Modulation of mucosal antiviral immune response by immunobiotic lactic acid bacteria – Part I: the intestinal mucosa

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Viruses are the most important cause of severe mucosal infections worldwide especially in high risk populations such as in infants, young children, elderly and immunocompromised hosts. A significant improvement in the knowledge of how the host immune response contributes to the pathogenesis of viral infections has been made during the last decade. This understanding of host response and molecular pathogenesis of viral infections has been critical for the development of vaccines, antivirals and other disease intervention approaches such as probiotic functional foods. Lactic acid bacteria (LAB) are technologically and commercially important and have various beneficial effects on human health. Several studies have demonstrated that certain LAB strains can exert their beneficial effect on the host through their immunomudulatory activity. These strains, termed immunobiotics, have been used for the development of probiotic foods with the ability to stimulate mucosal antiviral immunity. In this review we examine the current scientific literature concerning the advances in our understanding of how commensal microorganisms are able to modulate intestinal viral immunity and affect the outcome of viral diseases. Research from the last decade demonstrates that immunobiotic LAB represent a promising resource for the development of prevention strategies against viral infections that could be effective tools for medical application.

Keywords lactic acid bacteria, immunobiotics, intestinal viral infection

1. Introduction

Viruses are the most common etiological agents of acute infectious diseases and therefore, researchers have actively investigated how to control viral diseases during decades. Great efforts have been made worldwide to improve the development of universal vaccines and to reduce the global mortality from viral infections. The ability to grow cells from humans and other animals in the laboratory helped researchers generate vaccines against a range of viruses. A seminal moment in vaccinology occurred in 1979 when the World Health Organization formally announced the eradication of smallpox, which, said the authors, was a result of an unprecedented collaboration between governments, donors, industry and health professionals. However, despite decades of research, current prophylactic and therapeutic strategies to combat viral infections remain largely inadequate. In fact, despite the remarkable advances in therapies, diagnostic tools, prevention campaigns and intensive care, viral infections are still among the primary causes of death worldwide, and there have been no significant changes in mortality in the last decades [1].

Although a strong immune response is launched against viruses, some virally encoded proteins can interfere with the host's ability to efficiently recognize and clear virus, while others induce or alter specific immune responses to benefit viral replication or spread within the host. Therefore, in some cases modulation of host immunity allows survival of virus [2-4]. In addition, the number of immunocompromised patients has increased significantly in the past years. Viral infections in immunocompromised hosts have the ability to cause severe disease at much higher rates than in the healthy population [5]. Then, a better understanding of how viruses modulate the immune system in both healthy and immunocompromised hosts may help to control and prevent these infections.

Two of the most important strategies for the prevention of infectious diseases are healthy nutrition and the use of effective vaccines. Historically, deaths from infections have been reduced by improvements in nutrition. Besides, a large body of literature has established strong links between nutrition, immune function and infectious diseases. In addition, the development of vaccines and their massive use have enabled the eradication of numerous infectious diseases in various parts of the world. Lactic acid bacteria (LAB) can be used for both strategies. They have been used for the development of probiotic foods with the ability to stimulate the immune system, which would increase resistance to infections, even in immunocompromised hosts [6, 7]. On the other hand, the advances in the molecular biology of LAB have enabled the development of recombinant strains expressing antigens from various pathogens that have proved effective to induce protective immunity. Another goal in vaccine development is mucosal immunization, which is more patient friendly, mimics the natural infection route and does not require trained personnel for administration [8].

The probiotic approach represents a potentially effective and mild alternative strategy for the prevention and treatment of viral infectious diseases. Several studies have shown that different LAB strains can exert their probiotic abilities by influencing the host's immune system, thereby modulating antiviral immune responses. In addition, LAB pose no safety concerns and several have "generally recognized as safe" (GRAS) status, which are important conditions for immunocompromised hosts. The purpose of this chapter is to provide an overview of the scientific literature concerning studies in which immunoregulatory LAB (immunobiotics) have been used as antiviral-enhancing agents. Particular attention is given to the modulation of host immune responses and the possible mechanisms determining the effect on the immune system are also discussed. In addition, we described the advances in the biotechnological applications of immunobiotic LAB and the development of functional foods and recombinant vaccines designed to prevent intestinal, viral infections. Research from the last decade demonstrates that immunobiotic LAB represent a promising resource for the development of prevention strategies against viral infections that could be effective tools for medical application.

2. Modulation of intestinal antiviral immune response by immunobiotic LAB

Around 5.2 million children under five years old die yearly due to preventable infectious diseases like diarrhoea [9, 10]. Among these infectious diseases, viral gastrointestinal infections belong to the most frequent diseases suffered in childhood, especially in the developing world. Many different viruses can cause gastroenteritis in humans, including rotavirus, calicivirus, astrovirus and adenovirus [11, 12]. Rotavirus, a RNA virus, is the most common cause of severe dehydrating diarrhoea in children worldwide [13, 14]. Rotavirus infection causes a gastroenteritis characterized by acute diarrhea and vomiting. Although there is already a successful rotavirus vaccine in the market, the epidemic in the developing world is far from being controlled [14, 15]. Apart from being not affordable for low-income population groups, it has also been shown that protection induced by natural infection and vaccination is reduced in developing areas, where among other factors, children are infected at an early age and high viral challenge loads are usual [16].

Several studies have demonstrated that certain LAB strains can exert their beneficial effect on the host through their immunomodulatory activity. In this sense, some studies have centred on whether immunoregulatory probiotic LAB (immunobiotics) might sufficiently stimulate the intestinal immune system to provide protection against viral infections. It was reported that probiotics can exerts some beneficial effects in rotavirus intestinal infections such as shortening the duration of diarrhoea, reducing the number of episodes, lessening rotavirus shedding, normalizing gut permeability and increasing the production of rotavirus-specific antibodies [17-19].

The most studied probiotic bacteria in the prevention of viral intestinal disease is the known immunobiotic strain *Lactobacillus rhamnosus* GG. The antiviral activity of the GG strain in rotavirus gastroenteritis was evaluated more than 20 years ago by Isolauri *et al.* [20] in infants and children. Authors demonstrated that after oral rehydration, the patients receiving either a *L. rhamnosus* GG-fermented milk product or a *L. rhamnosus* GG freeze-dried powder presented a significantly shorter duration of diarrhea after commencing the therapy when compared to the placebo group. Later, Majamaa *et al.* [21] tested the effect of LAB on the recovery of children with acute rotavirus gastroenteritis. Children aged 6–35 months with rotavirus gastroenteritis were randomly selected to receive either *L. rhamnosus* GG, *L. acidophilus* or a preparation containing a combination of *S. thermophilus* with *L. delbrückii* subsp. *bulgaricus* twice daily for five days. Duration of diarrhea was reduced in children who received *L. rhamnosus* GG and the protective effect was associated with an improvement of intestinal IgA concentrations, the number of specific antibody-secreting cells to rotavirus as well as serum IgA antibody levels at the convalescent stage. The anti-rotavirus properties of *L. rhamnosus* GG have been well demonstrated in additional studies that indicate that consumption of 10^{10}–10^{11}cells of *L. rhamnosus* GG per day is able to shorten the diarrheal phase in children treated for rotavirus infection, and can significantly increase serum concentrations of IgA antibodies against rotavirus [20-25]. According to meta-analysis, the administration of *L. rhamnosus* GG compared with placebo, in hospitalized children, has the potential to reduce the overall incidence of healthcare-associated diarrhea, including symptomatic rotavirus gastroenteritis [26].

Other *Lactobacillus* and *Bifidobacterium* strains have been studied in the treatment of rotavirus gastroenteritis in children and infants (Table 1). *L. reuteri*, for instance, has been administered to hospitalized children with acute diarrhea, either 10^{10} to 10^{11} cfu or a matching placebo daily for the length of hospitalization or up to 5 days. *L. reuteri* was effective in shortening both the duration of rotavirus diarrhea and the number of days of illness [27]. The prophylactic feeding of *L. sporogenes* to newborn infants were randomized to receive a daily oral dose of 10^8 cfu or a placebo for one year. *L. sporogenes* had a preventive effect on the incidence and duration of acute rotavirus diarrhea [28]. Fang et al. [29] demonstrated that a minimal effective dose of 6 x 10^8 cfu for 3 days of *L. rhamnosus*, significantly reduced faecal shedding rotavirus concentration in paediatric patients. Although the administration of 10^{10} cfu of lyophilized *L. paracasei*s strain ST11 daily for 5 days had a clinically significant benefit in the management of non-rotavirus-induced diarrhea, ST11 treatment against severe rotavirus diarrhea was ineffective [30]. Children with rotavirus infection who received milk-based formula supplemented with either *B. animalis* Bb12, either alone or together with *Streptococcus thermophilus*, had fewer rotavirus infections when measured by salivary rotavirus-specific IgA antibody [31]. Moreover, *Saccharomyces boulardii* reduced the duration of diarrhea in infants when given to

hospitalized children within 72 hours after the onset of acute diarrhea although the number of rotavirus infections was similar between groups [32].

The beneficial effects of immunobiotics on viral-associated diarrheas have been well established in humans as described above (Table 1). However, few studies have evaluated their antiviral and anti-inflammatory effects in animals. Zhang et al. [33] studied the effect of probiotics on the response of gnotobiotic pigs to the challenge with human rotavirus. Although no differences were found between treated and control groups when virus replication was studied, the work suggested that LAB administration down-regulates the recruitment of viral-activated monocytes/macrophages into the intestinal tract thereby limiting the inflammation induced by the viral infection [33]. Afterwards, it was showed systemic toll like receptor (TLR) TLR2-, TLR3-, and TLR9-expressing monocyte/macrophage and dendritic cell (cDC) responses after virulent human rotavirus (HRV) infection, LAB colonization, and a combination of both [34]. Probiotic LAB induced strong TLR2-expressing APC responses in blood and spleen, HRV induced a TLR3 response in spleen, and TLR9 responses were induced by either HRV (in spleen) or LAB (in blood). LAB and HRV have an additive effect on TLR2- and TLR9-expressing antigen presenting cells (APC) responses, consistent with the adjuvant effect of LAB. LAB enhanced the interferon (INF)-γ and IL-4 responses in serum, but it had a suppressive effect on the TLR3- and TLR9-expressing CD14- APC responses in spleen and the serum IFN-α response induced by HRV [35]. Later, Azevedo et al. [36] demonstrated that probiotic LAB further enhanced the Th1 and Th2 cytokine responses to HRV infection as indicated by significantly higher concentrations of interleukin (IL) IL-12, IFN-γ, IL-4 and IL-10 in HRV-infected gnotobiotic pigs. LAB may also help to maintain immunological homeostasis during HRV infection by regulating transforming growth factor (TGF)-β production. In addition, recent studies by Maragkoudakis et al. [19], demonstrated that the known probiotics *L. casei* Shirota and *L. rhamnosus* GG were able to protect porcine and goat epithelial cells against rotavirus and transmissible gastroenteritis viruses challenges (Table 1).

Preidis et al. [37] used a neonatal mouse model of rotavirus diarrhea to gain insight into how probiotics ameliorate acute gastroenteritis by rotavirus. The probiotic *L. reuteri* strain enhancement of enterocyte proliferation, villus repopulation, and virus-specific antibodies may contribute to reduce diarrhea duration, and that nutritional status influences the host response to both beneficial microbes and pathogens [37].

Although these studies clearly demonstrate that immunobiotic LAB are able to beneficially modulate the outcome of rotavirus infection, the precise immunological mechanisms involved in the protective effect were not investigated. For example the role of intestinal epithelial cells (IECs), antigen presenting cells (APCs) or pattern recognition receptors (PRRs) in the immunoregulatory effect of LAB during rotavirus infection are not well established. Our research group has made some progress in this regard.

An initial and essential step in the viral infection cycle of rotavirus is entering and replicating in IECs of the small intestine [38]. Upon virus internalization, dsRNA molecules are generated in infected cells [38]. Viral dsRNA activate PRRs such as TLR3, retinoic acid-inducible gene 1 (RIG-I), and melanoma differentiation-associated protein 5 (MDA-5), which signal host cellular responses in order to try to control viral infection [38-40]. The production of cytokines and chemokines by IECs and immune cells after TLR3 activation is one of the major innate immune responses against dsRNA viruses such as rotavirus. Rotavirus dsRNA triggers the production of IL-8, interferon-inducible protein 10 (IP-10), IL-6, TNF-α and IL-15 in IECs via the TLR3-activated pathway inducing recruitment and activation of macrophages and NK cells and stimulating adaptive B- and T-cell immune responses. Since TLR3 responds to a synthetic dsRNA, poly(I:C) as well as viral dsRNA resulting in the induction of IFN-α/β and IFN- inducible genes transcription, it is thought that TLR3 plays a key role in anti-viral immune responses [41]. IFNs and IFN-regulated gene products produced after TLR3 activation play a key role in the host response for clearing viruses. Type I together with type II IFNs are able to limit rotavirus infection *in vitro* and their levels are augmented in rotavirus-infected children and animals [42-44]. Recently, it has been proposed that IFNs signalling is not only beneficial to the host, but it may also enhance rotavirus replication at the first stages of infection [45]. Nevertheless, other *in vivo* studies have shown a markedly increase in the virulence of certain strains of rotavirus when IFNs signalling was blocked during infection [46]. Furthermore, the fact that rotavirus has evolved mechanisms to manipulate IFNs signalling such as the type I IFNs damping NSP1 protein [47], strongly suggests that IFNs are crucial to limit infection.

Therefore, understanding how TLR3 is activated and regulated in immune cells and IECs can help to choose effective therapies for the prevention or treatment of viral diseases in humans and animals. For example, it could help to select immunobiotics capable of protecting against such diseases by increasing viral defenses and preventing unproductive inflammatory response. In this regards, our research group have used porcine intestinal epithelial (PIE) cells for the study of TLR3 activation on IECs and for the selection of LAB strains with specific immunomodulatory properties considering that approaches aiming to modulate pathways leading to IFNs production may provide valuable tools to increase natural viral defense mechanisms [48].

IECs rather than other cell types express TLR3 in many organs including the airways and the gastrointestinal tracts [49]. By immunohistochemical and flow cytometric analysis we observed an abundant intracellular expression of TLR3 in PIE cells [48]. This is in line with findings of Liu et al. [18] that demonstrated that the non-transformed porcine jejunum epithelial cell line (IPEC-J2) express TLR3 constitutively. Moreover, we evaluated the response of PIE cells to poly(I:C) challenge and found that monocyte quemotactic protein 1 (MCP-1), IL-8, TNF-α, IL-6 and both IFN-α and

IFN-β were up-regulated in PIE cells after stimulation [48]. In addition, considering that the interaction between IECs and immune cells is of fundamental importance for the type of immune response resulting from the contact with an intestinal antigen, we also analyzed the expression of cytokines in immune cells by using co-cultures of PIE cells and porcine PPs immune cells. After stimulation of co-cultures with poly(I:C) we observed an up-regulation of IFN-α, IFN-β, IFN-γ, IL-2 and IL-12p40 in immune cells [48]. Then, the activation of TLR3 in PIE cells would induce the expression and release of cytokines, which exert their action on the underlying immune cells, inducing the activation of APCs and effector lymphocytes. These results indicate that PIE cells could be a good tool to study *in vitro* immune response triggered by TLR3 on IECs and the interaction between IECs and immune cells [48].

We also showed that our *in vitro* systems could be used for the selection of immunobiotic LAB strains with anti-viral immune enhancing activities [48]. Among the lactobacilli strains evaluated by our group, *L. casei* MEP221106 was the strain with the highest capacity to improve IFN-β production in poly(I:C)-challenged PIE cells [48]. Moreover, *in vitro* co-culture experiments showed that *L. casei* MEP221106 is able to improve not only the production of IFN-β but also the levels of IFN-α, TNF-α, MPC-1 and IL-6 in PIE cells. In addition, immune cells in the co-cultures stimulated with *L. casei* MEP221106 showed and improved production of inflammatory and anti-viral cytokines when compared with control cells.

The modulation of cytokines induced by *L. casei* MEP221106 could have an important *in vivo* impact in viral intestinal infections such as those caused by rotavirus. Numerous studies have noted that rotaviruses are able to induce expression of type I IFNs in IECs and NK cells and that these responses contribute to innate immune-mediated clearance of viruses [14]. Type I and II IFNs are able to limit rotavirus infection *in vitro* and *in vivo* studies demonstrated that IFN-α administration is able to reduce rotavirus-associated diarrhea in cattle and pigs [50, 51]. In addition, it was showed in rotavirus-infected mice that DCs from PPs had an increased expression of IL-12/23p40, INF-β and TNF-α, as well as the regulatory cytokine IL-10, suggesting that DCs from PPs play a critical role in controlling the infection and, at the same time, avoiding an excessive inflammatory immune response [49]. Studies in mice also showed that $CD8^+$ T cells are responsible for shortening the course of rotavirus primary infection [52] and that $CD4^+$ T cells are involved in supplying help to $CD8^+$ T cells, but also appear to mediate active protection, via an IFN-γ–dependent pathway [53]. Therefore, our results suggest that *L. casei* MEP221106 would be capable of increasing anti-viral defenses in IECs (IFN-β and IFN-α) as well as the response of APCs (IL-1β, IL-6, IL-12 and IL-10) and $CD4^+$ effector cells (IFN-γ).

In addition, we showed that immune cells in co-cultures pretreated with *L. casei* MEP221106 induced higher levels of IL-10 when compared with control. It was reported that both purified viral dsRNA and poly(I:C) are able to induce severe mucosal damage via TLR3-dependent manner [54]. Moreover, it was showed that TLR3 is able to mediate harmful inflammatory responses in the intestine, thus contributing to the pathogenesis of viral infections [55]. While TLR3 recognition of dsRNA is required for clearance of viruses, it is believed that the degree and the duration of the pro-inflammatory cytokine secretion can become harmful to the host. Therefore, the improved production of IL-10 induced by *L. casei* MEP221106 would allow an efficient regulation of the inflammatory response and avoid tissue injury. As observed with other immunobiotic strains [56, 57], *L. casei* MEP221106 up-regulated the expression of both pro- and anti-inflammatory cytokines. The simultaneous up-regulation of both types of cytokines could be explained by the response of a distinct population of immune cells. It was showed that DCs from mice PPs can increase the mRNA expression of IFN-β, IL-12/23p40, TNF-α and IL-10 genes after the challenge with rotavirus [58]. The dome- resident $CD11c^+CD11b^+CD8^-$ DCs produce high levels of IL-10 upon stimulation while the interfollicular $CD11c^+CD11b^-CD8^+$ DCs and the dome $CD11c^+CD11b^-CD8^-$ DCs produce mainly type I IFN and IL-12 [59]. Further studies are required to evaluate the immunomodulatory activity of *L. casei* MEP221106 in order to conclusively asseverate that this bacterium is able to enhance antiviral immunity and protect against the inflammatory damage at the same time. These studies are currently under investigation in our laboratory.

Our group performed a randomized controlled trial in order to evaluate the effect of a probiotic yogurt containing the immunobiotic strain *L. rhamnosus* CRL1505 on both gut and non-gut related illnesses among children [60]. We demonstrated that administration of *L. rhamnosus* CRL1505 improved mucosal immunity and reduced the incidence and severity of intestinal and respiratory infections in children. When we studied the type of infectious events according to their location and symptoms, the frequency of them was consistent with the prevalence reported in Argentina. The most common infectious diseases were upper respiratory infections, followed by angina and then lower respiratory infections (acute bronchitis) and diarrhea [61-63]. We registered that 34% of children who consumed the probiotic yogurt showed some type of infectious event, while in the placebo group this value was higher reaching a 66% of children. These results demonstrate a significant reduction in occurrence of infectious events associated with consumption of *L. rhamnosus* CRL1505 [60]. We also evaluated the presence or absence of fever during infectious events as well as the need of antibiotic treatment in children who had infections, as indicators of severity. There was a significant decrease in the presence of fever in children who consumed probiotic yogurt as well as a slight decrease in the need for antibiotic treatment, indicating less serious infections in relation to the placebo group [60]. Although we did not evaluate etiology of intestinal infections in the clinical study, previous evaluations have shown that viral pathogens, such as rotavirus are considered the major viruses that can cause diseases in children [64]. Therefore, the findings of our study suggest that administration of *L. rhamnosus* CRL1505 may provide one of the potential

interventions to reduce the burden of common childhood morbidities, especially those associated to viral infections [60].

We used the porcine *in vitro* systems to gain insight into the mechanisms involved in the immunomodulatory effect of the CRL1505 strain, and concentrated our attention in the crosstalk between *L. rhamnosus* CRL1505, PIE cells and APCs in order to deepen our knowledge about the mechanisms, through which this strain may help preventing viral diarrhoea episodes. Moreover, we performed comparative studies with another immunobiotic strain, *L. rhamnosus* CRL1506, that is able stimulate intestinal immunity and not the respiratory defenses. Herein we show evidence of how IECs can be modulated by immunobiotic *L. rhamnosus* in a strain-dependent fashion to enhance antiviral responses.

There is a general concept that the overall effect of probiotics is strain-specific, but there are only a few comparative studies where at least two strains of the same species provide significant differences in their immunomodulatory potential [65]. Herein, we show that two strains, both *L. rhamnosus*, isolated from the same ecological niche and with similar technological properties [57, 66], are capable to induce differential antiviral defense phenotypes in IECs and APCs. We propose a model of action for each strain. In general terms, *L. rhamnosus* CRL1506 has a marked influence on IECs and antiviral innate defense mediated by type I IFNs, whereas *L. rhamnosus* CRL1505 stands out for its influence on APCs [76, 77].

Both *L. rhamnosus* CRL1505 and *L. rhamnosus* CRL1506 were able to induce IFN–α and –β in IECs and improve the production of type I IFNs in response to poly(I:C) challenge independently of TLR2 or TLR9 signalling [77]. However, *L. rhamnosus* CRL1506 showed a higher capacity to improve levels of IFN-α and IFN-β in IECs when compared with *L. rhamnosus* CRL1505, which is in line with our previously reported *in vivo* results, showing higher levels of IFN-α and IFN-β in intestinal fluids of CRL1506-treated than in CRL1505-treated mice [76]. Considering that type I IFNs up-regulate several genes involved in viral defense and genes of major importance for the development of a strong cellular response, we hypothesize that *L. rhamnosus* CRL1506 may play an important role in the improvement of innate immune responses against intestinal virus, especially in IECs.

In addition, both lactobacilli induced expression of IL-6 and TNF-α via TLR2 in IECs, being *L. rhamnosus* CRL1505 the stronger modulator of these cytokines. Furthermore, although both strains were able to significantly increase surface molecules expression and cytokine production in intestinal APCs, *L. rhamnosus* CRL1505 had a stronger effect both when applied alone or combined with a posterior poly(I:C) challenge [76, 77]. The improved Th1 response induced by *L. rhamnosus* CRL1505 was triggered by TLR2 signalling and included augmented expression of MHC-II and co-stimulatory molecules and expression of IL-1β, IL-6, and IFN-γ in APCs. Considering that TLR signalling is a crucial aspect of innate defense [67, 68], but if uncontrolled at mucosal surfaces, it would be pathological, it is important to highlight again the fact that IL-10 was also significantly up-regulated by *L. rhamnosus* CRL1505, suggesting that the inflammatory conditions may be held under control. These *in vitro* results are in line with our previous findings showing that *L. rhamnosus* CRL1505 was more efficient than Lr1506 for increasing the levels of IFN-γ, IL-10 and IL-6 in the intestine of mice [76, 77].

It was recently reviewed the emergence of TLR agonists as new ways to transform antiviral treatments by introducing panviral therapeutics with less adverse effects than IFN therapies [69]. The use of *L. rhamnosus* CRL1505 and *L. rhamnosus* CRL1506 as modulators of innate immunity and inductors of antiviral type I IFNs, IFN-γ, and regulatory IL-10 clearly offers the potential to overcome this challenge. To evaluate *in vitro* and *in vivo* the capacity of both strains to protect against rotavirus infection is an interesting topic for future research.

Table 1 Effect of immunobiotics on viral intestinal infections.

Strain	Viability	Host	Route	Challenge	Protective effect	Immunoregulatory effect	Ref.
L. rhamnosus GG	Viable	Human	Oral	Rotavirus	Shorter duration of diarrhea, less chance of a protracted course	Not studied	[70]
B. bifidum ATCC15696	Viable	Mice	Oral	Rotavirus	Early resolution of diarrhea	Increase of rotavirus-specific IgA in feces and in serum	[71]
B. infantis ATCC15697	Viable	Mice	Oral	Rotavirus	Early resolution of diarrhea	Increase of rotavirus-specific IgA in feces and in serum	[71]
L rhamnosus GG	Viable or inactivated	Human	Oral	Poliovirus	Not studied	Increase polio-1-specific IgG titers	[72]
L casei CRL431	Viable or inactivated	Human	Oral	Poliovirus	Not studied	Increase polio-1-specific IgG titers	[72]
L. rhamnosus 573L/1; 573L/2; 573L/3	Viable	Human	Oral	Rotavirus	Reduction of the diarrhoea duration	Not studied	[73]
L. casei Shirota	Viable	Pig (CLAB, PSI) Goat (GIE)	Co-culture	Coronavirus and Rotavirus	Highest protective effect on monolayers against viral disruption	Not studied	[19]
L. rhamnosus GG	Viable	Pig (CLAB, PSI) Goat (GIE) Human (TLT, H4)	Co-culture	Coronavirus and Rotavirus	Highest protective effect on monolayers against viral disruption	Not studied	[19]
E. faecium PCK38	Viable	Pig (CLAB)	Co-culture	Coronavirus and Rotavirus	Moderate protective effect on monolayers against viral disruption	Not studied	[19]
L. fermentum ACA-DC179	Viable	Pig (CLAB) Human (TLT)	Co-culture	Coronavirus and Rotavirus	Moderate protective effect on monolayers against viral disruption	Not studied	[19]
L. plantarum PCA236	Viable	Goat (GIE)	Co-culture	Coronavirus and Rotavirus	Moderate protective effect on monolayers against viral disruption	Not studied	[19]
L. acidophilus	Lyophilized	Human	Oral	Rotavirus	Diminished duration of diarrhea, fever and vomiting	Not studied	[74]
L. rhamnosus GR-1	Viable	Human	Oral	HIV associated diarrhea	Improved gut health and general well-being	Not studied	[75]

L. reuteri ATCC 23272	Viable	Pig	Oral	Human rotavirus	No enhanced protection	Enhanced Th1 and Th2 cytokines	[36]
L. acidophilus NCFM	Viable	Pig	Oral	Human rotavirus	No enhanced protection	Enhanced Th1 and Th2 cytokines	[36]
L. reuteri	Viable	Mice	Oral	Rotavirus	Enhancement of enterocyte proliferation, villus repopulation and reduction of diarrhea duration	Enhancement of virus-specific antibodies	[37]

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