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Review

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

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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 biorsurfactants. 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* sp. (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 Ca^{2+} 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 *fimH* 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 host 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-bactericidal 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. acidpohillus* LA14, *L. acidophillus* 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-Diaz 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.

Strains	Source	Mechanisms of action
L. rhamnosus EMC 1105 *L. gasseri* EMC 1930	Standard	Production of organic acids and inhibitory effect on proteolytic activities of *P. aeruginosa*, *E. coli* and *S. aureus* (Osama et al., 2017).
L. plantarum ATCC 1363 *L. acidophilus* ATCC 314 *L. casei* ATCC 25598	Homemade fermented milk	Lactobacilli supernatant had antimicrobial activity against the biofilm produced by ciprofloxacin-resistant uropathogenic *E. coli* strains in pasteurized milk, referred to lactic acid production. It was reported an important anti-adhesive effect, as well (Yeganeh et al., 2017).
L plantarum KSBT 56	Fermented milk product	*Lactobacillus* inhibited the growth, invasion and the biofilm formation of *Salmonella enteritidis* due to the production of organic acids and down regulation of virulence related genes (Das et al., 2013).
L. sakei and *L. curvatus* *L. helveticus* *L. casei*	Salami Goatcheese Ripened cheese	Reduce *Listeria monocytogenes*, *Salmonella* and *E. coli* O157:H7 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).
L. jensenii ATCC 25258 *L. rhamnosus* ATCC 7469	Standard	Anti-adhesive and antibiofilm abilities mediated by biosurfactant production against multidrug resistant *Acinetobacter baumannii, E. coli and S. Aureus* (Sambanthamoorthy et al., 2014).
L. hevelticus	Yak milk cheese	Antimicrobial and antiadhesive properties by the biosurfactant against various pathogenic and nonpathogenic microorganisms (Sharman and Saharan, 2016).
Lactobacillus strains	Vaginal samples and dairy products	Reduction of surface hydrophobicity and suppression of motility affected *E. coli* 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).
L. acidophilus La-5	Dairy products	Secretion of low molecular weight molecules that binds the autoinducers (AI-2 or AI-3) that altered the QS system in *E. coli* O157:H7, decreasing attachment to tissue culture cells (Mendellin-Peña and Griffiths, 2009).
L. plantarum CIRM653	Food	Production of strain-specific derived bioactive molecules cause destabilization of *Klebsiella pneumoniae* (multi-resistant) of pre-formed biofilm architecture, induced by transcriptional modifications of biofilm-related genes (Lagrafeuille et al., 2018).

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;

McFarland et al., 2018; Liu et al., 2018).

Meanwhile, it is worthy to highlight that those desirable effects trough probiotic administration encourage and indicate *Lactobacillus* species as promising therapy strategies against enteropathogens (Szajewska et al., 2016; Bustos and Chamorro, 2018). In the end, the real impact of probiotic administration on the microbial ecology of the gastrointestinal tract and on animal health is far from being understood (Ten Bruggencate et al., 2014; Suez 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.

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REFERENCES

Abdelhamid AG, Esaam A, Hazaa MM (2018). Cell free preparations of probiotics exerted antibacterial and antibiofilm activities against multidrug resistant *E. Coli.* Saudi Pharmaceutical Journal 26(5):603-607.

AL-Mamun M, Chowdhury T, Biswas B, Absar N (2018). Food poisoning and intoxication: A global leading concern for human health. Food Safety and Preservation pp. 307-352.

Arioli S, Koirala R, Taverniti V, Fiore W, Guglielmetti S (2018). Quantitative recovery of viable *Lactobacillus paracasei* CNCM I-1572 (L. casei DG®) after gastrointestinal passage in healthy adults. Frontiers in Microbiology 9:1720.

Balgir PP, Kaur B, Kaur T, Daroch N, Kaur G (2013). *In vitro* and *in vivo* survival and colonic adhesion of *Pediococcus acidilactici* MTCC5101 in human gut. BioMed research international 2013:1-9.

Bamidele O, Jiang ZD, Dupont H (2019). Occurrence of putative virulence-related genes, aatA, aggR and aaiC, of Enteroaggregative *Escherichia coli* (EAEC) among adults with travelers' diarrhea acquired in Guatemala and Mexico. Microbial pathogenesis 128:97-99.

Beltrán AR, Carraro-Lacroix LR, Bezerra CN, Cornejo M, Norambuena K,Toledo F, Araos J, Pardo F, Leiva A, Sanhueza C, Malnic G, Sobrevia L, Ramírez MA (2015). *Escherichia coli* heat-stable enterotoxin mediates Na^+/H^+ exchanger 4 inhibition involving cAMP in T_{84} human intestinal epithelial cells. PLoS One. 10(12):e0146042.

Bjarnsholt T, Ciofu O, Molin S, Givskov M, Hoiby N (2013). Applying insights from biofilm biology to drug development—can a new approach be developed?. Nature Reviews Drug Discovery 12(10):791-808.

Bustos SP, Chamorro JFV (2018). Probiotics in Acute, Antibiotic-associated and Nosocomial Diarrhea: Evidence in Pediatrics. Revista Colombiana de Gastroenterologia 33(1):41-48.

Carey CM, Kostrzynska M, Ojha S, Thompson S (2008). The effect of probiotics and organic acids on Shiga-toxin2 gene expression in enterohemorrhagic *Escherichia coli* O157:H7. Journal of Microbiological Methods 73(2):125-132.

Challinor VL, Bode HB (2015). Bioactive natural products from novel microbial sources. Annals of the New York Academy of Sciences 1354(1):82-97.

Chau ML, Hartantyo SH, Yap M, Kang JS, Aung KT, Gutiérrez RA, Ng LC, Tam CC, Barkham T (2016). Diarrheagenic pathogens in adults attending a hospital in Singapore. BMC Infectious Diseases 16: 32. https://doi.org/10.1186/s12879-016-1354-0

Chau TTH, Chau NNM, Le NTH, The HC, Vinh PV, To NTN, Ngoc NM, Tuan HM, Ngoc TLC, Kolader ME, Farrar JJ, Wolbers M, Thwaites GE, Baker S (2018). A double blind, randomized, placebo-controlled trial of *Lactobacillus acidophilus* for the treatment of acute watery diarrhea in vietnamese children. The Pediatric Infectious Disease Journal 37(1):35-42.

Chen C, Wang LP, Yu JX, Chen X, Wang RN, Yang XZ, Zheng SF, Yu F, Zhang ZK, Liu SJ, Li ZJ, Chen Y (2019). Prevalence of enteropathogens in outpatients with acute diarrhea from urban and rural areas, southeast China, 2010-2014. American Journal of Tropical Medicine and Hygiene tpmd19-0171. https://doi.org/10.4269/ajtmh.19-0171

Chew SY, Cheah YK, Seow HF, Sandai D, Than LTL (2015). Probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 exhibit strong antifungal effects against vulvovaginal candidiasis-causing *Candida glabrata* isolates. Journal of Applied Microbiology 118(5):1180-1190.

Choi SY, Rashed SM, Hasan NA, Alam M, Islam T, Sadique A, Johura F-T, Eppinger M, Ravel J, Huq A, Cravioto A, Colwell RR (2016). Phylogenetic diversity of *Vibrio cholerae* associated with endemic cholera in Mexico from 1991 to 2008. mBio 7(2):e02160-15.

Costa GN, Suguimoto HH, Miglioranzaand LHS, Gómez RJHC (2012). Antimicrobial activity of *Lactobacillus* and *Bifidobacterium* strains against pathogenic microorganisms "*in vitro*". Semina: Ciencias Agrárias 33(5):1839-1846.

Cui X, Shi Y, Gu S, Yan X, Chen H, Ge J (2017). Antibacterial and Antibiofilm Activity of Lactic Acid Bacteria Isolated from Traditional Artisanal Milk Cheese from Northeast China Against Enteropathogenic Bacteria. Probiotics and Antimicrobial Proteins 1-10.

Dallman T, Smith GP, O'Brien B, Chattaway MA, Finlay D, Gran, KA, Jenkins C (2012). Characterization of a verocytotoxin-producing enteroaggregative *Escherichia coli* serogroup O111:H21 strain associated with a household outbreak in Northern Ireland. Journal of Clinical Microbiology 50(12):4116-4119.

Das JK, Mishra D, Ray P, Tripathy P, Beuria TK, Singh N, Suar M (2013) *In vitro* evaluation of anti-infective activity of a *Lactobacillus plantarum* strain against *Salmonella enterica* serovar Enteritidis. Gut Pathogens 5(1):11.

Davoodabadi A, Dallal MMS, Lashani E, Ebrahimi MT (2015). Antimicrobial activity of *Lactobacillus spp*. isolated from fecal flora of healthy breast-fed infants against diarrheagenic *Escherichia coli.* Jundishapur Journal of Microbiology 8(12).

Do Carmo MS, Santos CI, Araújo MC, Girón JA, Fernandes ES, Monteiro-Neto V (2018). Probiotics, mechanisms of action, and clinical perspectives for diarrhea management in children. Food & Function 9(10):5074-5095.

Feltes MMC, Arisseto-Bragotto AP, Block JM (2017). Food quality, food-borne diseases, and food safety in the Brazilian food industry. Food Quality and Safety 1(1):13-27.

Fernandes MSM (2019). Atividade antimicrobiana e antibiofilme do sobrenadante de cepas de *Lactobacillus cell-free* sobre isolados de *Escherichia coli* farmacorresistentes. MS Thesis. Federal University of Ceará, CE, Brazil. 2019. http://www.repositorio.ufc.br/handle/riufc/40355

Fijan S (2014). Microorganisms with Claimed Probiotic Properties: An Overview of Recent Literature. Journal of Environmental Research and Public Health 11(5):4745-4767.

Floch MH, Walker WA, Sanders ME, Nieuwdorp M, Kim AS, Brenner DA, Qamar AA, Miloh TA, Guarino A, Guslandi M, Dieleman LA, Ringel Y, Quigley EM, Brandt LJ (2015). Recommendations for probiotic use - 2015 Update: Proceedings and consensus opinion. Journal of Clinical Gastroenterology 49(Suppl 1):S69-S73.

Gabliardi A, Totino V, Cacciotti F, Iebba V, Neroni B, Bonfiglio G, Trancassini M, Passariello C, Pantanella F, Schippa S (2018). Rebuilding gut microbiota ecosystem. Journal of Environmental Research and Public Health 15(8):1679.

Gálvez A, Abrioul H, Benomar N, Lucas R (2010). Microbial antagonists to food-borne pathogens and biocontrol. Current Opinion in Biotechnology 21(2):142-148.

Gómez NC, Ramiro JM, Quecan BXV, Franco BGDM (2016). Use of potential probiotic lactic acid bacteria (LAB) biofilms for the control of *Listeria monocytogenes*, *Salmonella typhimurium* and *Escherichia coli* O157:H7 biofilms formation. Frontiers in Microbiology 7:863.

Gutiérrez S, Martínez-blanco H, Rodríguez-Aparicio LB, Ferrero MA (2016). Effect of fermented broth from lactic acid bacteria oh pathogenic bacteria proliferation. Journal of Dairy Science 99(4): 2654-2665.

Guttenplan SB, Kearns DB (2013). Regulation of flagellar motility during biofilm formation. FEMS Microbiology Reviews 37(6):849-871.

Halder D, Mandal S (2016). Antibacterial potentiality of commercially available probiotic *Lactobacilli* and curd *Lactobacilli* strains, alone and in combination, against human pathogenic bacteria. Translational Biomedicine 7:1-7.

Haney EF, Trimble MJ, Cheng JT, Vallé Q, Hancock REW (2018). Critical assessment of methods to quantify biofilm growth and evaluate antibiofilm activity of host defence peptides. Biomolecules 8(2) 29.

Hegar B, Waspada IM, Gunardi H, Vandenplas Y (2015). A double blind randomized trial showing probiotics to be ineffective in acute diarrhea in Indonesian children. Indian Journal Pediatrics 82(5):410-414.

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminem S, Calder PC, Sanders AE (2014). The international scientific association for probiotics consensus statement on the scope and appropriate use of the term probiotic. Nature reviews Gastroenterology & Hepatology 11(8):506-514.

Hodges K, Gill R (2010). Infectious diarrhea: cellular and molecular mechanisms. Gut Microbes 1(1):4-21.

Hu S, Wang L, Jiang Z (2017). Dietary additive probiotics modulation of intestinal microbiota. Protein and Peptides Letters 24(5):382-387.

Hughes G, Webber MA (2017). Novel approaches to the treatment of bacterial biofilm infections. British Journal of Pharmacology 174:2237-2246.

Iacob S, Iacob DG and Luminos LM (2019). Intestinal Microbiota as a Host Defense Mechanism to Infectious Threats. Frontiers in Microbiology 9:3328.

Islam SU (2016). Clinical uses of probiotics. Medicine 95(5):e2658.

Jiang Y, Ye L, Cui Y, Yang G, Yang W, Wang J, Hu J, Gu W, Shi C, Huang H, Wang C (2017). Effects of *Lactobacillus rhamnosus* GG on the maturation and differentiation of dendritic cells in rotavirus-infected mice. Beneficial Microbes 8(4):645-656.

Kaur S, Sharma P, Kalia N, Singh J, Kaur S (2018). Anti-biofilm properties of the fecal probiotic Lactobacilli against *Vibrio* spp. Frontiers in Cellular and Infection Microbiology 8:120.

Lääveri T, Pakkanen SH, Antikainen J, Riutta J, Mero S, Kirveskari J, Kantele A (2014). High number of diarrhoeal coinfections in travellers to Benin, West Africa. BMC Infectious Diseases 14:81.

Lagrafeuille R, Miquel S, Balastrino D, Vareille-Delarbre M, Chain F, Langella P, Forestier C (2018). Opposing effect of *Lactobacillus* on *in vitro Klebsiella pneumoniae* in biofilm and in an *in vivo* intestinal colonization model. Beneficial Microbes 9(1):87-100.

Li J, Wang W, Xu SX, Magarvey NA, McCormick JK (2011). *Lactobacillus reuteri*-produced cyclic dipeptides quench *agr*-mediated expression of toxic shock syndrome toxin-1 in staphylococci. Proceedings of the National Academy of Sciences 108(8):3360-3365.

Liu J, Gu Z, Song F, Zhang H, Zhao J, Chen W (2019). *Lactobacillus plantarum* ZS2058 and *Lactobacillus rhamnosus* GG use different mechanisms to prevent *Salmonella* infection *in vivo*. Frontiers in Microbiology 10: 299. https://doi.org/10.3389/fmicb.2019.00299

Liu J, Hu D, Chen Y, Huang H, Zhang H, Zhao J, Gu Z, Chen W (2018). Strain-specific properties of *Lactobacillus plantarum* for prevention of *Salmonella* infection. Food and Function 9(7):3673-3682.

Liu W, Ran C, Liu Z, Gao Q, Xu S, Ringø E, Myklebust R, Gu Z, Zhou Z (2016). Effects of dietary *Lactobacillus plantarum* and AHL lactonase on the control of *Aeromonas hydrophila* infection in tilapia. MicrobiologyOpen 5(4):687-699.

Lixa C, Mujo A, Anobom CD, Pinheiro AS (2015). A structural perspective on the mechanisms of quorum sensing activation in bacteria. Anais da Academia Brasileira de Ciências 87(4):2189-2203.

Marchelletta RR, Gareau MG, McCole DF, Okamoto S, Roel E, Klinkenberg R, Guiney DG, Fierer J, Barrett KE (2013). Altered expression and localization of ion transporters contribute to diarrhea in mice with *Salmonella*-induced enteritis. Gastroenterology 145(6):1358-1368.

Markowiak P, Slizewska K (2017). Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients 9(9):1021.

Mathur H, Field D, Rea1 MC, Cotter PD, Hill C, Ross RP (2017). Bacteriocin-antimicrobial synergy: A medical and food perspective. Frontiers in Microbiology 8:1205.

McFarland LV, Evans CT, Goldstein EJC (2018). Strain-specificity and disease-specificity of probiotic efficacy: A systematic review and meta-analysis. Frontiers in Medicine 5:124.

Mendellin-Peña MJ, Griffiths MW (2009). Effect of molecules secreted by *Lactobacillus acidophilus* strain La-5 on *Escherichia coli* O157:H7 colonization. Applied and Environmental Microbiology 75(4):1165-1172.

Meng CY, Smith BL, Bodhidatta L, Richard SA, Vansith K, Thy B, Srijan A, Serichantalergs O, Mason CJ (2011). Etiology of diarrhea in young children and patterns of antibiotic resistance in Cambodia. The Pediatric Infections Diseases Journal 30(4):331-335.

Miquel S, Lagrafeuille R, Souweine B, Forestier C (2016). Anti-biofilm Activity as a Health Issue. Frontiers in Microbiology 7:592.

Misselwitz B, Barrett N, Kreibich S, Vonaesch P, Andritschke D, Rout S, Weidner K, Sormaz M, Songhet P, Horvath P, Chabria M, Vogel V, Spori DM, Jenny P, Hardt WD (2012). Near surface swimming of *Salmonella typhimurium* explains target-site selection and cooperative invasion. PLoS Pathogens 8(7):e1002810.

Moreira CG, Palmer K, Whiteley M, Sircili MP, Trabulsi LR (2006). Bundle-forming Pili and EspA are involved in biofilm formation by Enteropathogenic *Escherichia coli*. Journal of Bacteriology 188(11):3952-3961.

Nakazato G, Paganelli FL, Lago JC, Aoki FH, Mobilon C, Brocchi M, Stehling EG, Silveira WD (2011). *Lactobacillus acidophilus* decreases *Salmonella typhimurium* invasion *in vivo*. Journal of Food Safety 31:284-289.

Nascimento HH, Silva LEP, Souza RT, Silva NP, Scaletsky ICA (2014). Phenotypic and genotypic characteristics associated with biofilm formation in clinical isolates of typical enteropathogenic *Escherichia coli* (aEPEC) strains. BMC Microbiology 14(1):184.

Navaneethan U, Giannella RA (2008). Mechanisms of infectious diarrhea. Nature Reviews Gastroenterology & Hepatology 5(11):637-647.

Novik G, Savich V (2019). Beneficial microbiota. Probiotics and pharmaceutical products in functional nutrition and medicine. Microbes and Infection In Press. https://doi.org/10.1016/j.micinf.2019.06.004

Oelschlaeger TA (2010). Mechanisms of probiotic action – A review. International Journal of Medical Microbiology 300(1):57-62.

Oliveira NM, Martinez-Garcia E, Xavier J, Durham WM, Kolter R, Kim W, Foster KR (2015) Biofilm formation as a response to ecological competition. PLoS Biology 13(7):e1002191.

Osama DM, Elkhatib WF, Tawfeik AM, Mohammad M (2017). Antimicrobial, antibiofilm and immunomodulatory activities of *Lactobacillus rhamnosus* and *Lactobacillus gasseri* against some bacterial pathogens. International Journal of Biotechnology for Wellness Industries 6(1):12-21.

Paixão LA, Castro FFS (2016). A colonização da microbiota intestinal e sua influência na saúde do hospedeiro. Universitas: Ciências da Saúde 14(1):85-96.

Piescik-Lech M, Urbanska M, Szajewska H (2013). *Lactobacillus* GG (LGG) and smectite versus LGG alone for acute gastroenteritis: a double-blind, randomized controlled trial. European Journal of Pediatrics 172(2):247-253.

Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A (2019). Mechanisms of action of probiotics. Advances in Nutrition 10:S49-S66.

Prabhurajeshwar C, Chandrakanth RK (2017). Probiotic potential of Lactobacilli with antagonistic activity against pathogenic strains: an in vitro validation for the production of inhibitory substances. Biomedical Journal 40(5):270-283.

Quigley L, Coakley M, Alemayehu D, Rea MC, Casey PG, O'Sullivan Ó, Murphy E, Kiely B, Cotter PD, Hill C, Ross RP (2019). *Lactobacillus gasseri* APC 678 reduces shedding of the pathogen *Clostridium difficile* in a murine model. Frontiers in Microbiology 10:273.

Rabin N, Zheng Y, Opoku-Temeng C, Du Y, Bonsu E, Sintim HO (2015). Biofilm formation mechanisms and targets for developing antibiofilm agents. Future Medicinal Chemistry 7(4):493-512.

Ramos AN, Sesto Cabral ME, Noseda D, Bosch A, Yantorno OM, Valdez JC (2012). Antipathogenic properties of *Lactobacillus plantarum* on *Pseudomonas aeruginosa*: The potential use of its supernatants in the treatment of infected chronic wounds. Wound Repair and Regeneration 20(4):552-562.

Rätsep M, Kõljalg S, Sepp E, Smidt I, Truusalu K, Songisepp E, Stsepetova J, Naaber P, Mikelsaar RH, Mikelsaar M (2017). A combination of the probiotic and prebiotic product can prevent the germination of *Clostridium difficile* spores and infection. Anaerobe 47:94-103.

Rosenvinge EC, Maldarelli GA, Piepenbrink KH, Scott AJ, Freiberg JA, Song Y, Achermann Y, Ernst RK, Shirtliff ME, Sundberg EJ, Donnenberg MS (2016). Type IV pili promote early biofilm formation by *Clostridium difficile*. FEMS Pathogens and Disease 74(6):ftw061.

Ruiz MJ, Colello R, Padolaand NL, Etcheverría AI (2017). Efecto inhibitorio de *Lactobacillus* spp. sobre bactérias implicadas em enfermidades transmitidas por alimentos. Revista Argentina de Microbiologia 49(2):174-177.

Saldaña Z, Erdem AL, Schüller S, Okeke IN, Lucas M, Sivananthan A, Phillips AD, Kaper JB, Puente JL, Girón JA (2009). The *Escherichia coli* common pilus and the bundle-forming pilus act in concert during the formation of localized adherence by enteropathogenic *E. coli*. Journal of Bacteriology 191(11):3451-3461.

Sambanthamoorthy K, Feng X, Patel R, Patel S, Paranavitana C (2014). Antimicrobial and antibiofilm potential of biosurfactants isolated from lactobacilli against multi-drug-resistant pathogens. BMC Microbiology 14(1):197.

Saxelin M, Lassig A, Karjalainen H, Tynkkynen S, Surakka A, Vapaatalo H, Järvenpää S, Korpela R, Mutanen M, Hatakka K (2010). Persistence of probiotic strains in the gastrointestinal tract when administered as capsules, yoghurt, or cheese. International Journal of Food Microbiology 144(2):293-300.

Schiebel J, Böhm A, Nitschke J, Burdukiewicz M, Weinreich J, Ali A, Roggenbuck D, Rödiger S, Schierack P (2017). Genotypic and phenotypic characteristics associated with biofilm formation by human clinical *Escherichia coli* isolates of different pathotypes. Applied Environmental Microbiology 83(24):e01660-17.

Sharman D, Saharan BS (2016). Functional characterization of biomedical potential of biosurfactant produced by *Lactobacillus helveticus*. Biotechnology Reports 11:27-35. https://doi.org/10.1016/j.btre.2016.05.001

Sikorska H, Smoragiewicz W (2013). Role of probiotics in the prevention and treatment of meticillin-resistant *Staphylococcus aureus* infections. International Journal of Antimicrobial Agents 42(6):475-481.

Singh S, Singh SK, Chowdhury I, Singh R (2017). Understanding the Mechanism of Bacterial Biofilms Resistance to Antimicrobial Agents. The Open Microbiology Journal 11:53-62.

Sniffen JC, McFarland LV, Evans CT, Goldstein EJC (2018). Choosing an appropriate probiotic product for your patient: An evidence-based practical guide. PLoS One 13(12):e0209205.

Suez J, Zmora N, Zilberman-Schapira G, Mor U, Dori-Bachash M, Bashiardes S, Elinav E (2018). Post-antibiotic gut mucosal microbiome reconstitution is impaired by probiotics and improved by autologous FMT. Cell 174(6):1406-1423.

Szajewska H, Canani RB, Guarino A, Hojsak I, Indrio F, Kolacek S, Orel R, Shamir R, Vandenplas Y, Van Goudoever JB, Weizman Z (2016). Probiotics for the Prevention of Antibiotic-Associated Diarrhea in Children. Journal of Pediatric Gastroenterology and Nutrition 62(3):495-506.

Taverniti V, Koirala R, Via AD, Gargari G, Leonardis E, Arioli S, Guglielmetti S (2019). Effect of cell concentration on the persistence in the human intestine of four probiotic strains administered through a multispecies formulation. Nutrients 11(2):285.

Ten Bruggencate SJ, Girard SA, Floris-Vollenbroek EG, Bhardwaj R, Tompkins TA (2015). The effect of a multi-strain probiotic on the resistance toward *Escherichia coli* challenge in a randomized, placebo-controlled, double-blind intervention study. European Journal of Clinical Nutrition 69:385-391.

Ten Bruggencate SJM, Girard SA, Floris-Vollenbroek EGM, Bhardwaj R, Tompkins TA (2014). The effect of a multi-strain probiotic on the resistance toward *Escherichia coli* challenge in a randomized, placebo-controlled, double-blind intervention study. European Journal of Clinical Nutrition 69(3):385-391.

Thiagarajah JR, Donowirtz M, Verkman AS (2015). Secretory diarrhoea: mechanisms and emerging therapies. Nature Reviews Gastroenterology & Hepatology 12(8):446-457.

Tolker-Nielsen T (2015). Biofilm Development. Microbiology Spectrum 3(2):MB-0001-2014.

Turková K, Mavric A, Narat M, Rittich B, Spanova A, Rogelj I, Matijasic BB (2013). Evaluation of *Lactobacillus* strains for selected probiotic properties. Folia Microbiologica 58(4):261-267.

Vacheva A, Gerogieva R, Danova S, Mihova R, Marhova R, Kostadinova S, Vasileva K, Bivolarska M, Stoitsova SR (2012). Modulation of *Escherichia coli* biofilm growth by cell-free spent cultures from lactobacilli. Central European Journal of Biology 7(2):219-229.

Varavallo MA, Thomé JN, Teshima E (2008). Aplicação de bactérias probióticas para profilaxia e tratamento de doenças gastrointestinais. Semina: Ciências Biológicas e da Saúde 29(1):83-104.

Vieco-Saiz N, Belguesmia Y, Raspoet R, Auclair E, Gancel F, Kempf I, Drider D (2019). Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. Frontiers in Microbiology 10:57.

Vlasova AN, Chattha KS, Kandasamy S, Liu Z, Esseili M, Shao L, Rajashekara G, Saif LJ (2013). Lactobacilli and Bifidobacteria promote immune homeostasis by modulating innate immune responses to human rotavirus in neonatal gnotobiotic pigs. PLoS One 8(10):e76962.

Vuotto F, Longo F, Donelli G (2014). Probiotics to counteract biofilm-associated infections: promising and conflicting data. International Journal of Oral Science 6(4):189-194.

Wolska KI, Grudniak AM, Rudnicka Z, Markowska K (2016). Genetic control of bacterial biofilms. Journal of Applied Genetics 57(2):225-238.

World Gastroenteroly Organisation (WGO) (2017). World Gastroenterology Organization Global Guidelines – Probiotics and Prebiotics. http://www.worldgastroenterology.org/guidelines/global-guidelines/probiotics-and-prebiotics/probiotics-and-prebiotics-english

World Health Organization (WHO) (2002). Guidelines for the Evaluation of Probiotics in Food. Londres. https://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf

Wu H, Moser C, Wang Q, Hoiby N, Song Z (2015). Strategies for combating bacterial biofilm infections. International Journal of Oral Science 7(1):1-7.

Yeganeh M, Hosseini H, Mehrabian S, Torbatti ES, Zamir AM (2017). Antibiofilm effects of lactobacilli against ciprofloxacin-resistant uropathogenic *Escherichia coli* strains in pasteurized milk. Applied Food Biotechnology 4(4):241-250.

Zhang Z, Xiang Y, Li N, Wang B, Ai H, Wang X, Huang L, Zheng Y (2013). Protective effects of *Lactobacillus rhamnosus* GG against human rotavirus-induced diarrhoea in a neonatal mouse model. Pathogens and Disease 67(3):184-191.