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REVIEW

# Probiotics in respiratory virus infections

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**Abstract** Viral respiratory infections are the most common diseases in humans. A large range of etiologic agents challenge the development of efficient therapies. Research suggests that probiotics are able to decrease the risk or duration of respiratory infection symptoms. However, the antiviral mechanisms of probiotics are unclear. The purpose of this paper is to review the current knowledge on the effects of probiotics on respiratory virus infections and to provide insights on the possible antiviral mechanisms of probiotics. A PubMed and Scopus database search was performed up to January 2014 using appropriate search terms on probiotic and respiratory virus infections in cell models, in animal models, and in humans, and reviewed for their relevance. Altogether, thirty-three clinical trials were reviewed. The studies varied highly in study design, outcome measures, probiotics, dose, and matrices used. Twenty-eight trials reported that probiotics had beneficial effects in the outcome of respiratory tract infections (RTIs) and five showed no clear benefit. Only eight studies reported investigating viral etiology from the respiratory tract, and one of these reported a significant decrease in viral load. Based on experimental studies, probiotics may exert antiviral effects directly in probiotic–virus interaction or via stimulation of the immune system. Although probiotics seem to be beneficial in respiratory illnesses, the role of probiotics on specific viruses has not been investigated sufficiently. Due to the lack of confirmatory studies and varied data available, more randomized, double-blind, and placebo-controlled trials in different age populations investigating probiotic dose response, comparing probiotic strains/genera, and elucidating the antiviral effect mechanisms are necessary.

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## Introduction

Respiratory tract infections (RTIs) are a major cause of morbidity and mortality worldwide. Viral pathogens are the most common etiological agents of acute respiratory disease. The social and economic impact of viral respiratory disease is substantial, due to hospitalizations, medical costs, missed work, and school and day care absences. For instance, estimates show that viral respiratory tract illnesses (mostly common colds) cost US$40 billion annually in the United States alone [1].

There are over 200 different types of viruses which cause RTIs in humans. Human rhinoviruses (HRV) are the largest group of respiratory viruses, comprising over 150 serotypes [2]. In humans, the predominant illness caused by HRV is the acute upper RTI, also known as the common cold. The second most common viruses infecting humans are the human enteroviruses (HEV), which are associated with clinical manifestations ranging from mild respiratory symptoms to serious conditions [2]. Influenza viruses, respiratory syncytial virus (RSV), and adenoviruses are also major causative agents of both upper and lower RTIs [3–5]. In addition, many other viruses or virus groups cause RTIs, e.g., parainfluenza viruses and coronaviruses can cause a broad spectrum of respiratory diseases, ranging from mild upper RTIs to pneumonia [6]. In recent years, with the rapid development of high-throughput molecular techniques, several new viruses associated with respiratory diseases, such as human bocavirus, human

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metapneumovirus, and the new coronaviruses HKU1 and NL63, have been identified as well [7].

The prevention of viral respiratory infections is an important challenge to public health. Currently, the only effective antivirals and vaccines for the prevention and treatment of respiratory virus infections are available against influenza viruses and adenoviruses. For the viruses causing common cold (HRV, HEV), no effective therapies are available. Large varieties of etiologic agents and increasing antibiotic and antiviral resistance challenge the development of efficient therapies. Consequently, it is of importance to find alternative and safe ways to reduce the risk of these infections. Even partially effective therapy in the treatment and prevention of viral RTIs such as the common cold could have an impact on reducing morbidity and economic losses due to this illness.

Probiotics are defined as live microorganisms that confer a health benefit on the host [8]. The most common types of microbes used as probiotics are lactobacilli and bifidobacteria, which are generally consumed as part of fermented foods, such as yoghurts or dietary supplements. Criteria for probiotic bacteria include that the bacterial strain: (1) must be able to survive in the gastrointestinal tract and to proliferate in the gut; (2) should exert benefits to the host through growth and/or activity in the human body; (3) should be non-pathogenic and non-toxic; (4) provide protection against pathogenic microorganisms by means of multiple mechanisms; and (5) should be lacking transferable antibiotic resistance [9]. Different bacterial strains of the same genus and species, verified also by genomic information, may exert completely different effects on the host.

The most promising health effects of probiotics in human intervention studies include the amelioration of acute diarrhea in children, relief of children's milk allergy/atopic dermatitis, and relief of irritable bowel syndrome [10, 11]. Probiotics are likely to have an impact through gut mucosa by balancing the local microbiota by inhibiting the growth of pathogenic microorganisms [12], and by enhancing local and systemic immune responses [13]. They may also influence the composition and activity of microbiota in the intestinal contents. Considering the beneficial effects of probiotics in virus infections, specific probiotics have been suggested to be effective in alleviating the duration and severity of acute rotavirus gastroenteritis [14]. In addition, increasing evidence shows that probiotics are beneficial in RTIs [15], which, in most cases, are of viral origin. However, the mechanisms behind these effects are largely unknown.

## Aim

The aim of this review is to present the current knowledge of the health effects of probiotics on RTIs in humans, with a focus on viral respiratory infections. In addition, possible antiviral mechanisms of probiotics are discussed in context with studies conducted in vitro and in animal models.

## Methods

A PubMed and Scopus database search was performed up to January 2014 to review the relevant literature investigating the effects of probiotics on respiratory virus infections in cell culture, animal models, and clinical trials. The following search terms were used individually and in combination: 'probiotic', '*Lactobacillus*', '*Bifidobacterium*', '*Lactococcus*', 'respiratory infection', 'respiratory virus', and 'influenza virus'.

## Health effects of probiotics in respiratory virus infections

### Animal experiments

Animal experiments provide insight on the clinical effects of probiotics against respiratory virus infections (Table 1). In influenza virus infection in mice, the oral or intranasal administration of *Lactobacillus pentosus* strains [28–30], *L. casei* Shirota [16, 17], *L. plantarum* strains [18–20, 38], *L. delbrueckii* ssp. *bulgaricus* OLL1073R1 [39], *L. rhamnosus* GG [21, 23], *L. gasseri* TMC0356 [21, 22, 24], *Lactococcus lactis* ssp. *cremoris* FC [40], *L. brevis* KB [32], or *B. breve* YIT4064 [41] have reduced signs of infection, virus titer in the lungs or nasal washings, or increased body weight during infection and mice survival. In pneumovirus infection in mice, the virus-induced inflammation was suppressed and the mice were protected against lethal disease by *L. plantarum* NCIMB 8826 and *L. reuteri* F275 [35]. In addition, *L. rhamnosus* CRL1505 and *L. rhamnosus* CRL1506 protected mice against RSV infection [37].

### Clinical trials

#### *Children*

Altogether, five clinical trials have been conducted in children using *L. rhamnosus* GG as a probiotic [42–46]. In healthy children attending day care, *L. rhamnosus* GG reduced the number of children experiencing RTIs [42, 43], the number of upper and lower RTIs [43], and the number of antibiotic treatments or absences from day care [42]. In another study, no differences were reported between the *L. rhamnosus* GG and the control groups in the number of antibiotic treatments or respiratory symptom episodes [47]. However, in a subgroup with *L. rhamnosus* GG identification in feces, *L. rhamnosus* GG usage reduced the duration of RTIs. In hospitalized children, *L. rhamnosus* GG reduced the risk of

**Table 1** Immunomodulatory effects of probiotic bacteria in respiratory virus infections in animal experiments

| Probiotic strain/reference | Virus | Study design | Main findings |
|---|---|---|---|
| *L. casei* Shirota<br>[16] | IFV A/PR/8/34 (H1N1) | BALB/c mice, intranasal administration 3× daily for 3 days before infection | Mice survival rate ↑<br>IL-12, IFN-γ, TNF-α in MLN cells ↑<br>Virus titers in nasal wash ↓ |
| [17] | | BALB/c mice, oral administration 5×/week for 3 weeks before infection | Mice survival rate ↑<br>Pulmonary NK cell activity ↑<br>IL-12 production by MLN cells ↑<br>Viral titers in nasal wash ↓ |
| *L. plantarum* L-137<br>[18] | IFV A/FM1/47 (H1N1) | C57BL/6 mice, intragastric administration daily 7 days before and 6 days after infection | Viral titers in the lung ↓<br>IFN-β in sera ↑ |
| *L. plantarum* 05AM2<br>*L. plantarum* 06TCa8<br>*L. paracasei* ssp. *paracasei* 06TCa19<br>*L. paracasei* ssp. *paracasei* 06TCa22<br>*L. paracasei* ssp. *tolerans* 06TCa39<br>*L. plantarum* 06TCa40<br>*L. paracasei* ssp. *paracasei* 06TCa43<br>*L. plantarum* 06CC2<br>*L. delbrueckii* ssp. *lactis* 06TC3<br>*L. plantarum* 06CC9<br>[19] | IFV A/PR/8/34 (H1N1) | BALB/c mice, oral administration 2× daily for 10 days starting 2 days before infection | Effects only with *L. plantarum* 06CC2:<br>Body weight loss ↓<br>Virus yields in lungs ↓<br>Mice survival ↑<br>No. of macrophages and neutrophils in BALF ↓<br>TNF-α in BALF ↓<br>INF-α, IL-12, IFN-γ, NK cell activity ↑<br>mRNA IL-12 receptor, IFN-γ in Peyer's patches ↑ |
| *L. plantarum* DK119<br>[20] | | BALB/c mice, oral administration daily for 10 days before infection and 14 days after infection + experiments with nasal administration | Both administration routes:<br>Mice survival ↑<br>Lung viral loads ↓<br>BALF IL-12, IFN-γ ↑<br>BALF IL-4, IL-6, TNF-α ↓ |
| *L. gasseri* TMC0356<br>*L. rhamnosus* GG<br>[21] | | BALB/c mice, oral administration daily for 1 day, infection on day 14 | Effects with both bacteria:<br>Clinical symptom scores ↓<br>Pulmonary virus titers ↓ |
| [22] | | | Effects with *L. gasseri*:<br>Peyer's patches: mRNA IL-12, IL-15, IL-21 ↑<br>Lungs: mRNA IFN-γ, TNF, IL-12, perforin-1 ↑ |
| [23] | | BALB/c mice, intranasal administration 3× daily for 3 days before infection | *L. gasseri* TMC0356:<br>Morbidity ↓<br>Mice survival ↑<br>mRNA IL-1β, TNF, IL-10, MCP-1 ↑ |
| [24] | | | *L. rhamnosus* GG:<br>Accumulated symptoms ↓<br>Mice survival ↑<br>mRNA IL-1β, TNF, IL-10 + MCP-1↑ |
| *L. rhamnosus* (strain not provided)<br>[25] | IFV A/NWS/33 (H1N1) | BALB/c mice, sublingual administration for 10 days before infection | Mice mortality ↓<br>Lung lesion scores↓<br>Lung anti-IFV IgA ↑<br>Lung IL-12 ↑, IL-6+ TNF-α ↔<br>Lung CD4+, CD8+, CD25 expression ↑<br>Splenocyte NK cell activities ↑ |
| *L. fermentum-1*<br>*L. brevis-2*<br>[26] | | BALB/c mice, intranasal or oral administration for 21 days before infection | Mice survival ↑<br>Virus titer ↓<br>Lung IgA + IL-12 ↑<br>Lung TNF-α and IL-6 ↓<br>Lung IFN-γ ↔ |
| *L. fermentum* CJL-112<br>[27] | | BALB/c mice, intranasal administration for 21 days before infection | Effect in lungs:<br>IL-2, IFN-γ, IL-1β ↑<br>IL-4, IL-5 ↔<br>IL-10 ↓<br>Anti-influenza IgA ↑ |
| *L. brevis* KB290<br>[28] | IFV A/PR/8/34 (H1N1)<br>IFV A/PR8/34 H1N1 | BALB/c mice, oral administration 1× daily for 14 days before infection | Body weight loss ↓<br>Clinical symptom scores ↓<br>BALF IFV specific IgA ↑<br>Serum IFN-α ↑ |

**Table 1** (continued)

| Probiotic strain/reference | Virus | Study design | Main findings |
|---|---|---|---|
| *L. pentosus* S-PT84<br>[29] | | BALB/c mice, intranasal administration 1× daily for 3 days before infection | Mice survival ↑<br>Virus titer in BALF ↓<br>IL-12, IFN-γ in MLN cells ↑<br>BALF IL-12, IFN-α ↑<br>NK cell activity ↑ |
| *L. pentosus* b240<br>[30] | | BALB/c mice, oral administration for 3 weeks by gavage before infection | Mice survival ↑<br>Virus titers 7 days after infection ↓<br>Anti-IFV IgA, IgG BALF + plasma on day 7 ↑ |
| [31] | IFV A/California/04/2009 (H1N1) | BALB/c mice, oral administration daily for 5 weeks, IFV infection on day 21 | Mice survival ↑<br>Virus proliferation ↔<br>Lung histopathology ↔<br>Cytokines/chemokines ↔<br>Differential regulation of antiviral gene expression |
| *L. acidophilus* L-92<br>[32] | IFV A/PR/8/34 (H1N1) | BALB/c mice, oral administration daily for 21 days, infection on day 16 | Both bacteria:<br>- Body weight ↔<br>- Fatality ↔<br>Viable probiotic:<br>- Symptom score ↔<br>- Lung virus titers ↓<br>- Lung NK cell activity ↑<br>- Lung eotaxin, M-CSF, IL-1β, RANTES, IFN-α ↑<br>- Lung IgG ↓, IgA ↔<br>Nonviable probiotic:<br>- Symptom score ↓<br>- Lung virus titers ↓<br>- Lung NK cell activity ↑ |
| *B. longum* BB536<br>[33] | | BALB/c mice, oral administration daily for 2 weeks before infection | Symptom score ↓<br>Loss of body weight ↓<br>Lung virus titers ↓<br>Lung IL-10, IL-12 ↔<br>Lung IL-6, IFN-γ (↓) |
| *Bifidobacterium*<br>*Lactobacillus*<br>*Enterococcus*<br>(Bifico probiotic product)<br>[34] | IFV A FM1 (H1N1) | BALB/c mice were subjected to 8 days of oral neomycin administration, then infected intranasally with virus. Probiotic administration by gavage for 4 days after infection | Lung IFN-γ, IL-17 ↑, IL-4, IL-10 ↓<br>Probiotic treatment significantly restored initial levels of upregulation of TLR7, MyD88, IRAK4, TRAF6, and NF-kB mRNA expression |
| *L. plantarum* NCIMB 8826<br>*L. reuteri* F275<br>[35] | Pneumonia virus of mice J3666 | BALB/c and C57BL/6 mice, intranasal inoculation of 2 weekly doses 2 weeks before infection | Protection against virus infection ↑<br>Granulocyte recruitment ↓<br>CXCL10, CXCL1, CCL2,TNF↓<br>Virus recovery ↓ |
| [36] | | | Live *L. reuteri*:<br>Neutrophil recruitment ↑<br>CXCL1, CCL3, CCL2, CXCL10, TNF-α, IL-17A ↑<br>IFN-α, IFN-β, IFN-γ ↔ |
| *L. rhamnosus* CRL1505<br>*L. rhamnosus* CRL1506<br>[37] | Viral pathogen molecular pattern poly(I:C) + RSV A2 | BALB/c mice, nasal administration for 2 days before infection | BALF + serum IL-6, IFN-α,IFN-β, TNF-α, IL-10 ↑<br>Lung viral loads↓<br>Strains differentially modulated TLR3/RIG-I-triggered antiviral respiratory immune response |

Abbreviations for columns:

Probiotic strain: L = *Lactobacillus*; B. = *Bifidobacterium*

Virus: IFV = influenza virus; RSV = respiratory syncytial virus

Main findings: IL = interleukin; IFN = interferon; TNF = tumor necrosis factor; MLN = mediastinal lymph node; NK = natural killer cell; BALF = bronchoalveolar lavage fluid

↑ = significant increase; ↓ = significant decrease; ↔ = no significant effect

RTIs and duration of RTI episodes [42]. In preterm infants, *L. rhamnosus* GG reduced the incidence of RTIs [46]. In addition, a meta-analysis of four randomized controlled trials investigating the role of *L. rhamnosus* GG in the prevention of respiratory infections in children showed that *L. rhamnosus* GG has the potential to reduce the risk of upper RTIs, incidence of acute otitis media, and antibiotic use. There were no significant differences between the *L. rhamnosus* GG and the control groups in the incidence of lower RTIs [48].

There are seven studies conducted with probiotic bacteria other than *L. rhamnosus* GG. *L. casei rhamnosus* in children reduced the number of RTIs [49]. Also, *L. casei* DN114001 reduced the incidence rate for upper RTIs43 and decreased the duration (days) and incidence of only lower RTIs, but not upper RTIs [50]. *L. fermentum* CECT5716 with prebiotics in infants, however, reduced the incidence of both upper and lower RTIs [51]. The use of *B. animalis* ssp. *lactis* Bb12 in healthy newborns was able to reduce the number of RTIs as well, but was ineffective in reducing the occurrence of acute otitis media (AOM) or symptoms of otitis media [52]. In healthy infants, treatment with *L. reuteri* SD112, but not with *B. animalis* ssp. *lactis* Bb12, resulted in fewer days of absence from day care due to illness, lower number of days with fever, and clinical visits. Both strains were ineffective in reducing the incidence or duration of RTIs [53, 54]. In healthy children, *L. casei* CRL431 or *L. reuteri* DSM17938 did not reduce the incidence, number, or duration of acute RTIs or RTI episodes [55].

The effectiveness of several combinations of probiotics on RTIs has been investigated in four clinical trials. A combination of *L. rhamnosus* GG, *L. rhamnosus* Lc705, *B. breve* Bb99, and *P. freudenreichii* ssp. *shermanii* JS in otitis-prone children [56] or a combination of *L. rhamnosus* GG and *B. animalis* ssp. *lactis* Bb12 in healthy newborns [57] both reduced the occurrence of recurrent RTIs, but not the incidence of AOM. A combination of *L. acidophilus* and *B. bifidum* in healthy children reduced the duration of acute RTI symptoms, school absence, and the risk of upper RTI symptoms as well [58]. However, a combination of 12 bacteria including species of *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, and *Enterococcus* was not able to reduce the number of RTIs [49].

The viral etiologies of RTIs were investigated in only five studies. In preterm infants, *L. rhamnosus* GG decreased the incidence of rhinovirus-induced episodes, but not rhinovirus load [46]. In otitis-prone children, a combination of *L. rhamnosus* GG, *L. rhamnosus* Lc705, *B. breve* Bb99, and *P. freudenreichii* ssp. *shermanii* JS reduced human bocavirus load in the nasopharynx [59], but not picornaviruses [60]. In healthy children attending day care, *L. rhamnosus* GG was not able to decrease significantly respiratory viruses (HRV, HEV, influenza viruses, parainfluenza viruses, RSV, adenovirus, and human bocavirus) in the upper respiratory tract [47]. Healthy children receiving *L. casei rhamnosus* had significantly lower odds of viral infection diagnosed by a doctor and a significant difference in doctor-diagnosed RTI. However, specific viruses were not reported in that study [49].

### *Adults*

Probiotics' effectiveness in RTIs has been addressed in 13 studies in healthy adults, in athletes, and in individuals under stressful conditions. In healthy adults, *L. fermentum* CETC5716 reduced the number of RTIs and increased antigen-specific IgA formation after influenza virus vaccination [61]. In addition, a combination of *L. gasseri* PA16/8, *B. longum* SP07/3, and *B. bifidum* MF20/5 reduced the duration of RTI symptoms [62], duration of RTI episodes [63, 64], but not the severity of RTI symptoms [63, 64]. None of these trials reported the effects of combinations on respiratory virus load, although their viral etiology was studied. *B. animalis* ssp. *lactis* Bl-04 reduced the risk of an upper RTI episode [65]. A combination of *L. rhamnosus* GG and *B. animalis* ssp. *lactis* Bb12 reduced both the duration of upper RTI and the severity of RTI symptoms [66].

Altogether, seven trials have been conducted among athletes or stressed individuals, but they did not report studying the viral etiology. In male elite distance runners, *L. fermentum* VRI003 reduced the duration of RTI symptoms, but not the incidence of RTIs or the severity of symptoms [67]. In competitive cyclists, *L. fermentum* (PCC) had some decreasing effects on the symptoms of upper RTI in males, but not in females [68]. In rugby union players [69], a combination of *L. gasseri*, *B. bifidum*, and *B. longum* reduced the incidence of upper RTIs, but not the severity of symptoms. However, in marathon runners, *L. rhamnosus* GG did not decrease the number of RTI episodes or the severity or the duration of RTI symptoms [70]. In addition, in commando trainers, *L. casei* DN114001 was ineffective in reducing the incidence of RTIs or RTI symptoms [62–64, 71]. Similarly, *L. salivarius* did not lower the number of RTI episodes or reduce the severity or the duration of RTI symptoms in trainers [72]. However, in shift workers, *L. casei* DN114001 reduced the number of RTIs and increased the function of immune cell activity [73].

### *The elderly*

Only five studies have investigated the effects of probiotics on RTIs, but not on the occurrence of specific viruses, in the elderly. *L. casei* DN114001 decreased the duration of RTIs [74, 75], but had no effect on the incidence of RTIs [74]. *L. casei* Shirota did not have an effect on the number of upper RTIs or the severity of upper RTI symptoms, but probiotics decreased the duration of upper RTIs [76]. However, in another study, *L. casei* Shirota had no effect

on the duration of RTI symptoms [77]. A combination of *L. rhamnosus* GG, *L. rhamnosus* Lc705, *B. breve* Bb99, and *P. freudenreichii* ssp. *shermanii* JS was ineffective in lowering the number of RTIs and reducing the duration of RTI symptoms. However, the combination reduced the duration of RTI episodes [60].

The clinical trials in children, adults, and the elderly presented in this review are summarized in Table 2. A variety of probiotic strains have been used in these clinical trials, most of them belonging to the genus *Lactobacillus*. In addition, various combinations of probiotics have been used. Of 33 studies, altogether, 28 studies reported that probiotics had beneficial effects in the outcome of RTIs and five showed no clear benefit. Only eight studies, however, reported investigating the viral etiology. Of these, only one study showed a statistically significant reduction in the virus load in the probiotic group. A Cochrane systematic review by Hao et al. concluded that probiotics were better than placebo in terms of reducing the number of upper RTI episodes, the incidence of acute upper RTI episodes, and antibiotics used [15]. Although clinical trials show that the use of specific probiotics and probiotic combinations are beneficial in RTIs, there are also studies that report no clear advantage. In addition, several viruses can cause respiratory illnesses, but only a few studies have investigated probiotics' effectiveness on viral agents. The lack of consistent evidence between probiotic strains/genera and even within strains may be due to variation in study designs and reported outcome measures, the length of intervention, study populations used (children vs. adults) or bacterial doses ($10^6$–$10^{10}$ cfu), and matrices (milk, yoghurt, capsule) used. In addition, in the elderly, decreased immunity due to aging may partly explain the conflicting results [79].

## Possible mechanisms of actions of probiotics in respiratory virus infections

Clinical and animal studies have demonstrated that specific probiotics have antiviral effects, but the underlying mechanisms are unclear. Additionally, the strain-to-strain variation may be relatively large concerning strain properties and efficacy. Possible antiviral mechanisms of probiotics include: (1) hindering the adsorption and (2) cell internalization of the virus; (3) production of metabolites and substances with a direct antiviral effect; and 4) crosstalk (immunomodulation) with the cells in establishing the antiviral protection. The possible mechanisms of probiotics against respiratory viruses are presented in Fig. 1.

### Antagonism to respiratory viruses

The respiratory tract is covered by mucosal epithelial surfaces, which are constantly exposed to numerous microorganisms and serve as primary ports of entry for respiratory viruses. Virus attachment to a host cell is the first essential step in the disease process, and, therefore, interruption of this attachment could be beneficial to the host. Probiotic bacteria may bind directly to the virus and inhibit virus attachment to the host cell receptor. For instance, there is evidence that specific strains of lactobacilli are able to bind and inactivate vesicular stomatitis virus (flu-like virus) in vitro [81]. Probiotics may also show direct antimicrobial activity against pathogens by producing antimicrobial substances such as organic acids, hydrogen peroxide, biosurfactants, and bacteriocins [12]. In experimental studies in epithelial cells and macrophages, metabolic products of specific lactobacilli and bifidobacteria prevented vesicular stomatitis virus infection in a strain-specific manner [81]. In addition, metabolites of bacteria in yoghurts showed antiviral activity, inhibiting influenza virus replication [82]. The induction of low-level synthesis of nitric oxide may also be involved in the protective actions of probiotics against viruses in the respiratory cells, as shown in alveolar macrophages in vitro [27, 83, 84]. However, it should be noted that respiratory viruses infect cells with different mechanisms by using various receptors and, also, the antiviral effects of probiotics are strain-specific.

### Immunomodulation

#### *Cell-mediated immunity*

The induction of antiviral cytokines such as interferons (IFNs), as well as proinflammatory cytokines and chemokines, upon antigen recognition in epithelial cells or underlying effector cells [macrophages, dendritic cells (DCs), neutrophils] play a key role in virus infections by initiating cell-mediated viral elimination and adaptive immune responses. Probiotics may mediate their antiviral effects against respiratory viruses possibly by eliciting systemic immune responses via gut or enhancing cellular immunity in the airways with increased activity of natural killer cells and macrophages. In the gut epithelial cells and/or antigen-presenting cells, probiotics are recognized by toll-like receptors (TLRs) [85–88]. Probiotics may, therefore, modulate cytokine expression patterns through epithelial cells [89] and through underlying professional antigen-presenting cells, such as macrophages and dendritic cells [90–95].

Many experimental studies in vitro and in animals show that specific strains of probiotics are capable of providing protection against virus infections by stimulating antiviral, cytokine, and chemokine responses in the respiratory and gastrointestinal epithelial cells or immune cells. In murine DCs, *L. acidophilus* NCFM and *L. acidophilus* X37 induced the expression of viral defense genes (IFN-β, IL-12, IL-10) [96]. In human macrophages, *L. rhamnosus* Lc705 induced

**Table 2** Reported effects of probiotics in respiratory tract infections (RTIs) in clinical settings in children, healthy adults, and the elderly

| Study design | Subjects | Probiotics used | Main findings: probiotic vs. placebo |
|---|---|---|---|
| Children | | | |
| R DB PC 7 months [42] | 571 healthy children at day care centers (1–6 years) | *L. rhamnosus* GG in milk (on average, $10^8$ cfu) 3× daily | - Days with respiratory symptoms ↔<br>- No. of children with RTIs ↓<br>- Antibiotic treatments ↓<br>- Days of absence from day care ↓<br>- Age-adjusted results ↔ |
| R DB PC 7 months [45] | 523 healthy children at day care centers (2–6 years) | *L. rhamnosus* GG in milk (on average, $10^8$ cfu) 3× daily | - Days with respiratory symptoms/month ↔ (subgroup of completed cases:↓)<br>- Respiratory symptom episodes/month ↔<br>- Antibiotic treatments ↔ |
| [47] | | | Subgroup of children visiting study physician:<br>- Days with respiratory symptoms/month ↓<br>- Occurrence of respiratory viruses in the nasopharynx ↔<br>- RTI symptoms associated with viral findings ↔ |
| R DB PC 3 months [43] | 281 healthy children at day care centers (2–6 years) | *L. rhamnosus* GG ($10^9$ cfu) in milk daily | - No. of children with RTIs ↓<br>- No. of URTIs ↓<br>- No. of lower RTIs ↔<br>- No. of RTIs lasting >3 days ↓ |
| R DB PC during hospital stay [44] | 742 hospitalized children (≥12 months) | *L. rhamnosus* GG ($10^9$ cfu) in milk administered daily for duration of hospitalization | - Risk for RTIs ↓<br>- Risk for duration of RTI episodes lasting >3 days ↓<br>- Duration of hospitalization ↔ |
| R DB PC 57 days (3 days from birth) [46] | 94 preterm infants (gestational age >32+0 and <36+6 weeks) | Prebiotic GOS and polydextrose mixture or *L. rhamnosus* GG $1\times10^9$ cfu/day for 1–30 days and $2\times10^9$ cfu/day for 31 to 60 days stirred in 10 ml of liquid | Prebiotic and *L. rhamnosus* group:<br>- Incidence of RTIs ↓<br>- Incidence of HRV-induced episodes ↓<br>- HRV RNA load during infections ↔<br>- Duration of HRV RNA shedding ↔<br>- Duration/severity of HRV infections ↔ |
| R DB PC 6 months [56] | 309 otitis-prone children (10 months to 6 years) | Combination of *L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* 99, *P. freudenreichii* JS in capsules $8–9\times10^9$ cfu/capsule of each strain on 1 capsule daily | - Occurrence of AOM ↔<br>- Occurrence of recurrent (≥4) RTIs ↓<br>- *Moraxella catarrhalis* in the nasopharynx ↑ |
| [59] | | | - HBoV DNA in the nasopharynx after 3–6 months (studied in 152 children) ↓ |
| R DB PC 10–12 months [57] | 72 healthy newborns (<2 months) | Combination of *L. rhamnosus* GG, *B. animalis* ssp. *lactis* Bb12 $10^{10}$ cfu in capsules supplemented to infant formula once a day | During first 7 months of life:<br>- Incidence of AOM ↓<br>- Antibiotic treatments ↓<br>- No. of RTIs ↔<br>During first 12 months of life:<br>- Incidence of AOM ↔<br>- No. of recurrent RTIs ↓ |
| R DB PC 3 months [58] | 80 healthy children (8–13 years) | Combination of *L. acidophilus* (min. $10^9$/capsule) and *B. bifidum* (min. $10^9$/capsule) (strain information not provided) in capsules 2× daily | - Median duration of cold symptoms + school absence ↓<br>- Risk of fever, cough, rhinorrhea, school absence, and school absence related to common cold ↓ |
| R DB PC 6–7 months [52] | 109 healthy newborns (1 month old) | *B. animalis* ssp. *lactis* Bb12 ($10^9$ cfu/day) in tablet, 2×daily | - No. of RTIs ↓<br>- Occurrence of AOM ↔<br>- Symptoms of otitis media ↔ |
| R DB PC 3 months [53] | 201 healthy infants (4–10 months) | *L. reuteri* SD 112 ($10^7$ cfu/g) or *B. animalis* ssp. *lactis* Bb12 ($10^7$ cfu/g) in milk formula daily | *L. reuteri* vs. *B.* Bb12/control:<br>- No. of days with fever, clinic visits, child care absences, and antibiotic prescriptions ↓<br>Both bacteria:<br>- Rate and duration of RTIs ↔ |
| R DB PC 5 months [50] | 251 healthy school children (3–12 years) | *L. casei* DN 114001 2× daily in fermented yoghurt | - Incidence and duration (days) of RTI ↔<br>- Duration of lower RTIs ↓<br>- Incidence of lower RTI and fatigue ↓ |
| CR DB PC 3 months [78] | 638 healthy children (3–6 years) | *L. casei* DN 114001 ($1 \times 10^8$ cfu/g ) in fermented dairy yoghurt drink: 1× bottle daily | - Incidence rate for CIDs ↓<br>- Incidence rate for URTIs ↓<br>- Missed day care/school or parental missed work ↔ |
| R DB C 6 months | | | - Incidence of acute RTIs ↔ |

**Table 2** (continued)

| Study design | Subjects | Probiotics used | Main findings: probiotic vs. placebo |
|---|---|---|---|
| [55] | 494 healthy children (1–6 years) | *L. casei* CRL431($5 \times 10^8$ cfu/day) or *L. reuteri* DSM17938 ($5\times10^8$ cfu/day) in milk (low or regular calcium) | - No. of RTI episodes ↔<br>- Duration of acute RTIs↔ |
| R DB PC 6 months [51] | 215 healthy infants (6 months) | *L. fermentum* CECT5716 ($2\times10^8$ cfu/day)+GOS in formula daily | - Incidence ratio of URTIs ↓<br>- Incidence ratio of upper and lower RTIs↓ |
| DBRC 3–7 months [49] | 986 children (<5 years) | *L. casei rhamnosus*: 2 sachets ($2\times10^8$ cfu) daily or<br>*L. rhamnosus* T cell-1: 3 tablets ($1\times10^{10}$ cfu) daily or<br>combination of 12 bacteria (7× *Lactobacilli*, 3× *Bifidobacteria*, 1× *Streptococcus*, 1× *Enterococcus* 5 capsules daily ($10^9$ cfu/strain)<br>5 days a week | *L. casei rhamnosus*:<br>- Incidence of bacterial infections ↓<br>- Doctor-diagnosed viral infection in 3 months ↓<br>- Doctor-diagnosed RTI in 3 and 7 months ↓<br>*L. rhamnosus* T cell-1:<br>- Incidence of bacterial infections in 7 months ↓<br>Combination:<br>- No. of RTIs ↔ |
| Adults | | | |
| R DB PC 1 month + 5 months follow-up (intramuscular anti-influenza vaccine) [61] | 50 healthy adults (22–56 years) | *L. fermentum* CECT5716 in capsule ($10^{10}$ cfu/day): 2 weeks before and 2 weeks after vaccination | - No. of RTIs ↓<br>- Antigen-specific IgA ↑ |
| R DB PC C-O 1 month [67] | 20 healthy elite male distance runners | *L. fermentum* VR1003 ($1.3\times10^{10}$ cfu/day), 3× capsules 2× daily | - Incidence of RTIs ↔<br>- No. of days with respiratory symptoms ↓<br>- Severity of symptoms ↔ |
| R DB PC 11 weeks [68] | 99 competitive cyclists (26–45 years) | *L. fermentum* PCC® (minimum $10^9$ cfu/day) in capsules: 1× daily | - URTI illness load ↔<br>- Self-reported symptoms of lower RTI ↔ (↓ in men) |
| R DB PC 4 months [73] | 1,000 shift workers (18–65 years) | *L. casei* DN 114001 ($10^{10}$ cfu/g) in yoghurt drink, 2×100-g bottle daily | - Cumulated number of CIDs ↓<br>- Proportion of volunteers experiencing at least 1 CID ↓<br>-No. of CIDs in the subgroup of smokers ↓<br>- Leukocyte, neutrophil, and natural killer cell counts and activity ↑ |
| R DB PC 1 month [71] | 47 healthy men in French commando training | *L. casei* DN 114001 in milk, 3× 100 ml/day during training | - Incidence of RTIs ↔<br>- Proportion of rhinopharyngitis ↑<br>- Symptoms of infection ↔ |
| R DB PC 3 months [70] | 141 marathon runners (22–69 years) | *L. rhamnosus* GG in milk 2× bottles daily ($4\times10^{10}$ cfu) or capsules 2× daily ($10^{10}$ cfu) | - No. of RTI episodes (during training or 2 weeks after marathon) ↔<br>- No. of healthy days ↔ |
| R DB PC 4 months [72] | 66 healthy training adults (18–35 years) | *L. salivarius*($2\times10^{10}$ cfu) powder in water daily for 16 weeks | - No. of RTI episodes ↔<br>- Severity and duration of URTI symptoms ↔ |
| R DB PC 3 months [63] | 479 healthy adults (18–67 years) | Combination of *L. gasseri* PA16/8 ($4\times10^7$ cfu/tablet), *B. longum* SP07/3 ($5\times10^6$ cfu/tablet), *B. bifidum* MF20/5 ($5\times10^6$ cfu/tablet), vitamins, minerals, 1 tablet daily | - Duration of RTI episode ↓<br>- Severity of RTI symptoms ↔<br>- Duration of fever ↓ |
| R DB PC 3–5 months [64] | | | - Number of RTI episodes ↔<br>- Duration of RTI episodes ↓<br>- Severity of RTI symptoms ↔ |
| R DB PC 3–5.5 months [62] | 477 healthy adults (23–49 years) | | - Viral-induced incidence and duration of RTI ↔<br>- Days with fever ↓<br>- Duration of RTIs ↔ |
| R DB PC over 150 days [65] | 460 physically active adults (18 to 60 years) | *B. animalis* ssp. *lactis* Bl-04 $2\times10^9$ cfu in sachet per day or *L. acidophilus* NCFM + *B. animalis* ssp. *lactis* Bi-07 $5\times10^9$ cfu in sachet per day | Both bacteria groups:<br>- 0.7+0.9 month delay in the median time to an illness episode<br>- Duration of RTIs ↔<br>Only *B. animalis*:<br>- Risk of URTI episode ↓ |
| R DB PC 1 month [69] | 30 rugby union players | Combination of *L. gasseri* ($2.6\times10^9$ cfu), *B. bifidum* | - Incidence of URTI ↔<br>- Incidence of any symptoms ↓<br>- Severity of symptoms ↔ |

**Table 2** (continued)

| Study design | Subjects | Probiotics used | Main findings: probiotic vs. placebo |
|---|---|---|---|
| | | ($0.2\times10^9$ cfu), and *B. longum* ($0.2\times10^9$ cfu) 1× capsule daily | |
| R DB PC 3 months [66] | 198 healthy college students (18–25 years) | Combination of *L. rhamnosus* GG+ *B. animalis* ssp. *lactis* Bb12, 1× powder/stick ($2\times10^9$ cfu) daily | - Median duration of URTI ↓<br>- Severity of URTI ↓<br>- No. of missed work days ↔<br>- Missed school days ↓ |
| Elderly | | | |
| R C (pilot) 3 weeks [74] | 260 healthy elderly (>60 years) | *L. casei* DN 114001 in fermented yoghurt drink | - Incidence of RTI ↔<br>- Duration of RTI ↓ |
| R DB PC 3 months [75] | 1,072 elderly (≤70 years) | *L. casei* DN 114001 ($\leq10^{10}$ cfu/g) in yoghurt drink, 2×100 g daily | - Cumulative number or severity of CID ↔<br>- Average duration per episode of CID ↓<br>- Cumulative duration of CID ↓<br>- Average duration per episode of URTI ↓<br>- Cumulative duration of URTI ↓ |
| R DB PC 5 months [76] | 154 elderly (74–92 years) | *L. casei* Shirota ($4\times10^{10}$ cfu) in milk 1×80 ml daily | - No. of persons diagnosed with acute URTIs ↔<br>- No. of acute URTI events ↔<br>- Severity of URTIs ↔<br>- No. of acute URTI events/total days of observation ↔<br>- Mean duration of URTI per infection event ↓ |
| R DB PC 176 days [77] | 737 healthy people aged >65 years in nursing homes | *L. casei* Shirota (>6.5 × $10^9$ live bacteria/bottle) in milk, 2× daily | - Duration of RTIs ↔<br>- No. of participants with RTI symptoms ↔<br>- Influenza vaccination immune response ↔ |
| R DB PC 5 months [60] | 265 institutionalized elderly (>65 years) | Combination of *L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* 99, *P. freudenreichii* JS (8-9 × $10^9$ cfu/ capsule of each strain), 2× daily | - No. of RTIs ↔<br>- Duration of RTI episodes ↓<br>- Duration of RTI symptoms ↔ |

Abbreviations for columns:

Study design and duration: R DB PC = randomized double-blind placebo-controlled; CR = cluster-randomized; C-O = cross-over

Probiotics used: L = *Lactobacillus*; B = *Bifidobacterium*; P = *Propionibacterium*; cfu = colony-forming units; GOS = galactooligosaccharides

Main findings: probiotic vs. placebo: RTI = respiratory tract infection; URTI = upper respiratory tract infection, AOM = acute otitis media; CID = common infectious disease; Ig = immunoglobulin; HBoV = human bocavirus; HRV = human rhinovirus

↑ = significant increase; ↓ = significant decrease; ↔ = no significant effect

type I interferon-dependent gene activation, which correlated with the prevention of influenza A virus replication and the production of viral proteins [97]. In influenza infection in mice, orally administered probiotic product containing *Bifidobacterium*, *Lactobacillus*, and *Enterococcus* regulated the TRL7 signaling pathway [34] and *L. pentosus* b240 regulated antiviral gene expression against the infection [31]. In addition, orally ingested probiotics strains of *Lactobacillus* [17, 19, 20, 22, 26, 28, 32] and *Bifidobacterium* [33] have enhanced cytokine production in the lungs or serum against viruses. There is also evidence that intranasally administrated probiotics protect against respiratory virus infection in mice by stimulating innate immune responses directly in the respiratory epithelium [20, 23, 24, 26, 27, 29, 35–37, 98]. Additionally, sublingual administration of *L. rhamnosus* protected against influenza virus infection by enhancing mucosal secretory IgA production, T and NK cell activity, and lung IL-12 levels [25]. Table 1 summarizes the effects of probiotic bacteria on cell-mediated immunity upon respiratory virus challenge in animal models.

*Humoral immunity*

Data from animal studies indicate that strains of lactobacilli and bifidobacteria provide protection against respiratory virus infections also by inducing the synthesis of virus-specific immunoglobulins in the respiratory secretions and in serum [25, 30, 39, 41]. In addition, studies in healthy human subjects suggest that specific probiotics may enhance the immunogenicity of viral vaccines. *L. rhamnosus* GG was effective in inducing protective immune response against the H3N2 strain in influenza virus vaccine [99]. Moreover, *L. fermentum* CECT5716 ingestion in adults resulted in lower influenza-like illness, increased proportion of NK cells in blood, significantly higher TNF-α, and increased anti-influenza-specific IgA and IgM after influenza vaccination [61]. The consumption of *B. animalis* ssp. *lactis* Bb12 or *L. paracasei* ssp. *paracasei L. casei* 431431 also showed significantly greater increase in influenza virus vaccine-specific IgG antibodies in plasma and secretory IgA in saliva [100]. In the elderly, the consumption of fermented yoghurt with *L. casei* DN-114 001

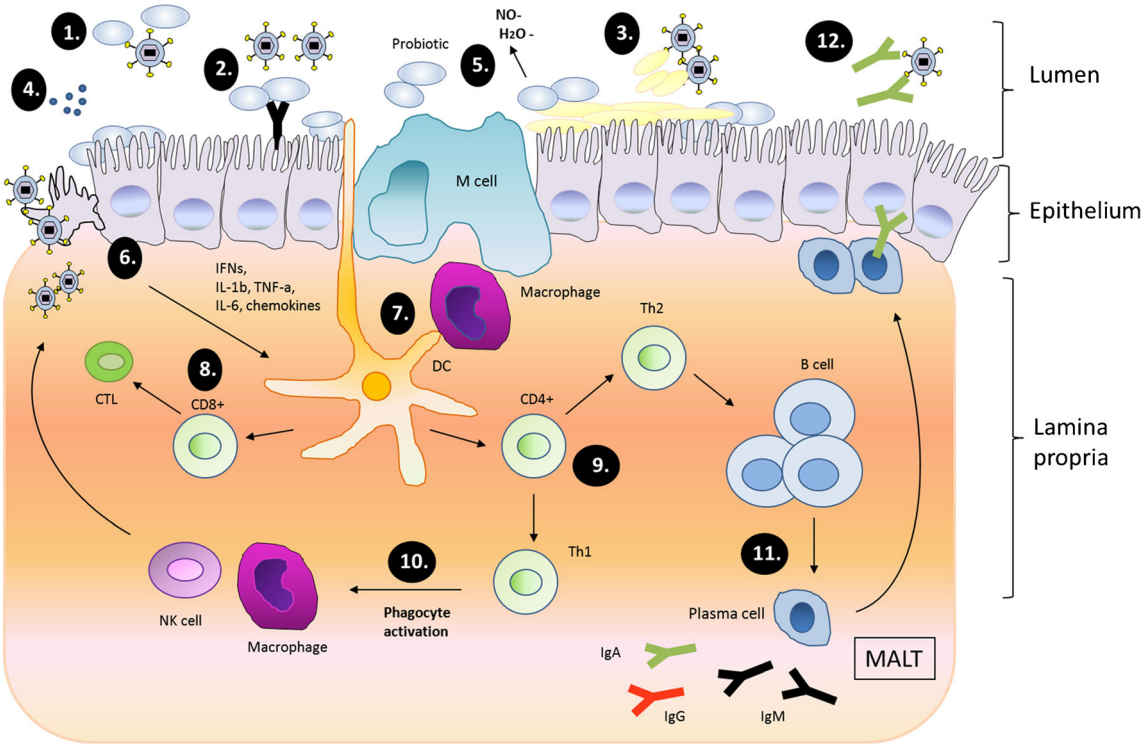


**Fig. 1** Schematic presentation of possible antiviral effect mechanisms of probiotics in respiratory virus infections (adapted from Lehtoranta [80]). *1* Probiotic bacteria may bind directly to the virus and inhibit virus attachment to the host cell receptor. *2* Adhesion of probiotics on the epithelial surface may block viral attachment by steric hindrance, cover receptor sites in a non-specific manner, or by competing for specific carbohydrate receptors. *3* Probiotics may induce mucosal regeneration: intestinal mucins may bind to viruses, and inhibit their adherence to epithelial cells and inhibit virus replication. *4* Probiotics also show direct antimicrobial activity against pathogens by producing antimicrobial substances. *5* Induction of low-grade nitric oxide (NO) production and dehydrogenase production may have antiviral activities. *6* Modulation of immune response through epithelial cells. *7* Modulation and activation of immune responses through macrophages and dendritic cells (DCs). *8* Upon activation, CD8+ T lymphocytes differentiate into cytotoxic T lymphocytes (CTLs), which destroy virus-infected cells. *9* CD4+ T lymphocytes differentiate into Th1 and Th2 cells. *10* T-helper cells type 1 (Th1) activates phagocytes, promoting virus killing. *11* Th2-cells induce proliferation of B-cells, which travel to secondary lymphatic organs in mucosa-associated lymphoid tissue (MALT) and differentiate into immunoglobulin (Ig)-producing plasma cells, which may migrate back to the infection site. *12* Secretory antibodies neutralize the virus

increased significantly influenza-specific antibody titers after influenza vaccination, especially against influenza B virus [101]. These studies suggest that orally ingested lactobacilli and bifidobacteria have an adjuvant-like effect on the humoral responses.

## Safety

Probiotics are frequently part of the normal gastrointestinal microbiota, and, therefore, probiotic therapy is generally considered as safe [102]. However, probiotic therapy has raised potential safety concerns, including systemic infections, toxic or metabolic effects on the gastrointestinal tract, and the transfer of antibiotic resistance in the gastrointestinal microbiota [103]. In rare cases, some studies have reported *Lactobacillus* septicemia in children [104], in immunocompromised subjects [105], and detrimental effects in subjects with hepatitis [106]. However, the European Food Safety Authority (EFSA) has concluded that there are no specific safety concerns regarding *Lactobacillus*, *Bifidobacterium*, or *Propionibacterium* strains, as they have a long history of safe use in food [107]. In addition, for instance in Finland, increased consumption of probiotic products containing *L. rhamnosus* GG has not resulted in a significant increase in *Lactobacillus* bacteremia [108] and *L. rhamnosus* GG consumption is regarded as safe in immunocompromised human immunodeficiency virus (HIV)-infected patients [108]. It should be taken into consideration that the safety of probiotics has not been as systematically investigated as in drugs, and the safety evaluation is partly based on long-term experience.

## Summary and conclusions

The aim of this review was to summarize the current literature investigating the effects of probiotics in respiratory virus infections in cell models, in animal models, and in humans. In addition, possible antiviral mechanisms of probiotics in

respiratory virus infections were discussed. Probiotic therapy may offer an interesting alternative in the alleviation or prevention of viral respiratory tract infections (RTIs), which cause a significant health and economic burden to humans. Based on this review, clinical trials in human subjects show promising data demonstrating that specific probiotics are able to shorten the duration or reduce the risk of respiratory infections. However, only a few clinical studies have actually investigated the effects of probiotics on specific viruses, which are the most common agents causing RTIs. Thus, more clinical research should be targeted to revealing which probiotics or their combinations would be the most effective ones against RTI viruses.

There are also contradictory data on probiotic use in the prevention of RTIs. The variability in the outcomes between clinical trials studying probiotics' role in RTIs may be explained by the use of different probiotic strains, bacterial dose, and matrices. In addition, it should be noted that the effects of probiotics are highly strain-specific and the adequate amount of bacteria transferred into the effector sites in the gut may be crucial. Due to the lack of confirmatory studies and varied data available, more randomized, double-blind, and placebo-controlled clinical trials in different age populations investigating probiotic dose response, comparing probiotic strains, and elucidating the mechanisms of effects are necessary.

As many animal studies show that probiotic administration through the nose is able to reduce viral titers and relieve clinical symptoms, nasal bacteriotherapy for viral RTIs in humans could be worthy approach for consideration in the future. Probiotics' ability to enhance local and systemic innate immunity during virus infection in animal experiments is a likely, yet unverified, effect mechanism behind beneficial effects, and an interesting area of future research. The inclusion of serological and immunological diagnostics, such as the identification of virus-specific immunoglobulins and cytokines, in clinical research would have clear benefits in providing valuable information on the effects of probiotics in respiratory virus infections.

**Conflict of interest** None.

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