their lifecycle involves different distinct stages, from bacterial attachment and adherence to maturation and the release of cells from the matrix^{7,8} (Figure 1). Beyond guarding the bacterial cells, biofilms ease the distribution of antibiotic resistance via stimulating horizontal gene transfer.⁹ In the course of biofilm formation, various bacterial species display social behaviors and communicate with each other through a quorum sensing (QS) mechanism.¹⁰

QS is a bacterial cell-to-cell communication that regulates gene expression coordination and detection of cellular density

that is mediated by hormone-like small organic compounds called auto-inducers (AIs). Using these signaling molecules, bacteria collectively regulate the expression of virulence factors, the production of secondary metabolite, biofilm development and communications with host and other microbes based on population density. During the process of QS, signaling molecules bind to new bacterial receptors and lead to the transcription of genes within a single bacterial species and between different bacterial species that enable intraspecies and interspecies communications.

Treatment Strategies for Combating Bacterial Biofilm Infections

Currently, biofilm infection therapy is a complex challenge for clinicians. Antibiotic treatment is insufficient in combating against biofilm-related infections; however, understanding the nature of biofilms helps us support our efforts to fight with biofilm infections. Biofilm treatment can include the elimination of infected foreign bodies, the choice of well-penetrating and sensitive antibiotics, early administration of high dosage antibiotics/combinations and the usage of biofilm dispersal and/or anti-QS agents.¹¹ In the following sections, we have a brief view of the biofilm-battling strategies, then a close look at the impacts of probiotics and their derivatives on biofilms will be discussed.

Prescribing Antibiotics

Different antibiotics like lincosamides, rifamycins, tetracyclines, macrolides, etc. penetrate better than β -lactam, glycopeptides, aminoglycosides and polymyxin into the cells and tissues.¹¹ The combination therapy of antibiotics also is better than antibiotic monotherapy against biofilm infection.¹² Beyond the proper selection of antibiotics, appropriate duration of antibiotic treatment is essential. Despite a superior ability of fluoroquinolones for Gram-negative bacteria and rifampicin for Gram-positive bacteria to counteract biofilms,¹³ the entire eradication of biofilm infection is still challenging. Antimicrobial agents used for treatment of infections are not effective on biofilm forming bacteria, since they induce a selective pressure on the pathogens which triggers development of resistance to certain agents.¹⁴

Suppressing of Quorum Sensing

Targeting the Quorum sensing mechanisms has been a striking strategy to control infection in which bacterial virulence is

Table I Activity of Probiotics Against Oral Biofilms

Biofilm Former	Study	Probiotics	Probiotic's Mechanism of Action	Ref.
<i>Campylobacter rectus</i> ,*	CT	<i>L. acidophilus</i> La-5, <i>Bifidobacterium</i> Bb-12 and <i>L. rhamnosus</i> GG	↓Concentration of bacteria in supragingival and subgingival plaques	[19]
Periodontitis	CT	<i>Bifidobacterium animalis</i> subsp. Lactis with ozenges as adjuvant	↓Pro-inflammatory cytokine levels, delayed the recolonization of periodontal pockets.	[6]
Dental biofilms	CT	<i>S. salivarius</i> M18	↓ Level of halitosis in patients with orthodontic braces	[68]
Supragingival plaque	Human	Lozenges containing two strains of <i>L. reuteri</i>	<i>L. reuteri</i> did not affect gingival inflammatory reaction, the plaque accumulation and the composition of the supragingival plaque.	[69]
<i>Streptococcus mutans</i> Cariogenic bacterium	In vitro	<i>L. crispatus</i> BCRC 14618, <i>L. pentosus</i>	↓ Biofilm formation associated with sucrose-dependent cell-cell adhesion and the gtfC level of enzyme in the biofilm.	[70]
<i>S. mutans</i>	In vitro	<i>L. fermentum</i> , <i>L. paracasei</i> , <i>L. paracasei</i> , and <i>L. paracasei</i>	Probiotics produce bioactive factors that decreased in <i>S. mutans</i> biofilms.	[71]
<i>S. mutans</i>	In vitro	<i>L. salivarius</i> strains	↓ <i>S. mutans</i> growth, ↓Expression of <i>S. mutans</i> virulence genes gtfB, gtfC, and gtfD gtfS and EPS production	[72]
<i>S. mutans</i> with <i>C. albicans</i>	In vitro	<i>L. salivarius</i>	Secretory factors inhibited the formation of biofilm and fungal morphological transformation, ↓ <i>C. albicans</i> pathogenicity	[73]
<i>Candida albicans</i> ,	In vitro	<i>L. fermentum</i> 20.4, <i>L. paracasei</i> 28.4, and <i>L. rhamnosus</i> 5.2	↓ ALS3, HWPI, CPHI and EFG1 expression level.	[74]
<i>Candida glabrata</i>	In vitro	<i>L. rhamnosus</i> GR-1 and <i>L. reuteri</i> RC-14	↓ EPA6 and YAK1 expression (biofilm-related genes)	[75]
<i>S. mutans</i>	In vitro	<i>Bifidobacterium bifidum</i> , <i>L. acidophilus</i> , <i>L. brevis</i> , <i>L. casei</i> , and <i>L. rhamnosus</i> GG	↓Glucan production by ↓expression of gtfS by <i>S. mutans</i> Inhibits growth of other oral biofilm-forming bacteria	[20]
<i>S. mutans</i> , Streptococci strains	In vitro	Commercial probiotic lactobacilli strains	With aggregation and growth inhibition to interfere with biofilm.	[76]
<i>S. mutans</i> strains, multispecies biofilms	In vitro	<i>L. casei</i> Shirota, <i>L. casei</i> LCO1, <i>L. plantarum</i> ST-III and <i>L. paracasei</i> LPC37	These strains are able to prevent the <i>S. mutans</i> and multispecies biofilms growth.	[77]
<i>S. mutans</i> , <i>S. sobrinus</i>		<i>L. kefirifaciens</i> , <i>L. plantarum</i> , <i>L. rhamnosus</i> , <i>L. johnsonii</i>	Suppression of all biofilm-associated genes encode carbohydrate metabolism and regulatory biofilm and adhesion proteins.	[78]
<i>S. mutans</i>		<i>L. casei</i> , <i>L. reuteri</i> , <i>L. plantarum</i> , <i>L. salivarius</i>	↓Expression of genes involved in acid tolerance, QS and EPS production. <i>L. salivarius</i> had peroxide-dependent antimicrobial and antibiofilm activities.	[42]
<i>S. mutans</i> , <i>S. sanguinis</i> ,#	In vitro	<i>L. rhamnosus</i> GG	↓Counts of <i>S. sanguinis</i> and <i>C. albicans</i> , ↓Biofilm-forming ability of <i>F. nucleatum</i> , ↓Adhesion of <i>S. mutans</i>	[79]
<i>A. actinomycetemcomitans</i> strains	In vitro	<i>L. acidophilus</i> ##	Lipase is an effective factor in the biofilm degradation.	[80]
<i>Candida albicans</i>		Combinations of <i>L. plantarum</i> , <i>L. helveticus</i> , and <i>Streptococcus</i> <i>salivarius</i>	↓Expression of <i>EFG1</i> , <i>HWPI</i> , <i>ALS3</i> and <i>SAP5</i> involved in biofilm formation, yeast–hyphae transition, virulence, and host cell invasion	[43]

(Continued)

Table 1 (Continued).

Biofilm Former	Study	Probiotics	Probiotic's Mechanism of Action	Ref.
<i>Candida tropicalis</i> , <i>Candida krusei</i> and <i>Candida parapsilosis</i>	In vitro	<i>L. gasseri</i> and <i>L. rhamnosus</i> supernatant	Disrupts mature biofilm formation, inhibits the mixed biofilms and damages the cells on silicone surface.	[81]
<i>C. albicans</i> , <i>C. tropicalis</i> , and <i>C. krusei</i> .	In vitro	<i>L. pentosus</i> strain LAPI	Probiotic had anti-Candida activity and antibiofilm property.	[43]
<i>S. aureus</i> strains 9P and 29P	In vitro	<i>L. casei</i> LBI	Biosurfactants could disperse the preformed biofilms.	[27]

Notes: **Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Campylobacter rectus*, *Parvimonas micra*, *Fusobacterium nucleatum* ssp. *Nucleatum*, *Treponema denticola*, *Prevotella intermedia*, *Porphyromonas gingivalis*, #*Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *C. albicans*, ##*L. plantarum*, *L. casei* subsp. *Rhamnosus*, *L. delbrueckii* subsp. *Casei*, *L. fermentum*, *L. fermentum*, *Lactococcus lactis*, *L. casei*, *Leuconostoc fructosum*, *Leuconostoc mesenteroides*.

Abbreviations: CT, Clinical trial; S, *Streptococcus*; C, *Candida*; gtf, Glucosyltransferases; QS, quorum sensing; EPS, exopolysaccharides.

attenuating to be easily cleared by the host immune system and not to establish an effective infection. Quorum sensing inhibitory compounds are a new generation of antimicrobial agents; however, they have not been largely successful. Several strategies are available to disturb bacterial QS. One of these strategies is the inactivation of LuxR homologs using N-acyl homoserine lactone (AHL) antagonists that competes with the native AHL to bind to the LuxR-type receptor. By inhibiting AHL-binding, the LuxR homolog would not be activated and the expression of virulence factor gets switched off. The suppression of the AHL synthesis is yet another strategy.¹⁵ While QS suppression has been studied as novel anti-infective strategy, evidence shows the development of bacterial resistance against QS-suppressing agents.¹⁶ In addition, the toxicity of some QS-suppressing compounds, such as nanoparticles, limits their biomedical usage.¹⁷

Probiotics Fight Against Biofilm Formation

Due to the insufficiency of well-known approaches, the development of novel biofilm-fighting strategies would be valuable in the clinic. Recent evidence indicates that probiotics have opened a new horizon to fight with infectious biofilms. Since probiotics cannot induce the strong selective pressure on resistant isolates than conventional antibiotics and also they are less cytotoxic than QS-suppressing agents, they can be considered as ideal option for new anti-virulence agents. Using different mechanisms, probiotics can hinder the activity of pathogenic bacteria and their adhesion to surfaces. Moreover, they prevent QS, biofilm formation and the survival of biofilm pathogens, interfere with biofilm integrity/quality and finally lead to biofilm eradication (Tables 1 and 2). Some of these molecular mechanisms include the secretion of antagonistic substances (e.g., surfactants, bacteriocins, exopolysaccharides (EPS),

organic acids, lactic acid, fatty acids, enzymes (amylase, lipase) and hydrogen peroxide) and the generation of unfavorable environmental conditions for pathogens (e.g., pH alteration as well as competition for surface and nutrients), Figure 2. Probiotics competitive adhesion to human tissues or medical equipment prevents the colonization of harmful bacteria. Moreover, by decreasing the environmental pH, indole production (a signal molecule in QS) and biofilm biomass, probiotics prevent pathogenic biofilm formation (Tables 1 and 2).

The probiotic strains can be isolated from numerous sources such as human, animal, plant, environment and foods.^{18,19} Then, they can be identified and characterized by microbiological, biochemical and molecular-based techniques. *Streptococcus salivarius*, *S. oralis*, *L. rhamnosus*, *L. fermentum*, *L. plantarum*, *L. casei*, *L. acidophilus*, *L. brevis*, *L. sporogenes*, *L. salivarius*, *L. delbrueckii*, *L. pentosus*, *Bifidobacterium lactis* and *B. longum* are the most reported probiotic strains that exert anti-biofilm activity (Tables 1 and 2).

Several in vitro biofilm models have been developed by attaching bacteria on adhesive surfaces.²⁰ All of these models lack features of the host immune competence and environment. So, animal models take into account since it is practically impossible to study the development of infectious diseases in humans (reviewed comprehensively in Ref [21]). MRSA mouse model²² and rabbit model of ischaemic and infected wounds²³ were developed. Moreover, a removable in vivo abutments was developed that mimicked dental implants.²⁴ To address in vitro and in vivo problems, a novel human plasma biofilm model was developed for studying the impact of probiotics on pathogens that mimicked a biofilm-challenged human wound milieu.²⁵

Table 2 Activity of Probiotics and Their Products Against Biofilms

Biofilm Former	Probiotics	Probiotic's Mechanism of Action	Ref.
<i>C. albicans</i> , [#]	<i>L. rhamnosus</i> supernatant	Secretes biosurfactants that disrupt the physical membrane structure or protein conformations; results in cell lysis, destroys the hyphae formation and interferes with the interaction between the cells and material.	[41]
<i>Vibrio cholera</i> and <i>V. parahaemolyticus</i>	<i>L. spp.</i> L13 (KY780504), ^{###}	Inhibited the adherence of <i>Vibrio spp.</i> to the epithelial cells and dispersed the preformed- <i>V. cholerae</i> biofilms	[54]
<i>P. aeruginosa</i>	<i>Pediococcus acidilactici</i> M7 strain isolated from newborn faeces	Lactic acid produced by the strain: - Inhibited the RhI system signaling molecule (C4-HSL) ↓Virulence factors regulated by the RhI including protease, pyocyanin, elastase, and biofilm production - Did not reduce/inhibit the Las system signaling molecule (3-oxo-C12-HSL)	[44]
<i>B. subtilis</i> BM19	<i>L. acidophilus</i> ATCC 4356	Bacteriocin from this probiotic inhibits the growth of <i>B. subtilis</i> BM19 planktonic cells and biofilm formation	[82]
<i>Propionibacterium acnes</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> , <i>E. coli</i>	<i>L. delbrueckii</i> subsp. <i>Bulgaricus</i> , ^{####}	Due to organic acid production, all probiotics except <i>L. delbrueckii</i> , had antimicrobial activity. Probiotics inhibit the AHL production and prevent biofilm formation, <i>P. innocua</i> was able to destroy pre-formed biofilms of <i>E. coli</i> , <i>P. aeruginosa</i> and <i>S. aureus</i>	[45]
<i>P. aeruginosa</i> PAOI, MRSA and their hospital-derived strains	<i>L. plantarum</i> F-10 supernatant	↓QS signals, ↓Oxidative stress in wound healing stages, Co-aggregated with all pathogens, inhibited the virulence factors (motility, activity of protease and elastase, production of pyocyanin and rhamnolipid)	[83]
<i>E. coli</i> ATCC35218	EPS-Lp from <i>L. plantarum</i> and EPS-B from <i>Bacillus spp.</i> ,	EPSs: ↓cell surface hydrophobicity level, ↓indole production, prevent biofilm formation, ↓efflux pumps involved in bacterial adhesion and antimicrobial resistance.	[84]
<i>Staphylococcus aureus</i> , [*]	<i>Streptococcus salivarius</i> 24SMB and <i>oralis</i> 89a	↓pH and ↓biofilm biomass prevent the biofilm formation of selected pathogens, disperse the pre-formed biofilms, secret diffusible molecules that are implied in their anti-biofilm activity	[85]
EHEC, <i>P. aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>S. epidermidis</i>	<i>E. coli</i> Nissle 1917	Secretes DegP, a bifunctional protein with protease and chaperone activity outside the cells and controls other biofilms.	[86]
<i>S. aureus</i>	<i>L. fermentum</i> TCUESC01 and <i>L. plantarum</i> TCUESC02	Inhibition of biofilm by alteration of the <i>ica</i> operon (<i>icaA</i> and <i>icaR</i>) involved in the biofilm matrix synthesis.	[87]
<i>C. albicans</i> , <i>C. tropicalis</i> , <i>C. krusei</i> .	<i>L. pentosus</i> strain LAPI	Probiotic indicated an anti-Candida activity and antibiofilm property	[88]
<i>C. albicans</i>	<i>Pediococcus acidilactici</i> HW01	It has antifungal agent against <i>C. albicans</i> by reducing the growth and biofilm formation.	[89]
Clinical <i>Salmonella</i> species and uropathogenic <i>E. coli</i>	<i>L. rhamnosus</i> GG	Lectins are involved in the adhesion capacity of <i>L. rhamnosus</i> to vaginal and gastrointestinal epithelial cells.	[90]
<i>Cronobacter sakazakii</i>	<i>L. casei</i> , <i>L. sporogenes</i> , ^{**}	With antimicrobial activity, production of bioactive molecules to limit the emerging infections.	[91]
<i>P. aeruginosa</i> PAOI	<i>L. fermentum</i> (KT998657) isolated from neonatal fecal samples	↓Biofilm forming due to postbiotics (bacteriocin and EPS), bacteriocins make pores in the cell membrane, change membrane integrity of cells, and cause cell death, EPS alter the matrix and restrict cell assembly, cell-cell interaction and <i>Pseudomonas</i> attachment to form biofilms.	[26]
<i>C. glabrata</i>	<i>L. rhamnosus</i> GR-1, <i>L. reuteri</i> RC-14	↓EPA6 and YAK1 expression (biofilm-related genes)	[75]

Notes: [#]*Candida tropicalis*, *Streptococcus salivarius*, *R. dentocariosa*, *Staphylococcus epidermidis*, ^{###}*L. plantarum* L14(KY582835), *L. spp.* L18 (KY770976), *L. fermentum* L32 (KY770983), *L. spp.* S30 (KY780503), *L. pentosus* S45 (KY780505), *L. spp.* S49 (KY770966) isolated from the fecal samples of healthy children, ^{####}*Bifidobacterium animalis* subsp. *Lactis*, *L. acidophilus*, *L. brevis*, *Bifidobacterium lactis*, *L. salivarius* *Bifidobacterium longum* subsp. *Infantis*, *L. plantarum*, *L. acidophilus*, *L. casei*, *Propioniferax innocua*, *L. casei* subsp. *Rhamnosus*, MRSA: methicillin-resistant *Staphylococcus aureus*, ^{*}*Streptococcus pyogenes*, *Propionibacterium acnes*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus epidermidis*, ^{**}*L. sporogenes*, *B. mesentericus*, *C. butyricum* *L. sporogenes*, *S. faecalis*, *L. sporogenes*, *S. faecalis*, *Clostridium butyricum*, *Bacillus mesentericus*.

Abbreviations: L, *Lactobacillus*; S, *Streptococcus*; P, *Pseudomonas*; C, *Candida*; EPS, exopolysaccharides; NEC, necrotizing enterocolitis; E, *Escherichia*; EHEC, enterohemorrhagic *E. coli*; QS, quorum sensing; A, *Aggregatibacter*.

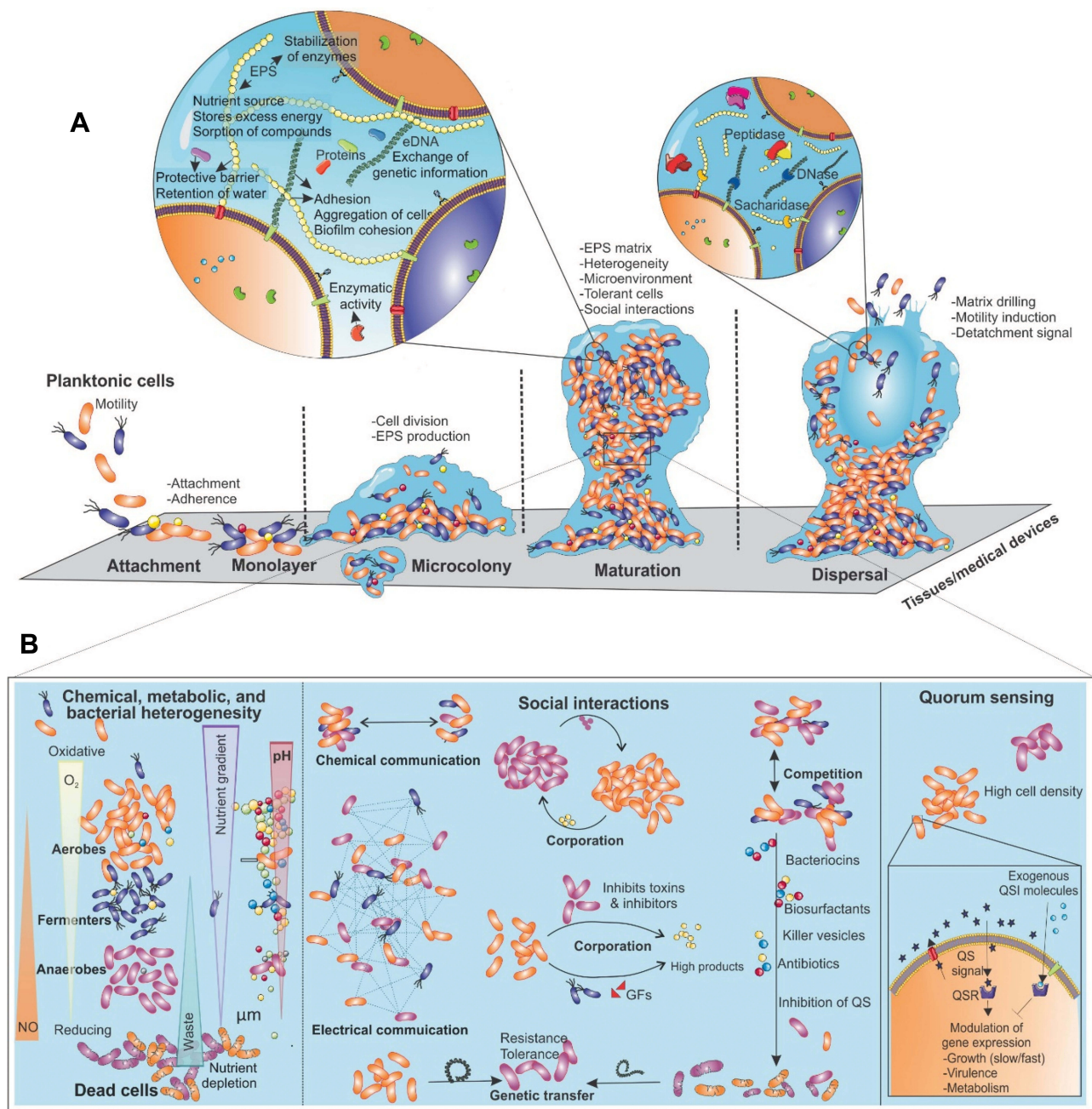


Figure 1 The stages and complex structure of bacterial biofilms. **(A)** Different stages are involved in biofilm formation, during which a series of changes happen. These stages include initial attachment, microcolony formation, maturation and dispersion. Detachment allows bacteria to colonize in new niches. **(B)** The formation of the EPS matrix leads to the establishment of stable gradients of nutrition, pH, waste products and oxygen that make different localized habitats at a small scale. Social connections in biofilms include positive (competition or cooperation) and negative (competition) interactions between bacterial cells that result in remodeling of the biofilm community. Cooperation is mediated by electrical and chemical communications between cells in biofilms while competition is mediated by different killing strategies such as producing bacteriocins, antibiotics, enzymes and growth inhibition mechanisms like preventing QS and depletion of nutrient.

Abbreviations: EPS, extracellular polymeric substance; GFs, growth factors; NO, nitric oxide; QS, quorum sensing.

Probiotic Products Against the Different Pathogenic Biofilms

Lactobacillus species produce different exometabolites such as EPSs, bacteriocins,²⁶ oxygen reactive species (ROS) and biosurfactants with anti-biofilm activity.^{27,28} The polysaccharides produced by LAB possess anti-biofilm,²⁹ immune

system stimulatory and antioxidant effects.³⁰ The EPS of *Lactobacillus* spp. was effective in both Gram-positive (e.g., *Listeria monocytogenes* and *S. aureus*) and Gram-negative (e.g., *P. aeruginosa* and *Salmonella typhimurium*) bacteria. The results displayed that the biofilm removal ability is related to EPS concentration.³¹

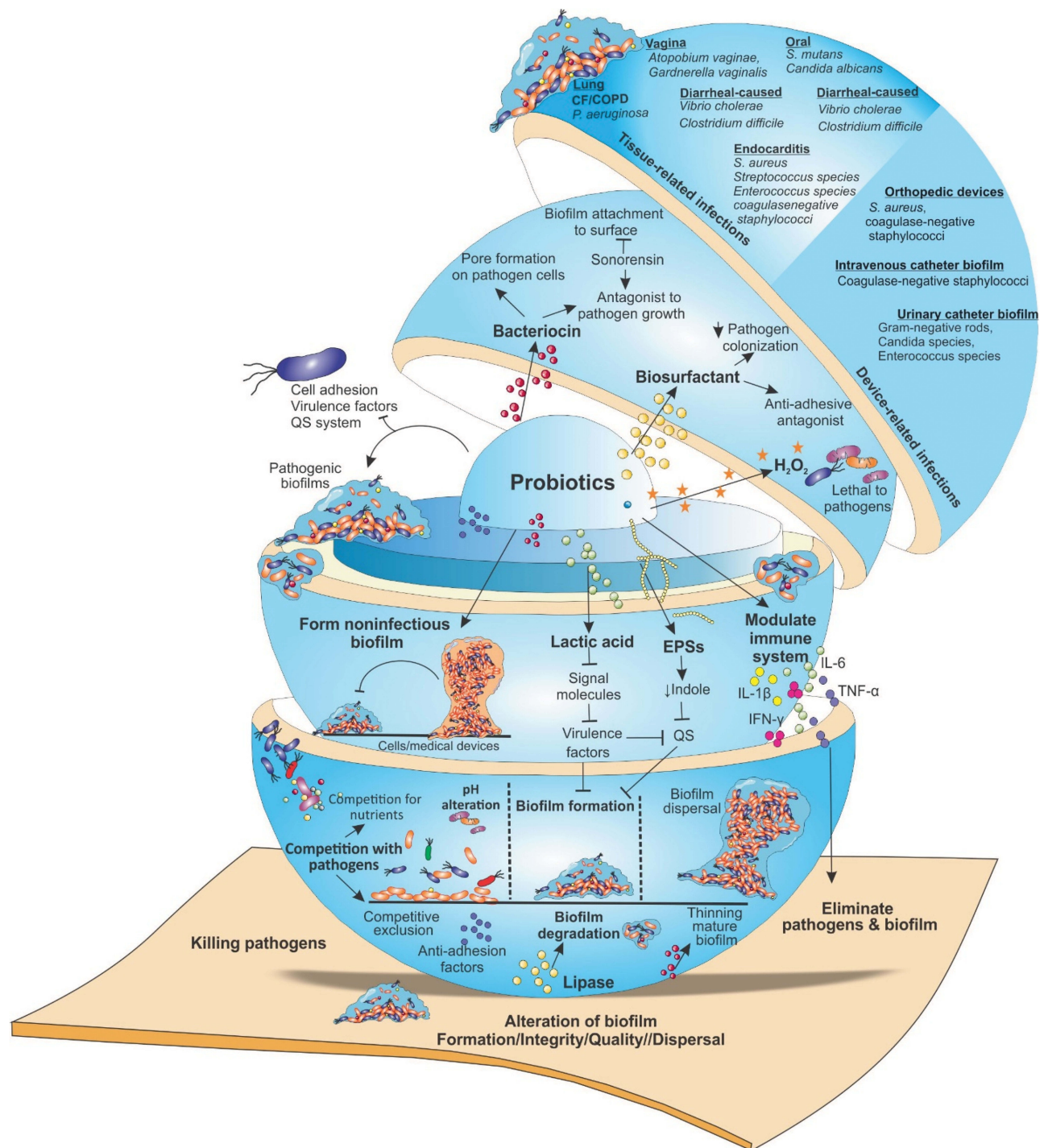


Figure 2 Targeting microbial biofilms by probiotics. Probiotics employ different mechanisms by which interfere with the activity of pathogenic bacteria. They produce antagonistic substances such as, surfactants, bacteriocins, EPS, organic acids, lactic acid, fatty acids, enzymes (lipase, amylase) and hydrogen peroxide that can hinder the activity of pathogenic bacteria and their adhesion to surfaces. Moreover, they prevent QS, biofilm formation and the survival of pathogens as well as interfere with biofilm integrity/quality, finally, lead to biofilm eradication. Furthermore, probiotics generate unfavorable environmental conditions for pathogens (e.g., pH alteration as well as competition for surface and nutrients). Their competitive adhesion to human tissues or medical devices (catheters, prostheses, or other medical devices), prevent the colonization of harmful bacteria. Additionally, by modulating host immune responses and formation of non-pathogenic biofilms, they target pathogenic biofilms that prevent the biofilms formation by certain pathogenic bacteria. **Abbreviations:** CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; EPS, extracellular polymeric substance; QS, quorum sensing; UTI, urinary tract infection.

The anti-biofilm activity of bacteriocins has been demonstrated in different reports. *L. brevis* DF01 bacteriocin prevents biofilm formation but does not eradicate the

established *Escherichia coli* and *S. typhimurium* biofilms.³² The mechanisms of biofilm inhibitory effects of bacteriocin are not well understood. Some of the bacteriocins eradicate

biofilm by the induction of pore-formation on the bacterial cell surface, leading to ATP efflux, while some others have biological activity by proteolytic enzymes.³³ Subtilisin A, a cyclic bacteriocin (lantibiotic protein) synthesized by *Bacillus subtilis*, is another derivative of probiotics. It has a net cationic charge that generally targets the surface receptors rather than binding to bacterial cells electrostatically. Beside the antimicrobial activity of subtilisin against *Gardnerella vaginalis* and *L. monocytogenes*, its anti-biofilm effect was reported against *G. vaginalis* alone and with natural antimicrobial agents.^{34–36} Given the wide-ranging activities of subtilisin, Chikindas et al observed its anti-QS effect in *E. coli* O157:H7, *L. monocytogenes* ScottA and *G. vaginalis* ATCC 14018. Subtilisin led to the inhibition of 60% of *E. coli*, 80% of *L. monocytogenes* and 90% of *G. vaginalis* biofilms.³⁷ Likewise, sonorensin, a bacteriocin produced by *Bacillus sonorensis* MT93, was able to decrease *S. aureus* biofilms cell viability, inhibit biofilm attachment and formation, and cause the thinning of mature biofilms.³⁸

Due to exometabolites formation, *Lactobacillus* species also inhibit *Candida albicans* biofilm by inhibiting the initial stage of colonization and hypha formation.³⁹ Lactobacilli that produce biosurfactant had antimicrobial, anti-adhesive properties and aggregation ability against pathogenic biofilm formation.⁴⁰ *L. rhamnosus* producing biosurfactants could disrupt the physical membrane structure or protein conformations; resulting in cell lysis.⁴¹ Furthermore, biosurfactants significantly decrease the adhesion and biofilm generation of bacteria in a dose-dependent manner.²⁸

Probiotics Influence Gene Expression of Pathogenic Biofilms

The mechanism by which probiotics prevent the biofilms formation is fairly unclear. Several in vitro studies have shown that the expression of genes involved in cell adhesion, QS, virulence factors and biofilm formation can be influenced by probiotics. Wasfi and coworkers assessed the *Lactobacillus* spp. effect on the gene expression of *S. mutans* in a co-cultured condition. They focused on genes involved in EPSs formation (*gtfB*, *sacB* (ftf), *gtfC* and *gtfD*), signal transduction systems (*vicR*, *comC*, *vicK* and *comD*) and stress survival (*atpD* and *aguD*). Results revealed that there was an overall significant decrease in the expression of these genes among different groups, in both biofilm-forming and planktonic cells. Additionally, by producing organic acid and peroxide, probiotics led to a decline in cell adherence and preformed biofilm.⁴²

Moreover, EPS produced by *L. acidophilus* A4 considerably could inhibit biofilm formation of *E. coli* O157: H7 by reducing the expression of genes related to chemotaxis (*cheY*) and curli formation (*csgA*, *csgB* and *crl*).²⁹ Burton et al clarified a mechanism of biofilm inhibition of *C. albicans* using the combination of *L. plantarum* SD5870, *Streptococcus salivarius* DSM 14685 and *L. helveticus* CBS N116411. The expression of some *C. albicans* genes such as *ALS3* (adhesin/invasin), *HWP1* (a critical hyphal wall protein for biofilm formation), *EFG1* (hyphae-specific gene activator) and *SAP5* (secreted protease) are affected by these probiotics. The results showed that these probiotics are effective in inhibiting the biofilm formation and also removing of the preformed biofilms of *C. albicans*.⁴³ Therefore, it is rational to claim that probiotics and their derivatives can be used as both prophylactic and treatment biodrugs.

Some probiotics have also inhibitory effects on QS systems that inhibit the QS-dependent physiologic behaviors of bacteria.⁴⁴ Lactic acid produced by probiotics had shown an inhibitory effect on QS by suppressing short-chain AHL production and biofilm formation of *P. aeruginosa* that is regulated by QS.⁴⁴ Probiotics also secrete organic acid as QS antagonists that interfere with AHLs production at the gene expression level and prevent biofilm formation.⁴⁵

Biosurfactants isolated from *L. plantarum* and *Pediococcus acidilactici* could inhibit the adhesion and biofilm formation of *S. aureus* CMCC 26003 in a dose-dependent manner in vitro. The molecular mechanism of biosurfactants is mediated by affecting the expression of biofilm-related (*cidA*, *sarA*, *icaA*, *dltB*, *sortaseA*, and *agrA*) genes and interfering with the release of signaling molecules (AI-2) in QS systems.²⁸ Similarly, *S. mutans* produce extracellular glucans by glucosyltransferases (gtfs) that are vital for the initiation and progression of dental caries. Biosurfactant produced by *L. fermentum* could decrease the *S. mutans* *gtfB/C* gene expression, the process of attachment and biofilm formation.⁴⁶ The impacts of probiotics on gene expression of pathogens are further summarized in Tables 1 and 2.

Probiotics Modulate the Host Immune Responses to the Biofilms

The host immune responses against biofilms are mediated by various cellular receptors, chemokine and cytokine expression, that can be different based on the stage of biofilm.⁴⁷ Probiotics and their secreted soluble factors are speculated to be recognized by the toll-like receptors (TLRs) on epithelial

cells; and thereafter exert their immunomodulatory effects on intestinal and systemic immunities.⁴⁸ Moreover, probiotics can modify innate immune functionality in different ways, some of which include the secretion of immunomodulatory metabolites, lipids and proteins, receptor expression, micro-RNAs induction and production of negative regulatory signaling molecules (reviewed in Ref. [49]). Therefore, by modulating the immune responses, probiotics can impact biofilms indirectly. *Streptococcus thermophilus* strains (ST1342, ST1275, and ST285) can activate monocyte cells to secrete IL-1 β , TNF α , IL-6 and IFN- γ that activate the innate immune responses in order to eliminate pathogens. Strain ST1342 could induce high levels of IL-1 β secretion that has both anti-viral and anti-bacterial activities.⁵⁰ Likewise, it was mentioned that the probiotic *L. paracasei* DG utilized generally in commercial probiotic products, possess immune-stimulatory activities by enhancing of TNF α , IL-6 and CCL20 expression in the human monocyte leukemia cell line.⁵¹ *Lactobacillus* sp. could induce IFN- γ production and inhibit IL-10 production and exert immunomodulatory effect on *S. mutans* in human-cultured cells. Detailed knowledge of the immune mechanisms, the cytokine and receptor expression profiles and bacterial defense mechanisms under biofilm formation is needed for demonstrating the effects of probiotics on the immune system to fight against microbial biofilm.

The Activity of Probiotics Against Different Types of Clinical Biofilms

Probiotic Influence the Dental Biofilms

Tooth plaque, as a multispecies biofilm organized by microbes, forms complex communities and plays an important role in different dental diseases such as periodontal diseases and tooth decay.⁵² The effect of *Lactobacillus* sp. against the formation of biofilm and gene expression of *S. mutans* was studied.⁴² Comelli et al selected the dairy probiotics that were capable of reducing the carcinogenicity of dental plaque. They showed that *Lactococcus lactis* NCC2211, as a nonpathogenic dairy probiotic, could be incorporated into a biofilm; so, imitating the dental plaque and it could be able to modify the growth of the cariogenic *S. sobrinus* OMZ176.⁵³ The inhibitory effects of probiotics on oral biofilms and their molecular mechanisms are summarized in Table 1.

Probiotics Against the Diarrhea-Causing Pathogens

Kaur et al screened the *Lactobacillus* spp. abilities to inhibit the formation of biofilm and disperse the preformed biofilms

of *Vibrio parahaemolyticus* and *V. cholerae* in vitro. They demonstrated that the pH non-neutralized culture supernatant (CS) of seven isolates of *Lactobacillus* spp. could prevent the biofilm formation of *V. cholerae*. The result displayed that CS of *Lactobacillus* spp. has a dispersion effect on *V. cholerae* biofilm.⁵⁴ A meta-analysis was done on the impact of probiotics on the prevention of *Clostridium difficile*-related diarrhea. The analysis demonstrated that probiotics such as *Saccharomyces* and *Lactobacillus* could significantly lower the risk of *C. difficile*-associated diarrhea development.⁵⁵

Interference of Probiotics in Wound Biofilm

P. aeruginosa is an opportunist Gram-negative bacterium and the most frequent pathogen isolated from chronic infections. This pathogen changes the response of the host immune system, inflammation and processes of wound healing.⁵⁶ Ramos et al studied the effect of *L. plantarum* supernatants (Lps) on the biofilm formation of *P. aeruginosa*. They found that LPS interferes with the biological action of AHL and inhibits the normal activity of *P. aeruginosa* QS. Moreover, it is capable of causing the interruption of a preformed *P. aeruginosa* biofilm.⁵⁷ Likewise, co-culturing of *L. fermentum* with *S. aureus* and *P. aeruginosa* prevented the growth and biofilm formation of both pathogenic bacteria. Moreover, in the presence of *L. fermentum* supernatant, a thin layer of *S. aureus* biofilm was formed across the surface of glass rather than the thicker biofilm layer of the control.⁵⁸

Probiotic Biofilms Against Pathogenic Biofilm

The formation of biofilm by probiotics is considered to be a beneficial strategy against pathogenic biofilms since they compete with pathogens for nutrients and space with different mechanisms of action. Moreover, probiotic biofilms can stimulate the colonization and longer stability of probiotics in the host mucosa that prohibit colonization of pathogenic bacteria. Only some of *Lactobacillus* strains such as *L. reuteri*, *L. rhamnosus*, *L. fermentum* and *L. plantarum* can form biofilm on abiotic surfaces (glass or polystyrene).⁵⁹ The EPS production by some biofilm-former probiotics can prevent the biofilms formation of certain pathogenic bacteria.

In line with this subject, Gómez and coworkers tested the protective effect of biofilms with bacteriocinogenic (*L. curvatus* MBSa3, *L. sakei* MBSa1, *L. lactis* VB94

and *L. lactis* VB69) and non-bacteriocinogenic (*Weissella viridescens* 113, *L. helveticus* 354, *L. lactis* 368, and *L. casei* 40) lactic acid bacteria to fight against *E. coli* O157:H7, *Salmonella typhimurium* and *L. monocytogenes*. Results show a prevention in biofilm formation of these pathogenic bacteria in 24, 48 and 72h of exposure.⁶⁰ Moreover, biofilms of probiotic *E. coli* Nissle 1917 on silicone substrates could decrease the colonization of the pathogenic *E. faecalis* 210.⁶¹ Likewise, *L. kunkeei* biofilm reduces the infection of *P. aeruginosa* by affecting biofilm formation and/or their stability.⁶² Furthermore, biofilms of probiotic formed by *Bifidobacterium infantis* and *L. reuteri* can be utilized as efficient bacteria to delay the *L. monocytogenes* growth.¹⁸

L. brevis 104/37, *L. plantarum* 118/37 and 6E could effectively eradicate *staphylococcal* biofilms. Yet, only *L. rhamnosus* ATCC 7469 and *L. plantarum* 2/37 could form their own biofilms to replace with the pathogenic ones.⁶³ Additionally, the *L. plantarum* WCFS1 and NA7 biofilms produce extracellular molecules with immunomodulatory and growth inhibitory properties against food pathogens (*S. aureus*, *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella enterica*). All the studied *Lactobacillus* strains had an anti-inflammatory effect in the in vitro, while just *L. fermentum* NA4 displayed a protective effect in vivo. Hence, *Lactobacillus* in biofilm status exerts beneficial probiotic properties in a strain-dependent manner.⁶⁴ The progress of the new technologies for the encapsulation of biofilms that covers in the double coated capsules has developed a new generation of probiotics.⁵⁹ *L. rhamnosus* GG microcapsules, as effective inhibitors of transcriptional activators of the *luxS* QS system, could prevent biofilm formation and disturb the mature biofilms.⁶⁵

Future Perspective

Biofilm infection therapy has been a complex challenge for clinicians. Better understanding and hacking into bacterial biofilms help scientists develop robust strategies. Recently, the immune system and probiotics relationships have been reported in defending the host against the colonization of pathogenic species. In fact, probiotics yield different compounds, ranging from peroxides and fatty acids to highly specific bacteriocins, to kill or hinder pathogenic bacteria. Recently, clinical trials and in vitro studies have provided evidence on the impact of the probiotics on different medical fields (wound, oral, intestinal and vaginal infections) to fight against pathogenic biofilms via a counteraction, competition and gene silencing of

pathogenic factors. All data together signify a great ability of probiotics to be used both in prevention and treatment of pathogenic biofilm infections.

In fact, in vitro studies on adhesion, the secretion of extracellular anti-biofilm factors, metabolic activity, the growth inhibition, co-aggregation, the prevention of biofilm formation and the eradication of mature biofilm have recommended possible roles for probiotic in modifying the biofilms microbial ecology. On the other hand, biofilm-forming probiotic strains can exchange resident biofilm pathogens with a non-pathogenic variant that produce bacteriocin;⁴⁰ however, their molecular mechanisms have been poorly examined.

Challenges with the Management of Biofilms by Probiotics

Data demonstrate that probiotics and their derived-products can be hopeful strategy to manage biofilms. It should be noticed that data are still scarce and there is not enough evidence to consider probiotics as bio-drugs to inhibit pathogenic biofilm formation and/or disperse preformed biofilms. Confounding results may be related to the diversity in delivery vehicle, dose, assessment of efficacy and viability, and particularly to the variability in selection of strains. It has been revealed that the impacts of probiotics are strains-specific, different strains of even one probiotic species can present an altered impact on the host and pathogenic biofilm since the host molecular signaling reprogramming extremely tend to depend on the bacterial strain and cell context. No two probiotics look like each other and different strains may exert different effects. Additionally, under various circumstances, even the same strains may function differently. Therefore, an ideal strain of probiotic for interfering and competing with pathogenic biofilms should be screened and identified at the molecular level for specific pathophysiological states, particularly in the context of definite infection and microbial targets.

Additionally, characterization and evaluation of safety aspects (blood hemolytic activity and resistance to antibiotics) of strains should be performed before their clinical administration. The essential criteria for selection of potential probiotic strains are proposed to be their adhesion to epithelial cells and mucus along with their co-aggregation with pathogens.⁶⁶ Furthermore, other criteria including potential antimicrobial activity against pathogens, survival in the human GI conditions and inhibition of colon cancer define a strain as

a probiotic. Moreover, their viability and stability during production and storage processing are also important issues in the clinical application of probiotics. Resistance in probiotics has been a focus of researchers. A major concern in this area would be the increased risk of transferable drug resistance(s) genes from probiotics to other bacterial population.⁶⁷ Therefore, it is essential to assess their non-transferable or transferable antibiotic resistance at the genome level. It seems that the use of cell-free supernatants of probiotics can address most of the aforementioned concerns.

Getting reliable enough in vivo and human study results are needed for transferring this treatment strategy in human subjects. In the near future, it would be quite possible to employ the probiotics or their products to develop an innovative safe therapy for biofilm-related infection.

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Disclosure

All of the authors declare that there are no personal, commercial, political, and any other potential conflicting interests related to the published paper.

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