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. 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. Methicillin-resistant Staphylococcus aureus (MRSA), Streptococcus mutans, Pseudomonas aeruginosa, S. epidermidis and Gardnerella vaginalis are the most common biofilm formers in the clinic. 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.
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. 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. 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.

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. The formation of biofilm is not accidently, 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 (Figure 1). Beyond guarding the bacterial cells, biofilms ease the distribution of antibiotic resistance via stimulating horizontal gene transfer. In the course of biofilm formation, various bacterial species display social behaviors and communicate with each other through a quorum sensing (QS) mechanism.

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. 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. The combination therapy of antibiotics also is better than antibiotic monotherapy against biofilm infection. 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, 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.

### Suppressing of Quorum Sensing

Targeting the Quorum sensing mechanisms has been a striking strategy to control infection in which bacterial virulence is
| Biofilm Former                   | Study   | Probiotics                                                                 | Probiotic's Mechanism of Action                                                                 | Ref.   |
|---------------------------------|---------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|--------|
| Campylobacter rectus,*          | CT      | L. acidophilus Lo-5, Bifidobacterium Bb-12 and L. rhamnosus GG             | ↓ Concentration of bacteria in supragingival and subgingival plaques                                | [19]   |
| Periodontitis                   | CT      | Bifidobacterium animalis subsp. Lactis with ozenges as adjuvant             | ↓ Pro-inflammatory cytokine levels, delayed the recolonization of periodontal pockets.             | [6]    |
| Dental biofilms                 | CT      | *S. salivarius M18                                                          | ↓ Level of halitosis in patients with orthodontic braces                                          | [68]   |
| Supragingival plaque            | Human   | Lozenges containing two strains of L. reuteri                              | L. reuteri did not affect gingival inflammatory reaction, the plaque accumulation and the composition of the supragingival plaque. | [69]   |
| Streptococcus mutans           | In vitro| L. crispatus BCRC 14618, L. pentosus                                      | ↓ Biofilm formation associated with sucrose-dependent cell-cell adhesion and the gtfC level of enzyme in the biofilm. | [70]   |
| S. mutans                      | In vitro| L. fermentum, L. paracasei, L. paracasei, and L. paracasei                 | Probiotics produce bioactive factors that decreased in *S. mutans* biofilms.                      | [71]   |
| S. mutans                      | In vitro| *S. salivarius* strains                                                    | ↓ *S. mutans* growth, ↓Expression of *S. mutans* virulence genes gtfB, gtfC, and gtfD gfts and EPS production | [72]   |
| S. mutans with C. albicans     | In vitro| L. salivarius                                                              | Secretory factors inhibited the formation of biofilm and fungal morphological transformation, ↓ C. albicans pathogenicity | [73]   |
| Candida albicans,              | In vitro| L. fermentum 20.4, L. paracasei 28.4, and L. rhamnosus 5.2                | ↓ ALS3, HWP1, CPH1 and EFG1 expression level.                                                      | [74]   |
| Candida glabrata               | In vitro| L. rhamnosus GR-1 and L. reuteri RC-14                                     | ↓ EPA6 and YAK1 expression (biofilm-related genes)                                               | [75]   |
| S. mutans                      | In vitro| Bifidobacterium bifidum, L. acidophilus, L. brevis, L. casei, and L. rhamnosus GG | ↓Glucan production by ↓expression of gfts by *S. mutans* Inhibits growth of other oral biofilm-formatting bacteria | [20]   |
| S. mutans, Streptococci strains| In vitro| Commercial probiotic lactobacilli strains                                  | With aggregation and growth inhibition to interfere with biofilm.                                 | [76]   |
| S. mutans strains, multispecies biofilms | In vitro | L. casei Shirota, L. casei LC01, L. plantarum ST-I11 and L. paracasei LPC37 | These strains are able to prevent the *S. mutans* and multispecies biofilms growth.               | [77]   |
| S. mutans, S. sobrinus          | L. kefiranofaciens, L. plantarum, L. rhamnosus, L. johnsonii               | Suppression of all biofilm-associated genes encode carbohydrate metabolism and regulatory biofilm and adhesion proteins. | [78]   |
| S. mutans                      | L. casei, L. reuteri, L. plantarum, L. salivarius                           | ↓Expression of genes involved in acid tolerance, QS and EPS production. L. salivarius had peroxide-dependent antimicrobial and antibiofilm activities. | [42]   |
| S. mutans, S. sanguinis,#       | In vitro| L. rhamnosus GG                                                            | ↓Counts of *S. sanguinis* and *C. albicans*, ↓Biofilm-forming ability of F. nucleatum, ↓Adhesion of *S. mutans* | [79]   |
| A. actinomycetemcomitans strains| In vitro| L. acidophilus###                                                          | Lipase is an effective factor in the biofilm degradation.                                        | [80]   |
| Candida albicans               | Combinations of L. plantarum, L. helveticus, and Streptococcus salivarius | ↓Expression of EFG1, HWP1, ALS3and SAPS involved in biofilm formation, yeast--hyphae transition, virulence, and host cell invasion | [43]   |

(Continued)
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.\(^\text{15}\) While QS suppression has been studied as novel anti-infective strategy, evidence shows the development of bacterial resistance against QS-suppressing agents.\(^\text{16}\) In addition, the toxicity of some QS-suppressing compounds, such as nanoparticles, limits their biomedical usage.\(^\text{17}\)

**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.\(^\text{18,19}\) 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. acidophillus, 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.\(^\text{20}\) 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 model\(^\text{22}\) and rabbit model of ischaemic and infected wounds\(^\text{23}\) were developed. Moreover, a removable in vivo abutments was developed that mimicked dental implants.\(^\text{24}\) 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.\(^\text{25}\)

### Table 1 (Continued).

| Biofilm Former | Study | Probiotics | Probiotic’s Mechanism of Action | Ref. |
|----------------|-------|------------|-------------------------------|------|
| *Candida* tropicalis, *Candida* krusei and *Candida* parapsilosis | In vitro | L. gasseri and L. rhamnosus supernatant | Disrupts mature biofilm formation, inhibits the mixed biofilms and damages the cells on silicone surface. | [81] |
| *C. albicans*, *C. tropicalis*, and *C. krusei.* | In vitro | L. pentosus strain LAP1 | Probiotic had anti-Candida activity and antibiofilm property. | [43] |
| *S. aureus* strains 9P and 29P | In vitro | L. casei LBL | Biosurfactants could disperse the preformed biofilms. | [27] |

**Notes:** *Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Campylobacter rectus, Porvimonas micra, Fusobacterium nucleatum ssp. Nucleatum, Treponema denticola, Prevotella intermedia, Parphyromonas 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; gfs, Glucosyltransferases; QS, quorum sensing; EPS, exopolysaccharides.
| Biofilm Former                     | Probiotics                          | Probiotic's Mechanism of Action                                                                 | Ref.       |
|-----------------------------------|-------------------------------------|------------------------------------------------------------------------------------------------|------------|
| C. albicans, ^t^                 | L. rhamnosus 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]       |
| V. cholera and V. parahaemolyticus | L. spp. L13 (KY780504), ^mm^      | Inhibited the adherence of V. cholera spp. to the epithelial cells and dispersed the preformed V. cholerae biofilms | [54]       |
| P. aeruginosa                     | Pediococcus acidilactici M7 strain isolated from newborn faeces | Lactic acid produced by the strain: - Inhibited the Rh1 system signaling molecule (C4-HSL). | [44]       |
|                                  |                                     | | Virulence factors regulated by the Rhl including protease, pyocyanin, elastase, and biofilm production | | - Did not reduce/inhibit the Las system signaling molecule (3-oxo-C12-HSL) | | | |
| B. subtilis BM19                  | L. acidophilus ATCC 4356            | Bacteriocin from this probiotic inhibits the growth of B. subtilis BM19 planktonic cells and biofilm formation | [82]       |
| Propionibacterium acnes, P. aeruginosa, S. aureus, E. coli | L. delbrueckii subsp. Bulgaricus, ^mm^ | Due to organic acid production, all probiotics except L. delbrueckii, had antimicrobial activity. Probiotics inhibit the AHL production and prevent biofilm formation, P. innocua was able to destroy pre-formed biofilms of E. coli. P. aeruginosa and S. aureus | [45]       |
| P. aeruginosa PA01, MRSA and their hospital-derived strains | L. plantarum 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]       |
| E. coli ATCC35218                 | EPS-Lp from L. plantarum and EPS-B from Bacillus spp., | EPSs; Indole production, prevent biofilm formation, Efflux pumps involved in bacterial adhesion and antimicrobial resistance. | [84]       |
| Staphylococcus aureus, *^a^      | Streptococcus salivarius 245MB and orals 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. P. aeruginosa, Staphylococcus aureus, S. epidermidis | E. coli Nissle 1917 | Secretes DegP, a bifunctional protein with protease and chaperone activity outside the cells and controls other biofilms. | [86]       |
| S. aureus                        | L. fermentum TCUESC01 and L. plantarum TCUESC02 | Inhibition of biofilm by alteration of the ica operon (icaA and icaR) involved in the biofilm matrix synthesis. | [87]       |
| C. albicans, C. tropicalis, C. krusei | L. pentosus strain LAP1 | Probiotic indicated an anti-Candida activity and antibiofilm property | [88]       |
| C. albicans                      | Pediococcus acidilactici HV01      | It has antifungal agent against C. albicans by reducing the growth and biofilm formation. | [89]       |
| Clinical Salmonella species and uropathogenic E. coli | L. rhamnosus GG | Lectins are involved in the adhesion capacity of L. rhamnosus to vaginal and gastrointestinal epithelial cells. | [90]       |
| Cronobacter sakazakii            | L. casei, L. sporogenes, ^t^       | With antimicrobial activity, production of bioactive molecules to limit the emerging infections. | [91]       |
| P. aeruginosa PA01               | L. fermentum (KT998657) isoleted from neonatal feecal 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 Pseudomonas attachment to form biofilms. | [26]       |
| C. glabrata                      | L. rhamnosus GR-1, L. reuten RC-14 | | EPA6 and YAK1 expression (biofilm-related genes) | [75]       |

Notes: t: Candida tropicalis, Streptococcus salivarius, R. dentocariase, Staphylococcus epidermidis, ^mm^L. plantarum L14(KY582835), L. spp. L18 (KY770976), L. fermentum L32 (KY770983), L. spp. S30 (KY780503), L. pentosus 545 (KY780505), L. spp. 549 (KY770966) isolated from the fecal samples of healthy children, ^mm^Bifidobacterium animals 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, ^t^Streptococcus pyogenes, Propionibacterium acnes, Streptococcus pneumoniae, Maracella catarrhalis, Staphylococcus epidermidis, ^mm^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.
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 typhymurium) bacteria. The results displayed that the biofilm removal ability is related to EPS concentration.31
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. 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.\textsuperscript{33} Subtilosin A, a cyclic bacteriocin (lantibiotic protein) synthesized by \textit{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 subtilosin against \textit{Gardnerella vaginalis} and \textit{L. monocytogenes}, its antibiofilm effect was reported against \textit{G. vaginalis} alone and with natural antimicrobial agents.\textsuperscript{34–36} Given the wide-ranging activities of subtilosin, Chikindas et al observed its anti-QS effect in \textit{E. coli O157:H7}, \textit{L. monocytogenes ScottA} and \textit{G. vaginalis ATCC 14018}. Subtilosin led to the inhibition of 60\% of \textit{E. coli}, 80\% of \textit{L. monocytogenes} and 90\% of \textit{G. vaginalis} biofilms.\textsuperscript{37} Likewise, sonorenisin, a bacteriocin produced by \textit{Bacillus sonorenensis MT93}, was able to decrease \textit{S. aureus} biofilms cell viability, inhibit biofilm attachment and formation, and cause the thinning of mature biofilms.\textsuperscript{38}

Due to exometabolites formation, \textit{Lactobacillus} species also inhibit \textit{Candida albicans} biofilm by inhibiting the initial stage of colonization and hypha formation.\textsuperscript{39} Lactobacilli that produce biosurfactant had antimicrobial, anti-adhesive properties and aggregation ability against pathogenic biofilm formation.\textsuperscript{40} \textit{L. rhamnosus} producing biosurfactants could disrupt the physical membrane structure or protein conformations; resulting in cell lysis.\textsuperscript{41} Furthermore, biosurfactants significantly decrease the adhesion and biofilm generation of bacteria in a dose-dependent manner.\textsuperscript{28} Moreover, EPS produced by \textit{L. acidophilus} A4 considerably could inhibit biofilm formation of \textit{E. coli O157: H7} by reducing the expression of genes related to chemotaxis (cheY) and curli formation (csgA, csgB and crl).\textsuperscript{29} Burton et al clarified a mechanism of biofilm inhibition of \textit{C. albicans} using the combination of \textit{L. plantarum} SD5870, \textit{Streptococcus salivarius DSM 14685} and \textit{L. helveticus} CBS N116411. The expression of some \textit{C. albicans} genes such as \textit{ALS3} (adhesin/invasin), \textit{HWP1} (a critical hyphal wall protein for biofilm formation), \textit{EFG1} (hyphal specific gene activator) and \textit{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 \textit{C. albicans}.\textsuperscript{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.\textsuperscript{44} Lactic acid produced by probiotics had shown an inhibitory effect on QS by suppressing short-chain AHL production and biofilm formation of \textit{P. aeruginosa} that is regulated by QS.\textsuperscript{44} Probiotics also secret organic acid as QS antagonists that interfere with AHLs production at the gene expression level and prevent biofilm formation.\textsuperscript{45} Biosurfactants isolated from \textit{L. plantarum} and \textit{Pediococcus acidilactici} could inhibit the adhesion and biofilm formation of \textit{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 (\textit{cidA}, \textit{sraA}, \textit{icaA}, \textit{dlb}, \textit{sortaseA}, and \textit{agrA}) genes and interfering with the release of signaling molecules (AI-2) in QS systems.\textsuperscript{28} Similarly, \textit{S. mutans} produce extracellular glucans by glucosyltransferases (gtfs) that are vital for the initiation and progression of dental caries. Biosurfactant produced by \textit{L. fermentum} could decrease the \textit{S. mutans} gtfB/C gene expression, the process of attachment and biofilm formation.\textsuperscript{46} The impacts of probiotics on gene expression of pathogens are further summarized in Tables 1 and 2.

### 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 \textit{Lactobacillus} spp. effect on the gene expression of \textit{S. mutans} in a co-cultured condition. They focused on genes involved in EPSs formation (\textit{gtfB}, \textit{ sacB} (flh), \textit{gtfC} and \textit{gtfD}), signal transduction systems (\textit{vicR}, \textit{comC}, \textit{vicK} and \textit{comD}) and stress survival (\textit{atpD} and \textit{agpD}). 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.\textsuperscript{42}

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.\textsuperscript{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. 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-1β, TNFα, IL-6 and IFN-γ that activate the innate immune responses in order to eliminate pathogens. Strain ST1342 could induce high levels of IL-1β secretion that has both anti–viral and anti-bacterial activities. Likewise, it was mentioned that the probiotic *L. paracasei* DG utilized generally in commercial probiotic products, possess immune-stimulatory activities by enhancing of TNFα, IL-6 and CCL20 expression in the human monocyte leukemia cell line. *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. The effect of *Lactobacillus* sp. against the formation of biofilm and gene expression of *S. mutans* was studied. 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. 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. 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.

**Interference of Probiotics in Wound Biofilm**

*P. aeruginosa* is an opportunistic 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. 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. 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.

**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). 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. Moreover, biofilms of probiotic *E. coli* Nissle 1917 on silicone substrates could decrease the colonization of the pathogenic *E. faecalis* 210. Likewise, *L. kunkeei* biofilm reduces the infection of *P. aeruginosa* by affecting biofilm formation and/or their stability. Furthermore, biofilms of probiotic formed by *Bifidobacterium infantis* and *L. reuteri* can be utilized as efficient bacteria to delay the *L. monocytogenes* growth.

*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. 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. 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. *L. rhamnosus* GG microcapsules, as effective inhibitors of transcriptional activators of the *luxS* QS system, could prevent biofilm formation and disturb the mature biofilms.

**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; 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. 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. 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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