Review

Probiotics *Lactobacillus* strains: A promising alternative therapy against to biofilm-forming enteropathogenic bacteria?

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Received 12 June, 2019; Accepted 24 September, 2019.

Biofilms formation stands out in context of persistent intestinal infections caused by Enterobacteriaceae, which are associated with a high resistance to antimicrobial agents’ and phagocytosis by host defense cells. Hence, understanding the mechanisms involved in this process becomes major for the development of new preventive and therapeutic strategies. Lactic acid bacteria, including species of the genus *Lactobacillus*, have been associated with the prevention or dispersion of biofilms formed by pathogenic microorganisms. This effect is often associated with the antimicrobial substances production, among them organic acids, bacteriocins, hydrogen peroxide and biosurfactants. However, the antibiofilm action of *Lactobacillus* seems to be strain-specific and may not be demonstrated by strains of the same genus. Thus, diet supplementation with beneficial microorganisms represents a possible strategy for prevention and treatment of intestinal infectious diseases, such as persistent or acute diarrhea caused by enteropathogenic bacteria. However, *in vitro* and *in vivo* further studies are needed to clarify the efficacy of different probiotic candidates, including commercially available products.

**Keyword:** Enterobacteria, biofilm, *lactobacillus*, antimicrobials.

INTRODUCTION

The term biofilm describes a lifestyle characterized by microbial adhesion with production of extracellular polymer substances, constituting a gelatinous network that protects the cells and its associated with numerous cases of infections in human beings (Schiebel et al., 2017). In a liquid environment the primary event of biofilm formation, mainly in Gram-negative bacteria such as *Escherichia coli* and *Salmonella*, is related to the flagellar apparatus, which provide an initial approach between the bacterial cell and the surface (Misselwitz et al., 2012; Guttenplan and Kearns, 2013). Afterwards, three sequential steps, initial microbial adhesion, attachment either by exopolysaccharide production and cell surface structures, and colonization by growth of attached organisms, guarantee their survival in complex environments (Tolker-Nielsen, 2015).

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The biofilm formation is considered as an essential factor in the pathogenesis of various enteropathogenic bacteria such as enteroaggregative E. coli (EAEC), which has been frequently implicated with several kinds of diarrhea (Meng et al., 2011; Dallman et al., 2012; Lääveri et al., 2014; Bamidele et al., 2019). Thus, diseases related with the ability of bacteria to form a structured microbial community are generally difficult to treat due to intrinsic biofilm resistance against antimicrobial agents (Haney et al., 2018; Bjarnsholt et al., 2013). In the attempt to explore new forms that may contribute to the prevention and treatment of infectious intestinal diseases, probiotics are standing out in merit of their protective effect against bacterial pathogens (Ramos et al., 2012; Sikorska and Smoragiewicz, 2013; Osama et al., 2017). Lactic acid bacteria (LAB), including Lactobacillus species, have been associated with the prevention or dispersion of biofilms formed by enteropathogenic microorganisms (Kaur et al., 2018; Miquel et al., 2016). Therefore, this review aims to describe the biotechnological application of Lactobacillus strains as probiotic agents and their role to counteract with enteropathogenic microbial biofilms, which may represent a promising alternative therapy.

**Infectious intestinal diseases and enteropathogens**

There is a complex beneficial relationship in intestine environment between host and gut microbiota that seeks a homeostatic equilibrium in which both are favored (Paixão and Castro, 2016). Thus, the interruption of this synchrony may result in the prevalent growth of pathogenic microorganisms, causing tissue inflammation and immunological regulation failures (Iacob et al., 2019). The ingestion of contaminated food or water are among the various causes of intestinal dysbiosis (Gabliardi et al., 2018). Food-borne outbreaks caused by several Enterobacteriaceae have been reported in Brazil, with emphasis on classical microorganisms such as *E. coli* and *Salmonella* spp. (Feltes et al., 2017).

Although many microorganisms cause foodborne infections, the severity of the disease, clinical manifestations, and duration of symptoms differ significantly (AL-Mamun et al., 2018). For instance, *Vibrio cholerae* and some strains of *E. coli* are capable to induce secretory diarrhea by exotoxins production (Choi et al., 2016; Chau et al., 2016; Chen et al., 2019). It triggers the intracellular cAMP and cGMP levels and result in the activation of the cyclic transmembrane conductance regulator nucleotide (CFTR) of Cl⁻ channels, increasing fluid secretion into intestinal lumen (Thiagarajah et al., 2015). Furthermore, the high production of cAMP, cGMP and Ca²⁺ by host cells induced through enterotoxins inhibits the sodium-hydrogen antiporter (NHE3), which is the main exchanger channel responsible for maintaining of cellular sodium balance (Beltrán et al., 2015; Hodges and Gill, 2010). On the other hand, bacteria such as *Campylobacter jejuni*, *Shigella* spp. and enteric *Salmonella* invade epithelial cells and trigger a massive neutrophilic infiltrate into the mucosa, followed by cellular transmigration into intestinal lumen (Navaneethan and Giannella, 2008). Thus, a response is established with recruitment of immune cells and release of cytokines, which characterizes inflammatory diarrhea (Hodges and Gill, 2010). It should be noted that enteropathogenic bacteria also alter the expression of transport proteins, as well, influencing the absorption of Na⁺ and Cl⁻ (Marchelletta et al., 2013).

Intestinal microbiota may act as a very competitive environment, since it can host over 2000 bacterial species (Novik and Savich, 2019). However, high nutrients availability combined with a constant microorganisms’ influx makes the gastrointestinal environment ideal for development of sessile communities by enteropathogens, called biofilms, fact closely related to chronic intestinal infections (Rosenvinge et al., 2016). Biofilm formation represents an ability developed by the most microorganisms, which facilitates colonization on new surfaces with increased tolerance to environmental stresses (Miquel et al., 2016). Thus, the morphology, cellular density, as well as their physiological state are associated to wide range of antibiotic resistance (Singh et al., 2017).

The development of the biofilm starts from a response of the planktonic cells to environmental signals in order to raise survival chances, altering the expression of hundreds of genes (Oliveira et al., 2015). In short, this process begins by the cell attachment to (a)biotic surface, reversibly adhered. Then, irreversibly attachment happens after cell proliferation with microcolonies formation, and polymer matrix production. Finally, maturation is achieved, followed by detachment or dispersion of mature biofilm parts, which may determine the appearance of cellular clusters on new colonization sites (Tolker-Nielsen, 2015).

External bacterial structures play an important role in biofilms formation by enteropathogens, which may include fimbriae, flagella and capsules (Rabin et al., 2015). The first one stands out for facilitating bacterial aggregation and adhesion on several substrates, being very common in Gram-negative organisms. These cellular interactions are also triggered by the presence of specific components encoded by plasmids, such as adhesins and curli, potent inducers of the inflammatory response in the host (Wolska et al., 2016).

Genetic mechanism directly influences the ability to form cell agglomerates and, consequently, the pathogenesis of each bacterial species (Wolska et al., 2016). Schiebel et al. (2017) related the expression of *timH* and *agn43* genes in pathogenic *E. coli* strains with the ability to form biofilms by encoding type 1 fimbriae and Ag43 antigen. It is known that adhesins are essential for initial cell adhesion on the surface and Ag43 is
capable of promoting auto cell-to-cell aggregation (Schiebel et al., 2017). Nascimento et al. (2014) also associate the participation of type 1 fimbriae with strong biofilm production by enteropathogenic E. coli strains (EPEC). Also in EPEC isolates, bundle-forming pilus (BFP) and the EspA filament are involved in the formation of microcolonies on epithelial cells and abiotic surfaces (Saldaña et al., 2009 and Moreira et al., 2006).

Probiotics microorganisms

Probiotics are living microorganisms that, when administrated in adequate amounts, confer a health benefit on the host, and can be found in foods or as dietary supplements and medications (WHO, 2002; Hill et al., 2014). The most commonly used are those belonging to Lactobacillus and Bifidobacterium genus, which are commensals bacteria that live in or on human bodies (Chew et al., 2015; Novik and Savich, 2019). Currently, the main claim for use of probiotic bacteria are: helping healthy microbiota maintenance, reducing the numbers or colonization of pathogenic bacteria, promoting the digestion of lactose by intolerant individuals, relieving constipation and increasing the absorption of vitamins and minerals (Novik and Savich, 2019). In addition, due to evidence of antimicrobial effect against several pathogens, the interest on the metabolic performance of these organisms has increased in recent years (Abdelhamid et al., 2018; Do Carmo et al., 2018; Osama et al., 2017).

Probiotics strains can affect pathogenic microorganisms through different mechanisms, such as enhancing the intestinal barrier function, increasing mucin production and modulating the immune system activity (Miquel et al., 2016; Hu et al., 2017; Vieco-Saiz et al., 2019). Other factors such as metabolites production, nutrients competition and suppression of toxin production are also involved in probiotic action (Markowiak and Slizewska, 2017). All of those effects can be triggered by metabolites, cell wall components, DNA fragments, as well as the adhesion of probiotic cells to epithelium (Oelschlaeger, 2010).

Recent in vitro studies concluded that the use of LABs, specially Lactobacillus species, are related to positive results against enteropathogens (Turková et al., 2013; Ruiz et al., 2017; Prabhurajeshwar and Chandrakanth, 2017; Kaur et al., 2018). However, when it comes to clinical trials, the effects against bacterial infections are related to regular consumption of food sources, such as yogurts and curds, as well as supplementation (Varavallo et al., 2008; Halder and Mandal, 2016; Prabhurajeshwar and Chandrakanth, 2017).

Other effect associated to probiotics strains is the inhibition of virulence-related gene expression, such as toxins production (Rätsep et al., 2017). Thus, the efficacy of certain strains for the treatment of diarrheal diseases is probably associated with their ability to protect the host against toxins action, including those produced by cyanobacteria and fungi (Oelschlaeger, 2010). Carey et al. (2008) reported that 15 different Lactobacillus strains were able to inhibit the expression of shiga-toxin production by EHEC O157:H7, from the production of organic acids in sub-bacterial concentrations with consequent pH reduction. Rätsep et al. (2017), testing the combination of xylitol with Lactobacillus plantarum detected the suppression of spores' germination and outgrowth into vegetative toxin producing cells of C. difficile, which reduces the colonization of gut with the pathogen.

Enterobacteria and Lactobacillus Interactions

Lactobacillus genus corresponds to an important group of microorganisms related to ferment dairy products, as starters or as secondary microbiota, as well as food preservation (Ruiz et al., 2017). Several species of this group have been accepted with GRAS (Generally Recognized as Safe) status, which identifies a microorganism or microbial derivatives as safe for use in food industry (Cui et al., 2017; Gabliardi et al., 2018). Lactobacillus is frequently found in environments with low molecular oxygen tension, such as intestinal and urinary tract of humans, sharing their habitat with several types of potentially pathogenic microorganisms, among them pathogenic enterobacteria (WGO, 2017; Ruiz et al., 2017). Thus, these microorganisms have antagonistic properties against to pathogenic bacteria through metabolites production that render a hostile environment, such as organic acids, hydrogen peroxide, biosurfactants and bacteriocins (Fijan, 2014; Davoodabadi et al., 2015; Yeganeh et al., 2017; Abdelhamid et al., 2018; Fernandes, 2019; Vieco-Saiz et al., 2019).

Although lactic and/or acetic acids are considered to have low acidity, it is noteworthy the bactericidal effect against numerous pathogens, especially under conditions with nutrient limitation (Fijan, 2014). In these conditions, acids in the non-dissociated form penetrate the cytoplasm, where they dissociate and decrease the intracellular pH, interfering on cellular metabolic processes (Hughes and Webber, 2017). In addition, these acids increase the permeability of the outer membrane of Gram-negative organisms, compromising their integrity, what may potentiate the action of other antimicrobial substances such as bacteriocins (Gálvez et al., 2010). Hydrogen peroxide produced by many strains of Lactobacillus is also capable of inducing stresses in the outer membrane of some bacteria, such as uropathogenic E. coli (UPEC) which affects the structure of fimbriae and prevent their cell adhesion ability (Costa et al., 2012).

In addition, Halder and Mandal (2016) have shown that Lactobacillus from different species, individually, have
demonstrated excellent in vitro inhibition of enterobacteria growth, such as E. coli and K. pneumoniae. Also, when tested different strains combination from the same genus, they showed a synergistic effect against E. coli (Halder and Mandal, 2016). The use of isolated species, as well as blends containing different strains combined have been shown to be useful in the treatment of gastrointestinal diseases in vivo (Vuotto et al., 2014). Nevertheless, bactericidal capacity does not necessarily predict an antibiofilm action (Kaur et al., 2018). Considering the increasing ability of pathogens to generate persistent infections related to biofilms formation, probiotics administration may be able to modulate and prevent the proliferation of invasive microorganisms in vivo (Vuotto et al., 2014).

**Antibiofilm strategies**

The trend in health promotion through natural means leads to interest in non-chemical antibiotic agents, including microbial products, capable of reducing bacterial biomass (Challinor and Bode, 2015; Miquel et al., 2016). Two mechanisms are verified to be able of modulating the formation of these communities: destabilization of mature biofilms irreversibly attached or the inhibition of bacterial surface attachment (Miquel et al., 2016). In this perspective, in vitro and in vivo studies shown that probiotics are useful to modify the composition of the exopolymeric matrix, affecting the primary cell adhesion and/or colonization by exclusion/competition, or even trigger a cellular dispersion from biofilm (Gutiérrez et al., 2016).

Recent researches highlight the antibiofilm feature of Lactobacillus genus. Osama et al. (2017) demonstrated antimicrobial and antibiofilm action of Lactobacillus rhamnosus and Lactobacillus gasseri strains against Pseudomonas aeruginosa, E. coli and Staphylococcus aureus, three pathogens commonly involved in persistent infections associated with biofilm formation. As also, Abdelhamid et al. (2018) identified effective results in the biofilm eradication from multidrug resistant (MDR) E. coli isolates through bioactive compounds acting as antimicrobials. Fernandes (2019) demonstrated that cell free supernatant produced by standard and commercial probiotic strains (L. acidophilus LA14, L. acidophilus ATCC 4356 and L. rhamnosus ATCC 9595) exerted strong bactericidal and antibiofilm action against MDR E. coli isolated from fish fillet samples.

However, the results obtained are attributed to several mechanisms of action which requires further investigations. For instance, an effective strategy to avoid the first step of biofilm formation it’s through to biosurfactants use, that impairs microbial adhesion modifying the physicochemical cell surface properties (Gómez et al., 2016; Sharman and Saharan, 2016; Kaur et al., 2018). The antibiofilm activity is also related to the production of bacteriocins, which are antimicrobial peptides produced by certain bacteria, that act suppressing biofilm formation and have a high applicability as food bioconservatives, since they have a broad spectrum against many food spoilage microorganisms, among them E. coli (Mathur et al., 2017; Novik and Savich, 2019).

The most of bacteriocins secreted by Lactobacillus belong to class II, heat stable, whose effect is related to the membrane destabilization with pores formation, plasma content extravasation and consequent cell death (Paixão and Castro, 2016). It is already recognized that bacteriocins action is potentiated under acidic conditions, which highlights the importance of organic acids secreted by various probiotic strains (Gálvez et al., 2010). The assembly of these antimicrobial peptides is controlled according to population density and communication between the cells through Quorum Sensing (QS), a process that bacteria use to coordinate gene expression and allow the production of virulence factors (Lixa et al., 2015).

QS control has become one of the purposes in the development of new strategies for the treatment of bacterial biofilm infections (Wu et al., 2015). Through QS, bacteria tend to produce low molecular weight chemical signals called autoinducers (AIs) that, when diffusing into the medium might be internalized to induce the differential genes expression that alter cellular metabolism (Lixa et al., 2015). Several enterobacteria are recognized for producing and responding to these AIs, so they can express virulence factors, succeed in colonization, and consequently to establish intestinal infections. Therefore, the use of probiotics emerges also due to produce small biologically active molecules capable of interfering on bacterial pathogens QS (Li et al., 2011; Liu et al., 2016). Some elucidations about the main mechanisms of action by Lactobacillus strains are able to exert an antibiofilm action are summarized in Table 1.

Although numerous in vitro evidences about antimicrobial activity of Lactobacillus strains, relevant actions of these probiotic preparation have been also observed by classical in vivo infectious models, as bacterium- and/or rotavirus-infected animals (Nakazato et al., 2011; Quigley et al., 2019; Jiang et al., 2017; Vlasova et al., 2013; Zhang et al., 2013). Normally, the probiotics effects have been related to normalization of intestinal microbial communities, competitive exclusion of pathogens associated with gut epithelia, bacteriocin production, production of short-chain fatty acids and modulation the activity of the immune system (Plaza-Díaz et al., 2019). Therefore, investigations performed by in vivo models are strongly recommended to help in the clarification of the mechanisms of action by each probiotic candidate.

In human beings, probiotic Lactobacillus strains can survive after oral administration and efficiently colonize
Table 1. *In vitro* evaluation of the mechanisms of action involved with antibiofilm feature of *Lactobacillus* genus against enterobacteria.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Source</th>
<th>Mechanisms of action</th>
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<tbody>
<tr>
<td><em>L. rhamnosus</em> EMC 1105</td>
<td>Standard</td>
<td>Production of organic acids and inhibitory effect on proteolytic activities of <em>P. aeruginosa</em>, <em>E. coli</em> and <em>S. aureus</em> (Osama et al., 2017).</td>
</tr>
<tr>
<td><em>L. gasseri</em> EMC 1930</td>
<td>Standard</td>
<td>Lactobacilli supernatant had antimicrobial activity against the biofilm produced by ciprofloxacin-resistant uropathogenic <em>E. coli</em> strains in pasteurized milk, referred to lactic acid production. It was reported an important anti-adhesive effect, as well (Yeganeh et al., 2017).</td>
</tr>
<tr>
<td><em>L. plantarum</em> ATCC 1363 <em>L. acidophilus</em> ATCC 314 <em>L. casei</em> ATCC 25598</td>
<td>Homemade fermented milk</td>
<td><em>Lactobacillus</em> inhibited the growth, invasion and the biofilm formation of <em>Salmonella enteritidis</em> due to the production of organic acids and down regulation of virulence related genes (Das et al., 2013).</td>
</tr>
<tr>
<td><em>L. sakei</em> and <em>L. curvatus</em></td>
<td>Salami Goat cheese Ripened cheese</td>
<td>Reduce <em>Listeria monocytogenes</em>, <em>Salmonella</em> and <em>E. coli O157:H7</em> biofilm formation. This effect was attributed to biosurfactant and bacteriocin production, as well as mechanisms of pathogens exclusion through their trapping (killing of cells embedded in biofilms) (Gómez et al., 2016).</td>
</tr>
<tr>
<td><em>L. helveticus</em></td>
<td>Yak milk cheese</td>
<td>Anti-adhesive and antibiofilm abilities mediated by biosurfactant production against multidrug resistant <em>Acinetobacter baumannii</em>, <em>E. coli</em> and <em>S. Aureus</em> (Sambanthamoorthy et al., 2014).</td>
</tr>
<tr>
<td><em>L. casei</em></td>
<td>Yeast samples foods</td>
<td>Antimicrobial and antiadhesive properties by the biosurfactant against various pathogenic and nonpathogenic microorganisms (Sharman and Saharan, 2016).</td>
</tr>
<tr>
<td><em>L. jensenii</em> ATCC 25258</td>
<td>Standard</td>
<td>Reduction of surface hydrophobicity and suppression of motility affected <em>E. coli</em> phenotypic characteristics important in the contacts with the substratum during the early stages of biofilm settlement. The results also suggested the peptides or protein factors also contributed to antibiofilm effect (Vacheva et al., 2012).</td>
</tr>
<tr>
<td><em>L. heveiticus</em></td>
<td>Vaginal samples and dairy products</td>
<td>Secretion of low molecular weight molecules that binds the autoinducers (AI-2 or AI-3) that altered the QS system in <em>E. coli O157:H7</em>, decreasing attachment to tissue culture cells (Mendellin-Peña and Griffiths, 2009).</td>
</tr>
<tr>
<td>Lactobacillus strains</td>
<td>Dairy products</td>
<td>Production of strain-specific derived bioactive molecules cause destabilization of <em>Klebsiella pneumoniae</em> (multi-resistant) of pre-formed biofilm architecture, induced by transcriptional modifications of biofilm-related genes (Lagrangeille et al., 2018).</td>
</tr>
</tbody>
</table>

Different parts of gastrointestinal tract, but the maintenance of the strain into gut environment seems to depends on intake frequency of probiotic preparation (Saxelin et al., 2010; Balgir et al., 2013; Arioli et al., 2018; Taverniti et al., 2019). That may explain when some of these probiotic strains come to randomized clinical trials, the results are occasionally inconclusive, and these intriguing outcomes put on doubt the clinical value of this treatment method (Chau et al., 2018; Ten Bruggencate et al., 2015; Hegar et al., 2015; Piescik-Lech et al., 2013). However, it must be considered that the anti-infectious action of *Lactobacillus* is not equally effective for all disease prevention or treatment indication. Several factors are involved on efficacy of probiotic strains, among them adequate doses for appropriate periods, besides diseases type and mechanisms of action of strains (Liu et al., 2019; Islam, 2016; Floch et al., 2015). In other words, the correct choose of probiotic must be strain-specific, and should not be generalized even among strains into the same species (Sniffen et al., 2018;
Conclusion

In conclusion, the biotechnological application of Lactobacillus strains as probiotics presents effective results to control microbial biofilms formed by enteropathogenic bacteria, representing a promising alternative for medicine use. The inhibitory effect seems to be strain specific and is referred to metabolic compounds, such as organic acids, hydrogen peroxide, bacteriocins, biosurfactants and QS inhibitors. Although, in vitro activity does not always correspond to in vivo results, which shows that further clinical trials are needed to predict select real beneficial strains.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENT

The authors show gratitude to the institutions University Center INTA and Federal University of Ceara for support during the course of this research.

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The Mechanism of Bacterial Biofilm Resistance to Antimicrobial Agents


difficile
gasseri

Biofilm formation mechanisms and targets for developing antipathogenic properties of 

Antipathogenic properties of 


