Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens

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Abstract

The gastrointestinal tract is a complex ecosystem that associates a resident microbiota and cells of various phenotypes lining the epithelial wall expressing complex metabolic activities. The resident microbiota in the digestive tract is a heterogeneous microbial ecosystem containing up to $1 \times 10^{14}$ colony-forming units (CFUs) of bacteria. The intestinal microbiota plays an important role in normal gut function and maintaining host health. The host is protected from attack by potentially harmful microbial microorganisms by the physical and chemical barriers created by the gastrointestinal epithelium. The cells lining the gastrointestinal epithelium and the resident microbiota are two partners that properly and/or synergistically function to promote an efficient host system of defense. The gastrointestinal cells that make up the epithelium, provide a physical barrier that protects the host against the unwanted intrusion of microorganisms into the gastrointestinal microbiota, and against the penetration of harmful microorganisms which usurp the cellular molecules and signalling pathways of the host to become pathogenic. One of the basic physiological functions of the resident microbiota is that it functions as a microbial barrier against microbial pathogens. The mechanisms by which the species of the microbiota exert this barrier effect remain largely to be determined. There is increasing evidence that lactobacilli and bifidobacteria, which inhabit the gastrointestinal microbiota, develop antimicrobial activities that participate in the host’s gastrointestinal system of defense. The objective of this review is to analyze the in vitro and in vivo experimental and clinical studies in which the antimicrobial activities of selected lactobacilli and bifidobacteria strains have been documented.

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1. Introduction

The gastrointestinal tract is a complex ecosystem that associates a resident microbiota and cells of various phenotypes lining the epithelial wall. The term microbiota as been defined by Savage [1] as the collective societies of bacteria assembled on the mucosal surfaces of an individual. Mammals are born without any such microorganisms [2]. The colonization of gastrointestinal tract starts immediately at birth. The first bacteria to colonize the gut originate from the birth canal and include aerobic and anaerobic bacteria, such as Escherichia coli, Clostridium spp., Streptococcus spp., Lactobacillus spp., Bacteroides spp., and Bifidobacterium spp. All the components of the gastrointestinal ecosystem are necessary for the gut to develop its specific intestinal functions [3]. The resident microbiota in the digestive tract is a heterogeneous microbial ecosystem containing up to $1 \times 10^{14}$ colony-forming units (CFUs) of bacteria [4–7]. The microbiota differs quantitatively and qualitatively at different points along the gastrointestinal tract. The stomach has few resident microorganisms, however lactobacilli have been isolated from the microbiota resident in the stomach [8]. The microbial profile of the digestive tract is typified by the absence of anaerobic microorganisms in the stomach and conversely their overwhelming predominance in the distal colon. Moreover, different microbial communities may be located in the intestinal lumen, in the mucus covering the epithelium, in the crypt spaces or in the different cells lining the epithelium, and in addition, some species adhere whereas others do not. The number of bacterial species in the intestinal microbiota has been estimated to be about 400. Aerobic, facultative and anaerobic bacteria all inhabit the gastrointestinal microbiota. The proportion of anaerobic bacteria gradually increases from the proximal to distal regions, and 99% of the inhabitants located in the large intestine are anaerobes. Currently, only 20–40% of bacterial species present in the gastrointestinal tract have been cultured or characterized. There are few detailed reports about the species and strains that compose the human intestinal microbiota. Identification of the species present in the gastrointestinal microbiota is in progress, as a result of the introduction of higher resolution molecular techniques based on 16S rRNA or rDNA, and of technological innovations, such as selective media that now make it possible to grow bacteria that had not previously been cultured.

The intestinal microbiota plays an important role in normal gut function and maintaining host health. Little is known about how members of the indigenous microbiota interact with their mammalian hosts to establish mutually beneficial relationships. Midtvedt and co-workers and Gordon and co-workers [9–17] have recently gained important new insights into the mechanism by which members of the intestinal microbiota influence intestinal functions by means of cross-talk with the epithelial cells. For example, the intraluminal microbiota influences the release of biologically active gastrointestinal peptides and contributes to regulating gastrointestinal endocrine cells and the epithelial structure [9]. Colonization of germ-free mice by Bacteroides thetaiotaomicron VPI-5482, a component of the intestinal flora, has revealed that this commensal bacterium modulates expression of genes involved in several important intestinal functions, including nutrient absorption, mucosal barrier fortification, xenobiotic metabolism, angiogenesis, and postnatal intestinal maturation [15,16]. The colonization of germ-free mice with the VPI-5482 strain of B. thetaiotaomicron restored the fucosylation program, whereas an isogenic strain carrying a transposon insertion that disrupts its ability to use L-fucose as a carbon source did not [10,12]. In line with this observation, comparison of gut glycosylation patterns in germ-free and conventional mice have revealed both quantitative and qualitative differences in the cellular and subcellular distribution of glycans [17]. It has been observed that this strain also has the capacity to change the galactosylation process in cultured human mucin-secreting HT29-MTX cells as a result of post-translational regulation, via a mechanism that involves a
soluble, heat labile, low molecular weight factor [18]. Interestingly, in colonized germ-free mice, a B. thetaiotaomicron strain increased matrilysin production [14], a matrix metalloprotease expressed in Paneth cells and found involved in innate host defence, as matrilysin-null mice have an impaired ability to activate prodefensins and to kill exogenous bacteria in their small intestine [19]. Finally, it has also been reported that the normal colonization of the mammalian intestine with commensally microbes has an influence on the development of the humoral and cellular mucosal immune systems during neonatal life, and in maintaining the physiologically normal steady state of inflammation in the gut throughout life [20,21].

2. The gastrointestinal defence ecosystem of the host

The host is protected from attack by potentially harmful microorganisms by the physical and chemical barriers created by the gastrointestinal epithelium [22–25]. The cells lining the gastrointestinal epithelium and the resident microbiota are two partners that properly and/or synergistically function to promote an efficient host system of defence. The gastrointestinal cells [26–28] that make up the epithelium [29–33], provide a physical barrier that protects the host against the unwanted intrusion of microorganisms into the gastrointestinal microbiota, and against the penetration of harmful microorganisms which can hijack the cellular molecules and signalling pathways of the host to become pathogenic [34–37].

The intestinal mucosa has a surface coating of mucus that is secreted by the specialized goblet cells [25,38,39]. There are two secretory pathways in intestinal mucin-secreting polarized cells, the first of which is the regular vesicular constitutive pathway of mucin exocytosis, in which no storage occurs since the small vesicles transporting the mucins through the constitutive pathway are guided directly to the cell surface by microtubules and undergo immediate exocytosis of their contents. The second pathway for mucin exocytosis involves the packaging and storage of mucins in large vesicles, from which mucin release is regulated by specific stimuli involving signalling molecules The entrapment of bacteria within the mucus which contains secretory immunoglobulins, coupled with peristalsis, results in the rapid expulsion of bacteria from the intestine.

Together with the innate defences of the host, the constituents of the intestinal immune system of defence reinforce the barrier function of the gastrointestinal epithelium [3,40,41]. Cross-talk mobilizes the cells lining the intestinal epithelium and gut-associated immune cells, allowing the host to sense the microbial environment in order to promote a strong defence response when required, by releasing signalling molecules such as cytokines and chemokines, that leads to the recruitment of leukocytes, and initiates the attraction of immune cells. How the host gut distinguishes between its commensal microbiota and the entervirulent bacteria and viruses to activate its innate and/or adaptive mucosal immunity has been recently reviewed by Didierlaurent et al. [42]. In innate mucosal immunity, the host defence mechanisms are activated mainly as a result of the specific recognition of pathogen-associated molecular patterns (PAMPs). In contrast, the endogenous bacterial species of the microbiota all share signature molecules, known as microbe-associated molecular patterns (MAMPs). Epithelial and monocytic cells are specialized cells that sense the environment within the gut by means of their pattern-recognition receptors (PRR), which form a heterogeneous family of receptors [43]. It should be pointed out that we still do not know why the commensal organisms expressing MAMPs do not trigger pro-inflammatory host responses under normal conditions.

One of the defence mechanisms present in the gastrointestinal tract of the host is the first line of chemical defence involving the production of antimicrobial peptides [44–54]. This chemical antimicrobial system of defence functions in the mucosa of the gastrointestinal tract, airways, gingival, cornea, reproductive tract and urinary tract [48,49]. Antimicrobial peptides, first identified in the skin and subsequently in polymorphonuclear neutrophils (PMNs) and macrophages, are produced in the intestine by Paneth cells located in the basal portion of intestinal crypts, underlying the zone of intestinal epithelial cell division. By releasing preformed antimicrobial peptides contained in numerous epically located eosinophilic secretory granules, the chemical defence system contributes directly to the innate immunity of the crypt microenvironment and probably by diffusing the peptides secreted, into the lumen. Antimicrobial peptides known as defensins were first identified by Ouellete [24] in mouse small intestinal cells, and subsequently these antimicrobial peptides have been shown to be produced by human intestinal cells [47,50,51,53,55]. The bactericidal activity of cryptdins requires proteolytic activation of precursors by matrix metalloproteinase-7 (MMP-7; matrilysin), expressed in Paneth cells and found involved in innate host defence since matrilysin-null mice have an impaired ability to activate prodefensins and to kill exogenous bacteria in their small intestine [19]. There are two major groups of defensins, α- and β-defensins, which differ in the arrangements of their disulfide bonds. Six human α-defensins (HD-1 to HD-6) and two β-defensins (hBD-1 and hBD-2) have been reported. HD-5 and HD-6 were identified in the Paneth cells, and hBD-1 was detected in Paneth cells and found involved in innate host defence since matrilysin-null mice have an impaired ability to activate prodefensins and to kill exogenous bacteria in their small intestine [19]. There are two major groups of defensins, α- and β-defensins, which differ in the arrangements of their disulfide bonds. Six human α-defensins (HD-1 to HD-6) and two β-defensins (hBD-1 and hBD-2) have been reported. HD-5 and HD-6 were identified in the Paneth cells, and hBD-1 was detected in a variety of epithelial cells. Interestingly, recent observations indicate that in response to attack by pathogenic bacteria, the host engage its first line of chemical defence...
by increasing the production of antimicrobial peptides, such as the α- and β-defensins [56–59]. In parallel, it is significant that enteric pathogens have developed sophisticated strategies to survive in the gastrointestinal tract by evading the innate mucosal defences. Both Salmonella [60] and Shigella [61] are able to down-regulate host antimicrobials by decreasing the expression of α-defensins and LL-37 in colonic epithelial cells, colonic human biopsy specimens and in infected mice. Moreover, intracellular survival of Salmonella depends on the ability of the bacteria to resist the activity of cationic antimicrobial peptides within the phagolysosome [62–64].

3. The resident microbiota as a partner of the gastrointestinal defence ecosystem of the host

One of the basic physiological functions of the resident microbiota is that it functions as a microbial barrier against microbial pathogens. The mechanisms by which species of the microbiota exert this barrier effect remain largely to be determined. Recent reports have provided new insights into the activity of members of the intestinal microbiota against enteropathogens. Nicaise et al. [65] have recently documented the mechanism(s) of the immune response of the intestinal microbiota by examining the regulation of interleukin-1 (IL-1), IL-6, tumour necrosis factor α (TNF-α) and IL-12 production in macrophages from germ-free and from flora-associated mice, conventional, conventionalized and E. coli-mono-associated mice. The findings show that the intestinal flora can modulate bone marrow and spleen macrophage cytokine production in a differential manner. Intestinal flora enhancing IL-12 production in the spleen is also potentially important, since this cytokine is implicated in determining the relative levels of Th1 and Th2 responses, and plays a pivotal role in defending the host against intracellular microorganisms. Hudault et al. [66] have shown that resident E. coli had a barrier effect when colonizing the gut of gnotobiotic C3H/HeOuJc mice orally infected by a lethal strain of S. typhimurium, but the mechanism of action remains to be determined. In addition, it has been established that E. coli participates in antibacterial defence by producing large proteins named colicins that function by forming pores in the cell membrane permeable to nuclease activity against DNA, tRNA and tRNA targets [67]. A potent antibacterial substance can be produced through a mechanism involving both intestinal bacteria and exocrine pancreatic secretions. Ramare et al. [68] have observed that when a human intestinal strain of Peptostreptococcus colonized the gut of gnotobiotic rats, it produced an antibacterial substance that, following cleavage by trypsin, was active against several Gram-positive bacteria, including potentially pathogenic Clostridium spp. such as C. perfringens, C. difficile, C. butyricum, C. septicum, and C. sordelli. Similarly, the E1 strain of Ruminococcus gnavus, a Gram-positive, strictly anaerobic strain isolated from a human faecal sample, was able to produce a trypsin-dependent antibacterial substance, called ruminococcin A that is a class IIA antibiotic, and is also active against various pathogenic clostridia [69,70].

It became possible to identify autochthonous species when the genera Lactobacillus and Bifidobacterium were described [5,71–73]. Species of both genera were occasionally found, even in the stomach. The composition of the bifidobacteria microbiota differed in infants and adults and at other stages in the host’s life. Marked inter-individual variations have been found in microbial composition at genus and species levels [74]. For example, the faecal microbiota of children was found to be bacteriologically less complex whereas advancing age was associated with decreased bifidobacteria and increased bacteroides species diversity. It has been postulated that changes in gut microbial composition with age may alter the metabolic capacity of the gut microbiota, and that this has important implications for the occurrence of diseases. There is increasing evidence that lactobacilli and bifidobacteria, which inhabit the gastrointestinal microbiota, develop antimicrobial activities that participate in the host’s gastrointestinal system of defence. Appropriate experimental evidence and properly-conducted clinical trials are now beginning to be published, particularly concerning the effectiveness of selected lactobacilli and bifidobacteria strains in the prevention and treatment of infectious bacterial and viral diarrhoea [75–88], Helicobacter pylori gastroenteritis [87–90] and urogenital infections [91–99]. The objective of this review is to analyze the in vitro and in vivo experimental and clinical studies in which the antimicrobial activity of selected lactobacilli and bifidobacteria strains has been documented.

4. Antimicrobial activity by lactobacilli and bifidobacteria against gastrointestinal microbial pathogens

4.1. In vitro demonstration of antimicrobial activity

Pioneering studies by Reid et al. [91,100–102] have demonstrated experimentally that Lactobacillus strains express adhesiveness properties that enable them to inhibit the adhesion of bacterial pathogens to host cells. On the basis of this demonstration, it has been suggested that lactobacilli and bifidobacteria may use the same mechanism to combat gastrointestinal microbial pathogens.

In vitro studies using cultured human intestinal cell lines have investigated the adhesiveness and the bacterial
interference effect of lactobacilli and bifidobacteria. These cell models, which express various specific characteristics of several of the cell phenotypes that line the intestinal epithelium, have been extensively used to study specific human intestinal cell functions [26,103]. For example, T84 cells are known to exhibit crypt cell morphology and functions. Depending on the culture conditions, the parent human intestinal epithelial cell lines HT-29 and Caco-2 have been shown to undergo morphological and functional differentiation in vitro, which is characteristic of the mature enterocytes of the small intestine. Different HT-29 cell subpopulations, such as the absorptive HT-29 glc− subpopulation, and mucus-secreting HT29-MTX and HT29-FU subpopulations, have been obtained in different culture conditions. Moreover, clones of the HT-29 and Caco-2 cell lines have been established, such as the absorptive Caco-2BB2, Caco-2/TC7 cells and HT29-19A cells, and the mucin secreting HT29-C1.16E cells. Moreover, these cell models form junctional complexes, and so constitute a monolayer that mimics the intestinal epithelial barrier [31], that pathogenic microorganisms have to cross before they can infect the systemic circulation of the host and spread within the organism. Further investigations of the pathophysiological mechanisms of gastric and enterovirulent bacteria and viruses are now being carried out using cultures of polarized and fully differentiated human intestinal cell models [103] that form these junctional complexes.

4.1.1. Adhesiveness properties

The adhesiveness of intestinal lactobacilli and bifidobacteria strains has been investigated using the Caco-2 and HT29-MTX cell lines. Chauvière et al. [104] observed that not all strains of lactobacilli examined, adhere onto enterocyte-like Caco-2 cells, indicating that adhesiveness is strain-specific. Some strains of lactobacilli, including *L. acidophilus* BG2F04 and LB, *L. johnsonii* La1, *L. rhamnosus* DR20, *L. acidophilus* HN017, *L. casei* subsp. *rhamnosus* Lcr35, *L. casei* *rhamnosus* GG, *L. casei* Shirota, *L. rhamnosus* LC-705 adhere to the enterocyte-like Caco-2 cells [105–111]. Heat-killed *L. acidophilus* LB bacteria were also found to adhere as effectively as the live strain to both undifferentiated and differentiated Caco-2 cells [112]. *L. acidophilus* NCFM bacteria have been observed adhering onto Caco-2 and mucus-secreting HT-29 cell culture systems [113]. Like lactobacilli, *Bifidobacterium* strains also display adhesiveness [114]. *B. adolescentis*, *B. angulatum*, *B. bifidum*, *B. breve*, *B. catenulatum*, *B. infantis*, *B. longum*, *B. pseudocatenulatum* *B. breve* 4, *B. infantis* 1 and *B. lactis* DR10 bacteria have been found adhering to the brush border of Caco-2 cells, and to the mucus secreted by HT29-MTX cells [110,115–117]. It has been postulated that strains of *B. longum* isolated from human intestine have been shown to be able to adhere to Caco-2 cells, probably as a result of their autoaggregative and hydrophobicity properties [118].

An interaction has been reported between *L. acidophilus* BG2F04 and *L. johnsonii* La1 bacteria and the mucus secreted by a subpopulation of human cultured HT29-MTX cells [105,106]. Surprisingly, *Bifidobacterium* bacteria were found to bind significantly less well to mucus isolated from elderly subjects, than to mucus from other age groups, suggesting that lower adhesion may be a contributory factor in the decreased colonization of elderly subjects by bifidobacteria [119]. It has also been observed that in the presence of *L. casei* *rhamnosus* GG or *L. bulgaricus* bacteria, the adhesion of *B. lactis* Bb12 bacteria to mucus was increased, whereas the other *Lactobacillus* strains tested had no effect on the adhesiveness of this *B. lactis* strain [120]. Pertinently, Ouwehand et al. [121,122] consider that adhesion to cultured human intestinal cells and isolated mucins is not sufficient to describe the mucosal interactions of lactobacilli and bifidobacteria. These authors have proposed that adhesiveness of lactobacilli and bifidobacteria were better investigated using human intestinal tissue pieces. Among the strains examined that have been described adhering onto Caco-2 cells, *L. casei* *rhamnosus* GG bacteria adhered well to colonic tissue, whereas *L. johnsonii* La1 and *L. casei* Shirota bacteria adhered to a lesser extent.

The microbial adhesion process of lactobacilli and bifidobacteria includes passive forces, electrostatic interactions, hydrophobic steric forces, lipoteichoic acids; and specific structures, such as lectin-covered external appendages. Among the twenty-five strains of lactobacilli they examined, Chauvière et al. [104] observed that seven of them adhered, and that only three of them, including *L. acidophilus*, possessed calcium-independent adhesiveness. Similarly, *L. johnsonii* La1 bacteria exhibited high calcium-independent adhesiveness to human, enterocyte-like Caco-2 cells in culture, and to the mucus secreted by the homogeneous cultured human goblet cell line HT29-MTX [106]. The bacterial component involved in the adhesion of *L. acidophilus* LB and BG2F04 bacteria was protease-resistant and bacterial-surface-associated [104,105,123]. An adhesion-promoting protein with a molecular mass of 29 kDa, which is present on the cell surface of *L. fermentum* 104R bacteria and can be isolated from the culture supernatant fluid of the strain, has been isolated and characterized. It has been shown to be involved in the binding of this strain to mucus from the small intestine of piglets and to partially purified gastric mucin [124–127]. Lipoteichoic acid has been identified as a factor responsible for the adhesion of the *L. johnsonii* La1 bacteria. This molecule has been isolated from the bacterial cell wall and from the culture supernatant, and is responsible for the concentration-dependent inhibition of La1 adhesion to Caco-2 cells [128]. *L. animalis*
and *L. fermentum* bacteria have lectin-like protein structures on their surfaces, and *L. animalis* bacteria has been shown to have ribitol teichoic acids in its cell wall [129]. The *L. acidophilus* UO 001 and *L. gasseri* UO 002 bacteria adhered to Caco-2 cells through glycoproteins in *L. gasseri* and carbohydrates in the case of *L. acidophilus* [130]. The genes encoding the aggregation-promoting factor (APF) protein from six different strains of *L. johnsonii* and *L. gasseri* have recently been identified and sequenced [131]. Both species harbour two *apf* genes, *apf1* and *apf2*, which are oriented in the same way and encode proteins containing 257–326 amino acids. All these genes have been found to exhibit very strong sequence conservation, with the exception of their central regions. Primer extension analysis has revealed that *apf1* and *apf2* harbour a putative promoter sequence that is conserved in all of the genes. These genes are transcribed, reaching their maximum expression during the exponential phase, and APF proteins are located on the cell surface. In addition, the amino acid composition and physical properties of APF proteins, and the genetic organization of the genes encoding these proteins have been found to be quite similar to those of S-layer proteins. However, the involvement of APF proteins in the adhesion of the *L. johnsonii* and *L. gasseri* bacteria remains to be demonstrated. In conclusion, lactobacilli and bifidobacteria display various surface characteristics that are involved in their interactions with intestinal epithelial cells. The 2.26-Mb genome sequence of an infant-derived strain of *B. longum* has been recently determined by Schell et al. [132] and 1730 possible coding sequences have been identified in the 60%/40% GC circular chromosome. Interestingly, the authors have identified polypeptides that showed homology to most major proteins needed for production of glycoprotein-binding fimbriae, structures that could possibly be important for adhesion and persistence in the gastrointestinal tract.

### 4.1.2. Bacterial interference in vitro

Pathogens have developed several mechanisms of association to ensure that they remain associated with the gut mucosa and withstand the flow of the intestinal chyme. Pathogenic bacteria in particular form close associations with the intestinal mucosa that require specialized factors encoded by the bacteria, and if they cannot do this, they are rapidly eliminated from the gut. In elegant experiments, Reid et al. [91,100–102] have demonstrated that uroagalactobacilli compete with uropathogens. Similarly, lactobacilli and bifidobacteria of intestinal origin have the capacity to interfere with the adherence of pathogenic bacteria onto intestinal cells.

Adherent live or heat-killed *L. acidophilus* LB and live *L. johnsonii* La1 bacteria in the presence of spent culture supernatant, showed dose-dependent inhibition of adhesion to the brush border of Caco-2 cells of diarrheagenic ETEC bearing CFA/I or CFA/II adhesive factors, *Salmonella enterica* serovar Typhimurium, EPEC, *Yersinia pseudotuberculosis* and *Listeria monocytogenes* in a concentration-dependent manner [112,133,134]. *L. casei* subsp. *rhamnosus* Lc35 organisms have been reported to inhibit the adherence of EPEC, ETEC and *Klebsiella pneumoniae* to Caco-2 cells [109]. *L. rhamnosus* DR20 and *L. acidophilus* HN017 strains inhibited colonization of the intestinal cell monolayer by *E. coli* O157:H7, and also reduced cell invasion by this enterovirulent strain [110]. *Lactobacillus* strains including *L. rhamnosus*, *L. gasseri*, *L. casei* and *L. plantarum*, developed inhibitory activity against enterohemorrhagic *E. coli* (EHEC) infecting the human colon epithelial cell line, C2BB1 [135]. Interestingly, while the adhesion and colonization of EHEC was not affected by any of the *Lactobacillus* strains tested, the internalization of EHEC into the cell line was markedly suppressed, particularly by *L. rhamnosus* strains, without affecting the viabilities of EHEC and host cells. Simple competition at certain receptors was unlikely, because the suppressive effect on EHEC internalization was strictly dependent on viable *L. rhamnosus* bacteria and could not be observed with the conditioned medium or killed *L. rhamnosus*. These findings suggested that an avid interaction between living *L. rhamnosus* bacteria and the host cells probably resulted in a negative modulation of the internalization process of EHEC. As a result of their adheresiveness to Caco-2 cells, *L. casei rhamnosus* GG and *L. casei* Shirota bacteria were able to compete with *E. coli* and *Salmonella* spp., probably by means of steric hindrance [136]. Human lactobacilli strains, *L. acidophilus* and *L. fermentum* isolated from chicken intestine, were able to produce exclusion and competition in cultured intestinal cells if they were infected by *S. pullorum* and *S. typhimurium*, but not if they were infected by *S. enteritidis* [137]. Strains of *L. fermentum* spp., *L. acidophilus* spp. and *L. salivarius* spp. isolated from the gastrointestinal tract of piglets at the time of weaning, were found to affect the attachment of enterotoxigenic *E. coli* to porcine enterocytes by coaggregating with *E. coli* [138]. When examining the inhibitory effect of *L. johnsonii* La1 and *L. acidophilus* LB cultures on invasion of Caco-2 cells by a wide range of diarrheagenic bacteria, it has been observed that these strains produce a dose-dependent inhibition of cell-entry by enteropathogenic *E. coli*, *Y. pseudotuberculosis*, and *S. enterica* serovar Typhimurium [112,133,134]. *L. acidophilus* UO 001 and *L. gasseri* UO 002 strains are able to inhibit the growth of certain enteropathogens: *Salmonella*, *Listeria* and *Campylobacter*. Finally, strongly adherent *L. gasseri* bacteria were found to inhibit the attachment of *E. coli* O111 to intestinal Caco-2 cells under the condition of exclusion [130]. *L. acidophilus* CYC 10051 and *L. kefiranofaciens* CYC 10058 strains isolated from kefir, adhered onto human enterocyte-like
Caco-2 cells, develop antimicrobial activities against enteropathogenic bacteria and were able to inhibit *S. typhimurium* attachment to Caco-2 cells [139]. Examination of the antimicrobial activity against *Clostridium difficile* of a high number of lactobacilli and bifidobacteria strains isolated from healthy Korean infants shows that only 8 of the 109 strains showed activity against *C. difficile* and that 19 strains were active against *E. coli* O157:H7, but none against *S. aureus* [140]. Interestingly, antimicrobial activity against both *C. difficile* and *E. coli* O157:H7 was restricted to four strains.

*L. casei rhamnosus* GG, *L. casei* Shirota, *L. rhamnosus* LC-705 and *L. johnsonii* La1 strains as well as an *L. rhamnosus* sp. strain isolated from human faeces, were able to reduce the S-fimbria-mediated adhesion of *E. coli* and the adhesion of *Salmonella* to human intestinal glycoproteins extracted from faeces, which constitute a mucin-like model [141]. By adhering to the intestinal mucin, *B. lactis* LKM512 inhibits *C. perfringens* adhesion [142]. There are increasing reports indicating that several lactobacilli strains are able to inhibit the adherence of pathogenic bacteria to intestinal epithelial cells through their ability to increase the production of intestinal mucus. By incubating *L. plantarum* 299v with HT-29 cells, the increased expression levels of mucus *MUC2* and *MUC3* mRNA was shown to be accompanied by quantitative inhibition of the cell-attachment of EPEC, an effect that could be mimicked by adding purified exogenous *MUC2* and *MUC3* mucins [143]. *L. casei rhamnosus* GG and *L. casei* Shirota strains were able to compete with and to exclude pathogenic *E. coli* and *Salmonella* spp. adhering onto human intestinal mucus glycoproteins and Caco-2 cell surfaces were studied [144]. Moreover, *L. casei rhamnosus* GG mediated the up-regulation of epithelial *MUC-2* in human enterocyte Caco-2 cells, and thus increased the inhibition of bacterial translocation [145].

The inhibitory effect of *B. breve* and *B. infantis* strains isolated from human adult subjects, on the colonization of an intestinal cell monolayer by a variety of diarrhoea pathogens demonstrates the marked and concentration-dependent inhibition of the association between enteroxygenic, enteropathogenic, diffusely adhering *E. coli* and *S. enterica* serovar Typhimurium strains and enterocytic Caco-2 cells [115]. *B. lactis* DR10 inhibited the binding of *E. coli* O157:H7 to an intestinal cell monolayer, and also reduced the invasiveness of this pathogenic strain [110]. Moreover, *Bifidobacterium* spp. CA1 and F9 strains isolated from infant stools, inhibited the entry of *S. enterica* serovar Typhimurium into Caco-2 cells [146].

Since the lactobacilli and bifidobacteria do not express pathogen-adhesive factors, it can be postulated that they were able to inhibit pathogenic attachment by means of steric hindrance at human enterocytic pathogen receptors, a mechanism first postulated by Reid et al. [91,100–102]. Consistent with this hypothesis are observations that *L. casei* sp. and *L. johnsonii* La1 strains express binding specificities for carbohydrates such as O-linked oligomannosides and gangliotriosylceramides and gangliotetraosylceramides (asialo-GM1) [147,148], which are known to be expressed on the cell surface adhesins of several enteropathogenic *E. coli* [149]. Competitive binding has been reported between several species of *Bifidobacterium* sp. BL2928 and ETEC-expressing colonization factor antigen (CFA) II, to gangliotetraosylceramide (asialo GM1 or GA1), a common bacterium-binding structure [150]. The factor produced by BL2928 bacteria in the culture supernatant fluid that inhibits the binding of *E. coli* to GA1 is a proteinaceous molecule or molecules(s) with a molecular weight around or over 100,000 and a neutral isoelectric point. In addition, removal of the S-layer protein from *L. crispatus* JCM 5810 cells reduced the adhesion capacity of lactobacilli, and the isolated S-layer protein was found to inhibit the adhesion of *E. coli* to both Matrigel and immobilized laminin, suggesting a competitive mechanism of inhibition [151]. Altogether, these results corroborate the hypothesis that selected strains of lactobacilli and bifidobacteria could compete with enteropathogens for the same carbohydrate receptors in the gut.

In conclusion, some selected adhesive lactobacilli and bifidobacteria strains develop antagonistic activity against adhesion of gastrointestinal pathogens onto cultured human intestinal cells that mimic the situation in vivo (Table 1). However, as pertinently underlined by Reid and Burton [152], it is difficult to extrapolate this in vitro effect to the gastrointestinal situation in vivo. Indeed, in vivo the resident microbiota and mucus, and the peristaltic flow that continuously washes the gastrointestinal epithelium, could efficiently modify adhesion of exogeneous *Lactobacillus* and *Bifidobacterium* bacteria. To ascertain that the antagonistic activities observed in vitro by adhering *Lactobacillus* and *Bifidobacterium* bacteria also develop in vivo, blocking the colonization of epithelium, or inhibiting invasiveness, appropriate infectious animal models should be used. However, some pathogenic bacteria and viruses involved in diarrhoea in humans cannot be investigated since they are highly specific for human tissues, mainly as the result of structural and functional differences between the human and murine intestinal epithelia.

### 4.2. Demonstration of antimicrobial activity in infectious animal models

The antibacterial activity of lactobacilli and bifidobacteria has been investigated principally using two infected mouse models. The first involves gnotobiotic mice, in which the microbiota is missing and the epi-
the digestive tract of HFA mice, L. casei the bacterium [156]. In contrast, when inoculated into pigs challenged by [154,155]. Both the jejunum and the ileum of gnotobiotic bacteria have been shown to colonize the gut of gnotobiotic mice, established themselves in all the segments of the bacteria given orally to C3H/He/Oujco, gnotobiotic intake of the in vitro adherent in vivo [158]. In C3H/He/Oujco gnotobiotic mice, oral also have the ability to colonize the gastrointestinal tract established itself [157].

4.2.1. Adhesiveness properties and persistence in the gut

Hautefort et al. [153] have pointed out that in vitro adhesion to intestinal cells of lactobacilli and bifidobacteria is not sufficient to ensure the persistence of living bacteria in the digestive tract of mice. The ability of several lactobacilli and bifidobacteria strains, already shown to adhere in vitro, to colonize the gastrointestinal tract of mice in vivo has been investigated. The in vitro adhering L. casei rhamnosus GG and L. johnsonii La1 bacteria given orally to C3H/He/Oujco, gnotobiotic mice, established themselves in all the segments of the gut [107,133]. The L. salivarius and L. plantarum 299v bacteria have been shown to colonize the gut of gnotobiotic BALB/c mice and IL-10-/- mice, respectively [154,155]. Both the jejunum and the ileum of gnotobiotic pigs challenged by Lactobacillus spp. were colonized by the bacterium [156]. In contrast, when inoculated into the digestive tract of HFA mice, L. casei DN-114 001 survived, but was eliminated with the same kinetics as an inert transit marker, indicating that it does not establish itself [157].

It has been observed that some bifidobacteria strains also have the ability to colonize the gastrointestinal tract in vivo [158]. In C3H/He/Oujco gnotobiotic mice, oral intake of the in vitro adherent B. infantis 1, and Bifidobacterium CA1 and F9 bacteria is followed by the establishment of high levels of adhering, viable bacteria in the mucosa of various segments of the gut, as well as in the intestinal contents [146]. Bifidobacteria strains isolated from breast-fed infants, formula-fed infants, or premature babies were predominantly B. adolescentis, and were present in the gut of conventional mice challenged daily, and remained present up to 5 days after feeding, even if feeding was interrupted [159].

Peyer’s patches have been shown to be involved in the first step in the induction of a mucosal immune response [40,41,160]. Strains of Lactobacillus have a range of properties that make them attractive candidates for oral vaccination purposes, e.g. their long history of safe use by humans, adjuvant properties, mucosal adhesive properties and low intrinsic immunogenicity. Potential applications of live lactobacilli for use as vectors in vaccines to deliver protective antigens to the mucosal surfaces have been suggested [161–166]. In line with this objective, it has been recently reported that lactobacilli are able to interact with Peyer’s patches [167].

4.2.2. Activity against Helicobacter pylori

An antagonist effect of lactobacilli against H. pylori infection has been also demonstrated in a murine model. In an H. pylori-infected gnotobiotic murine model, an L. salivarius strain that produces a large amount of lactic acid and completely inhibits the growth of H. pylori in a mixed culture, suppressed H. pylori, and reduced the H. pylori-induced inflammatory response [168]. In conventional mice, oral treatment with the spent culture supernatant of the human L. acidophilus LB conferred protection against infection by H. felis, reducing the urease activity of H. felis in the stomach, inhibiting colonization of the stomach by H. felis, and abolishing the H. pylori-induced gastric histopathological lesions [169].

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Selected lactobacilli and bifidobacteria strains with adhesiveness properties, developing in vitro bacterial interference activity against bacterial pathogens</th>
</tr>
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<tbody>
<tr>
<td>Strain</td>
<td>Adhesiveness</td>
</tr>
<tr>
<td>L. acidophilus LB</td>
<td>[104,112]</td>
</tr>
<tr>
<td>L. acidophilus HN017</td>
<td>[110]</td>
</tr>
<tr>
<td>L. johnsonii La1</td>
<td>[106,108,121,122,128,148]</td>
</tr>
<tr>
<td>L. rhamnosus DR20</td>
<td>[110]</td>
</tr>
<tr>
<td>L. casei subsp. rhamnosus Lcr35</td>
<td>[109]</td>
</tr>
<tr>
<td>L. casei Shirota</td>
<td>[136]</td>
</tr>
<tr>
<td>L. casei GR-1</td>
<td>[91,100–102]</td>
</tr>
<tr>
<td>L. fermentum RC-14</td>
<td>[91,100–102]</td>
</tr>
<tr>
<td>L. plantarum 299v</td>
<td>[143]</td>
</tr>
<tr>
<td>Bifidobacterium spp. CA1 and F9</td>
<td>[115,146]</td>
</tr>
<tr>
<td>B. lactis DR10</td>
<td>[110]</td>
</tr>
</tbody>
</table>

Bacterial interference activity against bacterial pathogens includes competitive exclusion and/or inhibition of invasiveness activity of pathogens in vitro using cultured human epithelial cells and/or isolated intestinal mucus.
4.2.3. Activity against Salmonella

The most common infectious model used to investigate the antibacterial activity of lactobacilli and bifidobacteria is that of gnotobiotic or conventional mice infected by Salmonella. L. johnsonii La1 [133] and GG [107] strains, which colonize the gut of gnotobiotic C3H/He/Oujo mice, develop antibacterial activity when the mice are orally infected by S. enterica serovar Typhimurium C5, increasing the survival of mice. Similarly, L. acidophilus has been found active against experimental infections with S. flexneri and S. enteritidis serovar Typhimurium in gnotobiotic mice [170]. The L. rhamnosus HN001 has the ability to confer immune enhancement and protection on BALB/c mice orally challenged with S. enterica serovar Typhimurium 1772 [171]. Interestingly, infected and treated mice produced higher serum and intestinal tract titres of anti-Salmonella antibodies, showed greater overall survival of infection and had significantly lower mean pathogen burdens in spleen and liver than controls. In addition, blood and peritoneal leukocytes obtained from HN001-fed mice exhibited significantly higher ex vivo phagocytic capacity than those from control mice. The spent culture supernatant of the human L. acidophilus strain LB given daily following infection is active against S. enterica serovar Typhimurium C5 infecting conventional C3H/He/Oujo mice, reducing the levels of viable Salmonella in the faeces [172]. Furthermore, L. salivarius CTC2197 colonizing the gastrointestinal tract of Leghorn chickens, is able to prevent S. enteritidis colonization [173]. In contrast, it has been observed that Salmonella survives and persists at a higher level in the faeces of conventional mice treated with L. casei rhamnosus GG given daily [107].

In the axenic C3/He/Oujo mouse, human Bifidobacterium spp. CA1 and F9 bacteria colonized the intestinal tract and protected the mice against a lethal infection of S. enterica pathovar Typhimurium C5, suggesting that they could contribute to the “barrier effect” produced by the indigenous microbiota [146]. B. lactis HN019 feeding in BALB/c mice conferred protection against S. enterica pathovar Typhimurium, and the protection included a 10-fold increase in the survival rate, a significantly higher post-challenge food intake and weight gain, and reduced translocation of the pathogen to the spleen and liver [174]. Similarly, the protective effect of B. lactis HN019 against E. coli O157:H7 in mice resulted in a lower cumulative morbidity rate, significantly higher proportions of phagocytically active cells in the blood and peritoneum, and higher intestinal tract IgA anti-E. coli antibody responses [175]. The anti-infectious activity of B. breve Yakult against S. enterica serovar Typhimurium has been investigated using an opportunistic, antibiotic-induced murine infection model. Intestinal growth and subsequent extra-intestinal translocation of the pathogen were inhibited by B. breve colonization [176]. In contrast, B. bifidum ATCC 15696 and B. catenulatum ATCC 27539T strains have no effect, even when they reached high population levels similar those of the B. breve strain. Crude cecal extract from B. breve-colonized mice displayed growth-inhibiting activity against S. enterica LT-2 in vitro, whereas that from the cecum of mice colonized by the ineffective B. bifidum displayed much less activity. B. longum inhibited bacterial translocation from the gastrointestinal tract of E. coli C25 in antibiotic-decontaminated specific-pathogen-free (SPF) mice and germ-free mice [177]. Moreover, in a piglet model developing naturally acquired diarrhoea, the administration of B. lactis HN019 led to less severe weaning diarrhoea, accompanied by higher E. coli, blood leukocyte phagocytic and T-lymphocyte proliferative responses, and higher gastrointestinal tract pathogen-specific antibody titres [178].

4.2.4. Activity against Escherichia coli

Rodent and non-rodent animal species infected by pathogenic strains of E. coli have been used to investigate the in vivo activity of lactobacilli. It has been shown that, after challenge, L. rhamnosus HN001-fed mice infected with E. coli O157:H7, exhibited lower cumulative morbidity and bacterial translocation rates, and significantly higher intestinal anti-E. coli IgA responses and blood leukocyte phagocytic activity [171]. L. plantarum 299v antagonizes E. coli-induced injury and intestinal permeability in rats [179]. The colonization pattern of Shiga toxin-producing E. coli in juvenile rabbits is inhibited by the L. casei Shirotta, probably as a result of enhanced local immune responses [180]. A culture condensate of B. longum sp. inhibits the translocation of E. coli from the gastrointestinal tract in antibiotic-decontaminated specific-pathogen-free (SPF) mice and germ-free mice [177]. However, not all the lactobacilli and bifidobacteria strains developing antibacterial activity in vitro were active in vivo. Indeed, despite the fact that Lactobacillus sp. inhibits EPEC in vitro, inoculation of gnotobiotic pigs with this strain failed to prevent enteropathogenic E. coli colonization of the mucosa of both the jejunum and ileum, although Lactobacillus sp. bacteria did adhere to the jejunum and ileum, and produced both lactic acid and acetic acid in the jejunum contents [156]. Similarly, the preventive administration of L. casei subsp. casei to E. coli-infected piglets had no inhibitory effect on the adherence of E. coli to the jejunal mucosa of either gnotobiotic or conventional piglets [181].

4.2.5. Activity against Listeria monocytogenes

Administration of L. casei sp. to mice injected with L. monocytogenes showed that the growth of L. monocytogenes in the liver of these mice was suppressed [182]. Similarly, ingesting viable L. casei Shirotta significantly
reduced the numbers of *L. monocytogenes* in the tissues of orally infected Wistar rats, probably by increasing cell-mediated immunity [183]. The enhanced host resistance to *L. monocytogenes* infection induced by *L. casei* may be mediated by macrophages migrating from the blood stream to the reticuloendothelial system in response to *L. casei* administration before or after infection with *L. monocytogenes*.

4.2.6. Activity against rotavirus

A few studies have been reported in which the antagonistic activity of lactobacilli and bifidobacteria against rotavirus was investigated in vivo. Mouse pups born to and nursed by dams fed *B. breve* YIT4064 and immunized orally with rotavirus were more strongly protected against rotavirus-induced diarrhea than those born to and nursed by dams immunized with rotavirus only. Indeed, observed increase in antirotavirus IgA level in faeces, mammary gland and in the intestine of dams, suggests that oral administration of *B. breve* YIT4064 induces protection against infection [184]. In pathogen-free suckling rats infected with SA11 rotavirus and supplemented daily with milk fermented by *L. casei* DN-114 001, which increases brush-border enzyme activities in mouse small intestine [185], the histological lesions in the small intestine, such as cellular vacuolization, were greatly reduced, and accompanied by a low level of rotavirus infection in all sections of the intestine, and a decrease in the clinical signs of diarrhoea [186]. *B. bifidum* and *B. infantis* strains modulated the course of rhesus rotavirus (RRV) infection in mice by mediating the associated mucosal and humoral immune responses. In a piglet model developing naturally acquired diarrhoea, the administration of *B. lactis* HN019 led to lower concentrations of faecal rotavirus [178]. Levels of Rotavirus-specific IgA in faeces and serum were higher post infection in bifidobacteria-treated RRV-infected mice than in RRV-infected mice that had not received bifidobacteria [187]. These findings suggest that selected strains of lactobacilli and bifidobacteria may act by modulating early mucosal and strong humoral rotavirus-specific immune responses, and thus mitigate the severity of rotavirus-induced diarrhea.

4.3. Clinical demonstration of the activity of lactobacilli and bifidobacteria

On the basis of evidence from the in vitro and in vivo studies discussed above it would appear that the antimicrobial activity of lactobacilli and bifidobacteria is a strain-specific property, and cannot be extrapolated to other strains. Following on from these in vitro and in vivo experimental studies, well-designed, double-blind, placebo-controlled clinical trials have been conducted in order to demonstrate that a selected lactobacilli or bifidobacteria strain does actually display these properties in humans developing the disease(s). As recently revisited by the Joint FAO/WHO Expert Consultation on the Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria, probiotic strains are defined as live microorganisms which, when consumed in appropriate amounts in the food, confer a health benefit on the host [188]. The probiotics most often being investigated at present are the lactic acid bacteria, particularly *Lactobacillus* spp. and *Bifidobacterium* spp. In parallel, pharmaceutical preparations of probiotics contain selected living or heat-killed, lyophilized, lactobacilli and bifidobacteria are used as biotherapeutic agents. These pharmaceutical preparations have the advantage of providing a stable and reproducible preparation of bacteria, which correspond to the pharmacologically established dose-efficacy of lactobacilli and bifidobacteria.

As pertinently underlined by Boriello et al. [189], although lactobacilli are extremely rare causes of infection in humans, strains used for new probiotics should be chosen from the commensally flora of humans and should not carry intrinsic resistance to antibiotics that would prevent treatment of a rare probiotic infection and these strains should be sent to reference centres for molecular characterization and confirmation of safety [189]. Adhesiveness properties and autoaggregation capacities are likely to be involved in the aetiology of lactobacillemia since a high capacity of adhesion for lactobacilli strains has been proposed as a risk, particularly for *L. rhamnosus* strains. *L. rhamnosus* PHLS A103/70 and *L. casei* PHLS A357/84 strains, as well as the probiotic *L. rhamnosus* GG, showed moderate infectivity in a rabbit infective endocarditis model showed, whereas the virulence of the probiotic *L. casei* Shirota and type strains such as *L. acidophilus* ATCC 4356(T) and *L. gasseri* DSM 2043(T) was negligible. The ability to adhere to human intestinal mucus has been tested by Apostolou et al. [190], for *Lactobacillus* bacteria of clinical blood culture, human faecal and for lactobacilli strain of dairy origin used as probiotics. The blood culture isolates were found to adhere better than the dairy strains. Of the nine clinical, 10 faecal and three dairy *L. rhamnosus* strains, blood culture isolates adhered better than the faecal strains. In consequence, the safety of lactobacilli and bifidobacteria strains should be tested before use as probiotics since strain-specific difference in safety should be observed [191].

4.3.1. Clinical evaluation of adhesiveness properties or persistence in the gut

Although it is believed that maximum effect is achieved if the organisms adhere to intestinal mucosal cells, there is little evidence that exogenously administered lactobacilli and bifidobacteria do in fact do this. A few well-designed human pharmacokinetic studies have
been conducted in order to investigate the persistence of live Lactobacillus bacteria in the gut and their colonization of it. They seem instead to pass through into the faeces without having adhered or multiplied. Clinical studies have shown that L. johnsonii L1, L. acidophilus NCFB 1748, L. casei Shirota, L. rhamnosus 19070-2, L. reuteri DSM 12246, L. casei rhamnosus GG, L. delbrueckii subsp. lactis CHCC 2329, and L. casei subsp. alactus CHCC 3137 bacteria survived passage through the human gut when administered in humans. The rate of survival has been estimated to be 20–40% for selected strains, the main obstacles to survival being gastric acidity and the action of bile salts [192–194]. However, survival seems to be strain-dependent. For example, L. casei rhamnosus GG bacteria persisted in the faeces of volunteers for four to seven days after feeding had stopped [195], whereas L. rhamnosus DR20 does not persist [196]. In addition, L. casei rhamnosus GG was recovered during colonic biopsies from patients who had consumed a whey drink fermented with this strain [197–201]. However, L. casei rhamnosus GG bacteria appeared relatively poor colonizers in infants and they do appear to affect neonatal intestinal colonization patterns [202]. Interestingly, a wild-type L. crispatus strain, showing a cell aggregation phenotype, and its spontaneous non-aggregating mutant, have been compared for their in vivo colonization and adhesion potential using colonoscopy patients as volunteers in feeding trials [203]. The authors observed that the wild type was recovered from both faecal samples and biopsies taken from the colon, whereas the mutant strain was not detected.

4.3.2. Diarrhoea

Diarrhoeal diseases continue to be a major cause of morbidity and mortality worldwide. The cornerstone of treatment recommended by the World Health Organization (WHO) remains the use of oral rehydration solution (ORS). As emphasised by Guandalini et al. [204], despite dramatic progress in the understanding of the pathophysiology of diarrhoea, the list of drugs available is indeed short. The addition of a medication to the WHO protocol for the treatment of acute diarrhoea in children is controversial. Recently, in addition to ORS, several new therapeutic strategies, including selected Lactobacillus strains and biotherapeutic agents containing selected, heat-killed Lactobacillus strains, have demonstrated considerable potential for promoting a more rapid recovery from acute, watery diarrhoea by children with microbial enteritis [85].

Overall, clinical trials have indicated that the selected Lactobacillus strains used in these clinical studies are a safe and effective treatment for children with acute infectious diarrhoea. A randomized, placebo-controlled trial of the effect of L. rhamnosus 19070-2 [205,206] and L. reuteri DSM 12246 [207,208] strains on acute diarrhoea in children showed that the mean duration of the diarrhoea was reduced. L. casei rhamnosus GG has proved effective in preventing and/or treating acute diarrhoeal disease in children in several clinical trials, most of which were randomized and placebo-controlled. The general conclusion is that oral administration of live GG bacteria reduced the duration of diarrhoea in children by half [197,209–214]. The clinical efficacy of lyophilized heat-killed L. acidophilus LB bacteria in the presence of its spent culture supernatant as an adjunct to ORS therapy, has been assessed in children aged 3 to 24 months with acute diarrhoea and mild or moderate dehydration [215]. The mean duration of diarrhoea was shorter with L. acidophilus LB versus placebo, particularly in the children who had received no antibiotic therapy before inclusion. Unfortunately, most of these clinical studies were conducted in small groups of patients. More significantly, a multicenter, double-blind, placebo-controlled investigation conducted to evaluate the efficacy of L. casei rhamnosus GG administered in the oral rehydration solution to patients with acute-onset diarrhoea showed a highly significant shortening in the duration of diarrhoea [204,216]. In a meta-analysis [217], the authors concluded that, where adequately randomized, blind and controlled, the clinical trials in which the treatment groups received Lactobacillus strains, and the control groups received an appropriate placebo demonstrated that both the severity and the duration of diarrhoea were significantly reduced.

In contrast to paediatric clinical reports showing substantial evidence of the clinical benefits from lactobacilli therapy in patients with viral gastroenteritis, data in support of the use of probiotic strains for the prevention of adult travellers’ diarrhoea are more limited and less convincing [81]. Some placebo-controlled, double-blind studies have shown that the L. casei rhamnosus GG reduces the incidence of travellers’ diarrhoea [218–220]. In contrast, a placebo-controlled, double-blind study conducted in subjects travelling on holiday showed an overall incidence of 46.5% of diarrhoea in the placebo group versus an incidence of 41.0% in the group given L. casei rhamnosus GG [221].

The administration of antimicrobial agents, therapeutically or as prophylactically, causes disturbances in the ecological balance between the host and the normal microbiota, leading to intestinal colonization by potentially pathogenic microorganisms and overgrowth by opportunistic microorganisms already present, followed by diarrhoea and fungal infections [222]. Clinical trials carried out to assess the therapeutic efficacy of lactobacilli strains in preventing diarrhoea are less convincing or even contradictory. For example, children receiving L. casei rhamnosus GG twice daily orally throughout their hospital stay, showed a lower risk of nosocomial diarrhoea than those receiving the placebo, although the
prevalence of rotavirus infection was similar in the two groups [223]. The prophylactic use of \textit{L. casei} \textit{rhamnosus} GG to prevent diarrhoea in children showed that there were significantly fewer episodes of diarrhoea in the treated group than in the placebo group, but that the duration of diarrhoea episodes was similar in both groups [213]. \textit{L. casei} DN-114 001 given to children during three one-month periods of supplementation, each month on supplementation being followed by a month without supplementation, showed that the incidence of diarrhoea was no different, but the severity of diarrhoea over the six-month study was significantly lower than that in a control group receiving milk [224]. In contrast, a multicenter, randomized, double-blind trial, conducted over four months on 928 children, showed that administration of \textit{L. casei} DN-114 001 fermented milk lowered the incidence of diarrhoea less significantly than yogurt [225].

There are a few clinical studies that have examined the preventative potential of lactobacilli against antibiotic-associated diarrhoea and contradictory results have been obtained. According to Siitonen et al., [226], subjects receiving \textit{L. casei} \textit{rhamnosus} GG yoghurt with erythromycin had less diarrhoea than those taking pasteurized yoghurt. In contrast, it has been observed that the incidence of diarrhoea in a group of patients treated with the \textit{L. casei} \textit{rhamnosus} GG group was lower than in the placebo group within 2 weeks of antimicrobial therapy [227]. A placebo-controlled trial examining the efficacy of \textit{L. casei} \textit{rhamnosus} GG in reducing the incidence of antibiotic-associated diarrhoea when co-administered with an oral antibiotic in children with acute infectious disorders showed that, compared to the placebo group, \textit{L. casei} \textit{rhamnosus} GG treatment significantly reduced the overall stool frequency and increased stool consistency during antibiotic therapy by the tenth day [228]. A prospective, randomized, placebo-controlled trial of \textit{L. casei} \textit{rhamnosus} GG in combination with standard antibiotics for the treatment of \textit{C. difficile} infection showed that patients seemed to have less recurrent \textit{C. difficile} diarrhoea and early symptomatic improvement when using the \textit{L. casei} \textit{rhamnosus} GG [229]. In contrast, the efficacy of \textit{L. casei} \textit{rhamnosus} GG in preventing antibiotic-associated diarrhoea in 267 adult patients showed no additional difference in the rate of occurrence of diarrhoea between treatment and placebo patients in a subgroup analysis of those treated with beta-lactam or with non-beta-lactam antibiotics [230]. In conclusion, analysis of published randomized, double-blind, placebo-controlled trials suggest that lactobacilli and bifidobacteria strains can be used to prevent antibiotic-associated diarrhoea, but the evidence for beneficial effects is still not conclusive, since the published studies are flawed by the lack of placebo controls, and by peculiar population features [231,232].

4.3.3. \textit{Helicobacter pylori} infection

Acid suppression plus two antibiotics is the reference treatment for \textit{H. pylori}. Reported eradication rates of around 65–80% have been obtained with combined rabeprazole, clarithromycin and amoxicillin treatment. However, antibiotic therapy is usually associated with the risk of significant adverse effects, as a result of its effects on the intestinal microbiota. Despite the fact that lactobacilli have a very short transit time in the stomach, the use of lactobacilli in anti-\textit{H. pylori} therapy could reduce the risks of adverse effects. Pre-clinical studies have shown that \textit{H. pylori} growth is inhibited by lactobacilli. The need for new strategies for \textit{H. pylori} eradication using selected \textit{Lactobacillus} strains, as an alternative or complementary to antibiotic therapy, has recently claimed the attention of many investigators [89]. Trials in which fermented milk products or whole cultures of lactobacilli were used tended to show better results than when the probiotic was taken in the form of bacteria alone. However, as pertinently analyzed by Hamilton-Miller [233], not all the clinical studies published were randomised, double-blind and placebo controlled, and some involved only small numbers of patients. Six out of fourteen patients with \textit{H. pylori} infection who were given milk containing live \textit{L. acidophilus} NAS showed eradication of \textit{H. pylori} [234]. \textit{L. gasseri} OLL2716 has been found to be effective against \textit{H. pylori} infection in children [235]. In a randomized, double-blind, controlled clinical trial, the effect of a drinkable, whey-based, \textit{L. johnsonii} La1 culture supernatant on \textit{H. pylori} infection in volunteers showed a marked decrease in breath test values immediately after treatment with La1 supernatant in both the omeprazole and the placebo group [236]. Interestingly, breath test values remained low 6 weeks after treatment, despite the fact that the persistence of \textit{H. pylori} infection was confirmed in gastric biopsies. In subjects with a \textit{H. pylori} infection who were receiving \textit{L. johnsonii} La1-acidified milk twice a day for 3 weeks; esophagastroduodenoscopy, biopsies and the determination of urease activity showed a decrease in \textit{H. pylori} density in the antrum and the corpus, and the reduction of inflammation and gastritis activity in the antrum, and of gastritis activity in the corpus [237]. Frequent ingestion of \textit{L. johnsonii} La1 results on a suppressive effect on \textit{Helicobacter pylori} colonization in asymptomatic volunteers [238]. In addition, the regular ingestion of a product containing \textit{L. johnsonii} La1 bacteria has been found to modulate the \textit{H. pylori} colonization in children infected by this pathogen [239]. \textit{L. gasseri} OLL2716 ingested in the form of a yogurt daily for an 8-week period, was effective both in suppressing \textit{H. pylori} and in reducing gastric mucosal inflammation, measured by the urea breath test and assays of serum pepsinogens [240]. Clarithromycin (CAM)-resistant \textit{H. pylori} sometimes offers serious problems with eradication by antibiotics.
L. gasseri OLL2716 inhibited in vitro the growth of CAM-resistant and CAM-susceptible H. pylori and suppressed H. pylori-associated IL-8 production [241]. Interestingly, in an in vivo model of mice infected with these CAM-susceptible or CAM-resistant H. pylori, the bacterial gastric colonization was significantly decreased. L. casei Shirota inhibited H. pylori growth in vitro by a lactic acid-independent mechanism and decreased urease activity in subjects (64%) with L. casei supplementation [242]. The addition of heat-killed L. acidophilus LB bacteria with its spent culture supernatant increased the eradication rate of a standard anti-H. pylori therapy to 88% [243]. In contrast, the L. johnsonii Lal-acidified milk did not improve the antibiotic effect in patients who had received clarithromycin treatment [237]. However, it should be noticed that not all the in vitro active strains are effective in vivo. Indeed, L. acidophilus, L. bulgaricus strains which inhibit the growth of H. pylori in vitro, had no effect on the eradication of H. pylori in twenty-six out of twenty-seven subjects [244]. Future trials should address more particular the type of patient (asymptomatic volunteers, symptomatic patients) and criteria of success (breath test, histology, culture, serology).

In conclusion, there is a low number of selected lactobacilli and bifidobacteria strains for which the antagonistic activities against microbial gastrointestinal pathogens have been demonstrated in appropriate infectious animal models and in well performed, randomised, placebo-controlled and double-blind clinical trials (Table 2).

5. Antimicrobial activity by lactobacilli against urovaginal microbial pathogens

Urinary tract infections (UTI) remain a common problem, particularly in the female population. Bacterial adherence to the urovaginal epithelium is recognized as an important mechanism in the initiation and pathogenesis of urovaginal tract infections. The uropathogens originate predominantly in the intestinal tract, and initially colonize the periurethral region before ascending into the bladder, resulting in symptomatic or asymptomatic bacteriuria. Urovaginal pathogens display virulence characteristics that enable them to resist the normally efficient defence mechanisms of the host.

Reid et al. [91,100–102] have demonstrated experimentally that selected Lactobacillus strains of urovaginal origin have adhesive properties that enable them to inhibit and/or prevent the colonization of uroepithelial cells by uropathogens. Complete or partial inhibition of the adherence of Gram-negative uropathogens has been obtained by preincubation of the uroepithelial cells with bacterial cell wall fragments isolated from the Lactobacillus strains GR-1, GR-2, GR-3 and A-60 [100,102]. Competitive exclusion was most effective with whole viable cells, but Lactobacillus cell-wall preparations suggested that lipoteichoic acid was responsible for the adherence of the Lactobacillus cells to uroepithelial cells, but that steric hindrance was the main factor in preventing the adherence of uropathogens [100]. This conclusion was also supported by blocking studies using reconstituted lipoteichoic acid-peptidoglycan, which was more effective at blocking adherence than either lipoteichoic acid or peptidoglycan alone. These reports, together with the fact that L. fermentum ATCC 14931, L. jensenii ATCC 25258, L. plantarum ATCC 14917, and L. reuteri JCM 1112 strains have no significant antimicrobial activity against uropathogenic E. coli, clearly demonstrate that the anti-bacterial effect of lactobacilli is strain-dependent [245].

In a chronic urinary tract infection model developed in female rats, the persistent adherence of L. casei GR-1 bacteria incorporated into agar beads produced no

Table 2

| L. acidophilus LB          | [169,172]  |
| L. johnsonii Lal          | [133]      |
| L. rhamnosus DR20         | [323,436]  |
| L. rhamnosus HN001        | [171]      |
| L. casei rhamnos G6       | [107]      |
| L. casei DN-114 001       | [185,186]  |
| L. casei Shirota          | [180,183,242,245,266,291,312–314] |
| L. casei GR-1             | ND         |
| L. fermentum RC-14        | [273]      |
| B. lactis strain HN019    | [174,175,178] |
| B. breve strain Yakult    | [176]      |

Antimicrobial activity in infectious animal models

| L. acidophilus LB          | [215,243]  |
| L. johnsonii Lal          | [236–239]  |
| L. rhamnosus HN001        | ND         |
| L. casei rhamnos G6       | [197,204,209–214,216,221,223,226–230] |
| L. casei DN-114 001       | [224,225]  |
| L. casei Shirota          | [242]      |
| L. casei GR-1             | [91,97,152,248,249,251] |
| L. gasseri Shirota        | [97,152,248,249,251] |

Antimicrobial effect in clinical studies

a Only against H. pylori infection.
b Only on patients with diarrhoea.
c In patients with diarrhoea or H. pylori infection.
d In patients with UTI.

ND, not documented.

* Only against H. pylori infection.

** Only on patients with diarrhoea.

*** In patients with diarrhoea or H. pylori infection.

**** In patients with UTI.
uropathogenic bacteria in the bladder and kidney tissues up to 60 days after challenge, and no immune response [101]. This finding indicates that in vivo this *Lactobacillus* strain appeared to prevent uropathogens from colonizing the urinary tract. *L. fermentum* CRL 1058 could reduce UTI by uropathogenic *E. coli* in mice [246]. Indeed, the reinforcement of lactobacilli led to the quicker elimination of the pathogen. In a murine UTI model, the antimicrobial activity of the intraurethrally administered living and heat-killed *L. casei* Shirataki bacteria against *E. coli* positive for type 1 and P fimbriae dramatically inhibited both *E. coli* growth and the inflammatory response in the urinary tract [245].

Several experimental and clinical studies have assessed the potential use of lactobacilli for the prevention or treatment of certain genitourinary tract infections, such as bacterial vaginosis, vaginitis, or urinary tract infections [247]. The main goal of therapy with biotherapeutic agents should be to prevent overgrowth by a pathogen until such time as the normal microbiota can be re-established. In addition, *Lactobacillus* therapy is considered as “natural” and devoid of side effects, in contrast to conventional pharmaceutical treatments. *L. rhamnosus* GR-1 and *L. fermentum* RC-14 strains are well-characterized lactic acid-producing strains which are effective in preventing and treating urogenital infections in women. In healthy women randomized to receive one of three lactobacilli strains in encapsulated form, the use of *L. rhamnosus* GR-1 and *L. fermentum* RC-14 strains correlated with a healthy vaginal flora, whereas the ingestion of *L. casei rhamnosus* GG failed to have any effect [248]. In healthy women in whom a capsule containing either a combination of strains GR-1 and RC-14 or the strain GG was inserted vaginally for 3 consecutive nights, a randomly amplified polymorphic DNA analysis showed that GR-1 and/or strain RC-14 bacteria were found to persist in the vaginal tract for up to 19 days after vaginal instillation, whereas *L. casei rhamnosus* GG bacteria were detectable only for up to 5 days post administration [249]. These data have provided molecular biology-based evidence that the selected strains RC-14 and GR-1 persist in the human vagina, and may be more appropriate for vaginal colonization than the intestinal isolate, strain GG. Interestingly, in premenopausal women, the vaginal bacteria examined by PCR/denaturing gradient gel electrophoresis, by sequencing of the V2–V3 region of the 16S rRNA gene and by randomly amplified polymorphic DNA analysis of isolated lactobacilli, demonstrated that the exogenous strains could be detected for up to 21 days in some subjects receiving vaginally inserted capsules containing viable lactobacilli [250]. Moreover, it has been demonstrated in women suffering from recurrent urinary tract infections, and treated twice weekly with intravaginal and perineal implantation of strain GR-1, that these organisms colonized the epithelium, and prevented the emergence of coliform bacteria in most instances, but did not appear to affect enterococcal colonization [91]. Finally, a randomized, placebo-controlled trial of 64 healthy women given daily oral capsules of strains GR-1 and RC-14 for 60 days showed no adverse effects, and restoration from asymptomatic bacterial vaginosis microbiota to a normal lactobacilli colonized microbiota occurred in 37% women during lactobacilli treatment compared to 13% on placebo [251].

### 6. Mechanisms of the antimicrobial effects

Many mechanisms have been postulated by which lactobacilli and bifidobacteria could produce antimicrobial activity. In addition to their competitive inhibition of the epithelial and mucosal adherence of pathogens and inhibition of epithelial invasion by pathogens, lactobacilli and bifidobacteria also show antimicrobial activity by producing antimicrobial substances and/or stimulating mucosal immunity.

#### 6.1. Production of \( \text{H}_2\text{O}_2 \)

The production of \( \text{H}_2\text{O}_2 \) by *Lactobacillus* spp. may be a non-specific antimicrobial defence mechanism of the normal vaginal ecosystem [98,152]. Optional \( \text{H}_2\text{O}_2 \)-producing *Lactobacillus* spp. are found in the vagina of most normal women but much less often in that of women with bacterial vaginosis, whereas anaerobic *Lactobacillus* spp. which do not produce \( \text{H}_2\text{O}_2 \), have been found to be increased in women with bacterial vaginosis [252–255]. *L. crispatus* and *L. jensenii*, the most common lactobacilli in the female genital tract, inhibit gonococci by producing \( \text{H}_2\text{O}_2 \) [256]. When examining the antimicrobial activity of twenty-two *Lactobacillus* strains of vaginal origin, Aroucheva et al. [257] observed that approximately 80% of the strains produced \( \text{H}_2\text{O}_2 \), lactic acid, organic acid and bacteriocins, and these strains were active against several, but not all, *Gardnerella vaginalis* strains. *L. brevis* CD2, *L. salitarius* FV2 and *L. gasseri* MB335 adhered to epithelial cells, displacing vaginal pathogens; and produced high levels of \( \text{H}_2\text{O}_2 \), coaggregated with pathogens and inhibited the growth of *G. vaginalis* [258]. *L. crispatus* F117 and *L. paracasei* strains F2 and F28, which generated the highest \( \text{H}_2\text{O}_2 \) level, inhibited *S. aureus* growth in a plate assay [259,260]. *L. delbrueckii* V11007 produces at least three growth-inhibiting factors, other than lactic acid, one of which has been identified as \( \text{H}_2\text{O}_2 \) [261].

#### 6.2. Production of acids

By producing metabolites such as acetic and lactic acids, and thus lowering the pH, a large number of
lactobacilli inhibit the growth of bacterial pathogens [262]. It has been observed that the mechanism by which *L. casei rhamnosus* GG impeded the invasion of Caco-2 cells by *S. enterica* serovar Typhimurium, without modifying the viability of the pathogen, was abolished after the *Lactobacillus* culture had been neutralized to pH 7, which suggested a pH-dependent mechanism [107,263]. However, the inhibition of the growth of *S. sonnei* is not due to pH alone, but results from the presence of *Lactobacillus*-inhibiting substance(s) that are extracellular and diffusible [264]. *L. lactis*, *L. casei Shirota* or *L. acidophilus* YIT 0070 strains reduced the growth of *E. coli* O157:H7 by lactic acid production and pH reductive effect [265,266]. The cell-free *L. casei* subsp. *rhamnosus* Lcr35 supernatant inhibited the growth of nine human pathogenic bacteria: enterotoxigenic *Escherichia coli* (ETEC), enteropathogenic *E. coli* (EPEC), *K. pneumoniae*, *S. flexneri*, *S. typhimurium*, *E. cloacae*, *P. aeruginosa*, *E. faecalis* and *Clostridium difficile* [109]. *Lactobacillus* strains isolated from the human digestive tract have been found to inhibit the growth of four species known to be anaerobic bacterial etiologic agents of gastroenteric infections, *H. pylori*, *Campylobacter jejuni*, *C. coli* and *C. difficile* [267]. *L. acidophilus* inhibited the growth of clinical isolates of *H. pylori* in vitro, an activity shown by lactic acid [268]. *L. acidophilus*, *L. casei* subsp. *rhamnosus*, *L. bulgaricus*, and *B. bifidus* strains have been found to inhibit the growth of *H. pylori*, an in vitro effect apparently due to the production of lactic, acetic and hydrochloric acids [269]. *Lactobacillus* strains isolated from chicken intestine were able to inhibit the growth of *S. enteritidis*, *S. pullorum*, *S. typhimurium*, and *S. enteritidis*, probably by producing organic acids [270]. The *L. plantarum* VTT-E-78076 secretes antibacterial compounds that inhibited the growth of the Gram-negative test organisms, *Pantoaea agglomerans* VTT-E-90396 and *Fusarium avenaceum* VTT-D-80147, and the active fraction included benzoic acid, 5-methyl-2,4-imidazolidinedione, tetrahydro-4-hydroxy-4-methyl-2H-pyran-2-one and 3-(2-methylpropyl)-2,5-piperazinedione [271]. The observation that these compounds were more active in the presence of lactic acid, is consistent with the observation by Alakomi et al. [272] that the lactic acid produced by lactobacilli acts as a permeabilizer of the Gram-negative bacterial outer membrane, allowing other antimicrobial substances produced by the host to penetrate and increasing the susceptibility of pathogens to these antimicrobial molecules.

6.3. Production of biosurfactants

*Lactobacillus fermentum* RC-14 and the biosurfactants it secretes have been reported to inhibit infections of surgical implants in rat [273], caused by *S. aureus*, a major cause of community- and hospital-acquired infections. In addition, *Lactobacillus* strains have been found to be highly adherent to catheters, and strain RC-14, together with the biosurfactant is produces, has been reported to produce significant inhibition of the adherence of *S. aureus* to surgical implants [274,275]. The stationary-phase biosurfactants from *L. casei* subsp. *rhamnosus* 36 and ATCC 7469, *L. fermentum* B54, and RC-14 strains were investigated further to determine their capacity to inhibit the initial adhesion of uropathogenic *Enterococcus faecalis* 1131 to glass in a parallel-plate flow chamber [276]. The initial deposition rate of *E. faecalis* on glass with an adsorbed biosurfactant layer from strains RC-14 or B54 was significantly decreased. The surface activity of the biosurfactants and their inhibiting activity were retained after dialysis (molecular weight cut-off, 6-8 kDa) and freeze-drying. The biosurfactants from strains RC-14 and B54 contained the most protein, whereas those from *L. casei* subsp. *rhamnosus* 36 and ATCC 7469 strain have relatively high polysaccharide and phosphate contents.

6.4. Immunomodulation

Besides its role as a barrier to potential pathogens, the intestinal flora is also thought to protect the host by priming the immunological defence mechanisms. Scientific attention is increasingly being focussed on the mechanism(s) of the innate immune response of the host to various components of the autochthonous microbiota, including lactobacilli and bifidobacteria. The interdependency between the epithelium and adjacent lymphoid cells is such that the epithelium is considered to have a central role in the mucosal immune system and is an active participant in both the afferent and efferent limbs of the mucosal immune response [277]. The molecular crosstalk between the epithelium and adjacent lymphocytes is just one aspect of a more complex network of intercellular signalling within the intestinal mucosa and upon which the integrity of the mucosa is dependent. Lymphoepithelial communication is bidirectional, and mediated in large part by shared ligands and receptors. The chemical messengers involved include cytokines, growth factors, local hormones, and products of arachidonate metabolism. Disruption of any aspect of the mucosal microenvironment is generally associated with impaired mucosal defence and inflammation.

Among the mechanisms suggested by which the selected *Lactobacillus* and *Bifidobacterium* strains may act against microbial pathogens, recent experimental reports have focused on immune stimulation and/or modulation, and evidence is accumulating that these selected strains could influence the immune response in a strain-dependent manner [278–282]. However, the mechanisms of interaction of lactobacilli and bifidobacteria with the intestinal epithelial cells linked to the
mucosal immune response system [40,41,160] are less well understood, as is how these bacteria activate immunocompetent cells.

Lactobacilli and bifidobacteria strains have been shown to activate cells to secrete both inflammatory and anti-inflammatory cytokines. H$_2$O$_2$-producing lactobacilli, a component of the normal vaginal flora, increase TNF-α and IL-1β production, activate NF-κB in THP-1 cells, and increase TNF-α production by human monocytes, suggesting the presence in the vaginal fluid of agent(s) derived from indigenous bacteria that can influence the physiology of the vagina and host defences [283]. It is known that _L. casei_ sp. and _L. fermentum_ sp. strains can induce cytokine responses in human peripheral blood mononuclear cells [284,285]. Bacterial cell walls are capable of inducing production of a proinflammatory cytokine, TNF-α, and an anti-inflammatory cytokine, IL-10, but not that of IL-4 or interferon-γ (IFN-γ). _L. acidophilus_ DDS-1 has been shown to induce the production of IL-1β and TNF-α [286]. _L. helveticus_ was able to release peptide compounds that may have important implications for the modulation of the cellular immune response [287,288]. _L. acidophilus_ TMC 0356 significantly increased the production of IL-6, IL-10, IL-12, and TNF-α, but not that of IL-1β in the murine macrophage-like cell line J774.1 [289]. _L. rhamnosus_ sp. induced higher levels of IL-10 in human blood mononuclear cells, or monocytes than _L. plantarum_ or _L. paracasei_ subsp. _paracasei_ strains whereas the highest levels of IL-12 produced by _L. paracasei_ subsp. _paracasei_ strains were roughly 10 times higher than those obtained by stimulation with _L. rhamnosus_ or _L. plantarum_ strains, suggesting that it may be able to stimulate cell-mediated immunity [290]. _L. casei_ Shirotia induced a marked increase in the production of IL-12, probably by macrophages, which in turn stimulated the production of IFN-γ, but not that of IL-4 or IL-5 [291]. _L. acidophilus_ and _L. casei_ strains potentiates IL-6 and IL-12 production by peritoneal cells whereas _L. acidophilus_ upregulated IFN-γ and nitric oxide (NO). In contrast, _L. helveticus_, _L. gasseri_, _L. reuteri_, and _Bifidobacterium_ strains attenuated the production of IL-6, IFN-γ, and NO by peritoneal cells. TNF-α was not detectable in peritoneal cultures. None of the bacteria altered ex vivo production of cytokines or NO by Peyer’s patch or spleen cell cultures [292]. The effect of in vitro exposure to heat-killed cells of _Bifidobacterium_, _L. acidophilus_, _L. bulgaricus_, _L. casei_, _L. gasseri_, _L. helveticus_, and _L. reuteri_ strains on cytokine and NO production has been examined in the RAW 264.7 macrophage cell line and in murine cultures composed of peritoneal, spleen, and Peyer’s patch cells [293]. Both the cell wall and cytoplasmic fractions of lactobacilli were able to stimulate cloned macrophages to produce significant amounts of TNF-α, IL-6, and NO [294]. _L. casei rhamnosus_ GG induced in J774 macrophages a low level production of NO in the presence of IFN-γ, corresponding to an IFN-γ-dependant increase in iNOS mRNA and protein levels, by a mechanism involving activation of transcription factor NF-κB. The observation that lipoteichoic acids (LTAs) also induced NO formation in J774 cells in the presence of IFN-γ suggests a general induction by lactobacilli [295]. In the murine macrophage-like cell line, J774.1, cultured in the presence of 27 strains of heat-inactivated bifidobacteria, He et al. [296] found that _B. adolescentis_ and _B. longum_, known as adult-type bifidobacteria, induced significantly more pro-inflammatory cytokine secretion of IL-12 and TNF-α than did the infant-type bifidobacteria, _B. bifidum_, _B. breve_, and _B. infantis_. In contrast, _B. adolescentis_ did not stimulate the production of anti-inflammatory IL-10 cytokine as did the other bifidobacteria tested.

On the basis that cytokines secreted by human enterocytes play a critical role in mucosal and systemic immunity, lactobacilli and bifidobacteria strains developing adhesion onto human intestinal Caco-2 cells, have been investigated for their potential to stimulate proinflammatory cytokine secretion. Lactobacilli and bifidobacteria strains that adhered in a strain-dependent manner to Caco-2 cells and HT29/19A, failed to lead to the production of pro-inflammatory cytokines, IL-6 or IL-8 [297–299]. Strains of _L. rhamnosus_, _L. delbrueckii_, and _L. acidophilus_ suppressed the production of TNF-α, TGF-β, RANTES and IL-8 by stimulated HT-29 cells in a strain-dependent manner [300]. _L. plantarum_ 299v. in the presence of the proinflammatory cytokine TNF-α, exerted a protective effect by downregulating IL-8 secretion in the human HT-29 colonic epithelial cell line [301]. Co-culture of enterocyte-like Caco-2 cells with human blood leukocytes challenged with a _L. saki_ strain induced the expression of IL-8, MCP-1, IL-1β, and TNF-α mRNA [302]. Normal colonic specimens obtained from patients with neoplasm and inflamed ileal specimens obtained from patients with Crohn’s Disease (CD) have been cultured with either nonpathogenic _E. coli_ ECOR-26, _L. casei_ DN-114 001, _L. casei_ DN-114 056, _L. casei_ ATCC-334, or _L. bulgaricus_ LB-10 strains [303]. Lactobacillus strains significantly reduced TNF-α, whereas _E. coli_ increased it. These effects were observed both in normal and inflamed mucosa. In combination studies, _L. casei_ DN-114 001 prevented TNF-α stimulation by _E. coli_. _L. casei_ DN-114 001 also reduced IL-8 release via a TNF-α-independent pathway. _L. casei_ DN-114 056 or _E. coli_ increased IL-10 release in the presence of neutralizing anti-TNF-α.

Dendritic cells (DC) play a pivotal immunoregulatory role in the Th1, Th2, and Th3 cell balance, and are present throughout the gastrointestinal tract. _Lactobacilli_ in the gut flora differentially activate DC [304]. Substantial differences have been found between strains with regard to their capacity to induce IL-12 and TNF-α...
production in the DC. Similar, but less pronounced, differences have been observed among lactobacilli with regard to the induction of IL-6 and IL-10. All the strains investigated up-regulated surface MHC class II and B7-2 (CD86), which are indicative of DC maturation. The lactobacilli with the greatest capacity to induce IL-12 were those most effective in producing DC maturation. *L. reuteri* DSM12246, a poor IL-12 inducer, inhibited IL-12, IL-6, and TNF-α induction by the otherwise strong cytokine inducer *L. casei* CHCC3139, whereas IL-10 production remained unaltered [304]. The Th1/Th2/Th3-driving capacities of the gut DC may be modulated by the composition of the gut microbiota, including the lactobacilli [304].

*Lactobacillus johnsonii* La1 produced less induction of proinflammatory cytokines, but increased transforming growth factor beta mRNA in leukocyte-sensitized Caco-2 cells [302]. This indicates that, in contrast with enterovirulent bacteria inducing adhesion-dependent production of proinflammatory cytokines, several lactobacilli strains, even those with strong adhesiv properties [106], are not very likely to trigger an inflammatory response in human enterocytes. Neither *L. johnsonii* La1 nor *L. acidophilus* La10, nor their LTAs stimulated intestinal epithelial cells, even in the presence of the soluble form of CD14 [305]. Interestingly, both LTAs inhibited the sCD14-mediated lipopolysaccharide (LPS) responsiveness of intestinal epithelial cells, suggesting that these lactic acid strains could temper and prevent an exaggerated inflammatory response. *L. johnsonii* La1-mediated activation of CD3(−) CD16(+)/CD56(+) human peripheral blood NK cells, including expression of the activation antigen CD69 and secretion of IFN-γ, required cell contact-dependent co-stimulation by autologous monocytes [306]. It has been shown in monocolonized mice that bacterial translocation was higher for *L. johnsonii* La1 than for *L. paracasei* ST11 and that *L. johnsonii* La1 behaves like a stronger antigen, resulting in greater induction of antibodies (secretory and systemically) [307]. *L. paracasei* ST11 was overall a poorer inducer of a humoral response, and its antibody isotopes corresponded more to a Th1 T cell helper. *L. acidophilus* Ke-10 was able to restore the proliferation reaction of lymphocytes and their capacity to produce IL-2 in a radiation-induced rat model of immune deficiency [308]. In humans, *L. johnsonii* La1 increased the humoral immune response to an attenuated *Salmonella typhi* Ty21a challenge [309]. In healthy volunteers receiving a fermented milk product supplemented with *L. johnsonii* La1 or *B. bifidum* Bb12 strains for 3 weeks, phagocytosis of *E. coli* sp. by leukocytes isolated from the blood, was enhanced in both groups [310,311]. This increased phagocytic activity persisted for 6 weeks after ingesting the strains, even though the fecal lactobacilli and bifidobacteria levels had returned to those prior to consumption.

In mice, oral administration of the *L. casei* Shirota or *B. breve* YIT4064 strains activated the humoral immune system [312]. The *L. casei* Shirota has been shown to induce the production of several cytokines, such as IFN-γ, IL-1β and TNF-α in mice [313]. Oral administration of *L. casei* Shirota has been found to enhance innate immunity by stimulating the activity of splenic NK cells and oral feeding with killed bacteria was able to stimulate the production of Th1 cytokines, resulting in repressed production of IgE antibodies against ovalbumin in experimental mice [314]. The natural killer (NK) activity of blood mononuclear cells and splenocytes in aged mice fed on a diet containing *L. casei* Shirota was significantly increased [315]. Oral administration of *L. casei* Shirota or *B. breve* YIT4064 strains to infants increased anti-rotavirus IgA production, and also significantly reduced the frequency of rotavirus shedding in stool samples [312].

*Lactobacillus casei rhamnosus* GG, prevents cytokine-induced apoptosis in intestinal epithelial cell lines in culture by activating the anti-apoptotic Akt/protein kinase B, and by inhibiting the pro-apoptotic p38/mitogen-activated protein kinase by stimulating the production of TNF-α, IL-1β, IL-1α, or INF-γ [316]. The effect of the GG strain involves molecule(s) present in the supernatant of the culture broth. *L. casei rhamnosus* GG diminishes production of TNF-α by the murine macrophage line, RAW 264.7 (NO−), and alter the TNF-α/IL-10 balance, in vitro [317]. Interestingly, when media conditioned by *L. casei rhamnosus* GG are co-incubated with LPS or LTA, TNF-α production is significantly inhibited, indicating that soluble molecules were produced and able to inhibit TNF-α production in activated macrophages. Veckman et al. [318] have recently observed that *L. casei rhamnosus* GG promotes in human macrophages an enhanced mRNA expression of inflammatory chemokine ligands (CCL2/monocyte chemoattractant protein-1 (MCP-1), CCL3/macrophage-inflammatory protein-1α (MIP-1α), CCL5/regulated on activation, normal T expressed and secreted, CCL7/MCP-3, CCL19/MIP-3β, and CCL20/MIP-3α and CXC chemokine ligands CXCL8/IL-8, CXCL9/monokine induced by IFN-γ, and CXCL10/IFN-γ-inducible protein 10), suggesting that GG bacteria can stimulate efficient inflammatory chemokine gene expression including those that recruit Th1 cells to the site of inflammation. Bacteria-induced CCL2, CCL7, CXCL9, and CXCL10 mRNA expression was partially dependent on ongoing protein synthesis. The expression of these chemokines and of CCL19 was dependent on bacteria-induced IFN-α/β production. *L. casei rhamnosus* GG, as a function of the time after administration, enhances T-cell proliferation at the optimal concanavalin A (ConA) concentration and B-cell proliferation at the optimal and supraoptimal concentrations of lipopolysaccharide B-cell proliferation, but decreases
marginally the T-cell proliferation at the optimal ConA concentration [319]. In healthy volunteers receiving *L. casei rhamnosus* GG or placebo for 7 days, an attenuated *Salmonella typhi* Ty21a oral vaccine given to mimic an enteropathogenic infection results in a greater increase in specific IgA among the subjects receiving the vaccine in combination with strain GG [320]. Orally administered *L. casei rhamnosus* GG in conjunction with D x RRV rhesus-human reasortant live oral rotavirus vaccine promotes a slight increased response with regard to rotavirus-specific IgM-secreting cells as compared with placebo [321]. When examining the effect of oral administration of *L. casei rhamnosus* GG on the cellular immune response to intestinal bacteria in a small number of healthy volunteers, Schultz et al. [322] have found that the activation response of CD4+ T-lymphocytes towards isolated and heat-inactivated intestinal bacteria was increased after the probiotic treatment. Additionally, TNF-α, IL-6 and, in part, IFN-γ cytokine secretion, following stimulation with whole stool preparations and single members of the flora, was significantly decreased, whereas the IL-10 and, in part, IL-4 cytokine secretion was increased. In contrast, the activation response of CD4+ T-lymphocytes following stimulation with whole 'non-self' intestinal flora was higher than with 'self' intestinal flora.

Feeding of mice with *L. rhamnosus* HN001, *L. rhamnosus* DR20, *L. acidophilus* HN017, *B. lactis* HN019 or *B. lactis* DR10 strains resulted in a significant increase in the phagocytic activity of peripheral blood leukocytes and peritoneal macrophages compared to that in control mice [323]. *L. acidophilus* treatment in mice enhanced ex vivo basal proliferation and B-cell response at suboptimal and optimal concentrations of LPS, and conversely *L. casei*, *L. gasseri* and *L. rhamnosus* strains inhibited both basal proliferation and mitogen-stimulated lymphoproliferation, particularly at supra-optimal concentrations of ConA and LPS [324]. *L. casei* could prevent enteric infections by stimulating secretory IgA in malnourished animals [325]. The antigenic effect of *L. acidophilus*, *L. casei*, and *L. delbrueckii* subsp. *bulgaricus* on the gut immune system examined in BALB/c mice shows that a dose-dependent increase of the Bcl2 protein develops [326]. Moreover, analysis of the cytokine-producing cells in the lamina propria of gut showed that TNF-α and INF-γ values, determined in macrophages cultured from Peyer’s patches, were enhanced. In parallel, an increase in interleukins IL-10 and IL-4 has been observed mainly in mice fed with *L. delbrueckii* subsp. *bulgaricus*, *L. delbrueckii* subsp. *bulgaricus* and *L. acidophilus* enhanced the IgG1 response favouring Th2. In addition, oral administration of *L. casei*, *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus*, *L. plantarum*, and *L. rhamnosus* is followed by an increase in CD4+ cells, suggesting the possibility that the lactobacilli may interact with Peyer’s patches and enhance B- and T-cell migration [327]. Administration of *L. rhamnosus* HN001 to healthy middle-aged and elderly volunteers is followed by a relative increased proportion of PMN cells showing phagocytic activity and by a marked increase in NK cell tumour killing activity [328].

*Bifidobacterium lactis* HN019 may enhance aspects of cellular immunity in elderly subjects by increasing the proportions of total, helper (CD4+), and activated (CD25+) T lymphocytes and natural killer cells [329,330]. Moreover, the phagocytic capacity of mononuclear and polymorphonuclear phagocytes was also elevated after *B. lactis* HN019 consumption. Interestingly, Schell et al. [132], sequencing the genome of an infant-derived strain of *B. longum* have recently found a eukaryotic-type serine protease inhibitor (serpin) that could possibly be involved in the reported immunomodulatory activity of bifidobacteria.

### 6.5. Production of antimicrobial molecules

Recent reports have revealed that some intestinal lactobacilli and bifidobacteria produce antimicrobial substances that are active against these enterovirulent microorganisms. Bacteriocins are bactericidal proteinaceous molecules produced by bacteria. The bacteriocin family includes a wide variety of peptides and proteins in terms of their size, microbial targets, and mechanisms of action and immunity. Three classes of bacteriocins in lactobacilli have been defined by Klaenhammer [331], but recent results suggest that a fourth class of “complex bacteriocins” could exist. The class I bacteriocins, also known as lantibiotics, comprise small peptides (≤5 kDa) containing the unusual aminoacids lanthionine and β-methyllanthionine, and a number of dehydrated amino acids. The class-II bacteriocins are small, heat-stable, non-lanthionine-containing peptides (≤5 kDa), subdivided into three sub-classes, the *Listeria*-active peptides, the peptides requiring two components for activity, and the sec-dependent secreted peptides. The class-III bacteriocins are large, heat-labile proteins (>30 kDa). Comprehensive reviews have recently described the genetics, biochemistry and mechanism(s) of action of the class-I [332,333] and class-II bacteriocins [332,334–336]. Bacteriocins have a relatively narrow killing spectrum, and are only toxic to bacteria closely related to the strain producing them, including *Lactococcus*, *Streptococcus*, *Staphylococcus*, *Listeria* and *Mycobacterium*. It has been clearly established that the primary target for many of the bacteriocins is the cytoplasmic membrane of sensitive bacteria, in which discrete pores are formed by the dissipation of the proton motive force, resulting in a loss of energy. Other bacteriocins do not form membrane...
pores, but act by interfering with essential enzyme activities in susceptible bacteria. Despite the fact that bacteriocins from lactobacilli are generally recognized as being inactive against Gram-negative organisms, it has been reported that lacticins A164 and BH5 are active against the Gram-negative bacterium, \textit{H. pylori} [337], and that niisin is active against Gram-negative organisms by disrupting the outer membrane [338]. Moreover, \textit{L. acidophilus} IBB 801 produces a small bacteriocin, designated acidophilin 801, with an estimated molecular mass of less than 6.5 kDa which displays a narrow inhibitory spectrum against the Gram-negative pathogenic bacteria \textit{E. coli} Row and \textit{S. panama} 1467 with a bactericidal activity [339,340]. Bacteriocins have attracted great interest with regard to their potential use as food preservatives [341]. However, the use of bacteriocins to combat human gastrointestinal disorders resulting from Gram-positive pathogen infections is limited, because these infectious disorders are much less common than those produced by Gram-negative bacteria or rotaviruses, which are insensitive to bacteriocins.

Other antibacterial components produced by lactobacilli and bifidobacteria do not share the characteristics of bacteriocins. The inhibitory compound secreted by a \textit{L. casei} sp. strain was insensitive to proteolytic enzymes and to heat treatment [342]. \textit{L. casei rhamnosus} GG secretes a low molecular weight, heat-stable, inhibitory substance, which is distinct from lactic and acetic acids, and which is active against \textit{Clostridium} spp., \textit{Bacteroides} spp., \textit{Bifidobacterium} spp., \textit{Enterobacteriaceae}, \textit{Pseudomonas} spp., \textit{Staphylococcus} spp., and \textit{Streptococcus} spp. [343]. These characteristics suggest that this inhibitory molecule could be a non-bacteriocin; closely resembling a microcin, which has been associated previously with members of the \textit{Enterobacteriaceae} family. \textit{L. casei} subsp. \textit{rhamnosus} GR-1 and \textit{L. acidophilus} 76 that exert an inhibitory effect on pyelonephritogenic \textit{E. coli} produced a bactericidal substance that is neither lactic acid nor hydrogen peroxide, has a molecular weight greater than 12–14 kDa, is heat labile, not precipitated by up to 80% ammonium sulphate, extractable in chloroform, and that retains its activity under pH-buffered conditions [344]. \textit{L. delbrueckii} VI1007 produces at least three growth-inhibiting factors, other than lactic acid, one of which has been identified as a bacteriocin-like, heat- and proteinase-sensitive bactericidal molecule or complex with a molecular weight greater than 50 kDa [261]. \textit{L. acidophilus} NCFM produces antimicrobial compounds [113]. The spent culture supernatant of strains LB and La1 contained antibacterial components that were active against a wide range of Gram-negative and Gram-positive pathogens, such as \textit{S. aureus}, \textit{L. monocytogenes}, \textit{S. typhimurium}, \textit{S. flexneri}, \textit{K. pneumoniae}, \textit{P. aeruginosa}, and \textit{E. cloacae}, but inactive against species found in the normal gut flora, such as lactocabilli and bifidobacteria [133,172]. This antibacterial activity was insensitive to proteases and independent of lactic acid production [133,172]. The inhibitory molecules active against \textit{E. coli} O157:H7 secreted into the spent media by the \textit{L. rhamnosus} DR20, \textit{L. acidophilus} HN017, and \textit{B. lactis} DR10 strains were partially inactivated by treatments with lactate dehydrogenase, trypsin and proteinase K, suggesting that the overall inhibition may be due to a synergistic action of lactic acid and proteinaceous substances [110]. An antibacterial component produced by human \textit{Bifidobacterium} spp. CA1 and F9 strains was found to be a lipophilic molecule (or molecules) with a molecular weight(s) of less than 3500 Da [146]. The growth of \textit{Giardia intestinalis}, and its attachment to the human intestinal epithelial cell line Caco-2, are both significantly inhibited by \textit{L. johnsonii} La1, resulting in significant inhibition of the proliferation of \textit{G. intestinalis} trophozoites [345]. Although the effect is strongly pH dependent, it is not simply attributable to lactic acid, and partial characterization of the factors involved in the antigiardiasic action has shown that they have a low molecular mass and are inactivated by heating. It has been recently observed that the in vitro, anti-\textit{H. pylori} activity of \textit{L. acidophilus} CRL 639 results from an autolytic activity which is linked to a proteinaceous compound released after cell lysis [346]. Using HT29-MTX cells [347], which express MUC5AC mucin that functions as receptor for \textit{H. pylori} [348], it has been observed that \textit{L. acidophilus} LB, which secretes an active antibacterial substance (or substances) develops anti- \textit{H. pylori} activity regardless of pH and lactic acid levels [169]. Similarly, the culture supernatant of \textit{L. johnsonii} La1 interferes with the growth, urease activity, and adhesion to cultured human epithelial cells of \textit{H. pylori}, [236]. Another possible mechanism of the inhibition of \textit{H. pylori} adhesion has been proposed by Mukai et al. [349], who observed that \textit{L. reuteri} strains bind to the putative glycolipid receptor of \textit{H. pylori}. Finally, it should be noted that lactic acid, in addition to its antimicrobial effect resulting from the lowering of the pH, also functions as a permeabilizer of the outer membrane of Gram-negative bacteria, and so may potentiate the effects of other antimicrobial substances [272]. It has been demonstrated that the production of bacteriocins was growth associated, dependent on energy, temperature, pH value, complex nitrogen source and environmental conditions [350–354], and controlled by different regulatory mechanisms [355], including a cell-density dependent process involving a secreted peptide pheromone for quorum-sensing [355–357]; whereas no information exists on the mechanism(s) by which the production of non-bacteriocin antimicrobial molecules by lactobacilli and bifidobacteria was regulated.

Adherence to the epithelial intestinal cells is an important prerequisite for colonization by microorganisms, and for bacterial pathogens this is a pivotal step for virulence. Enterovirulent microorganisms [34–37,
358–360] use the host cell cytoskeleton to produce their deleterious effects on the host, affecting in turn specific intestinal functions that are known to be established and maintained through a cytoskeleton-dependent, polarized cell organization [26,27,103]. Molecules secreted by Lactobacillus strains have recently been shown to have the capacity to interfere with the cross-talk between enteric pathogens and the intestine. Indeed, the non-bacteriocin, heat-stable molecules secreted by lactobacilli strains decreased the ability of pathogens to cross the brush border of intestinal cells, probably by altering the expression and/or the functions at the membrane of bacterial virulence factors necessary for bacterial inter-enteric pathogens and the intestine. The secreted molecules produced by L. acidophilus LB isolated from resident human gastrointestinal microbiota, inhibited the signalling-dependent cell damage induced by Entamoeba histolytica [106,115,133,146,169,172, 297]. The secreted molecules produced by L. acidophilus LB isolated from resident human gastrointestinal microbiota, inhibited the signalling-dependent cell damage induced by S. enterica serovar Typhimurium in human intestinal Caco-2/TC-7 cells [297]. In addition, these secreted molecules have been found to protect Caco-2/TC-7 cells against the signalling-dependent structural and functional brush border lesions promoted by the entero-virulent diffuse-adhering E. coli (Afa/Dr DAEC) [361–364]. Indeed, L. acidophilus LB secreted molecules, blocked Afa/Dr DAEC-induced disassembly in brush border F-actin cytoskeleton and the induced loss in expression of functional brush border-associated proteins, sucrase-isomaltase, dipeptidylpeptidase IV, alkaline phosphatase, and fructose transporter [365]. In cultured intestinal epithelial T-84 cell monolayers infected with EPEC, viable L. plantarum bacteria were able to inhibit apical EPEC binding only when added to the cell monolayers before EPEC infection [366]. Moreover, the EPEC-induced transepithelial neutrophil migration across the cell monolayers was blocked only by the viable L. plantarum bacteria preincubated with host epithelia, but not by the L. plantarum culture supernatants. A similar mechanism of action has been recently reported for the yeast Saccharomyces boulardii, [367]. S. boulardii abrogates the alterations induced by an EPEC strain on transcellular resistance, inulin flux, and tight junction-associated ZO-1, inhibits the EPEC-induced tyrosine phosphorylation of the ERK1/2 mitogen-activated protein (MAP) kinase pathway, the cholera toxin-induced cAMP- and Ca2+-mediated Cl-secretion in IEC-6, HT29-D4 and T84 cells [368–370], and in rat isolated jejunal loop [371]. In addition, S. boulardii maintains the transmembrane electrical resistance of enterohemorrhagic E. coli (EHEC)-infected cells, abolishes the EHEC-induced MLC phosphorylation and inhibits NF-κB DNA binding activity, phosphorylation and degradation of IκB-α, and activation of the three members of a MAPK group (extracellular signal-regulated protein kinases 1 and 2, p38, and c-jun N-terminal kinase) [372].

In conclusion, the mechanisms including bacterial interference, production of antimicrobial substance(s), and/or immunomodulating activity, by which lactobacilli and bifidobacteria strains develop antagonistic activities against microbial gastrointestinal and uro vaginal pathogens, have been demonstrated for a low number of selected strains (Table 3).

7. Lactobacilli and bifidobacteria: what the future holds

7.1. Inflammatory bowel diseases: potential therapeutic relevance

The pathogenesis of inflammatory bowel disease (IBD), ulcerative colitis (UC) and CD remains elusive [373]. The current opinion is that the appearance and chronicity of IBD involves an extremely complex chain of events that includes the physiology and genetic elements of the host in addition to multiple factors related to the nature of the gut microbiota and/or to the emergence of “silent pathogens”, which are repressed when the gut microbiota is functioning normally. Moreover, in inflammatory states, interleukins regulate the intensity of the intestinal immune response, either directly or via the production of additional effector molecules. The resident luminal bacteria seem to be an

<table>
<thead>
<tr>
<th>Strain</th>
<th>Bacterial interference</th>
<th>Acid and pH effects</th>
<th>Antimicrobial substance(s)</th>
<th>Immunomodulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. rhamnosus DR20</td>
<td>[110]</td>
<td>ND</td>
<td>[110]</td>
<td>[323,328,436]</td>
</tr>
<tr>
<td>L. casei rhamnosus GG</td>
<td>[136,141,143–145,263]</td>
<td>[107,263]</td>
<td>[343]</td>
<td>[295,316–322]</td>
</tr>
<tr>
<td>L. casei subsp. rhamnosus Lcr35</td>
<td>[109]</td>
<td>[109]</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>L. casei Shirota</td>
<td>[136,141,144,245]</td>
<td>[266]</td>
<td>[344]</td>
<td>ND</td>
</tr>
<tr>
<td>L. casei GR-1</td>
<td>[91,100–102]</td>
<td>ND</td>
<td>[344]</td>
<td>ND</td>
</tr>
<tr>
<td>L. plantarum 299v</td>
<td>[143]</td>
<td>ND</td>
<td>ND</td>
<td>[301]</td>
</tr>
<tr>
<td>Bifidobacterium spp. CA1 and F9</td>
<td>[115,146]</td>
<td>ND</td>
<td>[146]</td>
<td>ND</td>
</tr>
<tr>
<td>B. lactis DR10</td>
<td>[110]</td>
<td>ND</td>
<td>[110]</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not documented.
important factor in their development and chronicity. There is evidence to suggest that inflammatory bowel diseases may represent an aggressive immunological response to the resident luminal flora, rather than an alteration in the normal flora. Indeed, an exaggerated immune response in the gut-associated lymphoreticular tissue is characteristic of IBD, UC and CD. Moreover, the resident luminal bacteria seem to be an important factor in the onset, development and chronicity of IBD. The role of microbiota in IBD has been investigated in transgenic rats expressing HLA-B27 and human β2-microglobulin (HLA-B27 rats) that spontaneously develop chronic colitis at 8 weeks of age, resembling human IBD [374]. The colonization of Bacteroides spp., Bifidobacterium spp. and Lactobacillus spp. was already detectable at high concentrations, whereas Clostridium spp. and Eubacterium spp. were not detected [374]. In parallel, the expression of proinflammatory cytokines (IL-1β, IL-8 and TNF-α) appeared at 8 weeks of age, and these were detectable until 17 weeks. A similar pattern was observed in the expression of Th1 cytokines (IL-2, IL-12 and IFN-γ) whereas the expression of Th2 cytokines (IL-4, IL-10 and TGF-β) was weak. The authors concluded that the development of colitis may be mediated by both the predominant expression of Th1 cytokines and the weakness of Th2 cytokine expression in the mucosa and that the colonization of gut by anaerobic bacteria, especially Bacteroides spp., may be initiating and promoting these cytokine responses.

In addition, although controversial, a causative link between microbial pathogens and IBD, UC and CD has been suggested resulting from the identification of retroviruses, and enterovirulent bacteria, such as Salmonella spp., Yersinia enterocolitica and Shigella spp. in biopsies of patients [375]. In particular, pathogenic E. coli strains present in the colon may play a crucial role in the pathogenesis of IBD [376–379] and several studies have demonstrated the presence of pathogenic E. coli strains in patients with CD and UC [380–384]. Seksek et al. [385] investigating potential differences in the faecal microflora between patients with colonic CD in remission, patients with active colonic CD, and healthy volunteers observed that Enterobacteria were observed significantly more frequently in CD than in health, and more than 30% of the dominant flora belonged to yet undefined phylogenetic groups. Recently, it has been reported that the enteroviral adhering E. coli (Afa/Dr DAEC) [361–364] induced proinflammatory responses such as an increase in IL-8 production and transepithelial migration of polymorphonuclear leukocyte (PMNL), followed by increased expression of MHC class I-related MICA and decay-accelerating factor (DAF, CD55) [386,387], a molecule found up-regulated on the surface of epithelial cells in colonic biopsies from IBD- [388–394] and CD-affected patients [387]. Interestingly, an antibacterial effect by molecules secreted by L. acidophilus LB has been observed against Afa/Dr DAEC infecting cultured human intestinal cells [112,365].

Various strategies to fortify or otherwise modify the enteric flora by dietary supplements containing lactobacilli and bifidobacteria have been conducted [395]. However, as recently pertinently underlined [396–401], demonstration of the therapeutic efficacy of probiotics needs rigorous research including scientific characterisation of the activities of individual organisms in appropriate animal models and detailed comparisons of efficacy amongst different bacterial strains under experimental conditions mimicking the future clinical trial conditions. In addition, therapeutic progress may require clarification of the mechanism of action of each selected probiotic strain used and of the synergistic action of strains when associated in a probiotic preparation containing lactobacilli and bifidobacteria strains.

Preliminary results of probiotic therapy in animal models with ulcerative colitis and pouchitis have been encouraging. For example, in colitis in IL-10 gene-deficient mice, restoring the Lactobacillus spp. to normal levels by L. reuteri resulted in lower levels of colonic mucosal adherent and translocated bacteria, and attenuated the development of the colitis [402] and L. plantarum 299v administered to IL-10-deficient mice decreased mucosal IL-12, IFN-gamma, and immunoglobulin G2a levels [403]. In IL-10 knockout mice the effect of feeding L. salivarius subsp. salivarius 433118 and B. infantis 35624, colonic and caecal inflammatory scores were significantly decreased in both groups of probiotic fed mice accompanied by a significantly reduced proinflammatory cytokine production by Peyers’ patches and splenocytes whereas TGF-β levels were maintained [404]. A controlled feeding trial in IL-10 knockout mice using the probiotic L. salivarius subsp. salivarius UCC118 results in a significant reduction of the faecal coliform and enterococci levels, and by reduced score in intestinal inflammation [405]. In the IL-10 gene-deficient mouse, the VSL#3 probiotic mixture (5 × 10^11 per gram of viable lyophilized bacteria of L. acidophilus subsp. L. salivarius UCC118 results in a significant reduction of the faecal coliform and enterococci levels, and by reduced score in intestinal inflammation [405]. In the IL-10 gene-deficient mouse, the VSL#3 probiotic mixture (5 × 10^11 per gram of viable lyophilized bacteria of four strains of lactobacilli, three strains of bifidobacteria, and one strain of S. salivarius subsp. thermophilus) completely restored normal physiological transport function and barrier integrity, in conjunction with a reduction in the mucosal secretion of TNF-α and IFN-γ [406]. Furthermore, it would appear that a soluble factor is released from a bacterium found in the VSL#3 mixture that can act directly on the epithelium to enhance barrier integrity. The activity of B. breve, B. bifidum and L. acidophilus strains measured in SAMP1/Yit strain mice results in a reduced histological injury score, and shows that the tissue contents of immunoglobulins such as IgG1 and IgG2a were lower in the inflammatory regions in the SAMP1/Yit group fed with lactobacilli and bifidobacteria [155]. In a germ-free colony of TCR-α
... mice colonized with a limited bacterial flora consisting of *L. plantarum*, *S. faecalis*, *S. faecium*, and/or *E. coli* strains, intestinal inflammation does not occur spontaneously [407]. In Ob/ob mice, a model for non-alcoholic fatty liver disease that develop intestinal bacterial overgrowth and overexpress TNF-α, a diet with VSL#3 probiotic adjunct for 4 weeks, reduced activity of Jun N-terminal kinase (JNK), a TNF-regulated kinase, and decreased the DNA binding activity of nuclear factor kappaB (NF-κB) [408]. In contrast, *L. casei rhamnosus* GG did not prevent colitis in *B. vulgatus* co-associated germfree B27 TG rats, but treated established disease in SPF rats receiving oral vancomycin and imipenem. Daily administration of the potential probiotic *L. plantarum* NCIMB8826 in healthy mice and in mice suffering from colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) is followed by a greatly reduction in the translocation of endogenous microflora to the mesenteric lymph nodes and spleen in the TNBS-treated mice [409]. In rats treated daily intragastrically with the probiotic preparations, VSL#3 or *L. casei rhamnosus* GG, 7 days before inducing colitis by intracolonic administration of either dinitrobenzene sulfonic acid (DNBS) or iodoacetamide, there was a significant decrease in the lesion area when colitis has been induced by iodoacetamide, but no effect on immune-mediated DNBS-induced colitis [410]. However, *L. rhamnosus* GG but not *L. plantarum* 299v prevented colitis relapse in antibiotic-treated rats with reduced gross and histological scores, caecal myeloperoxidase, IL-1β, and TNF whereas caecal IL-10 was increased [411].

Initial reports in irritable bowel syndrome (IBS) have resulted in encouraging results with the use of multiple-organism probiotic supplements. However, caution must still be applied to the use of probiotics in IBD and IBS because the reports and the number of patients treated are limited [412]. Moreover, because of strain-specific variability and clinical and therapeutic heterogeneity within CD and UC, it cannot be assumed that a given probiotic is equally suitable for all individuals [396–400]. In addition, the full potential of therapeutic manipulation of the enteric flora with probiotics may not be optimally realised until the composition and metabolic activities of the normal flora are better understood, although progress has been made in better characterization of the indigenous flora. From the data provided in clinical studies, the most promising probiotic preparation is VSL#3 containing 5 × 10^{11} per gram of viable lyophilized bacteria, however the mechanism of action remains unknown. When VSL#3 has been inoculated into humans, results indicated that VSL#3 bacteria seem able to colonize the large bowel [413]. Indeed, *B. infantis* Y1 and *B. breve* Y8 strains present in VSL#3 have been found by PCR analysis in faeces of patients affected by IBD and treated with VSL#3 for 2 months, similar to that observed with the healthy subjects [414]. In patients with ulcerative colitis that have been treated with VSL#3 for 12 months, faecal concentrations of *S. salivarius* subsp. thermophilus, lactobacilli and bifidobacteria increased significantly in all patients and remained stable throughout the study [415]. In this study, fifteen of 20 treated patients remained in remission throughout the study, one patient was lost to follow-up, while the remaining patients relapsed. Examination in a small group of patients of the effects of VSL#3 on gastrointestinal transit and symptoms of patients with Rome II IBS with predominant diarrhoea showed borderline significant differences in abdominal bloating scores and a reduction in abdominal bloating without an alteration in gastrointestinal or colonic transit. In contrast, no effect on other individual symptoms: abdominal pain, gas and urgency were observed [416]. Other clinical studies of the use of *L. casei rhamnosus* GG in IBD have produced contradictory results. Oral bacteriotherapy with *L. casei rhamnosus* GG (10^{10} CFU/ml, twice daily for 10 days) in children with CD increased the gut IgA immune response [417], a pilot study indicated that the strain GG appears to be effective in improving the CD clinical status of children with CD [418]. In contrast, patients that had randomly been assigned *L. casei rhamnosus* GG or placebo for 12 months showed no differences between the two groups with regard to endoscopic and clinical remission [419]. Finally, a clinical report demonstrated that administration of the biotherapeutic agent, heat-killed *L. acidophilus* LB with its spent culture supernatant, to patients with irritable bowel syndrome leads to a statistically significant therapeutic benefit evaluated on abdominal pain, bloating or gas, daily number of stools, consistency and mucus content [420].

Mucosal lesions of pouchitis are characterized by a neutrophil infiltrate and IL-8 is the main mediator involved in neutrophil recruitment and is down-regulated by IL-10. Moreover, histological lesions of pouchitis are associated with a mucosal imbalance between IL-8 and IL-10 [421]. VSL#3 has been evaluated in maintaining remission of chronic pouchitis [395,422]. Faecal concentrations of lactobacilli, bifidobacteria, and *S. thermophilus* increased significantly from baseline levels in the VSL#3-treated patients group, and only 15% of the treated-patients had relapses within the 9-month follow-up period, compared with 100% in the placebo group. Four randomized controlled trials of medical therapy in adult patients with pouchitis, showed that oral probiotic therapy with VSL#3 appears to be an effective therapy for maintaining remission in patients with chronic pouchitis in remission [423,424]. In contrast, a prospective, randomized, double-blind, placebo-controlled trial of *L. casei rhamnosus* GG supplementation conducted in a low number of patients indicated that intake of two gelatine capsules (0.5–1 × 10^{10} CFU/capsule) for 3 months, changed the pouch intestinal...
bacterial flora, but was ineffective as primary therapy for a clinical or endoscopic response [425].

7.2. Lactobacilli antimicrobial molecules: Potential therapeutic agents

Over the past decade, levels of bacterial resistance to antibiotics have risen dramatically, and “superbugs” resistant to most or all available agents have appeared in clinical practice. This means that there is a growing need to discover and introduce new drugs. An innovative therapeutic strategy has been developed over the past 10 years involving epithelium-derived antimicrobial peptides that function as the first line of chemical defence in the gastrointestinal tract and that have attracted interest as a source of innovative antibiotic molecules [44–54]. Extensive structure–function studies have been carried out on the ‘designer’ peptides as prototypes of innovative drugs that may be used in the future as antimicrobials [426–430]. However, a few of these peptide antibiotics have entered clinical trials to date, and have met with mixed success [44,426,428]. The finding that thousands of different antimicrobial peptides with variable lengths and sequences, all of which are active at similar concentrations, suggests that there is a general mechanism for killing bacteria rather than a specific mechanism that requires specific active structures. Although many studies suggest that bacterial membrane damage is a lethal event for bacteria, other studies point to a multihit mechanism, during which the peptide binds to several targets in the cytoplasmic region of the bacteria [431,432]. Some antibacterial molecules produced by selected Lactobacillus strains (Table 3) develop mechanism(s) of action that resembles the mechanism(s) by host antimicrobial peptides [433,434]. In the context of bacterial resistance to antibiotics, the nonbacteriocin, antibiotic-like molecules produced by selected lactobacilli and bifidobacteria strains are of interest in terms of innovative antimicrobial therapy.

8. Concluding remarks

The field of the use lactobacilli and bifidobacteria strains on treatment and prevention of microbial infections in humans has been clouded by exaggerated claims for therapeutic efficacy and generally, many of these claims have not been well substantiated. During the last decade, the scientific basis for the use of selected lactobacilli and bifidobacteria expressing strain-specific properties to combat microbial infectious diseases has been established. However, the level of scientific characterisation of individual strains is variable. Indeed, the rationale for using lactobacilli and bifidobacteria strains as probiotics or pharmaceutical preparations containing live or heat-killed bacteria, with or without spent culture supernatant, has been recently demonstrated only for a low number of selected strains in a coherent chain of well-designed experimental and clinical studies (Table 4). Moreover, the mechanisms of action of a large majority of these selected strains need to be determined. In particular, progress should be made in the understanding of how the selected lactobacilli and bifidobacteria strains kill the pathogens, antagonise the process of pathogenicity of microbial pathogens, and/or modulate the immune response to infection. To document these points, it should be necessary to purify and characterise at molecular level, the antimicrobial molecule(s) produced by the selected strains exerting killing and/or inhibitory activities, and for strains developing immunomodulation it should be necessary to more clearly establish the way by which these bacteria promote the effect in vivo.

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Table 4

Selected lactobacilli strains expressing antagonistic activities against microbial pathogens demonstrated in experimental and clinical studies

<table>
<thead>
<tr>
<th>Strain</th>
<th>Antagonistic activities in experimental in vitro and in vivo studies, with a proposed mechanism(s) of action</th>
<th>Therapeutic efficacy in clinical studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. acidophilus LB</td>
<td>[112,134,169,172,297,365,435]</td>
<td>[215,243]</td>
</tr>
<tr>
<td>L. casei DN-114 001</td>
<td>[185,186]</td>
<td>[224,225]</td>
</tr>
<tr>
<td>L. casei Shiroti</td>
<td>[136,141,144,180,183,242,245,266,291,312–315]</td>
<td>[242,437]</td>
</tr>
<tr>
<td>L. casei GR-1</td>
<td>[91,100–102,344]</td>
<td>[91,97,152,248,249,251]</td>
</tr>
<tr>
<td>L. fermentum RC-14</td>
<td>[100,102,273–276]</td>
<td>[97,152,248,249,251]</td>
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