Disease managing capacities and mechanisms of host effects of lactic acid bacteria

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ABSTRACT

Consumption of lactic acid bacteria (LAB) has been suggested to confer health-promoting effects on the host. However, effects of LABs have been reported to be species- and strain-specific and the mechanisms involved are subjects of discussion. Here, the possible mechanisms by which LABs induce antipathogenic, gut barrier enhancing and immune modulating effects in consumers are reviewed. Specific strains for which it has been proven that health is improved by these mechanisms are discussed. However, most strains probably act via several or combinations of mechanisms depending on which effector molecules they express. Current insight is that these effector molecules are either present on the cell wall of LAB or are excreted. These molecules are reviewed as well as the ligand binding receptors in the host. Also postbiotics are discussed. Finally, we provide an overview of the efficacy of LABs in combating infections caused by Helicobacter pylori, Salmonella, Escherichia coli, Streptococcus pneumoniae, and influenza virus, in controlling gut inflammatory diseases, in managing allergic disorders, and in alleviating cancer.

Introduction

Lactic acid bacteria (LAB) are a group of gram-positive microorganisms, which mainly produce lactic acid as end fermentation product during carbohydrate metabolism. They can be found in a wide range of foods such as vegetables, fruit, meat, and dairy products. In addition, many LAB species belong to indigenous microflora in various mucosal niches of both humans and animals (George et al. 2018). Certain LAB species and strains have been characterized as probiotics (George et al. 2018). Probiotics have been defined as living microorganisms, which when consumed in adequate amounts, can confer health benefits to the host (Hemarajata and Versalovic 2013). LAB is one of the most commonly applied sources of probiotics, and a variety of beneficial functions have been attributed to consumption of probiotic LAB strains (Bron, van Baarlen, and Kleerebezem 2012; Lebeer, Vanderleyden, and De Keersmaecker 2008). The majority of established probiotics come from the LAB species Lactobacillus, whereas certain LAB strains within the species Lactococcus and Streptococcus are also recognized for probiotic functionalities (Borchers et al. 2009).

Despite broad recognition as probiotic species, the mechanisms by which specific LAB strains contribute to health are still not completely understood (van Baarlen, Wells, and Kleerebezem 2013). The most proposed mechanisms include modulation of the composition and function of gut microbiome, improvement of intestinal barrier function, immune modulation, competitive adhesion for pathogens to mucosal sites, attenuation of virulent factors of pathogens, and production of bioactive compounds with anti-infectious or other functional properties. In the present review these potential mechanisms and the explicit modes of actions are discussed in detail. LAB may influence gut microbial communities via the provision of growth substrates for specific commensal microbes. LAB modulate gut mucus barrier via improving the production of mucus components, and enhance intestinal epithelial barrier via regulating tight junction (TJ) networks, lowering apoptosis, and supporting immune signaling in epithelial cells. The host immune function can be modified by LAB via modulating functional activities of different types of immune cells such as natural killer (NK) cells, phagocytes, dendritic cells (DCs), T cells, and B cells (Figure 1). In these sections examples of specific strains are also included for which it has been proven that well-being is enhanced by one or combinations of these mechanisms.

However, most strains probably act via several or combinations of mechanisms depending on which effector molecules they express. Current insight is that these effector molecules are either present on the cell wall of LAB or are excreted. In this review, these molecules are reviewed as well as their associated ligand binding receptors in the host. Finally, examples of LABs are critically discussed for management of pathogenic bacterial and viral infections, gastrointestinal inflammatory disorders, allergy, and cancer.
Although the mechanisms and exact ligand on LABs for management of these diseases remain to be identified, the studies provide a proof of principle. With our review we hope to contribute to design of more systemical studies to create effective formulations of LABs for reproducibly inducing health benefits.

Mechanisms involved in probiotic actions of LAB

Regulation of gut microflora by LAB derived fermentation products

Gut microbiota plays a vital role in maintaining gut immune equilibrium (Derrien and van Hylckama Vlieg 2015). Modulatory effects of LAB consumption on gut microbiota have been demonstrated (Hemarajata and Versalovic 2013). LAB can influence intestinal microbial communities by providing growth substrates to other species by fermenting carbohydrates and amino acids and formation of secondary metabolites which leads to enhanced richness of specific commensal bacteria (Derrien and van Hylckama Vlieg 2015; Pessione 2012). An example for this reciprocal nutritional relationship is that LAB-derived lactic acid can be utilized and converted to butyrate by Eubacterium hallii, which is a specie of importance for a balanced microbial community (Engels et al. 2016). Moreover, exopolysaccharide synthesized by a wide array of LAB species was found to serve as energy source for symbiosis in microbial populations (Ryan et al. 2015).

By modulating gut microbiota, LABs might contribute to prevention of disease such as to reduce chances on pathogenic infection, intestinal inflammation, cancer, and allergy. There are a number of clear examples demonstrating this (Kepert et al. 2017; Liu et al. 2011; Rodríguez-Nogales et al. 2017; Zhang et al. 2018). Salmonella translocation into liver could be attenuated by one-week pre-supplementation of a well-known probiotic strain Lactobacillus (L.) rhamnosus GG, which was partially attributed to the restoration of decreased gut microbial diversity (Zhang et al. 2018). Administration of a L. fermentum strain CECT5716 mitigated dextran sodium sulfate-induced colonic damage and suppressed colitis-associated aberrant changes in intestinal microbial composition (Rodríguez-Nogales et al. 2017). Liu et al. (2011) reported that a strain mixture of lactobacilli and bifidobacteria mitigated post-surgery infection in colorectal cancer patients accompanied by improvement of

Figure 1. Schematic representation illustrating key mechanisms through which LAB exert health-promoting effects. LAB can (A) modulate the composition and function of gut microbiota via (B) support of production of metabolites that can serve as growth substrates for specific commensal bacteria. LAB can combat pathogenic infections by (B) producing bioactive components with anti-infectious properties such as short chain fatty acids, bacteriocins, and biosurfactants, (C) inhibiting pathogen adhesion to the mucosal surface, and (D) attenuating the expression of virulent factors. Moreover, LAB can (E) modulate gut mucus barrier and (F) strengthen epithelial barrier. (G) Both innate and adaptive immunity can also be regulated by LAB.
intestinal microbial diversity. Moreover, lactobacilli-derived D-tryptophan was shown to beneficially regulate dysregulated gut microbiota, prevent allergic airway inflammation, and create a more balanced immune environment in the gut and lung of mice with experimental asthma (Kepert et al. 2017).

Production of bioactive or metabolic compounds with anti-pathogenic effects

LAB strains can produce molecules that reduce the possibility for pathogens to invade the host. The production of lactic acid and short chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, is an example of that. These LAB derived acids create an unfavorable acidic environment for invading pathogens and inhibit their growth (Derrien and van Hylckama Vlieg 2015). The lactic acid produced by LABs can also have direct anti-pathogenic effects by disrupting the cell membrane of pathogenic bacteria such as Escherichia (E.) coli, Listeria monocytogenes, and Salmonella (Wang et al. 2015). It has suggested that via production of lactic acid, LAB protect against pathogens such as Helicobacter (H.) pylori, Salmonella, and E. coli (Choi et al. 2018; Zheng et al. 2016). SCFAs production by LAB was also considered as a mechanism responsible for the effects of LAB on cancer such as colorectal cancer due to the apoptosis-promoting properties of SCFAs on cancer cells (dos Reis et al. 2017).

Apart from the aforementioned molecules, some LABs synthesize other bactericidal agents such as bacteriocins, biosurfactant, and hydrogen peroxide (Lebeer, Vanderleyden, and De Keersmaecker 2008). Strong in vivo evidence that LAB derived bacteriocins are responsible for protective effects on pathogenic infections was provide by van Zyl, Deane, and Dicks (2019), who observed that the mutant strain of L. plantarum 423 without ability to express bacteriocin plantaricin 423 could not confer protection against Listeria in mice. Also, for LAB derived biosurfactant and hydrogen peroxide proof was found for antipathogenic effects especially in the vagina. A vaginal biosurfactant-producing L. crispatus strain BC1 was demonstrated to antagonize urogenital pathogen Neisseria gonorrhoeae (Foschi et al. 2017). Stapleton et al. (2011) showed that 10-week intravaginal administration of a hydrogen peroxide-producing L. crispatus strain CTV-05 was effective in diminishing the recurrence of urinary tract infection in women.

Spatial competition to prevent pathogen adhesion to mucosa

Another proposed anti-pathogenic mechanism of LAB is prevention of adhesion of pathogens to the mucosal surface by competition for mucosal adhesion sites (Lebeer, Vanderleyden, and De Keersmaecker 2008; van Baarlen, Wells, and Kleerebezem 2013). This has been proven to be a mechanism by which L. sobrius, L. acidophilus, and S. thermophilus prevent adherence of pathogenic E. coli on epithelium (Resta-Lenert and Barrett 2003; Roselli et al. 2007). Many surface components that might contribute to the adhesion of LAB to mucosal surfaces and prevent pathogen adhesion have been identified. These include molecules such as mucus-binding proteins, S-layer proteins, and exopolysaccharide (Ryan et al. 2015; Van Tassell and Miller 2011). Singh et al. (2018) reported that Mubs5s6 protein, the last two domains of L. plantarum mucus-binding protein Lp_1643 effectively inhibited the adherence of enterotoxigenic E. coli to gut epithelium. L. johnsonii F0421-derived S-layer proteins were shown to dampen the adhesion of Shigella sonnei to human HT-29 gut epithelial cells (Zhang, Zhang, et al. 2012). Furthermore, it was demonstrated that exopolysaccharide molecules from L. reuteri DSM17938 and L. reuteri L26 prevented the attachment of E. coli to IPEC-1 porcine epithelial cell line (Kšonžeková et al. 2016).

Diminution of pathogenic virulence

In addition to production of bactericidal compounds and competition for adhesion sites, attenuation of expression of virulent factors has been proposed as another essential mechanism underlying the anti-pathogenic effects of LAB (Laughton et al. 2006; Li et al. 2011; Reid et al. 2011; Ryan et al. 2009). L. reuteri RC-14 can suppress the expression of staphylococcal exotoxin staphylococcal superantigen-like protein 11 and prevent its spreading in the host (Laughton et al. 2006). Li et al. (2011) observed that this same L. reuteri RC-14 dampened the expression of staphylococcal exotoxin toxic shock syndrome toxin-1 in vitro. Probably this was caused by the L. reuteri RC-14 derived cyclic dipeptides (Li et al. 2011). L. salivarius strains were demonstrated to inhibit H. pylori-induced infection not only by acid production but also by interfering with expression and secretion of Cag pathogenicity island genes of H. pylori (Ryan et al. 2009). In addition to the cytotoxic virulent factor Cag, expression of H. pylori adhesin such as sabA can be decreased by L. gasseri Kx110 A1 and L. brevis ATCC14869, via which adhesion of H. pylori to gastric epithelium was suppressed (de Klerk et al. 2016).

Modulation of gut barrier function

Improving the intestinal mucus barrier

LAB have been reported to contribute to mucus barrier function (Dykstra et al. 2011; Mack et al. 2003; Otte and Podolsky 2004; van Beek et al. 2016; Wang et al. 2014). Mucus layers covering intestinal epithelium are of pivotal importance for gut homeostasis and gut barrier function (Deplancke and Gaskins 2001). The primary constituent of mucus are mucins, which are produced by secretory epithelium goblet cell and form the gel structure contributing to the defensive function of mucus (Kim and Ho 2010). Aside from mucins, other bioactive factors including goblet cell-synthesized molecules such as intestinal trefoil peptides and resistin-like molecule β, secretory antibodies such as sIgA, and Paneth cell-produced antimicrobial molecules such as defensins and lysozymes are crucial mucus components. These mucus-associated components are trapped in the
mucus matrix and contribute to the rheological and defensive properties of mucus barrier (Kim and Ho 2010; McGuckin et al. 2011). In vitro studies demonstrate that lactobacilli or the probiotic strain mixture VSL#3 elevated the expression of mucins at mRNA and protein level (Mack et al. 2003; Otte and Podolsky 2004; Ren et al. 2018), which is suggested to be a possible mechanism underlying their conferred suppression of pathogenic E. coli adhesion to intestinal epithelium (He et al. 2017; Mack et al. 2003). Such an anti-pathogenic action of lactobacilli by up-regulation of mucin production can be achieved partly via fortifying mucus barrier, thereby diminishing the access of pathogen such as E. coli to Caco-2 intestinal epithelial cells (He et al. 2017). Also secretion of mucins such as MUC2 and MUC3 can lead to their binding with pathogens in the lumen of the gut and reduce pathogen invasion in the host (He et al. 2017; Mack et al. 2003). Furthermore, in vivo evidence suggest that L. rhamnosus GG restored declined MUC2 expression caused by Pseudomonas (P.) aeruginosa invasion, which might partially mediate L. rhamnosus GG-conferred protection of mice from P. aeruginosa-induced pneumonia (Khallova et al. 2017).

Not only direct interaction of LABs with intestinal cells but also LAB-derived molecules such as SCFA (Jung et al. 2015) and bioactive proteins (Wang et al. 2014) were shown to exert enhancing effects on mucin expression. L. rhamnosus GG-secreted p40 protein induced an epidermal growth factor receptor-dependent enhancement of mucin production both in vitro in LS174T human goblet cells and in vivo in mice, which might be the mechanism underlying the protective property of p40 on epithelial function (Wang et al. 2014). Moreover, LAB might also reinforce mucus barrier function via augmenting the production of other mucus elements such as defensins (Nocerino et al. 2017; Rizzo, Losacco, and Carratelli 2013) and sIgA (Nocerino et al. 2017; Zhang et al. 2010). L. paracasei CBA L74-fermented products potentiated the production of human defensins and sIgA, via which incidence of infection in children was declined (Nocerino et al. 2017). Promotion of human β-defensin 2 and 3 production was suggested to partly mediate L. crispatus ATCC33820-conferred protection on epithelial cells against urogenital pathogen Candida albicans-induced infection (Rizzo, Losacco, and Carratelli 2013). The mechanisms by which LABs contribute to mucus barrier function in the intestine are depicted in Figure 2.

Enhancing of gut epithelium function and gut epithelial barrier

A single layer of organized intestinal epithelial cells builds up a defensive barrier effectively separating the underlying immune system from luminal exogenous insults (Wells et al. 2011). Also, this part of the barrier function can be modulated by LAB via diverse mechanisms (Figure 2). The epithelial barrier is a semipermeable cellular filter solely allowing the entry of water, electrolytes, and dietary nutrients from the lumen side, whilst excluding intrusion of luminal microbes and detrimental antigens (Groschwitz and Hogan
Paracellular permeability, a pivotal regulator for epithelial barrier function, is regulated by interepithelial junctional complexes such as TJs, desmosomes, and adherens junctions (Groschwitz and Hogan 2009; Ohland and MacNaughton 2010). TJs are located at the apical site of the lateral membrane between two adjacent cells, and are composed of diverse transmembrane and adaptor proteins such as zonula occludens (ZO)s, occludins, and claudins (Groschwitz and Hogan 2009; Ohland and MacNaughton 2010). LAB can enhance epithelial barrier via regulation of these TJ protein function (Anderson et al. 2010; Karczewski et al. 2010; Madsen 2012; Mennigen et al. 2009). \textit{L. plantarum} strain WCFS1 has been shown to have such an effect in human. Administration of \textit{L. plantarum} WCFS1 enhanced the expression of TJ proteins ZO-1 and occludin in the duodenal epithelial cells of healthy volunteers (Karczewski et al. 2010). Others have suggested that TJ protein enhancement might be one of the most important mechanisms for LAB induced protection of intestinal inflammation, pathogenic invasion, and colorectal cancer (dos Reis et al. 2017; Lépine et al. 2018; Liu et al. 2011; Mennigen et al. 2009). There is ample proof that this mechanism is involved in beneficial health effects of LABs. For example, the VSL\#3 mixture was shown to reverse aberrant expression and redistribution of TJ proteins (i.e. claudin-1, -3, -4, and -5, occludin, and ZO-1) in mice with dextran sodium sulfate-induced colitis, which might contribute to the alleviation of gut inflammation (Mennigen et al. 2009). We previously found that \textit{L. acidophilus} W37 protected intestinal epithelial cells against \textit{Salmonella} invasion at least partially via up-regulating the expression of TJ-related proteins such as claudin-4, -15, and -16 (Lépine et al. 2018). Moreover, pre- and post-operative supplementation of \textit{L. plantarum} CGMCC1258, \textit{L. acidophilus} LA-11, and \textit{Bifidobacterium (B.) longum} BL-88 in colorectal cancer patients effectively diminished the occurrence of post-operative infection in conjunction with enhanced gut barrier integrity and TJ protein expression (Liu et al. 2011).

Ocludins, claudins, and ZO$s$ are the most frequently measured TJ proteins in most studies on epithelial barrier function regulation. Of note, apart from those vital TJ formation proteins occludin, ZO-1, ZO-2, and cingulin, Anderson et al. (2010) tested \textit{L. plantarum} MB452-induced alterations in basal expression level of a range of crucial regulators involved in TJ-correlated signalings by using global gene-expression analysis. \textit{L. plantarum} MB452 enhanced the expression of occludin, ZO-1, ZO-2, and cingulin, but
down-regulated the expression of cytoskeleton protein tubulin and proteasomes (Anderson et al. 2010). Moreover, it was shown that LAB not only modulate the expression of the TJ-proteins but can also modulate epithelial barrier function through influencing phosphorylation of TJ proteins (e.g. ZO-1 and occludins) and cytoskeletal proteins (e.g. actin and actinin) (Resta-Lenert and Barrett 2003).

In addition to modulation of TJ function, LAB can preserve epithelial barrier function by inhibition of epithelial apoptosis (Dykstra et al. 2011; Menningen et al. 2009; Yan et al. 2007). Yan et al. (2007) characterized two bioactive proteins p40 and P75 from L. rhamnosus GG, and showed that these two proteins effectively dampened tumor necrosis factor-induced epithelial apoptosis in colon epithelial cell lines and murine colon explants via evoking the apoptosis-antagonizing Akt pathway. In addition, VSL#3 administration was shown to prevent epithelial apoptosis in a murine acute colitis model (Menningen et al. 2009). In another study L. plantarum 299v was shown to stimulate both gene and protein expression of apoptosis-inhibiting HIAP2/cIAP1 in the jejunum of rats (Dykstra et al. 2011). Also, L. brevis SBC8803a and a soluble peptide from L. rhamnosus GG were shown to enhance the expression of cytoprotective molecules such as heat shock protein in intestinal epithelium, which is a pivotal mediator in extracellular stress defense of cells and avoider of cell-death (Tao et al. 2006; Ueno et al. 2011).

LABs can also change immune signaling of intestinal epithelial cells (Wan et al. 2016). Gut epithelial cells not only serve as elemental structural components of the gut epithelial barrier, but also initiate immune signals in response to extrinsic stimuli. It has been suggested that some LAB strain such as L. sobrius DSM 16698T or strain mixture such as VSL#3 can also modulate gut epithelial function by affecting epithelium-involved immune signals such as cytokines production (Lépine et al. 2018; Roselli et al. 2007; Wan et al. 2016). For example, the porcine L. sobrius strain DSM16698T potentiated IL-10 secretion in porcine IPEC-1 intestinal epithelial cells, which was demonstrated to fully mediate its protective actions against E. coli K88 invasion (Roselli et al. 2007). Enhancement of epithelial barrier by LAB was also suggested to mediate their beneficial properties on allergic disease. For instance, a gnotobiotic mouse model colonized with the mixture of L. casei LOCK919, L. rhamnosus LOCK0900, and L. rhamnosus LOCK0908 displayed attenuated allergic sensitization, which was suggested to be partly induced by the fortification of gut epithelial barrier function (Kozakova et al. 2016).

**Modulation of immune function by LABs**

**Effects on innate immunity**

Regulation of host immunity by LAB through their crosstalk with the immune system has been acknowledged as a fundamental mode of probiotic actions (Bron, van Baarlen, and Kleerebezem 2012; Lebeer, Vanderleyden, and De Keersmaecker 2010; van Baarlen, Wells, and Kleerebezem 2013). A large body of research substantiate that both innate and adaptive immune responses can be modified by LAB supplementation (Borchers et al. 2009; Cangemi de Gutierrez, Santos, and Nader-MacÂ As 2001; de Moreno de LeBlanc, Castillo, and Perdigon 2010; Delcenserie et al. 2008; Gill et al. 2001; Harata et al. 2010; Izumo et al. 2010; Lin et al. 2007; Nagai et al. 2011; Nanno et al. 2011; Ogawa et al. 2006; Pelto et al. 1998; Racedo et al. 2006; Schiffrin et al. 1995; Shu and Gill 2002; Takeda et al. 2006; Villena et al. 2009; Villena et al. 2005; Yasui, Kiyoshima, and Hori 2004). NK cells and phagocytes such as macrophages, neutrophils, and monocytes as cardinal participants in innate immunity, serve as crucial immunological defense barrier against exogenous invaders, and their functions can be modified by LAB (Figure 3) (Cangemi de Gutierrez, Santos, and Nader-MacÂ As 2001; de Moreno de LeBlanc, Castillo, and Perdigon 2010; Gill et al. 2001; Harata et al. 2010; Izumo et al. 2010; Lin et al. 2007; Nagai et al. 2011; Nanno et al. 2011; Ogawa et al. 2006; Olives et al. 2007; Pelto et al. 1998; Racedo et al. 2006; Schiffrin et al. 1995; Shu and Gill 2002; Takeda et al. 2006; Villena et al. 2009; Villena et al. 2005; Yasui, Kiyoshima, and Hori 2004). Animal studies on anti-pathogenic potentials of LAB confirm enhancement of phagocyte functions by LAB, causing enhanced clearance of various pathogens including Salmonella typhi-murium, Streptococcus (S.) pneumoniae, and E. coli (Cangemi de Gutierrez, Santos, and Nader-MacÂ As 2001; de Moreno de LeBlanc, Castillo, and Perdigon 2010; Gill et al. 2001; Lin et al. 2007; Racedo et al. 2006; Shu and Gill 2002; Villena et al. 2009; Villena et al. 2005). Animal studies showed that orally or intranasally administration of alive or heat-killed lactobacilli of differential species such as L. acidophilus, L. casei, L. fermentum, and L. rhamnosus augmented phagocytes functions at both the intestinal and extraintestinal level as defined by up-regulated activation of lung macrophages (Cangemi de Gutierrez, Santos, and Nader-MacÂ As 2001; Racedo et al. 2006), increased numbers of leukocytes and neutrophils in blood and bronchoalveolar lavage (BAL) (Villena et al. 2005), enhanced phagocytic activity of phagocytes from Peyer’s patches, spleen, peritoneum, blood, and BAL (de Moreno de LeBlanc, Castillo, and Perdigon 2010; Gill et al. 2001; Lin et al. 2007; Shu and Gill 2002; Villena et al. 2009; Villena et al. 2005). A human trial in healthy subjects also demonstrated that intake of L. acidophilus La1 potentiated the phagocytic activity of blood leukocytes (Schiffrin et al. 1995). Intriguingly, L. rhamnosus GG was found to distinctively impact expression of phagocytosis receptors in peripheral neutrophils in healthy participants and individuals with allergies (Pelto et al. 1998). In healthy groups, the expression of phagocytosis receptors was elevated by L. rhamnosus GG, whereas in milk-hypersensitive individuals LAB administration attenuated allergen challenge-induced heightened expressions of phagocytosis receptors (Pelto et al. 1998).

In addition to their influence on phagocyte activities, LAB were also suggested to augment NK cell function (Harata et al. 2010; Izumo et al. 2010; Nagai et al. 2011; Nanno et al. 2011; Ogawa et al. 2006; Olives et al. 2007; Takeda et al. 2006; Yasui, Kiyoshima, and Hori 2004).
casei Shirotai was shown to enhance pulmonary NK activity and to heighten IL-12 secretion by mediastial lymph node cells in an influenza infected infant mouse model (Yasu, Kiyoshiba, and Hori 2004). Moreover, in other murine influenza virus infection models some other LABs such as L. rhamnosus GG, L. pentosus S-PT84, and L. delbrueckii ssp. bulgaricus OLL1073R-1 potentiated NK function in lung or spleen, which might account for LAB-elicited protective actions against influenza virus infection (Harata et al. 2010; Izunou et al. 2010; Nagai et al. 2011). In healthy volunteers, consumption of L. casei ssp. casei JCM1134® initiated an increased peripheral NK frequency (Ogawa et al. 2006). L. fermentum CECT5716 also enhanced peripheral NK proportion in healthy subjects and fortified the immune efficacy of influenza vaccination (Olivares et al. 2007). Both in vivo and ex vivo L. casei Shirotai enhanced peripheral NK activity in healthy humans, which correlated with L. casei Shirotai’s capability of elevating peripheral IL-12 production (Nanno et al. 2011; Takeda et al. 2006). Apart from healthy subjects, L. casei Shirotai intake also boosted NK cell function in patients with bladder or colorectal cancer and thereby decreased the occurrence of relapse (Nanno et al. 2011).

Effects on adaptive immunity
Many LAB strains including the extensively studied VSL#3 mixtures influence adaptive immunity. This often starts with modulation of DCs (Figure 3). DCs are a crucial cell type participating in both innate and adaptive immune signaling. Different LAB strains have been suggested to divergently regulate the maturation and activation of DCs, which is accompanied by increased expression of major histocompatibility complex molecules as well as co-stimulatory molecules such as CD40, CD80, CD83, and CD86, and by enhanced cytokine secretion (Borchers et al. 2009; Smelt, de Haan, Bron, van Swam, Meijerink, Wells, Faas, et al. 2013). In vitro studies on LAB-induced modulation of DC function applied multiple types of DCs from humans and rodents including peripheral blood monocyte-derived DCs (MDDCs), enteric lamina propria (LP)-derived DCs (LPDCs), mesenteric lymph node (MLN)-derived DCs (MLN DCs), and bone marrow-derived DCs (BMDCs) (Borchers et al. 2009; Hart et al. 2004). It was proposed that DCs of different sources might react differently to LAB strains (Borchers et al. 2009). Despite this, specific LAB-mediated similar effects on polarization of DCs of different sources were obtained (Borchers et al. 2009; Drakes, Blanchard, and Czinn 2004; Hart et al. 2004). For instance, stimulation of both human LPDCs and human MDDCs by VSL#3 resulted in elevated IL-10 production (Hart et al. 2004). Another study also reported augmented IL-10 production level induced by VSL#3 in BMDCs (Drakes, Blanchard, and Czinn 2004). Our own previous in vivo study also demonstrated the strain-dependent effects of LAB on DC distribution and function which were different in the small and large intestine (Smelt, de Haan, Bron, van Swam, Meijerink, Wells, Faas, et al. 2013). Of the three tested LAB strains, i.e., Lactococcus (Le.) lactis MG1363, L. plantarum WCFS1, and L. salivarius UCC118, only L. salivarius UCC118 potentiated the regulatory CD103+ DCs population in the small intestine Peyer’s patches of healthy mice (Smelt, de Haan, Bron, van Swam, Meijerink, Wells, Faas, et al. 2013). The intestinal site-dependent effects were illustrated by the observation that L. salivarius UCC118 increased the activated CD86+ DCs proportion in the small intestine Peyer’s patches but reduced the activated CD80+ DCs proportion in the small intestine LP (Smelt, de Haan, Bron, van Swam, Meijerink, Wells, Faas, et al. 2013).

LAB-primed DCs can polarize T lymphocytes and instruct the differentiation of naive T cells into divergent T cell subsets, subsequently priming distinctive immune responses (Figure 3) (Borchers et al. 2009). Of note, it was validated both in vitro and in vivo that LAB-polarized DCs can trigger the generation of regulatory T cells (Foligne et al. 2007; Lebeer, Vanderleyden, and De Keersmaecker 2008; Smelt et al. 2012; Smits et al. 2005). Similar to the aforementioned effects of LAB on DC responses, we also observed strain- and small and large intestinal dependent effects of LAB on T cell differentiations and responses in healthy mice (Smelt et al. 2012). Only one out of the tested LAB strains, i.e., L. plantarum WCFS1, specifically augmented regulatory T cell population in the spleen but not in the MLN (Smelt et al. 2012). For T helper (Th) cell subsets, we found that only L. salivarius UCC118 induced a decline in Th17 cell proportion in MLN but not in the spleen (Smelt et al. 2012). Those LAB strains with abilities of eliciting regulatory cell subsets are promising candidates for managing inflammatory disorders.

Aside from DC and T cells, B cells are another important lymphocyte subpopulation in adaptive immunity (Delcenserie et al. 2008). A large number of studies indicate that LABs alter IgA and IgG production by B-cells (Figure 3). IgA, a major antibody isotype in the mucosal system, is a vital player in mucosal defense against noxious agents (Bron, van Baarlen, and Kleerebezem 2012). Animal experiments disclosed that LAB strains such as L. casei CRL431, L. pentosus b240, L. delbrueckii ssp. bulgaricus OLL1073R-1, and L. rhamnosus GG enhanced production of total or antigen-specific IgA at both intestinal and respiratory level, and/or at a systemic level such as on serum and plasma values, which might be a principal mechanism involved in LAB-exerted protection against pathogenic microorganisms such as Salmonella Typhimurium, influenza virus, S. pneumoniae, and E. coli (de Moreno de LeBlanc, Castillo, and Perdigon 2010; Kobayashi et al. 2011; Nagai et al. 2011; Racedo et al. 2006; Shu and Gill 2002; Villena et al. 2005; Villena et al. 2006; Villena et al. 2005; Zhang et al. 2010). Consumption of L. pentosus b240 prior to influenza virus infection in mice reduced the viral titers in the lung and up-regulated specific IgA responses in BAL (Kobayashi et al. 2011). Further, regulation of adaptive antibody responses by LAB is commonly concomitant with modification of innate immunity such as phagocytosis (de Moreno de LeBlanc, Castillo, and Perdigon 2010; Racedo et al. 2006; Shu and Gill 2002; Villena et al. 2009; Villena et al. 2006; Villena et al. 2005). However, the systemic and mucosal IgA production induced by LAB such as L. rhamnosus GG and L. fermentum CECT5716 is...
different in healthy and diseased individuals (Malin et al. 1996; Olivares et al. 2007). Besides IgA, also IgG can be altered by the administration of LAB strains such as by *L. pentosus* b240, *L. rhamnosus* GG, and *L. casei* CRL431, which might be an important mechanism mediating the anti-pathogenic effects of these LAB strains (Kobayashi et al. 2011; Nagai et al. 2011; Racedo et al. 2006; Villena et al. 2009; Villena et al. 2006). In the aforementioned study investigating the protective potentials of *L. pentosus* b240 for influenza virus infection in mice, specific IgG levels in BAL were also enhanced by *L. pentosus* b240 treatment (Kobayashi et al. 2011).

**Molecular basis for gastrointestinal mucosa-LAB interaction**

The previous sections review the different host cellular levels at which LABs might influence host health. LABs with specific cell-wall components interact with specialized receptor on host cells. The molecules on LABs that interact with gastrointestinal mucosal cells are often referred to as LAB-derived microbe associated molecular patterns (MAMPs) and the receptors on host cells are referred to as pattern recognition receptors (PRRs) (Lebeer, Vanderleyden, and De Keersmaecker 2010). Below we will review the current insight in the MAMPs on LABs responsible for PRR-signaling in the host. Such knowledge is essential in designing effective bacteria-based strategies to manage health and disease.

**Toll-like receptor (TLR)–LAB interaction**

TLR is the most widely studied PRR family and is regarded as a canonical player mediating LAB-initiated signaling responses in the host (Bron, van Baarlen, and Kleerebezem 2012; Lebeer, Vanderleyden, and De Keersmaecker 2010). TLRs are expressed on a wide range of cell lineages within the gut including on intestinal epithelium, subepithelial stromal cell, and subepithelial immune cell such as DC, macrophage, T cell, and B cell (Cario 2005; Wells et al. 2011). Distinctive expression levels, distribution patterns as well as signaling specificities of TLRs in various cell types of different gastrointestinal compartments are strategically designed to maintain mucosal homeostasis (Wells et al. 2011). The binding of ligands to TLR recruits particular adaptor molecules such as myeloid differentiation primary-response protein 88 (MyD88), and subsequently evokes downstream signaling cascades (Akira and Takeda 2004; Lebeer, Vanderleyden, and De Keersmaecker 2010). Adaptor protein MyD88 engages in all the TLRs signaling except TLR4-mediated type I IFNs induction and TLR3 pathway (Akira and Takeda 2004).

TLR2 is identified as one major TLR responsible for initiating LAB-induced signaling response via recognizing cell surface components of LAB such as peptidoglycan (PGN), wall teichoic acid (WTA), and lipoteichoic acid (LTA) (Bron, van Baarlen, and Kleerebezem 2012). For fully priming downstream signaling cascades, TLR2 forms heterodimers with different co-receptors such as TLR1 and TLR6 and thereby triggers differential immune responses (van Bergenheegouwen et al. 2013). TLR2/TLR1 heterodimer recognizes triacyl lipopeptides (Akira and Takeda 2004), and was proposed to elicit pro-inflammatory responses (DePaolo et al. 2012; DePaolo et al. 2008). In contrast, TLR2/TLR6 heterodimer is responsible for ligating diacyl lipopeptides and LTA (Akira and Takeda 2004), and engages in mounting regulatory responses (DePaolo et al. 2012; DePaolo et al. 2008). Therefore, variations in the structural characteristics of LAB-derived TLR2 ligands may result in the activation of different TLR2 pathways. In our previous study, among a number of LAB strains of various species only 6 strains such as *L. acidophilus* CCFM137, *S. thermophilus* CCFM218, *L. fermentum* CCFM381, *L. fermentum* CCFM787, *L. plantarum* CCFM634, and *L. plantarum* CCFM734 were confirmed to activate TLR and to signal via TLR2/TLR6 pathway (Ren et al. 2016).

In addition to TLR2, it was shown that TLR5 transduced immune signals toward flagellin-producing LAB strains such as *L. ruminis* ATCC27782 via flagellin–TLR5 interaction (Neville et al. 2012). Moreover, TLR9 was defined to mediate the probiotic mixture VSL#3-elicited alleviation of colitis in mice via interaction with this mixture-derived unmethylated CpG DNA (Rachmilewitz et al. 2004). These findings suggest that LAB-derived flagellin or CpG DNA could independently interact with corresponding TLRs without the requirement of viable or intact bacterial cell context (Neville et al. 2012; Rachmilewitz et al. 2004).

**Other PRRs–LAB interaction**

Apart from TLRs, other PRRs such as intracellular nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and C-type lectin receptors (CLRs) can also mediate certain LAB strains-initiated signaling responses. Well-defined NLRs such as NOD1 and NOD2 are differentially expressed in the gut. NOD1 is expressed in multiple cell types comprising intestinal epithelium and DC, whereas absence of expression of NOD2 in intestinal epithelial cells was reported (Wells et al. 2011). NOD1 is known to recognize PGN-derived structural motif γ-D-glutamyl-mesodiaminopimelic acid of specific lactobacilli, while NOD2 senses PGN muramyl dipeptide in all microbes including on LABs (Bron, van Baarlen, and Kleerebezem 2012; Kozakova et al. 2016; Lebeer, Vanderleyden, and De Keersmaecker 2010). Notably, NOD2-PGN interplay was defined to be essential in immuno-stimulatory and anti-inflammatory functions of specific LAB strains (Fernandez et al. 2011; Foligne et al. 2007; Zeuthen, Fink, and Frokiaer 2008). For example, TLR2 and NOD2 were suggested to act in synergism to prime regulatory DCs in response to a *L. rhamnosus* strain (Lr32), resulting in amelioration of inflammatory responses (Foligne et al. 2007). Further, *L. casei* LOCK0919, *L. rhamnosus* LOCK0908, and *L. rhamnosus* LOCK0909 were shown to stimulate both TLR2 and NOD2 pathways (Kozakova et al. 2016). These findings also correlate with the broadly accepted view that multiple rather than single PRR–MAMP
interactions dictate the ultimate tune of immune responses toward specific LAB (Lebeer, Claes, and Vanderleyden 2012).

LAB were reported to signal through DC-SIGN to confer inflammation-inhibiting effects (Konstantinov et al. 2008; Smits et al. 2005). DC-SIGN, a receptor of CLR family, can respond to carbohydrate structures on microbial cells (Konstantinov et al. 2008). DC-SIGN is primarily expressed on DCs, and is a key mediator in DC responses to environmental stimuli (Konstantinov et al. 2008). LAB strains from the species L. reuteri and L. casei were verified to ligate DC-SIGN, thereby modulating DC function to promote regulatory T cell responses (Smits et al. 2005). Moreover, S layer protein A of probiotic L. acidophilus NCFM was reported to drive the development of tolerogenic DC subtype via binding to DC-SIGN (Konstantinov et al. 2008).

**PRRs-LAB crosstalk and strain-dependency of LAB-mediated regulation**

Species- and strain-specific functional properties of LAB have been described in many studies (Cruchet et al. 2003; Sgouras et al. 2005; Yang et al. 2015; Youn et al. 2012). From the perspective of molecular communication of LAB with host gut mucosa, this species- and strain-specificity of LAB may partially arise from the distinctions in MAMPs expression on LAB cells, which result in different actions of LAB on PRR signaling pathways (Lebeer, Claes, and Vanderleyden 2012; Lee et al. 2013). PGN and TA (especially LTA), have been characterized as pivotal determinants for disparate cellular responses elicited by different LAB strains (Lebeer, Claes, and Vanderleyden 2012; Lee et al. 2013). Despite shared conserved structural features, slight structural differences of PGN or TA among diverse LAB strains give rise to varying functional activities of LAB (Fernandez et al. 2011; Lee et al. 2013; Smelt, de Haan, Bron, van Swam, Meijerink, Wells, Kleerebezem, et al. 2013). Anti-inflammatory effects conferred by L. salivarius Ls33 but not L. acidophilus NCFM was due to the existence of additional muropeptides in PGN structure of strain Ls33 (Fernandez et al. 2011). Subtle structural mutations of LTA in LAB cells such as switch from D-Ala to D-glucose substitution, deletion of D-Ala substitution and D-alanine esters, and LTA deletion have been shown to reverse immunomodulatory performances of LAB strains (Claes et al. 2010; Grangette et al. 2005; Smelt, de Haan, Bron, van Swam, Meijerink, Wells, Kleerebezem, et al. 2013). Our previous study showed that D-Ala substitution is vital for both the pro- and anti-inflammatory properties of L. plantarum WCFS1 in mice (Smelt, de Haan, Bron, van Swam, Meijerink, Wells, Kleerebezem, et al. 2013).

Previous studies showed that specific structural components separated from LAB cells were able to activate PRR (particularly TLR) signaling pathways. Moreover, some of these “independent components” interact with TLRs differently from when they are in the context of intact LAB cells (Kaji et al. 2010). This can be attributed to structural properties of LAB cells (i.e. spatial organization of cell surface components) that can influence the spatial accessibility of specific cell components to PRRs when they are in intact LAB cell context (Kaji et al. 2010). In addition, interactions between diverse PRRs triggered by different LAB ligands determine the final cellular host-response to LAB (Foligne et al. 2007; Kaji et al. 2010). This is also the reason why combinations of different LAB strains or their derived ligands may elicit different cytokine responses (Christensen, Frøkiaer, and Pestka 2002; Kaji et al. 2010). A better understanding of these molecular interactions is crucial for targeted modulation of host immune functions and defining effective mixtures of multiple strains or their derived bacterial ligands.

Aside from complexity in biochemical or conformational characteristics of LAB cell components, secreted metabolites or bioactive proteins from LAB strains can also mediate host-LAB interplay and contribute to strain-specific effects (Ren et al. 2020; Tao et al. 2006; Voltan et al. 2008; Wang et al. 2014; Yan et al. 2007). For instance, a L. crispatus strain-released hydrogen peroxide regulated PRR expression levels via activating peroxisome proliferator activated receptor-γ signaling (Voltan et al. 2008).

**Do LABs have to be alive to confer beneficial effects**

It has been shown that growth conditions and phase of harvesting, that is, log or stationary phase, of administered LAB affects the composition of the LAB cell surface and thereby with the presence of MAMPs. As a consequence this impacts the final effects of LAB on the host (van Baarlen et al. 2009). Some strains such as L. gasseri TMC0356, L. brevis SBC8803, and L. casei CRL431 do not even have to be alive when administered when they carry the beneficial ligands on their cell wall (Kawase et al. 2010; Kawase et al. 2012; Ueno et al. 2011; Villena et al. 2009; Villena et al. 2005). However, for other strains such as L. johnsonii La1, L. plantarum 299v, and L. rhamnosus, gastrointestinal transit tolerance and viability in vivo can impact their specific regulatory activities (Cruchet et al. 2003; Dykstra et al. 2011; Youn et al. 2012). For those strains whose viability is a determining factor in their clinical effects, direct interactions of host cells with intact bacterial cells or/and their produced functional metabolites in vivo probably are prerequisites for their beneficial effects. For some strains whose intracellular molecules such as CPG DNA-evoked signaling responses are parts of mechanisms of their actions, bacterial viability can also influence their effects since their intracellular ligands can only be released following lysis of bacterial cells and subsequently become accessible to PRRs expressed on host cells. Taken together, the impact of LAB survival ability on their modulatory effects in the host needs to be addressed for individual LAB strains.

Postbiotics is a new, emerging term proposed to define beneficial effects of microbial fermentation products as well as enhanced generation of supporting microbial cell components following bacterial lysis (Wegh et al. 2019). Previous findings have suggested that apart from probiotic bacteria the formation of postbiotic compounds such as organic
acids, enzymes, exopolysaccharides, and bacterial cell lysates may provide additional physiological benefits (Aguilar-Toalá et al., 2018; Ren et al. 2020; Verkhnyatskaya et al. 2019). Therefore, postbiotic compounds with bioactive properties may offer novel options for the development of nutraceutical products, dietary supplements, or functional foods. When compared to postbiotic products, traditional probiotic products require a more stringent control of manufacturing, transport, and storage to ensure optimal bacterial viability in products. Postbiotics probably suffer less from this and may also overcome the potential adverse effects of viable probiotic bacteria when applied in highly vulnerable populations such as premature babies and immunocompromised individuals, which endows postbiotics with a more favorable safety profile (Wegh et al. 2019). Nevertheless, considering the distinctive biochemical properties of different postbiotic compounds, the manufacturing process and storage methods of postbiotic products should be carefully optimized to achieve desirable functional efficacy.

**Health promotion and disease control by LABs**

**Efficacy of LABs in pathogenic infections**

In the previous sections, we have described the mechanisms by which LAB can contribute to reduction of pathogenic infections. Crucial mechanisms are regulation of gut microbiome, production of antibacterial metabolites or bacteriocides, inhibition of pathogen adhesion to gut mucosa, suppression of virulent factors, reinforcement of gut barrier function, and enhancement of host immunity. Proof of principle of administration of LABs to manage pathogenic disease have been shown for pathogenic bacteria such as *H. pylori* (Sgouras et al. 2004; Wang et al., 2004), *Salmonella* (Wu et al. 2018; Yang et al. 2017) and *E. coli* (Wang et al. 2018; Zhang et al. 2010), but also against viral infections such as influenza virus (Hori et al. 2001, 2002; Yasui, Kiyoshima, and Hori 2004) (Table 1). These studies will be reviewed below.

**Efficacy of LABs in reducing *H. pylori* gastric infection**

*Helicobacter pylori* is a pathogen affecting the stomach, and has been linked with enhanced chances to develop chronic gastritis, peptic ulcer disease, and gastric cancer (Kabir et al. 1997; Wang et al., 2004). Different in vitro experiments and in vivo animal experiments have been performed to select LAB strains that effectively suppress or prevent *H. pylori* infection. An effective strain was *L. salivarius* WB1004 that showed inhibitory capability on the adhesion of *H. pylori* to both murine and human gastric epithelial cells as well as on IL-8 induction in vitro (Kabir et al. 1997). This *L. salivarius* strain weakened colonization of *H. pylori* in the stomach in a gnotobiotic mouse model and by that prevented but also expedited recovery from *H. pylori* infection (Kabir et al. 1997). Moreover, postinfection administration of *L. gasseri* OLL2716 in *H. pylori*-infected germ-free mice effectively dampened the colonization of clarithromycin-resistant *H. pylori* (Ushiyama et al. 2003). Probiotic *L. casei* Shirota was also reported to exert long-term beneficial effects against *H. pylori* colonization and attenuated the associated gastritis (Sgouras et al. 2004). A mechanistic study showed that *L. johnsonii* La1 ameliorated *H. pylori*-induced gastritis probably through dampening proinflammatory chemotactic responses and attenuating subsequent intramuscular infiltration of lymphocytes and neutrophils (Sgouras et al. 2005).

In addition to experimental animal studies, LABs have also been tested in the fight against *H. pylori* in clinical trials (Cruchet et al. 2003; Francavilla et al. 2008; Gotteland et al. 2008; Hauser et al. 2015; Wang et al., 2004) (Table 1). Six-week consumption of a commercial yoghurt containing *L. acidophilus* La5 and *B. lactis* Bb12 by patients with *H. pylori* infection significantly reduced *H. pylori* density (Wang et al., 2004). Cruchet et al. (2003) showed that also *L. johnsonii* La1 has such an effect in children and demonstrated that it is important to administer this strain alive. Besides, probiotic LAB strains have been applied as an adjunct treatment to support the conventional antibiotics therapy against *H. pylori* infection and to ameliorate the side effects of antibiotic treatment (Francavilla et al. 2008; Hauser et al. 2015). Improvement of *H. pylori* eradication rate and alleviation of antibiotic therapy-associated adverse effects by a strain mixture of *L. rhamnosus* GG and *B. lactis* Bb12 were validated (Hauser et al. 2015). However, *L. reuteri* ATCC55730 intervention did not enhance the *H. pylori* eradication rate with antibiotic therapy in *H. pylori*-infected patients, but four-week treatment of this *L. reuteri* strain prior to antibiotic therapy decreased *H. pylori* density and alleviated *H. pylori*-induced gastrointestinal symptom (Francavilla et al. 2008). This illustrates species and strain dependent efficacy of LAB and the differences in preventing infection and contributing to enhanced clearance of the pathogens. As outlined in the preceding sections this is probably due to the different mechanism by which different LAB strains and species contribute to anti-pathogenic effects.

**Efficacy of LABs in reducing enteric *Salmonella* infection**

Another enteropathogen in which efficacy of LAB against infection and symptoms has been shown is *Salmonella*. Experimental animal studies examining the effects of LAB in fighting *Salmonella* infection exploited oral infection models, and mainly focused on *Salmonella* colonization in the gut (Abatemarco Júnior et al. 2018; He et al. 2019; Mian et al. 2016; Wu et al. 2018; Yang et al. 2017; Yu et al. 2017), *Salmonella* invasion into visceral organs (liver and spleen) (Abatemarco Júnior et al. 2018; He et al. 2019; Zhang et al. 2018), and local/systemic immunity against *Salmonella* (Abatemarco Júnior et al. 2018; Mian et al. 2016; Yang et al. 2017; Zhang et al. 2019) (Table 1). In many studies on efficacy of LABs against *Salmonella* infection administration of LAB prior to infection of animals was applied. Administration of *L. rhamnosus* strain GG before *Salmonella* challenge was shown to effectively reduce *Salmonella* colonization and translocation in weaned piglet models (Yang et al. 2017; Yu et al. 2017; Zhang et al. 2019; Zhang et al. 2018). Anti-*Salmonella* efficacy of this well-recognized probiotic strain may be ascribed to its role in inhibiting

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**Table 1**

| Study | Conditions | Outcome |
|-------|------------|---------|
| Cruchet et al. 2003 | Six-week consumption of a commercial yoghurt containing *L. acidophilus* La5 and *B. lactis* Bb12 | Reduced *H. pylori* density |
| Francavilla et al. 2008 | | |
| Gotteland et al. 2008 | | |
| Hauser et al. 2015 | | |
| Wang et al., 2004 | Six-week consumption of a commercial yoghurt containing *L. acidophilus* La5 and *B. lactis* Bb12 | Reduced *H. pylori* density |
| Cruchet et al. 2003 | | |
| Francavilla et al. 2008 | | |
| Hauser et al. 2015 | | |
| Wang et al., 2004 | | |
| Abatemarco Júnior et al. 2018 | | |
| He et al. 2019 | | |
| Mian et al. 2016 | | |
| Wu et al. 2018 | | |
| Yang et al. 2017 | | |
| Yu et al. 2017 | | |
| Abatemarco Júnior et al. 2018 | | |
| He et al. 2019 | | |
| Zhang et al. 2018 | | |
| Zhang et al. 2019 | | |
| Zhang et al. 2019 | | |
### Table 1. Effects of LAB on pathogenic infection in experimental animals and humans.

| Target pathogen | Bacterial strains tested; administration route; live/dead bacteria | Study design; timing, dose, and duration of LAB administration | Main findings | Reference |
|-----------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------|-----------|
| *Helicobacter (H.) pylori* | *Lactobacillus (L.) salivarius* WB1004; Oral inoculation; | GF BALB/c mice; Pre-infection: one inoculation of $5 \times 10^7$ CFU; Post-infection: $1 \times 10^8$ CFU/day for 3 days + $1 \times 10^6$ CFU/week for 3 weeks; | Pre- and post-infection: reduced *H. pylori* colonization in the stomach; reduced *Salmonella* colonization in the stomach; | Kabir et al. (1997) |
| *H. pylori* | *L. gasseri* OLL2716; Oral inoculation; *L. casei* Shirota; Oral inoculation; | GF BALB/c mice; Post-infection: $1 \times 10^7$ CFU/week for 4 weeks; SPF C57BL/6 mice, n = 25/group; Post-infection: $1 \times 10^8$ CFU/ml in the drinking water for 9 months; | Reduced *H. pylori* colonization in the stomach; reduced *Salmonella* colonization in the stomach; | Ushiyama et al. (2003) Sgouras et al. (2004) |
| *H. pylori* | *L. johnsonii* La1; Oral inoculation; | SPF C57BL/6 mice, n = 15/group; Post-infection: $1.5 \times 10^8$ CFU/day for 3 months; | No effects on *H. pylori* colonization in the stomach; Decreased lymphocytic and neutrophilic infiltration in gastric LP; Downregulated serum anti-*H. pylori* IgG levels; | Sgouras et al. (2005) |
| *H. pylori* | *L. acidophilus* La5 + *Bifidobacterium (B.) lactis* Bb12; Oral consumption; | H. *pylori*-positive adults, n = 59; $\geq 4.6 \times 10^7$ CFU/strain/day for 6 weeks; | Promoted *H. pylori* eradication (decreased *H. pylori* urease activity; UBT); | Wang et al. (2004) |
| *H. pylori* | *L. johnsonii* La1; Oral consumption; heat-killed/live bacteria; | H. *pylori*-positive children, n = 252; DB, randomized; $\geq 1.6 \times 10^7$ CFU/day for 4 weeks; | Only live LAB reduced *H. pylori* colonization (UBT); | Cruchet et al. (2003) |
| *H. pylori* | *L. rhamnosus* GG + *B. lactis* Bb12; Oral consumption; | H. *pylori*-positive adults with antibiotic therapy, n = 804; DB, randomized, PCT; $2 \times 10^7-2 \times 10^8$ CFU/day for 2 weeks; | Increased *H. pylori* eradication rate; Improved antibiotic therapy-related side effects; | Hauser et al. (2015) |
| *H. pylori* | *L. reuteri* ATCC55730; Oral consumption; | H. *pylori*-positive dyspeptic adults, n = 40; DB, randomized, PCT; Before antibiotic therapy: $10^8$ CFU/day for 4 weeks; | Decreased *H. pylori* colonization (UBT, HpSA test); No effects on *H. pylori* eradication rate; Alleviated dyspeptic/infection-related gastrointestinal symptoms; | Francavilla et al. (2008) |
| *Salmonella (S.) enterica* Serovar 4,[5],12:i:- | *L. rhamnosus* GG; Oral inoculation; | Newly weaned piglets, n = 7/group; Pre-infection: $1 \times 10^8$ CFU/day for 2 weeks; | Reduced *Salmonella* colonization in the jejunum mucosa; Alleviated infection-induced diarrhea and gut inflammation (reduced intestinal expression of CCL20 and IL-7RA); Enhanced mucosal anti-*Salmonella* immune response (increased expansion of CD4+ T-bet+ IFN$^+$ T cells in PB, increased intestinal T-bet expression, activated IL-22BP/IL-22/STAT3 pathway); | Yang et al. (2017) |
| *S. Infantis* | *L. rhamnosus* GG; Oral inoculation; | Newly weaned piglets, n = 6/group; Pre-infection: $1 \times 10^8$ CFU/day for 1 week; | LGG reduced infection-induced disturbance to ileal mucosal microbiota; LGG upregulated CD3 CD19 T-bet+ IFN$^+$ and CD3 CD19 T-bet+ IFN$^+$ cell populations in PB while reduced these two cell subsets in ileum; | Zhang et al. (2019) |
| *S. Infantis* | *L. johnsonii* LS31; Oral inoculation; | Newly weaned piglets, n = 6/group; Pre-infection: $1 \times 10^8$ CFU/day for 1 week; | Decreased *Salmonella* translocation to liver; Modulated the composition of ileal microbiome; Attenuated infection-induced autophagy of ileal epithelial cells via stimulating EGFR/Akt signaling pathway; | Zhang et al. (2018) |
| *S. Infantis* | *L. johnsonii* LS31; Oral inoculation; | | Inhibited *Salmonella* colonization in colon and jejunum; Decreased *Salmonella* translocation to spleen; Accelerated *Salmonella* clearance; | He et al. (2019) |
| Target pathogen                  | Bacterial strains tested; administration route; live/dead bacteria | Study design; timing, dose, and duration of LAB administration | Main findings                                                                 | Reference                           |
|---------------------------------|---------------------------------------------------------------------|-----------------------------------------------------------------|--------------------------------------------------------------------------------|-------------------------------------|
| S. Typhimurium                  | *L. acidophilus* ATCC4356; Oral inoculation;                         | SPF C57BL/6 mice, *n* = 8/group; Pre-infection: \(1 \times 10^6\) CFU every other day for 1 month; | Restored *Salmonella*-induced reduction in intestinal SCFA levels;            | Wu et al. (2018)                    |
|                                 |                                                                     | Conventional BALB/c mice, *n* = 10/group; 10^7 CFU/ml in drinking water starting 7 days before infection until 28 days after infection; | Ameliorated colitis and colonic crypt hyperplasia; Reduced *Salmonella*-induced decline in colonic goblet cell numbers and MUC2 mRNA levels; | Abatemarco Júnior et al. (2018)    |
| S. Typhimurium                  | *L. acidophilus* Rosell-52; Oral inoculation;                        |                                                                     | Conventional mice: increased survival rate;                                    |                                     |
|                                 |                                                                     |                                                                      | GF mice: reduced fecal *Salmonella* counts and *Salmonella* translocation to liver; alleviated ileal mucosal lesions; upregulated intestinal total IgA levels and Kupffer cell expansion in liver; enhanced hepatic IL-10 and TGF-β mRNA levels; |                                     |
| S. Typhimurium                  | *L. fermentum* 299v; Oral inoculation;                              | NIH mice, *n* = 13/treat group; Post-infection: 2.5 \(\times 10^6\) CFU/day with/without ofloxacin (20 mg/kg/day) for 8 days; | LAB + ofloxacin treatment: eradicated *Salmonella* from ileum, liver, and blood; inhibited granulomas development in liver and spleen; downregulated lipid peroxides levels in ileal mucosa; | Trususalu et al. (2008)             |
| Salmonella                      | *L. acidophilus*; Oral consumption;                                 | Participants (3–73 years old), *n* = 292; Consumed *L. acidophilus*-containing milk; | Accelerated *Salmonella* eradication;                                             | Alm (1983)                          |
| Salmonella                      | *L. plantarum* 299v; Oral consumption;                              | Symptomatic patients with *Salmonella* infection (5–68 years old), *n* = 149; DB, randomized, PCT; 5 \(\times 10^6\) CFU/day; | No acceleration of *Salmonella* clearance; No alleviation of post-infectious symptoms; | Lönnemark et al. (2015)             |
| *Escherichia* (E.) coli          | *L. rhamnosus* HN001; Oral inoculation;                             | BALB/c mice, C57BL/6 mice; 3 \(\times 10^7\) CFU/g in diet starting 1 week before infection until 1 week after infection; | Downregulated cumulative morbidity and *E. coli* translocation rate; Enhanced intestinal anti-*E. coli* IgA levels and phagocytic activity of blood leukocytes; | Shu and Gill (2002)                 |
| *E. coli*                       | *L. reuteri* HCM2; Oral inoculation;                                | SPF ICR mice, *n* = 5/group; Pre-infection: \(10^8\) CFU/day for 2 weeks; | Decreased *E. coli* colonization in jejunum; Mitigated *E. coli*-induced intestinal morphological damage; Maintained the relative abundance of dominant colonic bacteria; | Wang et al. (2018)                  |
| *E. coli*                       | *L. reuteri* TMW1.656, *L. reuteri* LTH5794; Oral inoculation;       | Weaning piglets, *n* = 6/group; *L. reuteri*-fermented feeds for 3 weeks; | Decreased intestinal *E. coli* colonization;                                     | Yang et al. (2015)                  |
| *E. coli*                       | *L. rhamnosus* GG; Oral inoculation;                                | Weaned piglets, *n* = 6/group; Pre-infection: \(10^9\) CFU/day for 1 week; | Decreased diarrhea rate and fecal coliform bacterial numbers; Enhanced total IgA in jejunum and ileum; Decreased serum IL-6 and IL-1β levels; Elevated fetal lactobacilli and bifidobacteria numbers; | Zhang et al. (2010)                 |
| *E. coli*                       | *L. acidophilus* NPS1; Oral inoculation;                            | Feedlot cattle, *n* = 448; \(10^6\) CFU/day; | Over a 2-year period, decreased *E. coli* fecal shedding;                      | Peterson et al. (2007)              |
| *E. coli*                       | *L. helveticus* Rosell-52 + *L. rhamnosus* Rosell-11 + *B. longum* Rosell-175 + *Saccharomyces cerevisiae var boulardii* CNCM I-1079; Oral consumption; | Healthy adult males, *n* = 30/group; DB, randomized, PCT; \(1 \times 10^{10}\) CFU/strain/day for 2 weeks before infection and for 2 weeks after infection; | No improvement of *E. coli*-triggered symptoms; No effects on fecal *E. coli* numbers; | Ten Bruggencate et al. (2014)       |
| *E. coli*                       | *L. reuteri* DSM17938; Oral consumption;                            | Infants with colic (< 3-month old), *n* = 167; DB, randomized, PCT; \(1 \times 10^8\) CFU/day for 1 month; | No effects on intestinal *E. coli* colonization;                                 | Sung et al. (2014)                  |
| Streptococcus (S.) pneumoniae    | *L. casei* CRL431; Oral inoculation;                               | Adult Swiss albino mice; Pre-infection: \(10^6\) CFU/day for 2/5/7 days; | 2 days of pretreatment exerted the most anti-infective effects (reduced *S. pneumoniae* counts in lung; enhanced levels of anti-pneumococcal serum IgG and BAL IgA); 2 days of pretreatment reduced lung inflammation, upregulated | Racedo et al. (2006)                |
### Table 1. Continued.

| Target pathogen | Bacterial strains tested; administration route; live/ dead bacteria | Study design; timing, dose, and duration of LAB administration | Main findings | Reference |
|-----------------|---------------------------------------------------------------------|----------------------------------------------------------------|--------------|-----------|
| *S. pneumoniae* | *L. casei* CRL431; Oral inoculation;                              | Malnourished Swiss albino mice, n = 5–6/group; Pre-infection: 10^9 CFU/day for 2/7/14/21 days; | LAB treatment facilitated the normalization of anti-infectious immunity; 2 days of pretreatment enhanced *S. pneumoniae* eradication from blood, mitigated lung injury, upregulated anti-pneumococcal IgA in BAL, and increased the number of leukocytes and neutrophils in blood and BAL; | Villena et al. (2005) |
| *S. pneumoniae* | *L. casei* CRL431; Intranasal inoculation, heat-killed/live LAB;   | Malnourished Swiss albino mice, n = 5–6/group; Pre-infection: 10^9 CFU/day for 2 days; | Both live and dead bacteria decreased *S. pneumoniae* counts in blood and lung, enhanced anti-pneumococcal antibodies in BAL (IgG, IgA) and serum (IgG), and increased the number and phagocytic activity of blood phagocytes; | Villena et al. (2009) |
| *S. pneumoniae* | *L. fermentum*; Intranasal inoculation;                           | Adult BALB/c mice; Pre-infection: 10^7 CFU/time every 12 h for 4 times; | Reduced *S. pneumoniae* counts in respiratory tract; Increased serum anti-pneumococcal antibodies; Elevated activated macrophage numbers in lung and lymphocytes in tracheal LP; | Cangemi de Gutierrez, Santos, and Nader-MacÃAs (2001) |
| *S. pneumoniae* | *L. pentosus* b240; Oral inoculation (heat-killed LAB in lyophilized power); | SPF CBA/J mice; Pre-infection: 12.5 mg/kg/day for 3 weeks; | Increased survival rate; Reduced *S. pneumoniae* counts in lung; Alleviated pulmonary inflammation and decreased inflammatory mediator (TNF-α, IL-6, MIP-2) levels in lung; | Tanaka et al. (2011) |
| *S. pneumoniae* | *L. rhamnosus* GG + *Bifidobacterium sp* B420 + *L. acidophilus* 145 + *S. thermophilus*; Oral consumption; | Healthy subjects, n = 209; *L. rhamnosus* GG (7.1 × 10^9 CFU/day) + *Bifidobacterium sp* B420 (8.4 × 10^9 CFU/day) + *L. acidophilus* 145 (3.2 × 10^9 CFU/day) + *S. thermophilus* (2.7 × 10^10 CFU/day) for 3 weeks; | Decreased nasal *S. pneumoniae* colonization; | Glück and Gebbers (2003) |
| *S. pneumoniae* | *L. casei* Shirota; Oral consumption;                             | Healthy adult males with low NK cell activity, n = 72; DB, randomized, PCT, 1.95 × 10^9 CFU/day for 4 weeks; | No effects on nasopharyngeal *S. pneumoniae* colonization; | Franz et al. (2015) |
| Influenza virus | *L. casei* Shirota; Intranasal inoculation, heat-killed LAB in lyophilized power; | BALB/c mice; Pre-infection: 20/200 µg/day for 3 days; | 200 µg/day treatment increased survival rate, downregulated influenza viral titer in upper respiratory tract, and enhanced production of IL-12, IFN-γ and TNF-α by MLN cells; | Hori et al. (2001) |
| Influenza virus | *L. casei* Shirota; Oral inoculation (heat-killed LAB in lyophilized power); | Aged BALB/c mice; Pre-infection: 0.05% (wt/wt) in diet for 4 months; | Decreased influenza viral titer in nasal washings; Enhanced NK cell activity of splenocytes and pulmonary cells; Upregulated IFN-γ and TNF-α production levels by nasal lymphocytes; | Hori et al. (2002) |
| Influenza virus | *L. casei* Shirota; Oral inoculation;                             | Neonatal and infant BALB/c mice; Pre-infection: 10^9.6 CFU/time for 17 times within 3 weeks; | Reduced influenza viral titer in nasal washings; Augmented pulmonary NK cell activity and IL-12 production by MLN cells; | Yasui, Kiyoshima, and Hori (2004) |
| Influenza virus | *L. rhamnosus* GG; Intranasal inoculation (heat-killed LAB in lyophilized power); | BALB/c mice; Pre-infection: 200 µg/day for 3 days; | Improved survival rate and accumulative symptom rate; Enhanced NK cell activity and mRNA expression levels of cytokines and chemokines (IL-1β, TNF-α, and MCP-1) in lung; | Harata et al. (2010) |
| Influenza virus | *L. gasseri* TM0356; Oral inoculation (heat- | | Reduced pulmonary influenza viral titer; | Kawase et al. (2012) |
infection-induced gut microbial changes (Zhang et al. 2019; Zhang et al. 2018), in enhancing intestinal epithelial barrier function (Zhang et al. 2018), and in fortifying innate/adaptive immunity against Salmonella (Yang et al. 2017; Zhang et al. 2019). Moreover, other LAB strains such as L. johnsonii L531 and L. acidophilus ATCC4356 were shown to restore declined SCFA production and to suppress goblet cell depletion during Salmonella infection (He et al. 2019; Wu et al. 2018), which are other possible mechanisms involved in the anti-Salmonella actions of LABs. Prophylactic efficacy of LAB against Salmonella infection was also studied and confirmed in both conventional and germ-free mice by Abatemarco J (2018).

Furthermore, the supportive effects of LAB on antibiotic therapy against Salmonella infection was also demonstrated (Trusulau et al. 2008). For example, supplementation of L. fermentum ME-3 with the antibiotic ofloxacin achieved complete Salmonella eradication in the liver, serum, and ileum of mice (Trusulau et al. 2008). Clinical efficacy of LAB against Salmonella infection was evaluated by Alm (1983), who observed reduced Salmonella colonization in Salmonella carrying patients by consumption of a L. acidophilus strain. However, another strain L. plantarum 299 v was shown to be ineffective in combating infection in Salmonella-infected patients (Lönnermark et al. 2015).

**Efficacy of LABs in reducing enteric E. coli infection**

Multiple animal infection models were used to study potential beneficial effects of LAB in fighting E. coli infection in the gut (Shu and Gill 2002; Wang et al. 2018; Zhang et al. 2010) (Table 1). Shu and Gill found that both pre- and post-infection administration of probiotic L. rhamnosus HN001 (DR20™) effectively decreased E. coli-induced morbidity in mice and reduced E. coli invasion to blood, liver as well as to spleen (Shu and Gill 2002). These beneficial effects might be attributed to strengthened humoral and cellular immunity by LAB supplement, which initiated stronger enteric specific IgA responses and phagocytosis in blood leucocytes (Shu and Gill 2002). Another study evaluated anti-E. coli efficacy of L. reuteri HCM2 in mice and observed that E. coli colonization and infection-induced inflammation in jejunum were dampened by this strain (Wang et al. 2018). Maintaining gut microbiome stability was suggested as one possible mechanism that mediated the anti-E. coli effects of L. reuteri HCM2 (Wang et al. 2018). Moreover, in post-weaning piglets alleviation of E. coli infection was achieved by supplementation of L. rhamnosus GG and reuteran-producing L. reuteri TMW1.656 (Yang et al. 2015; Zhang et al. 2010). In addition, effectiveness of L. acidophilus strains against E. coli infection was confirmed in feedlot cattles (Peterson et al. 2007).

In human trials the clinical efficacy of LAB against E. coli invasion was also evaluated (Ten Bruggencate et al., 2014) but to the best of our knowledge was not successful up to now. In healthy subjects, a mixture of L. helveticus Rosell-52, L. rhamnosus Rosell-11, B. longum ssp. longum Rosell-175, and Saccharomyces cerevisiae var boulardii CNCM I-1079 was shown to be ineffective in reducing the colonization of enterotoxigenic E. coli in the intestine and attenuating E. coli challenge-induced increased fecal output (Ten Bruggencate et al., 2014). Moreover, in infants with colic L. reuteri DSM17938 did not inhibit gut E. coli colonization (Sung et al. 2014).

**Efficacy of LABs in avoiding respiratory S. pneumoniae infection**

Streptococcus pneumoniae is one of the most common respiratory pathogens and the major cause of pneumococcal infectious disease (Villena et al. 2011). Pneumococcal infections still have a high occurrence and mortality rate despite currently available prophylactic and therapeutic approaches (Villena et al. 2011). Because of this low efficacy of current therapeutic approaches there have been many efforts to reduce S. pneumoniae infection with LABs. There is substantial evidence from animal experiments that specific LAB strains are capable to stimulate host innate and adaptive immunity against S. pneumoniae infection (Agüero et al. 2006; Racedo et al. 2006; Villena et al. 2009; Villena et al. 2005) (Table 1). Protective capabilities of L. casei CRL431 have been systemically evaluated in various murine pneumococcal infection models with different supplementation schemes (Racedo et al. 2006). Different pretreatment durations (2, 5, and 7 days) of L. casei CRL431 against S. pneumoniae challenge were tested (Racedo et al. 2006). Interestingly, it was found that a short duration of 2 days of LAB intake prior to S. pneumoniae-infection conferred the most effective protection in mice. This was illustrated by a more declined pneumococcal density as well as enhanced phagocytosis in lung, and increased specific IgG and IgA antibodies titers in serum and BAL compared with 5 and
The effectiveness of *L. casei* CRL431 in potentiating host immune defense against *S. pneumoniae* was also evaluated in malnourished mice (Agüero et al. 2006; Villena et al. 2009; Villena et al. 2005). These mice were subjected to protein-free diet for 21 days after weaning (Villena et al. 2005). After pneumococcal challenge, the mice were fed with live *L. casei* CRL431 and received protein containing diet again. The mice with a diet containing *L. casei* CRL431 had a faster recovery of impaired defense immunity caused by nutritional deficiency, a higher pathogen eradication rate, improved tissue damage in lung, and up-regulated phagocytosis of blood and BAL neutrophils (Villena et al. 2005). Similar beneficial effect on *S. pneumoniae* infection was obtained by intranasally administration of malnourished mice with either alive or heat-killed *L. casei* CRL431 (Villena et al. 2009). Since crosstalk between inflammation and coagulation processes play an important role in infection (Agüero et al. 2006), the influence of LAB treatment on inflammation and coagulation processes using the same *L. casei* CRL431 intervention strategy (Villena et al. 2005) was studied in a *S. pneumoniae* infection-caused pneumonia model without inducing sepsis (Agüero et al. 2006). It was observed that *L. casei* CRL431 supplementation normalized IL-4 production, heightened IL-10 levels in BAL, and improved blood coagulation (Agüero et al. 2006). Additionally, malnourished mice with dietary supplementation of yoghurt containing *L. bulgaricus* CRL423 and *S. thermophilus* CRL412 exhibited a quicker regaining of defective immune functions against pneumococcal infection (Villena et al. 2006). Furthermore, protective potentials of *L. fermentum* and *L. pentosus* were also demonstrated in *S. pneumoniae*-infected healthy mice (Cangemi de Gutierrez, Santos, and Nader-MacAAs 2001; Tanaka et al. 2011).

The efficacy of LABs in reducing respiratory colonization of *S. pneumoniae* was tested in clinical trials (Franz et al. 2015; Glück and Gebbers 2003). Three-weeks of consumption of a probiotic drink containing *L. acidophilus*, *L. rhamnosus* GG, *S. thermophilus*, and *Bifidobacterium* sp diminished the incidence of *S. pneumoniae* in the nasal microbiota of healthy participants (Glück and Gebbers 2003). However, it was shown that supplementation of *L. casei* Shirota could not decrease the pharyngeal colonization of *S. pneumoniae* in healthy subjects with low NK cell functions (Franz et al. 2015).

**Efficacy of LABs in reducing viral infections induced by influenza virus**

Influenza virus caused acute respiratory infection is characterized by high morbidity and fatality worldwide especially in susceptible subjects such as children and elderly (Kobayashi et al. 2011). Present anti-influenza treatments were not efficacious enough owing to rapid viral mutations, thus demanding novel generic treatment strategies (Nagai et al. 2011). LAB strains with recognized properties of strengthening host unspecific innate immunity gain extensive research interests for protecting hosts from influenza virus infection. Anti-influenza potential of probiotic *L. casei* Shirota has been explored in mice models of different ages (Hori et al. 2001, 2002; Yasui, Kiyoshima, and Hori 2004). Intranasal inoculation of adult mice with *L. casei* Shirota prior to influenza viral challenge reduced viral density in the respiratory tract and up-regulated survival rate of infected mice, which probably resulted from *L. casei* Shirota-induced Th1 cytokine responses (Hori et al. 2001). In both infant and elderly mice, prophylactic oral supplementation of *L. casei* Shirota was also shown to boost respiratory innate immunity and to protect mice against viral infection (Hori et al. 2002; Yasui, Kiyoshima, and Hori 2004). Mice administrated with probiotic *L. rhamnosus* GG via the intranasal route before influenza infection displayed improved general health during the influenza infection period (Harata et al. 2010). Moreover, *L. gasseri* TMC0356 was shown to confer beneficial effects against influenza (Kawase et al. 2010; Kawase et al. 2012). The importance of viability and inoculation route of *L. rhamnosus* for efficacy of influenza management was investigated by Youn et al. (2012), who reported that intranasal administration of live *L. rhamnosus* provided the most protection. In a clinical trial the supportive potential of *L. fermentum* CECT5716 derived from human breast milk on anti-influenza vaccination was examined (Olivares et al. 2007). It was found that 2-week consumption of this *L. fermentum* strain before and after vaccination enhanced NK cell frequencies, augmented specific IgA responses, and decreased the occurrence of influenza-like illness during a 5-month post-vaccination period (Olivares et al. 2007).

**Gastrointestinal inflammatory disorders**

Inflammatory bowel disease (IBD) is a common and prevalent gut inflammatory disorder characterized by recurrent intestinal inflammatory symptoms. Crohn’s disease (CD) and ulcerative colitis are two primary clinical forms of IBD and have distinctly different symptoms. Inflammation in ulcerative colitis is generally limited to the large intestine, whereas CD affects the whole gastrointestinal tract (Celiberto et al. 2017). The pathogenesis of IBD is broadly regarded as a combination of genetic predisposition, aberrant gastrointestinal microbiota ecology, and defective host gastrointestinal immune-sensitivity to commensal flora or other luminal antigens (Celiberto et al. 2017). Because of the influence of microbiota ecology and immunity, probiotic LAB strains with defined beneficial properties have received interests in IBD management (Celiberto et al. 2017).

**CD Management and LABs**

IL-10-deficient mice were found to spontaneously develop human CD-like colitis under conventional conditions. Microbes are involved in development of this CD-like colitis in mice as colitis was not observed in germ-free IL-10-deficient mice (Madsen et al. 2001). Several studies evaluated preventative and therapeutic benefits of various LAB strains in this genetically predisposed CD mouse model (Madsen et al. 2001; Madsen et al. 1999; McCarthy et al. 2003;
Schultz et al. 2002; Sheil et al. 2004) (Table 2). Administration of a single *L. reuteri* strain in IL-10 deficient mice boosted the colonization of intestinal lactobacilli and ameliorated colitis (Madsen et al. 1999). Subsequently, this research team examined therapeutic effect of VSL#3 in the IL-10-deficient murine model. It was observed that VSL#3 reversed colonic inflammatory responses in IL-10-deficient mice as well and possibly had such an effect through strengthening the intestinal epithelial barrier (Madsen et al. 2001). Also oral and subcutaneous administration of *L. salivarius* UCC118 were shown to mitigate colitis and to facilitate the establishment of an anti-inflammatory cytokine environment in an IL-10-deficient CD mouse model (McCarthy et al. 2003; Sheil et al. 2004). In addition, the prophylactic and curative efficacy of *L. plantarum* 299V was shown in IL-10 deficient mice with colitis (Schultz et al. 2002).

Also, in some clinical trials the protective potentials of specific probiotic LAB strains such as that of *L. rhamnosus* GG, VSL#3, and *L. johnsonii* LA1 were tested (Table 2). However, although some efficacy was shown there were discrepancies between efficacy of LAB strains (Bousvaros et al. 2005; Fedorak et al. 2015; Gupta et al. 2000; Malin et al. 1996; Marteau et al. 2006; Prantera et al. 2002; Van Gossum et al. 2007). *L. rhamnosus* GG was reported to stimulate intestinal IgA responses (Malin et al. 1996) and to restore intestinal barrier function in children with CD (Gupta et al. 2000). This strain however was not capable to delay or to prevent the recurrence of CD symptoms in either children or adults with CD remission (Bousvaros et al. 2005; Prantera et al. 2002). Notably, VSL#3 administration during and after the surgical treatment resulted in lower recurrence rate and attenuated mucosal inflammatory response (Fedorak et al. 2015). Besides, *L. johnsonii* LA1 intake was not efficacious to prevent endoscopic relapse in CD patients after surgery (Marteau et al. 2006; Van Gossum et al. 2007).

**Ulcerative colitis**

VSL#3 was clinically demonstrated to be efficacious in managing ulcerative colitis (Bibiloni et al. 2005; Miele et al. 2009; Sood et al. 2009; Tursi et al. 2004; Venturi et al. 1999) (Table 2). It was shown that VSL#3 abrogated clinical symptoms in patients with active mild-to-moderate ulcerative colitis when either applied alone or in combination with anti-inflammatory drug balsalazide (Bibiloni et al. 2005; Sood et al. 2009; Tursi et al. 2004). VSL#3 in combination with balsalazide was shown to be more effective in inducing remission than treatment with balsalazide or mesalazine in ulcerative colitis patients (Tursi et al. 2004). Another promising clinical trial with VSL#3 was conducted by Miele et al. (2009), who found that VSL#3 consumption in combination with regular medication significantly enhanced remission rate, declined recurrence rate, and was safe in children with ulcerative colitis within a one-year, placebo-controlled, double-blind clinical study. The probiotic strain *L. rhamnosus* GG however was not found to induce statistically significant effects in retaining ulcerative colitis remission when administrated alone or in combination with mesalazine in a 12-month clinical trial (Zocco et al. 2006).

In addition to oral consumption, rectal administration was also shown to be an effective route for probiotic application in ulcerative colitis patients (D’Incà et al. 2011; Oliva et al. 2012). *L. reuteri* ATCC55730 applied as rectal enema beneficially down-regulated rectal mucosal inflammatory responses in children with active mild to moderate ulcerative colitis (Oliva et al. 2012). In another study, rectal inoculation of *L. casei* DG together with oral consumption of 5-aminosalicylic acid favorably adjusted microbiota profile and mucosal cytokine signals in the colon, whereas oral supplementation of this strain combined with 5-aminosalicylic acid was ineffective (D’Incà et al. 2011). In the above two studies modulation of mucosal inflammatory signals by LAB strains might be one possible mechanism whereby LAB improving clinical status of ulcerative colitis patients. However not all trials were successful. A 52-week duration of treatment with a mixture of *L. acidophilus* La-5 and *B. animalis* BB-12 did not contribute to retaining remission in ulcerative colitis patients (Wildt et al. 2011).

**Pouchitis**

Pouchitis is a common IBD-related complication, which occurs in ulcerative colitis patients after ileal pouch-anal anastomosis (IPAA) surgery (Gionchetti et al. 2000). Clinical trials have proved the efficacy of probiotic LAB in managing pouchitis (Landy and Hart 2013) (Table 2). One-year supplementation of the probiotic mixture VSL#3 started straight away after IPAA in patients was suggested to effectively prevent the incidence of pouchitis (Gionchetti et al. 2003). Another study showed that daily VSL#3 consumption by patients who were at different stages after receiving IPAA weakened pouchitis clinical activities and enhanced mucosal regulatory T cell responses (Pronio et al. 2008). In addition, efficacy of VSL#3 intervention in inhibiting relapse of pouchitis was demonstrated in two clinical studies (Gionchetti et al. 2000; Mimura et al. 2004).

Probiotic *L. rhamnosus* GG was also reported to delay or prevent onset of pouchitis in patients with IPAA (Gosselin et al. 2004). However, this effect of *L. rhamnosus* GG was not confirmed in another clinical study (Kuisma et al. 2003). Eight-week consumption of a probiotic mixture of bifidobacteria, lactobacilli, and lactococci following antibiotic treatment strengthened gut barrier function in patients with severe pouchitis, which was suggested as one mechanism underlying the beneficial effects of LAB on pouchitis (Persborn et al. 2013).

**Allergic diseases**

The frequency and morbidity of allergic diseases have been rising over the past several decades especially in industrialized countries (Toh et al. 2012). A popular explanation for this is the “microbiota hypothesis,” previously referred to as the “hygiene hypothesis.” This theory explains the enhanced allergy frequency by aberrant microbial exposure during
### Table 2. Effects of LAB on gastrointestinal inflammatory disorders in experimental animals and humans.

| Target disease | Bacterial strains tested, administration route, live/dead bacteria | Study design, timing, dose, and duration of LAB administration | Main findings | Reference |
|----------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------|-----------|
| CD             | *Lactobacillus* (L.) *reuteri*; Rectal inoculation;           | SPF neonatal 129 Sv/Ev IL-10-deficient mice; Before onset of colitis: 3 × 10^7 CFU/day for 3 weeks; | Ameliorated colonic damage; Reduced colonic mucosal adhesion and translocation of aerobic bacteria; Increased colonic lactobacilli numbers; | Madsen et al. (1999) |
| CD             | VSL#3; Oral inoculation;                                      | SPF 129 Sv/Ev IL-10-deficient mice; Before onset of colitis: 2.8 × 10^8 CFU/day for 4 weeks; | Attenuated colitis (mucosal ulceration, epithelial hyperplasia, mononuclear and neutrophil infiltration into LP); Strengthened colonic epithelial barrier function; Inhibited intestinal production of TNF-α and IFN-γ; | Madsen et al. (2001) |
| CD             | *L. salivarius* UCC118, *Bifidobacterium* (B.) *infantis* 35624; Oral inoculation; | SPF 129 Ola × C57BL/6 IL-10-deficient mice, n = 10/group; Before onset of colitis: *L. salivarius* (4–7 × 10^5 CFU/day) or *B. infantis* (4–7 × 10^5 CFU/day) for 19 weeks; | *L. salivarius* group mitigated colitis andecal inflammation; *L. salivarius* group downregulated IFN-γ production by PP cells; | McCarthy et al. (2003) |
| CD             | *L. salivarius* UCC118; Subcutaneous inoculation;             | SPF 129 Ola × C57BL/6 IL-10-deficient mice, n = 10/group; Before onset of colitis: 1 × 10^5 CFU/week at weeks 2, 4, 6, 10, 14, and 18; | Attenuated intestinal inflammation; SPF mice: alleviated colonic inflammation; Reduced IFN-γ and IL-12 production by splenocytes; | Sheil et al. (2004) |
| CD             | *L. plantarum* 299v; Oral inoculation;                       | SPF 129 Ola × C57BL/6 IL-10-deficient mice, n = 10/group; After onset of colitis: 5 × 10^7 CFU/day for 4 weeks; | Enhanced intestinal IgA response; | Schultz et al. (2002) |
| CD             | *L. rhamnosus* GG; Oral consumption;                         | Children with CD, n = 14; 2 × 10^10 CFU/day for 10 days;        | Enhanced intestinal IgA response; | Malin et al. (1996) |
| CD             | *L. rhamnosus* GG; Oral consumption;                         | Children with active DC, n = 4; 2 × 10^10 CFU/day for 6 months;  | Reduced disease activity; Enhanced intestinal barrier function; | Gupta et al. (2000) |
| CD             | *L. rhamnosus* GG; Oral consumption;                         | Adult CD patients with curative resection, n = 45; DB, randomized, PCT; 1.2 × 10^10 CFU/day for 1 year; | No reduction of endoscopic relapse rate; No mitigation of recurrent endoscopic lesions; | Prantera et al. (2002) |
| CD             | *L. rhamnosus* GG; Oral consumption;                         | Children with CD, n = 75; DB, randomized, PCT; ≥ 2 × 10^10 CFU/day for 2 years; | No reduction of recurrence rate; No effects on remission maintenance; | Bousvaros et al. (2005) |
| CD             | VSL#3; Oral consumption;                                     | CD patients with curative surgery, n = 119; 1.8 × 10^12 CFU/day for 90/365 days; | Decreased endoscopic recurrence rates; Suppressed colonic mucosal expression of inflammatory cytokines; | Fedorak et al. (2015) |
| CD             | *L. johnsonii* LA1; Oral consumption;                        | Adult CD patients with surgical resection, n = 98; DB, randomized, PCT; Within 21 days following surgery: 4 × 10^9 CFU/day for 6 months; | No improvement of endoscopic relapse; | Marteau et al. (2006) |
| CD             | *L. johnsonii* LA1; Oral consumption;                        | Adult CD patients with surgical resection, n = 70; DB, randomized, PCT; | No prevention of endoscopic recurrence; | Van Gossum et al. (2007) |
| UC             | VSL#3; Oral consumption;                                     | Patients with active UC, n = 34; 3.6 × 10^12 CFU/day for 6 weeks; | Yielded a remission/response rate of 77%; | Bibiloni et al. (2005) |
| UC             | VSL#3; Oral consumption;                                     | Adult patients with active UC, n = 147; DB, randomized, PCT; 3.6 × 10^12 CFU/day for 12 weeks; | Increased remission rate; Reduced the incidence of stools and rectal bleeding; | Sood et al. (2009) |
| UC             | VSL#3; Oral consumption;                                     | Adult patients with active UC, n = 90; balsalazide treatment with/without 9 × 10^13 CFU/day for 8 weeks; | LAB accelerated remission and enhanced well-being status; LAB reduced stool frequency as well as disease activity (endoscopic and histological scores); | Tursi et al. (2004) |
| UC             | VSL#3; Oral consumption;                                     | Adult UC patients in remission, n = 20; 1.5 × 10^12 CFU/day for 12 months; | Maintained remission in most patients; Decreased fecal PH; | Venturi et al. (1999) |
early childhood, which leads to an inappropriate intestinal microecology and primes aberrant immune maturation characterized by a Th2-biased immunity (Kuitunen 2013; Toh et al. 2012). This emphasizes the essential role of gut microbiome in the pathogenesis of allergic diseases (Kuitunen 2013). Accordingly, probiotic LAB-interventions that specially target intestinal microflora have been extensively studied. Clinical attempts which demonstrate the proof of principle of LAB administration to manage allergy have been mainly focused on eczema and specifically IgE-associated eczema (Table 3), a common type of allergy that develops during early life (Kuitunen 2013). Most prospective
clinical evidence demonstrates that LABs can prevent rather than cure eczema, which is also acknowledged by the World Allergy Organization (Forsberg et al. 2016).

Of note, some systematic reviews and meta-analyses revealed that prenatal together with early postnatal LAB supplementation seems to provide the most preventative benefits when compared with a solely prenatal or postnatal administration strategy (Boyle et al. 2011; Forsberg et al. 2016; Kuitunen 2013; Kuitunen et al. 2009; Kukkonen et al. 2007; Toh et al. 2012; Wickens et al. 2008). This indicates that timing is of great importance for the efficacy of LAB intervention in eczema (Toh et al. 2012). In a clinical trial conducted by Kukkonen et al. (2007), a LAB mixture of 2 L. rhamnosus strains, 1 Bifidobacterium strain, and 1 Propionibacterium strain was given to a large cohort of pregnant women (n = 1223) 2–4 weeks prior to delivery. Their babies with high risk of allergic disorders also received this LAB mixture together with galacto-oligosaccharides as off birth until 6 months old. At the age of 2 years, it was shown that the LAB-supplementation prevented IgE-related diseases and allergen sensitization in children; alleviated allergic symptoms in mothers.

### Table 3. Effects of LAB on allergic disease in humans.

| Bacterial strains tested, administration route, live/dead bacteria | Study design, timing, dose, and duration of LAB administration | Main findings | Reference |
|---------------------------------------------------------------|---------------------------------------------------------------|--------------|-----------|
| **Lactobacillus (L.) rhamnosus**
  + L. rhamnosus LC705 + Bifidobacterium (B.) breve Bb99 + Propionibacterium freudenreichii ssp. shermanii JS; Oral consumption; | Pregnant women carrying children at high risk of allergy, n = 1223; DB, randomized, PCT; Prenatal stage: mothers received 2 capsules/day starting 2–4 weeks before delivery (L. rhamnosus GG, 5 × 10^8 CFU/capsule + L. rhamnosus LC705, 5 × 10^8 CFU/capsule + B. breve Bb99, 2 × 10^9 CFU/capsule + Propionibacterium freudenreichii JS, 2 × 10^9 CFU/capsule); Postnatal stage: children received 1 capsule/day since birth for 6 months; | At age 2 years: no effects on cumulative rate of allergic diseases; decreased the incidence of IgE-related diseases and eczema; At age 5 years: no effects on cumulative rate of allergic diseases; decreased the rate of IgE-related allergic diseases in cesarean-born children; | Kukkonen et al. (2007) Kuitunen et al. (2009) |
| **L. rhamnosus** HN001; B. lactis HN019; Oral consumption; | Pregnant women carrying children at risk of allergy, n = 512; DB, randomized, PCT; Mothers: L. rhamnosus (6 × 10^7 CFU/day) or B. lactis (9 × 10^7 CFU/day) from 35 weeks of gestation until 6 months after delivery; Children: same LAB treatment as mothers since birth until 2 years old; | At age 2 years: L. rhamnosus but not B. lactis reduced the cumulative eczema rate; At age 4 years: L. rhamnosus but not B. lactis reduced the cumulative rate of eczema and rhinoconjunctivitis; | Wickens et al. (2008) Wickens et al. (2012) |
| **L. rhamnosus** GG; Oral consumption; | Pregnant women with atopic diseases, n = 191; DB, randomized, PCT; Prenatal stage: mothers received 1 × 10^10 CFU/day starting 24 weeks of gestation; Postnatal stage: breast-feeding mothers and non-breastfeeding children received the same LAB treatment for 6 months; | At age 3 years: no effects on incidence of allergic sensitization and allergic diseases in children; alleviated allergic symptoms in mothers; | Ou et al. (2012) |
| **L. rhamnosus** GG; Oral consumption; | Pregnant women carrying children at high risk of allergy, n = 250; DB, randomized, PCT; Prenatal stage: mothers received 1.8 × 10^10 CFU/day starting 36 weeks of gestation; | At age 1 year: no effects on eczema rate in children; | Boyle et al. (2011) |
| **L. rhamnosus** LPR + B. longum BL999; Oral consumption; | Children at high risk of allergy, n = 253; DB, randomized, PCT; Postnatal stage: children received L. rhamnosus LPR (1.85 × 10^9 CFU/day) and B. longum BL999 (9.26 × 10^8 CFU/day) since birth for 6 months; | At age 1 year: no effects on the rate of eczema and allergen sensitization in children; | Soh et al. (2009) |
| **L. acidophilus** LAVR-A1; Oral consumption; | Children at high risk of allergy, n = 231; DB, randomized, PCT; Postnatal stage: children received 3 × 10^6 CFU/day since birth for 6 months; | At age 1 year: increased the incidence of allergen sensitization in children; no effects on the rate of atopic dermatitis; At ages 2.5 and 5 years: no effects on the incidence of allergic diseases and allergen sensitization; | Taylor, Dunstan, and Prescott (2007) Prescott et al. (2008) Jensen et al. (2012) |
| **Streptococcus (S.) thermophilus** 065 + B. breve CS0; Oral consumption (heat-killed bacteria in infant formula); L. paracasei F19; Oral consumption; | Children at high risk of allergy, n = 129; DB, randomized, PCT; Postnatal stage: children received S. thermophilus (3.84 × 10^9 CFU/g) and B. breve CS0 (4.2 × 10^9 CFU/g) since birth for 1 year; Children, n = 127; DB, randomized, PCT; 1 × 10^9 CFU/day from age 4 months to 13 months; | At age 2 years: decreased the rate of respiratory potentially allergic adverse event; no effects on the rate of cow’s milk allergy; At age 13 months: decreased the cumulative eczema rate; upregulated IFN-γ/IL-4 mRNA ratio in polyclonally stimulated PB T cells; | Morisset et al. (2011) West, Hammarström, and Hernell (2009) |

DB, double-blind; PB, peripheral blood; PCT, placebo-controlled trial;
which pregnant mothers carrying high-allergy risk children consumed *L. rhamnosus* HHN001, *B. animalis* subsp *lactis* HHN019 or placebo after week 35 of gestation. Probiotic intake continued during breast-feeding. Also, the infants received the probiotics from birth to 2 years old. It was shown that *L. rhamnosus* but not *B. animalis* effectively reduced the cumulative incidence of eczema (Wickens et al. 2008). An even more promising finding was that during the 4th-year of follow-up, a significant decrease was found in the cumulative occurrence of eczema and in the incidence of rhinoconjunctivitis by *L. rhamnosus* HHN001 administration (Wickens et al. 2012). However, another similar clinical study reported that consumption of *L. rhamnosus* GG by pregnant women with allergic disorders since the second trimester until 6 months after delivery or by their children without breast feeding from birth till 6 months old only alleviated atopic disorders in the mothers but not in the infants (Ou et al. 2012). This illustrates that the timing of administration and the strain applied are probably key in the clinical efficacy of the strain.

In general, to be effective LABs have to be given both pre- and postnatally as either prenatal or postnatal treatment alone elicited insufficient protection against allergy (Boyle et al. 2011). Only prenatal maternal intake of *L. rhamnosus* GG could not effectively inhibit eczema in infants during their first year after birth even though down-regulated levels of soluble CD14 and total IgA in breast milk was found by *L. rhamnosus* GG treatment (Boyle et al. 2011). Similarly, most clinical trials exploring only postnatal supplementation of LAB strains such as *L. rhamnosus* LPR and *L. acidophilus* LAVRI-A1 in high-risk individuals failed to confer adequate benefits against eczema based on both short-term (Soh et al. 2009; Taylor, Dunstan, and Prescott 2007) and longer-term follow-up periods (Jensen et al. 2012; Prescott et al. 2008). However, strain dependency of effects cannot be excluded in relation to pre- or post-natal administration of LABs. In clinical study by Morisset et al. (2011), it was shown that postnatal administration of heat killed *B. breve* C50 and *S. thermophilus* 065 in infant formula fed babies during the first year after birth effectively lowered respiratory allergic adverse events at both 1 and 2 years of age, although no significant decline in the prevalence of cow’s milk allergy was found. It cannot be excluded that the longer treatment duration, for example, 1 year of LAB administration (Morisset et al. 2011) as compared with the intervention length of others, for example, first 6 months might have led to different results (Jensen et al. 2012; Prescott et al. 2008; Soh et al. 2009; Taylor, Dunstan, and Prescott 2007). It should be noted that also different results with respect to allergy-modulating efficacy of LAB might be obtained in clinical trials using different study populations. Consumption of *L. casei* F19 by nonselected infants during their weaning period, that is, 4–13-month old, induced reduced eczema morbidity accompanied by augmented Th1-type responses (West, Hammarström, and Hernell 2009).

Collectively, even though promising clinical benefits of LAB intervention have been reported hitherto, inconsistent results in different investigations make it difficult to draw definitive conclusions as to the effectiveness of probiotic LAB application in allergy management (Forsberg et al. 2016; Kuitunen 2013). Variations in experimental setups such as inclusion of either high-risk, healthy or nonselected populations, differences in LAB strains applied, maternal or infants administration of LABs, differences in timing, for example, pre- and/or post-natal treatment, variations in doses and duration of LAB administration, and application of varying food matrices, for example, capsules, prebiotic supplement, and infant formula, might contribute to inconsistencies in reported results (Forsberg et al. 2016; Kuitunen 2013; Nermes, Salminen, and Isolauri 2013). More stringently-designed, standardized protocols are required to further validate the protective functions of probiotic LAB strains in allergic disorders (Forsberg et al. 2016).

**Cancer**

Cancer is becoming a globally prevalent clinical health burden. High fatality among cancer patients is still observed in most countries despite improved cancer therapies such as radiotherapy and chemotherapy. The increasing morbidity of various cancers, particularly colorectal cancer has been linked to unhealthy diet and lifestyle and is associates with intestinal microflora dysbiosis (Kahouli, Tomaro-Duchesneau, and Prakash 2013). Consequently, probiotic LAB strains with well-defined capabilities to adjust microbiota dysbiosis have been considered as potential beneficial dietary supplementations for cancer (dos Reis et al. 2017; Kahouli, Tomaro-Duchesneau, and Prakash 2013). The majority of in vitro studies on anti-cancer potentials of LAB strains of different species such as *L. acidophilus*, *L. brevis*, *L. casei*, *L. fermentum*, *L. plantarum*, *L. reuteri*, and *L. Lactis* focused on the effects of LAB on proliferation and apoptosis of cancer cell lines in vitro (So, Wan, and El-Nezami 2017). A kimchi-derived *Lc. lactis* strain KC24 was demonstrated to inhibit the proliferation of human HT-29 and LoVo colon carcinoma, AGS gastric carcinoma, MCF-7 breast carcinoma, and SK-MES-1 lung carcinoma cell lines (Lee et al. 2015). It was shown that *L. casei* ATCC393 significantly suppressed the proliferation and augmented the apoptosis of CT26 murine and HT29 human colon cancer cells (Tiptiri-Kourpeti et al. 2016). Such an in vitro cancer cell-inhibitory effect of *L. casei* ATCC393 was also confirmed in vivo in a colon carcinoma mouse model, in which inhibition of colonic tumor growth was observed (Tiptiri-Kourpeti et al. 2016). There are some other in vivo studies in animals exploring the direct tumor-inhibitory properties of LAB (Kahouli, Tomaro-Duchesneau, and Prakash 2013; Raifer 2004; So, Wan, and El-Nezami 2017). Administration of *L. acidophilus* NC9M before and after tumor initiation was shown to dampen colon cancer development in a murine colon carcinoma model (Chen et al. 2012). Notably, the anti-tumor effects of *L. acidophilus* ATCC3314 were found to be more effective in the small than in the large intestine (Urbanska et al. 2016). In the APC (Min/+) mouse model of intestinal tumorigenesis, supplementation of *L. acidophilus* ATCC3314 specifically reduced the number of tumors in
the small intestine but did not alter the amount of polyps in the large intestine (Urbanska et al. 2016).

At present, several clinical studies substantiate beneficial potentials of probiotic LAB in cancer management (Kahouli, Tomaro-Duchesneau, and Prakash 2013; So, Wan, and El-Nezami 2017) (Table 4). For instance, a 12-year clinical survey involving 45241 volunteers in Italy showed that high yoghurt consumption containing L. delbrueckii subsp bulgaricus and S. thermophilus seemed to reduce the incidence of colorectal cancer (Pala et al. 2011). Also, the efficacy of the probiotic LAB strain L. casei Shirota and the probiotic strain mixture of lactobacilli, bifidobacteria, and enterococci were also investigated in cancer patients prior to or following resection surgery for their potential for reducing relapse (Nanno et al. 2011), for mitigating postoperative complications (Kotzampassi et al. 2015; Liu et al. 2011; Zhang, Du, et al. 2012), and for improving other physiological health functions (Liu et al. 2011; Zhang, Du, et al. 2012). Administration of L. casei Shirota following surgery was shown to significantly reduce the postoperative relapse in patients with bladder or colorectal cancer probably via enhancing NK cell activity (Nanno et al. 2011). Pre-operative administration of L. acidophilus mixed with other probiotic strains, that is, B. longum and Enterococcus faecalis, reduced the incidence of post-operative infections accompanied by reinforcement of gut barrier function, up-regulation of serum IgG and sIgA levels, increase in fecal Bifidobacteria, and decrease in Escherichia (Zhang, Du, et al. 2012). Moreover, protective properties of a mixture of L. rhamnosus LC705 and a probiotic strain Propionibacterium freudenreichii ssp shermanii JS against potential cancer were found in still healthy populations as reflected by reduced activities of carcinogenic enzyme beta-glucosidase after consumption of this strain mixture (Hatakka et al. 2008). As illustrated in the aforementioned clinical studies on the efficacy of LAB for cancer management, possible involved mechanisms include gut microflora modulation, reinforcement of host immunity, diminution of the activity of carcinogenic agents, production of anti-carcinogenic factors, and enhancement of gut barrier function (dos Reis

### Table 4. Effects of LAB on intestinal cancer in experimental animals and humans.

| Bacterial strains tested, administration route, live/dead bacteria | Study design, timing, dose, and duration of LAB administration | Main findings | Reference |
|---------------------------------------------------------------|------------------------------------------------------|--------------|-----------|
| Lactobacillus (L.) casei ATCC393; Oral inoculation; **| BALB/c mice, n = 10/group; Before cancer induction; 1 × 10⁹ CFU/day starting from 10 days before cancer induction until 3 days after cancer induction; | Decreased colonic tumor volume; Upregulated TRAIL expression and inhibited the expression of anti-apoptotic protein Survivin in tumor tissues; | Tiptiri-Koupeti et al. (2016) |
| L. acidophilus NCFM; Oral inoculation; **| BALB/cByJ mice, n = 10-15/group; Before cancer induction; 1 × 10⁹ CFU/day for 14 days; After cancer induction; 1 × 10⁹ CFU/week for 3 weeks; | Decreased colonic tumor volume; Promoted tumor cell apoptosis; Improved structural damage in colon; Downregulated the expression of CXCR4 and MHC I class molecules in colon, MLN, and spleen; | Chen et al. (2012) |
| L. acidophilus ATCC314; Oral inoculation; **| C57BL/6 Apo^−/− mice, n = 24/group; Daily received encapsulated LAB in yoghurt; | Decreased the tumor numbers in small intestine but not in large intestine; High yoghurt intake resulted in reduction of CRC incidence; | Urbanska et al. (2016) |
| Streptococcus (S.) thermophilus + L. delbrueckii ssp bulgaricus; Oral consumption; **| Participants without CRC, n = 45241; Consumed standard yoghurt; | Reduced the relapse rate of bladder cancer and CRC; Decreased the incidence of post-surgical complications (pneumonia, surgical site infections, and anastomotic leakage); Reduced the hospitalization length; | Nanno et al. (2011) |
| L. casei Shirota; Oral consumption; **| Bladder cancer/CRC patients with surgical resection; LAB treatment after surgery; | | |
| L. acidophilus LA-5 + B. longum + Bifidobacterium (B.) lactis Bb12 + Saccharomyces boulardii; Oral consumption; **| Adult CRC patients with colorectal surgery, n = 164; DB, randomized, PCT; L. acidophilus LA-5 (7 × 10⁹ CFU/day) + L. plantarum (2 × 10⁸ CFU/day) + B. lactis Bb12 (7 × 10⁹ CFU/day) + Saccharomyces boulardii (6 × 10⁹ CFU/day) starting 1 day before operation until 15 days after operation; | Decreased post-surgical infection and promoted peristalsis recovery; Enhanced gut barrier function; Decreased bacterial translocation; Improved intestinal microbial variety; | Kotzampassi et al. (2015) |
| L. plantarum CGMCC1258 + L. acidophilus LA-11 + B. longum BL-88; Oral consumption; **| Adult CRC patients with colorectal surgery, n = 100; DB, randomized, PCT; L. plantarum CGMCC1258 (≥ 2 × 10¹¹ CFU/day) + L. acidophilus LA-11 (≥ 1.4 × 10¹¹ CFU/day) + B. longum BL-88 (≥ 1 × 10¹¹ CFU/day) starting 6 days before operation until 10 days after operation; | | |
| L. acidophilus + B. longum + Enterococcus faecalis; Oral consumption; **| Adult CRC patients, n = 60; DB, randomized, PCT; 1.89 × 10⁹ CFU strain/day starting 5 days until 3 days before surgical resection; | Decreased the rate of post-surgical infection; | Zhang, Du, et al. (2012) |
| L. rhamnosus LC705 + Propionibacterium freudenreichii ssp. shermanii JS; Oral consumption; **| Healthy adult males, n = 38; DB, randomized, PCT; 2 × 10¹⁰ CFU strain/day; | Decreased fecal β-glucosidase and urease activities; | Hatakka et al. (2008) |

CRC, colorectal cancer; CXCR4, C-X-C motif chemokine receptor 4; DB, double-blind; MHC, major histocompatibility complex; MLN, mesenteric lymph node; PCT, placebo-controlled trial; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.
et al. 2017; Kahouli, Tomaro-Duchesneau, and Prakash 2013). However, reproducible efficacious clinical protocols remain to be developed.

Concluding remarks
The well-established safety status and demonstrated health-promoting properties render LAB promising candidates to be applied as dietary supplements or medical interventions. The primary mechanisms underlying the functions of LAB include modulation of gut commensal microflora, production of bioactive or bactericidal molecules, preclusion of pathogen attachment, attenuation of virulent factors of pathogens, fortification of intestinal barrier function, and regulation of innate and adaptive immune function. The current available studies have confirmed the clinical efficacy of different LAB strains in reducing infections induced by H. pylori, Salmonella, and influenza virus, in managing gastrointestinal inflammatory disorders especially pouchitis, in controlling allergies specifically eczema, and in ameliorating the postoperative complications in cancer patients.

Despite these promising findings in the research field of LAB, only limited numbers of LAB strains have been approved to be applied as dietary supplements or medical interventions when considering the extensive LAB resources in nature. Lack of standard, highly efficient evaluation systems for screening suitable LAB strains for diverse intervention purposes is a major bottleneck that hampers the identification of proper strain candidates. Furthermore, inconsistent effects of specific strains or varied effects between different strains often exist. Mechanistic studies aiming at identification of effector factors and elucidating the relationship between specific LAB effector molecules and associated effects will undoubtedly contribute to our deeper understanding of strain specificity. This will also facilitate the establishment of more effective and targeted selection systems for LAB strains.

However, it should be noted that the observed modulatory effects of LAB intervention are most likely the combinations of responses elicited by various LAB effector molecules, which increases the complexity of LAB-host interaction. Another critical aspect is how particular LAB strains react to the in vivo environments. As discussed earlier, in vivo resistance or adaptivity of LAB strains to harsh gastrointestinal milieu determines final physiological responses evoked by LAB. Moreover, in vivo conditions were also suggested to specifically influence the expression profiles of specific genes in LAB, thus impacting LAB-conferred functions on the host (Bron, van Baalen, and Kleerebezem 2012).

Besides these LAB-related factors, host-associated factors such as age, gender, genotype, lifestyle factors, and dietary pattern play a vital part in shaping gut microbiota composition and immune status of the host, thereby affecting host responsiveness to specific LAB strains. Thus, establishing comprehensive repositories of well-defined characteristics for both individual strains and humans will contribute to more personalized LAB selection or consumption for targeted populations. Notably, factors in LAB intervention regimens including administration mode, dose, timing, and duration, and the parameters that measured should be more standardized in order to achieve a more consistent and comparable physiological efficacy of LAB consumption.

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