Mini Review

Probiotic Lactobacillus Strains and Their Antimicrobial Peptides to Counteract Biofilm-Associated Infections - A Promising Biological Approach

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Abstract
Biofilms keep the intimate relationship between human body and resident microbes. According to National Institutes of Health (NIH), the development of extracellular microbial communities, called biofilms contribute approximately 75% of pathogenic infections to human. The formation of biofilm confers several advantages during pathogen colonization and tolerates extreme conditions like exogenous stress caused by anti-infective agents. The interpretation and exploitation of anti-biofilm properties would help in future challenges, particularly in the control of human infections. The proven scientific evidence with regard to cellular association and exopolysaccharide production by probiotic bacteria could play an important role as anti-biofilm tools. These extracellular components may directly interact with the biofilms as they are actively transported to the bacterial environments via cytoplasmic membrane. The interactive ability of these extracellular metabolites to treat pathogenic biofilms is gaining significant research interest and their possibility to use as anti-biofilm agents. In this review, the extracellular probiotic bacterial markers and molecular approaches to control pathogenic biofilms have been reviewed and future perspectives and research interests are discussed as well.

Introduction
Gastrointestinal tract and biofilms - an overview

The term ‘biofilm’ represents a structured group of bacteria enclosed in a pre-formed polymeric matrix that adhered to living or inert surface [1] that will have the effect of microbiome functionality. Biofilm forming flora of the gastrointestinal tract comprising lactobacilli may have some protective mechanism. In contrast, adherent structured microbial cells in the respiratory tract and oral cavity are well-characterized and are associated with respiratory infections, periodontitis and dental caries [2,3]. Bacteria employ on a particular phenotype during formation of biofilm [4,5]. Biofilm-forming bacterial strains have the ability to populate to enhanced thermo tolerance and resistance to freeze-drying, and to compete resident biofilm-forming pathogens with a non-pathogenic property [6]. Several studies revealed the divergent gene expressions between biofilms and planktonic cells [7]. Moreover, biofilm will attain higher resistance to destruction by the use of bactericidal antibiotics [8]. The significant factors for the optimum functionality and survival are the colonization and an expression of the health-promoting properties of probiotics in digestive tract [9]. Bacteria must be of acid pH tolerant and bile toxicity resistant that is prevalent in the digestive tract to survive in the gut [10]. This extends and stabilizes gastrointestinal tract and helps to control pathogenic bacteria by competitive inhibition or steric barrier, in spite of a variety of defensive host cell immune responses [11]. Bacteria colonizing the digestive tract grow well in the form of biofilms [12] and the majority of the research work on probiotics is conducted on planktonic cells. Several researchers have investigated on the effect of diverse environmental factors on biofilm forming Lactobacillus strains isolated from diverse niches. Slama et al. [13] reported that the LAB strains isolated from Tunisian traditional fermented food showed a significant reduction of the biofilm formation by Listeria species. Similarly, Das et al. [14] Lakhtin et al. [15] Tahmourespou and Kermanshah [16] also reported the efficacy of the different LAB strains isolated from different fermented food products showed in their potential ability to reduce the biofilm formation by human pathogens. In the post-genomic era, rapid screening techniques such as Metagenomics, transcriptomics, proteomics and metabolomics, have very much helped to categorize probiotic strains [17] and to know the mechanisms by which several lactic acid-producing bacteria assist to maintain human health and the many functions associated to these species in the gut [18]. They provide nutrition, aid the host to digest foods, struggle for space and nutrients with potential pathogens and persuade the production of antimicrobial peptides through an interaction with intestinal epithelial cells.

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Progress on anti-biofilm approaches

An intensive study on microbial biofilms began only a couple of decades back with the rediscovery of natural biotic systems, predominant bacteria attached to surfaces [11]. Earlier Henrici [19] who reported “it is demonstrated that for the most bacteria in water are not only planktonic organisms, but also grow upon submerged surfaces”. Biofilms are comprised of either single or several microbial species and express on a variety of biotic and abiotic surfaces. Mixed-species of biofilms exist in most environments, but single-species biofilms last in a variety of pathogenic infections and on the surface of therapeutic embeds [20]. Biofilms of single species are vitally important in the current area of research. Biofilm forming Pseudomonas aeruginosa is the most premeditated single species, Gram-negative bacterium. Escherichia coli, Pseudomonas fluorescents and Vibrio cholera have also been studied for biofilm producing potential. The Gram-positive biofilm forming bacteria include Enterococcus species, Lactobacillus species, and Staphylococcus species have been investigated. Studies report that biofilms are on stable point in a life cycle that consists of instigation, maturation, maintenance, and dissolution [21]. Bacteria commence biofilm formation in response to temperature, growth conditions, nutrient availability, etc. Even though these conditions vary widely, the Gram-negative organisms, with the exception of Myxococcus Xanthus and E. coli OS17:H7, endure a shift-over from free-living planktonic to sessile, surface adhered cells in response to a nutrient-rich medium. These biofilms persist as long as the availability of fresh nutrients, but when they are deprived of nutrients, they detach from the surface and reform to a planktonic mode. Therefore O’Toole et.al. [21] proposed that the starvation response pathway is a part of the total biofilm developmental cycle. It is noteworthy that most microorganisms were able to prepare the transition in life on a biotic or a biotic surface, irrespective of their physiological parameters. Even though these factors are essential in the initial cell to cell interactions and also cell surface, they are not complete by themselves.

Recent investigations on the ability of some of the probiotic strains (Lactobacillus acidophilus DSM 20079, Lactobacillus plantarum 299v, Lactobacillus paracasei DSMZ 16671, Lactobacillus reuteri strains PTA 5289, Lactobacillus rhamnosus GG, and L. reuteri SD2112, etc.) to hinder the growth of S. mutants and the in vitro biofilm formation has been evaluated, and these results support that the antibacterial activity of Lactobacillus seems to be pH-dependent and strain-specific [22]. Lactobacilli have also been shown to reduce Streptococcal adhesion [23] not much on glass surfaces, but in particular on saliva-coated hydroxyapatite [24].

The anti-biofilm potential of some probiotics against biofilm forming enteropathogens has also been reported, despite the fact that the results obtained so far are very few and conflicting. On the other hand, there are studies evaluating that probiotics are able to inhibit biofilms of intestinal pathogens, but further different experimental results seem to support the improvement of biomass of the enteropathogens biofilm in the presence of probiotics [23]. Bacterial surfaces are heterogeneous, and can change greatly in response to their environmental factors. Therefore, a bacterium cannot be biologically modeled as a sphere with a homogeneous surface. A comprehensive understanding of the essential bacterial components, meticulous for biofilm development and the mechanisms that control their production and its activity are necessary.

Probiotic Lactobacillus for the biofilm eradication

The use of probiotics in the treatment of diarrheal diseases has been proposed for many years [24]. The presence of Lactobacillus species in the gastrointestinal tract has gained significance due to health-promoting effects [25]. The probiotic mechanism involves the diversity in function of the intestinal microbiota for nutrients, competitive inhibition of attachment of pathogens to the surface, production of antagonistic substances and modulation of intestinal immunity Preidis et al. [26] proved a transitory increase in phylogenetic diversity and constancy taxa of fecal microbiome 24 h following single probiotic L. reuteri gavages in mice. The diversity in microbial communities is associated with increased ecological stability [27]. One of the methods to screen potential probiotic strains is adhesion to the mucous and epithelial cells, an essential part of the immune modulation, pathogen elimination and enhanced contact with the gut mucosa [28]. To study the quantitative adhesion potential a range of methods such as radioactive labeling, quantitative culturing, fluorescence in situ hybridization-FISH [29], or in vitro model systems viz., immobilized mucus and cell-culture models [30,31] have been developed. However, studies report that probiotic lactobacilli do not colonize GIT permanently but are beneficial to the hosts only for a short period later they stopped being administered [32].

The genus Lactobacillus in rats, mice, chickens and pigs are autochthonous to the proximal gut region [33]. The epithelial adhesion formed by lactobacilli in parts of the stomach, esophagus and crop illustrates distinctive characteristics of the bacterial biofilm formation [34]. The probiotic bacteria get strongly adhered to the intestinal epithelium surface and became well-established in a matrix of extracellular polymeric material [35,36]. Living in a biofilm is a selective advantage for microbes from the ecological point of view that provide a protected niche permitting to interact directly with the host, and to longer survival in the GIT with greater metabolic and beneficial efficiency [37]. There are a number of genetic and environmental factors that affect the formation of these microbial structures within the GIT [38]. The hierarchically ordered genetic factors can control the chronological development of biofilm formation and these genetic switches generally turn on in response to the changes in external stimuli such as microbe-microbe interactions, shear stress, host-microbe interactions and the presence of oxygen [39]. Most of the adherent bacteria form in the natural environment in the form of surface-attached biofilms, where they are bound within a self-produced extracellular matrix that protects them against unfavorable environmental conditions [17].

Genes that are transferred horizontally between bacteria are contributing significantly to bacterial evolution. While gene transfer within a mono-species result in the formation of specific traits, interspecific transfer of a gene may cause entirely new genetic combinations, which rarely impose some serious health issues to human [40]. Biofilm formation is a result of interbacterial interactions. Biofilms can be both single and multispecies, but the development of a stable and mature biofilm is always the product of abundant social interactions that have evolved through adaptation [40]. Diverse probiotic LAB species have been used as therapy for different biofilm-forming pathogens (Table 1).
Inhibit biofilm formation of Lactobacillus jensenii degradation, oxidative stress (glutathione synthesis) and acid tolerance (urea recorded with high-frequency genes encoding pathways, improving reuteri TMW1 induce cell aggregation, in vitro biofilm formation and Antibiofilm cell surface adhesion peptides implicated in probiotic-pathogen interaction.

Table 2:

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Biofilm Forming Pathogen</th>
<th>LAB used to Control</th>
<th>Possible mode of action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Listeria monocytogenes, Salmonella Typhimurium and Escherichia Coli</td>
<td>Lactococcus lactis VB69, Lactobacillus lactis VB94, Lactobacillus sakei MBSa1, and Lactobacillus curvatus MBSa2</td>
<td>Pathogen Exclusion mechanism</td>
<td>[71]</td>
</tr>
<tr>
<td>2.</td>
<td>Candida albicans</td>
<td>Lactobacillus rhamnosus, Lactobacillus casei, and Lactobacillus acidophilus</td>
<td>Production of exometabolites</td>
<td>[72]</td>
</tr>
<tr>
<td>3.</td>
<td>Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus and Candida albicans</td>
<td>Lactobacillus helveticus</td>
<td>Production of Biosurfactants with antiadhesive potential</td>
<td>[73]</td>
</tr>
<tr>
<td>5.</td>
<td>Bacillus cereus and pseudomonas aeruginosa</td>
<td>Lactobacillus plantarum and Lactobacillus pentosus</td>
<td>Production of antibiotic metabolites from the Cell free supernatant</td>
<td>[75]</td>
</tr>
<tr>
<td>6.</td>
<td>Klebsiella pneumonia and Pseudomonas aeruginosa</td>
<td>Enterobacter faecalis</td>
<td>Production of bacteriocin like inhibitory substances</td>
<td>[76]</td>
</tr>
<tr>
<td>7.</td>
<td>Candida albicans</td>
<td>Enterobacter faecalis</td>
<td>Disruption of biofilm by exopolysaccharide production</td>
<td>[77]</td>
</tr>
<tr>
<td>8.</td>
<td>Acinetobacter baumannii, Escherichia coli, andStaphylococcus aureus (MRSA)</td>
<td>Lactobacillus jensenii and Lactobacillus rhamnosus</td>
<td>Production of Biosurfactants</td>
<td>[78]</td>
</tr>
<tr>
<td>9.</td>
<td>Listeria monocytogenes</td>
<td>Lactobacillus plantarum</td>
<td>Production of inhibitory compounds from the extracts</td>
<td>[79]</td>
</tr>
</tbody>
</table>

LAB Markers Responsible for Anti-Biofilm Property

Lactobacillus is a large component of GIT biofilm and has been very commonly used to identify bacterial feature that allow labacilli to survive in the GIT. Several genes encoding large cell surface proteins putatively involved in adhesion to the intestinal epithelium and biofilm formation are harbored in its genome [41]. Cell surface peptides of probiotic LAB were established to be effective as anti-biofilm agents (Table 2). The cell surface proteins MucBP and Lar 0958 are responsible in adhering to carbohydrate moieties [47]. The Exopolysaccharide (EPS) -producing enzymes, GtIA and Inu of L. reuteri TMW1 induce cell aggregation, in vitro biofilm formation and colonization in the mouse gastrointestinal tract [44]. L. reuteri also recorded with high-frequency genes encoding pathways, improving oxidative stress (glutathione synthesis) and acid tolerance (urea degradation, γ-amino butyrate, arginine pathway) [45]. In addition, expression of pathways altering the structure of the bacterial cell wall (Cyclopropane-fatty-acyl-phospholipid synthase, DltA) was related with acid resistance [46], Walter et al. [37] proved the inactivation of dltA gene from L. reuteri and reported a reduction in strain competitiveness in vivo; however the adherence was not altered. The LysM/YG proteins demonstrate the characteristics of proteins that persuade aggregation in lactobacilli, probably by the N-terminal LysM/YG proteins demonstrate the characteristics of proteins that persuade aggregation in lactobacilli, probably by the N-terminal

Table 2: Antibiobiofilm cell surface adhesion peptides implicated in probiotic-pathogen interaction.

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>LAB</th>
<th>Inhibitory peptides</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>L. johnsonii</td>
<td>LTA, elongation factor Tu (EF-Tu), and heat shock protein (GroEL)</td>
<td>Adherence</td>
<td>[80]</td>
</tr>
<tr>
<td>2.</td>
<td>L. brevis</td>
<td>S-layer proteins (SlpA)</td>
<td>Adherence, protection against stressors (low pH, bile, etc.), and enhancement of barrier function</td>
<td>[81]</td>
</tr>
<tr>
<td>3.</td>
<td>L. rhamnosus GG</td>
<td>Peptides NPSRQERR and PDENK</td>
<td>Antimicrobial activity</td>
<td>[82]</td>
</tr>
<tr>
<td>4.</td>
<td>L. rhamnosus</td>
<td>Fimbriae, mucus binding factor (MBF)</td>
<td>Adherence, protection against pathogens, and antiapoptotic effects on intestinal epithelial cells</td>
<td>[83]</td>
</tr>
<tr>
<td>5.</td>
<td>L. casei</td>
<td>EPS, sortase-dependent proteins (SrtA)</td>
<td>Maintenance of barrier function, increased mucus production, and immunomodulation</td>
<td>[84]</td>
</tr>
<tr>
<td>6.</td>
<td>Lysinibacillus kuasformis S9</td>
<td>Glycolipid</td>
<td>Inhibit biofilm formation of E. coli and Streptococcus mutants</td>
<td>[85]</td>
</tr>
<tr>
<td>7.</td>
<td>Lactobacillus rhamnosus</td>
<td>Unspecified protein</td>
<td>Inhibit biofilm formation of A. baumannii, E. coli, and S. aureus</td>
<td>[86]</td>
</tr>
<tr>
<td>8.</td>
<td>Lb. plantarum PA21</td>
<td>pMG36e-GFP</td>
<td>Antibiofilm activity</td>
<td>[87]</td>
</tr>
<tr>
<td>9.</td>
<td>Lactobacillus rhamnosus</td>
<td>surface antigen NLP/P60 (gi:199598074)</td>
<td>Human mucus binding protein</td>
<td>[88]</td>
</tr>
<tr>
<td>10.</td>
<td>L. acidophilus NCFM</td>
<td>acm8 (iba0176 ) N-acetylglycosaminidase, a surface protein</td>
<td>Intestinal adhesion and modulation of the mucosal immune system</td>
<td>[89]</td>
</tr>
</tbody>
</table>

in nature as these aggregates formed on mucosal cells and a biotic surface has comparable molecular properties [59]. However, in general molecular data of the biofilm mode of life obtained from the pre-formed aggregates on biotic surfaces, and biofilm host immune responses remain to be discovered. The potential strategy used to productively control biofilm formation as by the use of essential components of probiotics [60].

Conventional anti-biofilm therapies aim to target bacterial species without taking into consideration that most biofilm-related infections are due to mixed microbial biofilms [56]. As, there is no ideal system to totally eradicate biofilm, the solution would be the concurrent application of agents implementing synergic potential to both control the biofilms and kill bacteria [61]. A multidisciplinary approach is essential to elucidate the genetic networks, exploring complex community interactions and to replace them in their evolutionary and ecological context. Biofilm and mixed biofilm forming species modeling tools are to be made available, including heterotrophy parameters [62]. Three-dimensional system models of biofilm dynamics have been proposed as tools for studying mechanisms of protection against microbial inhibitors in biofilms [63]. They could be useful to investigate the effects of anti-biofilm compounds, in particular to assess their efficacy and to explore their impact on the emergence of new groups of resistant microbes [64].

Future directions and Concluding remarks

Several reports from medical device submissions have been received by the Food and Drug Administration that hold potential anti-biofilm agents [64]. However, existing in vitro and in vivo assays are not effective to predict biofilm effect in humans and it is necessary to introduce reliable and potential alternatives to clinical studies for the evaluation of anti-biofilm agents with standardized anti-biofilm methodologies and evaluation methods that can establish association with clinical outcomes. Potential targets with the elucidation of the mechanisms of action of several anti-biofilm and bactericidal agents have been depicted so far. It is important, considering bacterial anti-biofilm agents that will be an essential tool in the future to establish a frame to help industrial and academic institutions to explore their potential ability in agreement with health and nutrition policy [65]. Because of the administrative complications of these strategies, other potential applications such as vaccine therapy must be considered. FomA an outer membrane protein involved in bacterial cell aggregation is preferentially potential target for developing an oral vaccine against the bacterium Fusobacterium nucleatum, and this can be considered as a potent anti-biofilm vaccine [66]. Not only novel and specific vaccines are required, but it is necessary first to more fully explore the interactions between biofilm and the immune system of the host, a domain as yet unexplored [67]. The mechanism of probiotics comprises the diversity and function of the intestinal micro biota for nutrients, competitive inhibition of pathogen attachment, and production of antagonistic substances and modulation of intestinal immunity. On the other hand, consumption of traditional fermented foods, the rich source of Lactic Acid Bacteria (LAB) has the probiotic effect for healthy gut (Figure 1).

On the whole, the effect of probiotic counterparts in gene expression modification of pathogens within biofilm could represent an essential anti-biofilm target with a dual-purpose- to control bacterial colonization and to inhibit the expression of virulence...
factors. Some Lactobacilli can down-regulate the expression of the virulence genes of gut pathogens \[68,69,70\]. The literature suggests, that the virulence capability of bacteria is generally opposed to the biofilm formation potential \[71\], suggesting that biofilm forming bacteria stay within a specific ecological niche and induces adverse effects. Further experiments are needed to assess the in vivo potential of anti-biofilm agents that can assure to possess therapeutic benefits that are target specific, highly effective and safe alternatives \[64\]. However, in particular the potential side effects on beneficial bacteria of the host gut and the development of antimicrobial resistant substances should also be given consideration along with the risks and benefits for a healthy gut.

References


