

Oral probiotics in coronavirus disease 2019: connecting the gut–lung axis to viral pathogenesis, inflammation, secondary infection and clinical trials

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Abstract

Defined as helpful live bacteria that can provide medical advantages to the host when administered in tolerable amounts, oral probiotics might be worth considering as a possible preventive or therapeutic modality to mitigate coronavirus disease 2019 (COVID-19) symptom severity. This hypothesis stems from an emerging understanding of the gut–lung axis wherein probiotic microbial species in the digestive tract can influence systemic immunity, lung immunity, and possibly viral pathogenesis and secondary infection co-morbidities. We review the principles underlying the gut–lung axis, examples of probiotic-associated antiviral activities, and current clinical trials in COVID-19 based on oral probiotics.

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Gut–lung connectivity in infection and immunity

The spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) uses angiotensin converting enzyme 2 as the receptor for cell entry, which is highly expressed in gut and

lung tissues that produce well-described symptoms in coronavirus disease 2019 (COVID-19) [1,2]. In the gut, proximal and distal enterocytes are targets [3], and both the receptor and these cell types are known to be pathologically connected with intestinal inflammation and diarrhoea [4]. Nausea and diarrhoea are reported to be primary symptoms of COVID-19 even before the development of fever and respiratory symptoms, whereas abdominal pain continues to be reported frequently in patients admitted to intensive care [5]. The most severe cases of COVID-19 often involve pneumonia followed by acute respiratory distress syndrome [6], involving hypoxaemic respiratory distress concurrent with lung neutrophilia, mucus and fluid accumulation in bronchi, and bronchiectasis [7].

Besides the shared trait of direct viral targeting in both gut and lung, the two tissues share a relationship influencing inflammatory and immune responses via the gut–lung axis that can be responsive to probiotics through effects on commensal microbial flora. From birth, both gut and lungs share exposure to microbes through the oral route, a process that over time seeds a quasi-stable and complex gut flora, with growing

evidence for a much lower level of microbial species in the lung that are different between the upper and lower respiratory tracts [8–10]. Focusing on gut flora, microbe interactions and their products influence innate and adaptive immune signals and cells locally, systemically, and specifically in the lungs, where it has been shown that the gut microbiome affects susceptibility to asthma, lung allergic responses and chronic obstructive pulmonary disease [11,12]. Various insults can cause dysbiosis of the gut microbial flora, including antibiotic treatment or pathogenic infection, which can result in increased gut permeability, in turn leading to microbial translocation and systemic dispersal of toxins and inflammatory products to the circulatory system [13]. Therefore, whether due to pre-existing differences in gut flora, or differences induced upon infection, it is conceivable that the microbiome may influence differences in the inflammatory response between patients that could correspond with COVID-19 severity.

Probiotics improving anti-viral responses

Oral probiotics are live bacteria that can improve gut health in homeostasis, and can exhibit antiviral effects [14,15] via the gut–lung axis [16]. Upon delivery, probiotics are understood to adjust the crosstalk between commensal microbes and the mucosal immune framework, and in this way alter the basal and induced inflammatory balance in response to viral infections [14]. Regarding upper respiratory tract disease, some probiotics have shown anti-viral protective and therapeutic effects, lessening the severity and extent of tissue damage from infection and inflammation [17,18]. Diminished plasma titres of Epstein–Barr virus

and antibody titres against cytomegalovirus were observed in upper respiratory tract infections upon treatment with the lactic acid-producing bacterium *Lactobacillus casei*, by a mechanism dependent on Toll-like receptors [19]. Another probiotic bacterium, *Lactococcus lactis* JCM 5805, was demonstrated to have antiviral activity against influenza virus infection and to activate plasmacytoid dendritic cells using Toll-like receptor 9 [20]. Treatment with the probiotic *Bifidobacterium lactis* HN019 was reported to increase mononuclear leucocyte recruitment and elevate phagocytic and lytic activity [21]. The probiotic impact of *Lactobacillus gasseri* has been shown against respiratory syncytial virus infection in mice by a significant reduction of viral titre in the lungs along with decreases in several pulmonary pro-inflammatory cytokines, but increases in interferon types I and II [22]. Lactic acid-producing bacteria (LAB) have been used in probiotic settings via nasal and oral application, where modulation of cytokine profiles was associated with protection against respiratory syncytial virus [23,24], and wider application for respiratory infections has been suggested [25]. A clinical report in neonates demonstrated that probiotic treatment in early life was associated with decreased rates of subsequent respiratory tract infections [26]. Up to this point, many clinical trials have investigated the potential impacts of probiotics on viral infections (Table 1), with some current trials exploring the effectiveness of probiotics in the context of COVID-19 (Table 2).

Anti-inflammatory probiotics and COVID-19

Some probiotics enhance regulatory T-cell activity and reduce pro-inflammatory cytokine production [27–30]. For example,

TABLE 1. Probiotics, targeted viral infections, immunostimulatory mode of action, reported medicinal effects and supporting references

Probiotic bacteria (strain)	Viral infection	Immunostimulatory mode of action	Reported medicinal effects	Ref.
<i>Lactobacillus brevis</i> (KB290)	Influenza virus	Increased IFN- α production and augmentation of influenza-virus-specific immunoglobulin A production	Reduced risk of infection	[61]
<i>Lactobacillus rhamnosus</i> (GG)	Influenza virus	Increased IFN- γ production in serum	Reduced risk of infection	[62]
<i>Lactobacillus bulgaricus</i> (OLL1073R-1) and <i>Streptococcus thermophilus</i>	Rhinovirus	Increased IFN- γ production in serum	No significant difference	[63]
<i>Lactobacillus rhamnosus</i> (GG)	Rhinovirus	Not determined	Reduced incidence of respiratory tract infections (RTIs)	[64]
<i>Lactobacillus rhamnosus</i> (GG)	Rhinovirus	Not determined	Reduced incidence of RTIs	[65]
<i>Lactobacillus rhamnosus</i> (GG)	Rhinovirus, respiratory syncytial virus, parainfluenza virus 1	Not determined	Reduced number of days with symptoms	[66]
<i>Lactobacillus casei</i> (DN-114001)	Rhinopharyngitis, influenza virus	Increased expression of defensins	Decreased duration of common infectious diseases	[67]
<i>Lactobacillus rhamnosus</i> (M21)	Influenza virus	Increased IFN- γ and interleukin-2	Increased host resistance against influenza virus infection	[68]
<i>Bacillus subtilis</i> (OKB105)	Transmissible gastroenteritis virus	Inhibition of virus entry by competing with viral entry receptors	Reduced viral entry <i>in vitro</i>	[69]
<i>Bifidobacterium animalis</i>	Rhinovirus	Inhibition of CXCL8 response upon viral infection	Decreased viral titres in nasal lavage and viral shedding in the nasal secretions	[70]

Abbreviations: COVID-19, coronavirus disease 2019; IFN, interferon; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

TABLE 2. Current clinical trials of probiotics in coronavirus disease 2019 registered at [ClinicalTrials.gov](https://clinicaltrials.gov)

ClinicalTrials.gov identifier	Study title	Probiotic bacteria (strain)	Procedure synopsis
NCT04458519	Efficacy of intranasal probiotic treatment to reduce severity of symptoms in COVID-19 infection	<i>Lactococcus lactis</i> (W136)	Nasal irrigation with probiorinse
NCT04390477	Study to evaluate the effect of a probiotic in COVID-19	Not revealed	Dietary supplementation
NCT04366180	Evaluation of probiotic <i>Lactobacillus coryniformis</i> K8 on COVID-19 prevention in health-care workers	<i>Lactobacillus coryniformis</i> (K8)	Dietary supplementation
NCT04517422	Efficacy of <i>Lactobacillus plantarum</i> and <i>Pediococcus acidilactici</i> in adults with SARS-CoV-2 and COVID-19	<i>Lactobacillus plantarum</i> (CECT7481) <i>Lactobacillus plantarum</i> (CECT7484) <i>Lactobacillus plantarum</i> (CECT7485) <i>Pediococcus acidilactici</i> (CECT7483)	Not revealed

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

the anti-inflammatory activity of *Weissella cibaaria* (JW15) was assessed upon lipopolysaccharide challenge in mouse macrophages, where the probiotic was associated with reduced induction of interleukin-1 β (IL-1 β), IL-6 and tumour necrosis factor- α (TNF- α). When the stimulus was changed to heat-killed JW15, the same study observed diminished production of nitric oxide and prostaglandin E₂ using down-regulation of inducible nitric oxide synthase and cyclooxygenase 2 [31]. Human commensal strains *Lactobacillus rhamnosus* GG and GR-1 appear capable of anti-inflammatory effects using down-regulation of TNF- α production in human monocytes and mouse macrophages [32].

Evidence to date suggests that probiotics with anti-inflammatory or immunomodulatory properties might be predicted to have the most beneficial potential to prevent or alleviate COVID-19 symptoms (Fig. 1). Clinical investigations along with increasing data worldwide suggest that cytokine storm causing hyper-inflammation in the respiratory tract has an apparent causal, positive correlation with COVID-19 disease severity [33]. Blood plasma analysis of 41 individuals with confirmed COVID-19 in Wuhan, China revealed increased levels of various cytokines including IL-1 β , IL-7, IL-8, IL-9, IL-10, fibroblast growth factor, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, interferon- γ , IFN- γ -inducible protein-10, monocyte chemoattractant protein-1, macrophage inflammatory protein-1A and -1B, platelet-derived growth factor, TNF- α , and vascular endothelial growth factor in people with COVID-19 compared with healthy individuals [34]. Given these observations, inhibiting or down-regulating this cytokine response may create a healthier immune activation balance to reduce inflammatory symptoms while maintaining adaptive immune engagement against SARS-CoV-2 (Fig. 1).

Because probiotics have been studied and recommended in the context of respiratory tract infections, the hypothesis emerges that probiotics might play a positive role against COVID-19. For example, gut dysbiosis during influenza virus

infection has been shown to worsen lung pathology and aggravate secondary pneumococcal lung infections [35,36], and gut microbiota dysbiosis has been reported in some COVID-19 patients concurrent with decreases in natural probiotic bacterial species including *Lactobacillus* and *Bifidobacterium* [37]. Clinical transcriptome analyses from COVID-19 patients have also indicated a gastrointestinal disease course and potential systemic crosstalk between gut and lungs during SARS-CoV-2 infection [38]. Sufficient rationale has accumulated such that clinical trials of probiotics against COVID-19 are already underway, so far emphasizing probiotics with expected anti-inflammatory effects in the gut-lung axis (Table 2).

Possible roles of probiotic antimicrobial peptides

Probiotics can produce direct antimicrobial effects via metabolites and antimicrobial peptides, including bacteriocins, which could potentially contribute beneficial effects against SARS-CoV-2 as a membranous envelope virus. A species of genus *Lactococcus* is in a current clinical trial for probiotic activity against COVID-19 (Table 2), and this genus includes LAB whose anti-viral effects may be due in part to secreted metabolites and an enormous number of bacteriocins [39], one class of antimicrobial peptides considered to be guard peptides [40]. Nisin is one of the most widely studied bacteriocins and has been approved for many years as an FDA endorsed food additive. Antimicrobial peptides that can be expressed by LAB appear to contribute to probiotic antiviral effects against influenza A virus and other respiratory viruses [41,42]. From a different genus, the probiotic *Bacillus subtilis* strain was shown to produce an antiviral peptide, P18, which inhibited influenza infection both *in vitro* and *in vivo* [43]. Examples of probiotic lipopeptides include lipopeptide detergent-12, subtilisin, curvacin A, sakacin P and lactococcin Gb,

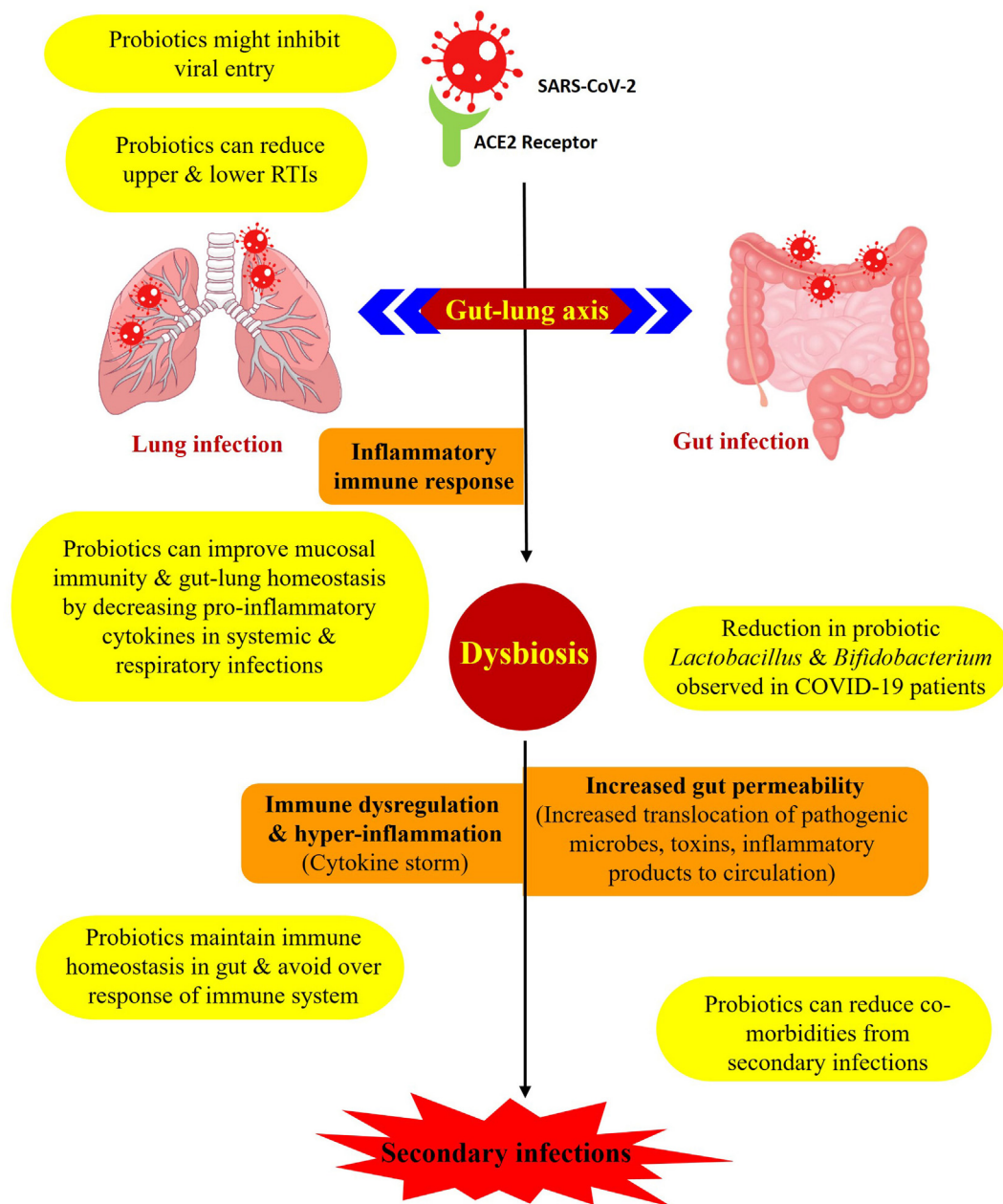


FIG. 1. Step-by-step progressive schematic illustration of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and involvement of the gut–lung axis. Potential targets or steps at which the action of probiotics might mitigate coronavirus disease 2019 (COVID-19) are represented, with specific mechanisms of action possible for probiotics highlighted in yellow.

which are well described extracellularly expressed products that may block the virus–cell fusion process or other steps of viral entry by mechanisms involving their amphiphilic nature [44,45]. If such probiotic products were to have direct access to SARS-CoV-2 virions in the gut, or perhaps in the lungs through disease-induced dysbiosis, gut permeability, and dissemination, it can be hypothesized that direct antiviral effects may be possible.

Possible probiotic protection against infections secondary to SARS-CoV-2

Because probiotics can mitigate problems of dysbiosis, inflammation and immune function, and can include direct antimicrobial activities, there may be the potential for a positive contribution against secondary infection co-morbidities in COVID-19. New

evidence is emerging that infections secondary to SARS-CoV-2 might contribute to COVID-19 pathology or severity [46–49]. Although an early study suggested relatively little concern across the COVID-19 patient population [50], recent reports are finding increased secondary infections in hospitalized individuals with severe disease, observations that may have some association with immunosuppressive drugs in current treatment regimens [51–53]. Outside the COVID-19 context, application of the probiotic strains *Lactobacillus rhamnosus* GG, *Bacillus subtilis* and *Enterococcus faecalis* during clinical trials, showed a significant improvement in patients with ventilator-associated pneumonia, including pathogens of various types, compared with placebo treatment [54,55]. In general, probiotic strains themselves, including LAB strains, are well known to be non-pathogenic and non-immunogenic, and therefore are considered safe and not a source of potential secondary infections, themselves [56]. Along with protective effects reported against influenza A virus, LAB have been reported to promote heterotypic immunity to secondary infections [57,58]. Furthermore, probiotics have been reported to provide some protection against biofilm-forming pathogens in the respiratory tract [59,60]. As secondary infections may rise to more prominence in COVID-19, perhaps commensurate with increasing anti-inflammatory regimens, treatment options that include probiotics may present an even more attractive modality that merits further investigation and attention in clinical trials.

Conflict of interest

There is no conflict of interest.

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