Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Clinical Nutrition ESPEN 51 (2022) 17–27

Contents lists available at ScienceDirect

Clinical Nutrition ESPEN

journal homepage: http://www.clinicalnutritionespen.com

Narrative Review

Probiotics: A gut response to the COVID-19 pandemic but what does the evidence show?

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ARTICLE INFO

Article history:
Received 6 June 2022
Accepted 17 August 2022

Keywords:
SARS-CoV-2
COVID-19
Gut microbiome
Lung microbiome
Probiotics
Respiratory tract infections
Supplements

SUMMARY

Since the global outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), research has focused on understanding the etiology of coronavirus disease 2019 (COVID-19). Identifying and developing prophylactic and therapeutics strategies to manage the pandemic is still of critical importance. Among potential targets, the role of the gut and lung microbiomes in COVID-19 has been questioned. Consequently, probiotics were touted as potential prophylactics and therapeutics for COVID-19. In this review we highlight the role of the gut and lung microbiome in COVID-19 and potential mechanisms of action of probiotics. We also discuss the progress of ongoing clinical trials for COVID-19 that aim to modulate the microbiome using probiotics in an effort to develop prophylactic and therapeutic strategies. To date, despite the large interest in this area of research, there is promising but limited evidence to suggest that probiotics are an effective prophylactic or treatment strategy for COVID-19. However, the role of the microbiome in pathogenesis and as a potential target for therapeutics of COVID-19 cannot be discounted.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) first identified in the city of Wuhan, China, in November 2019 is responsible for the coronavirus disease 2019 (COVID-19) that has resulted in almost 6.5 million deaths and over 600 million confirmed cases across the globe [1]. While vaccines have lowered the risk of death, hospitalization, and to some extent infection, the hunt for additional prophylactics and therapeutics is still ongoing. Among these, probiotics were deemed of interest due to their potential immunomodulatory and anti-inflammatory effects, as well as their putative protective effects against viral diseases [2,3]. Probiotics are generally defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit to the host" [4], although it should be noted that multiple definitions exist that are interpreted in various ways [5]. These discrepancies have contributed to differing regulation regarding probiotics in Europe and North America [6]. Despite these regulatory issues, interest surrounding probiotics and their potential benefits have not waned and probiotics are among the main products intentionally consumed by humans to beneficially affect the gut microbiome to improve health [7,8]. Research has continued to grow over the last decade with a fivefold increase in the number of publications published between 2010 and 2021 in PubMed alone relating to "probiotics". Indeed, over 300 manuscripts have been published on COVID-19 and probiotics alone since the emergence of the pandemic [9]. Among the areas of research being investigated, probiotics administration and their potential effects on the gut microbiome and subsequent health and protection against respiratory infections has been explored.

The human microbiome is the sum of all microbiotas present in our tissues and fluids. The microbiome is composed of diverse bacteria (bacteriome), fungi (mycobiome), protozoa, and even viruses (virome). The human gut microbiome alone accounts for a collection of between 100 million to 100 trillion commensal

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https://doi.org/10.1016/j.clnesp.2022.08.023

microorganisms [10], which feed on fiber, a crucial dietary component that has the potential to influence host immune health [11–13]. Exploration of the human microbiome has led to important discoveries in brain and neurological diseases, mood, depression [14,15] and various noncommunicable diseases. The microbiome provides a unique highway of communication between the gut and the lungs via the circulatory system [16–19], whereby the gut-lung axis has been linked to asthma [20] and various respiratory diseases [21,22]. Indeed, COVID-19 has also been linked to the gut-lung axis [23].

Coronaviruses that affect humans are generally associated with respiratory infections, and occasionally the gastrointestinal tract [24]. Angiotensin-converting enzyme 2 (ACE2) is the cellular gateway of the SARS-CoV-2 spike protein [24]. A recent study by Xu et al. conducted retrospective analysis of bulk tissue RNA of the digestive tract organs and lungs from a public database and observed that the digestive tract had higher ACE2 expression levels compared to the lung, and the expression of ACE2 in the lung increased with age [25]. In the gut, SARS-CoV-2 targets both the proximal and distal enterocytes [26] and there is evidence that SARS-CoV-2 can be prevalent in the mucous membranes of the oesophagus, stomach, and tissues of the small and large intestines [27,28]. Therefore, it is not surprising that SARS-CoV-2 infection induces gastrointestinal symptoms in adults and children [29,30]. Factors that affect gut health may also have negative consequences for a patient's nutritional status, which is now under investigation in relation to COVID-19 [31]. Indeed, many host factors can affect the microbiome including one's health, diet, exercise, and lifestyle choices [32,33].

SARS-CoV-2 enters the host via the respiratory system, which may implicate the lung microbiome in the etiology of COVID-19 [34]. Indeed, this has led researchers to question the role of the gut-lung axis and how its modulation may affect COVID-19 outcomes [35]. The aim of this review is to provide an update and cast a critical eye on the current state of research regarding the gut-lung axis in relation to the microbiome, probiotics, nutritional status, and their interactions in relation to COVID-19. Additionally, we discuss the current state of clinical trials investigating the modulation of the gut and lung microbiome via probiotics administration in humans. We highlight the need for a greater understanding of how probiotics affect the gut microbiome and immune function in response to viral infections.

2. The gut-lung microbiome axis and COVID-19

Attenuating the gut and/or lung microbiome is a strategy that has been previously explored for viral infections. Research has shown that lactic acid bacteria (LAB) can be used to treat oral [36], vaginal [37,38], and gastrointestinal infections [39] in animal and human studies. Probiotics have also been shown to be effective against respiratory infections. Administration of *Lactobacillus gasseri* to mice reduced respiratory syncytial virus (RSV) viral titers, increased interferon I and II, and decreased cytokines in the lung [40]. The latter findings are in agreement with other studies that suggest nasal and oral administration of LAB may exert anti-inflammatory effects against RSV in mice [41,42].

Considering nasal and oral routes of probiotic administration may be beneficial against viral infection *in vivo*, some speculate a mechanistic role for the gut-lung axis in amelioration of respiratory viral infections. As alluded to, microbial metabolites can affect host health on a system level by producing vital metabolic byproducts from dietary components [43,44]. One of the most studied of these microbiome-derived metabolites for their potential human health benefits are short chain fatty acids (SCFA), such as acetic acid, butyric acid, and propionic acid. SCFA act as the main sources of energy for intestinal epithelial cells and thereby help to strengthen the immune system through Toll-like receptor (TLR) expression, differentiation of T cells including $CD4^+$ T cells, and antibody expression [16]. Butyrate has been touted as a potential therapy to prevent the development of ARDS and the cytokine storm via immune response modulation and potentially proresolving factors [45]. In accordance, depletion of butyrate-producing microbiota may also be linked to a potential increased risk of COVID-19 in hypertension patients via down regulating pathways integral to SARS-CoV-2 infection, upregulating antiviral and TLR related genes, and modulating *Ace2* and *Tmprss2* gene expression as demonstrated in a gut epithelium organoid model [46]. Although these findings have not been validated in human studies, there is evidence that the gut microbiome of COVID-19 patients have impaired capacity to produce SCFA, which persists into recovery and correlates with host immune responses [47]. Contrariwise, some *in vitro* evidence casts doubt on the benefits of SCFA in the prevention of SARS-CoV-2 infection in gut tissue [48]. Though promising, alteration of the gut microbiome to favor synthesis of SCFA requires further research.

Because alterations to the gut microbiome have been previously implicated in the outcomes of patients with viral infections [49–51], the scientific community speculates that there could be a strong link associated with the risk factors for COVID-19 driven by poor diet consisting of high saturated fat, high sugar, refined carbohydrates and lesser amounts of fibers, unsaturated fat, and polyphenols, leading to a dysbiosis in gut microbial community [52–54]. Dysbiosis is a mechanism whereby an imbalance of the microbiome occurs because of disruption of the microbiota homeostasis. Many factors including host genetics, diet, supplementation, stress, infection, and/or medical interventions (such as antibiotics) influence the microbial community at compositional and functional levels [55]. The gut microbiome affects pulmonary health through a crosstalk via the gut-lung axis [56–59]. Consequently, the role of the gut and lung microbiomes in SARS-CoV-2 infection susceptibility and COVID-19 severity and their modulation by probiotics are under investigation.

A systematic review of COVID-19 and the microbiome determined that the human fecal microbiomes were altered by COVID-19, which was characterized by a greater prevalence of opportunistic pathogens [34]. The presence of such pathogens is a concern due to the potential for further dysbiosis or nosocomial infections. These findings are important, because it is known that the commensal microbiota can modulate virus-specific adaptive immune responses. Several studies have shown that germ free mice or mice orally treated with antibiotics have impaired responses to systemic and respiratory infections [60–63]. Numerous mechanisms have been proposed for how the microbiome may prime the lungs to control respiratory infections including production of type I interferons (IFNs) [64–66] or modulation of adaptive immune responses via activation of $CD4^+$ and $CD8^+$ T cells [67].

3. Targeting the gut-lung microbiome axis and its clinical significance

The oropharynx and gastrointestinal tract are physiologically interlinked and share similar microbial compositions, albeit the lung microbiota has a significantly lower biomass than the gut microbiota whose diversity depends on the conditions in the entire respiratory tract [19,68]. There has been no shortage of studies demonstrating a link between lung health and the lung and gut microbiomes [19,68–71]. As discussed, the microbiota and their metabolites play an important role in the development of the immune system both locally and systemically. However, alterations to the gut microbiome by diet, medical interventions or disease has

been shown to alter immune responses to the respiratory system [72]. It is irrefutable that an individual's diet and lifestyle contributes to a person's risk of non-communicable diseases [73–75]. The same is true of how nutritional status contributes to one's susceptibility to acute illnesses such as infectious diseases [76,77] as nutritional status is interlinked with immune function [78]. Indeed, deficiency of a single nutrient may weaken immune function [79]. There is already emerging evidence that a patient's nutritional status is an important factor in COVID-19 [31,80–82]. A bidirectional relationship exists, whereby the gut microbiome affects nutritional status and vice versa [83,84], which likely affects COVID-19 risk [85]. These links are mediated by metabolites generated by gut microbiota in response to factors such as nutrition or drug intake. These metabolites can act locally and systemically to affect multiple systems, including the respiratory system [72]. Therefore, the maintenance of the gut microbiome prior to, during, and post COVID-19 through appropriate dietary and therapeutic measures is critical for positive patient outcomes and recovery [51,86]. As a consequence, several guidelines have been devised to promote COVID-19 patient recovery [87].

In COVID-19 patients, several studies show that the lung [23,88,89] and gut microbiota [30,50] composition is altered versus non-COVID-19 patients, which in the case of the gut at least seems to stratify according to disease severity [51]. Depletion of the gut microbiome is associated with immune modulation and can remain low for up to 30 days [51]. In some cases, gut dysbiosis can persist six-months post infection [90]. This is important as gut dysbiosis has been linked to dysbiosis in the lung microbiome, both in animal studies and in humans and may potentially have long-term gut and lung health implications [21,91–93].

A systematic review highlighted that the majority of existing studies have determined that both the upper and lower respiratory tract microbiome is altered by COVID-19. These alterations are characterized by a greater prevalence of opportunistic pathogens [22]. Therefore, it is no surprise that studies have shown that respiratory tract co-infections are common among COVID-19 patients [94–96]. In the upper respiratory tract, it has been reported that pathogenic *Candida albicans*, *human alpha-herpesvirus,* RSV, and influenza were in abundance [94]. Other studies have also confirmed the presence of RSV and influenza A/B, along with rhinoviruses and enteroviruses in abundance [95]. These findings also highlight that fungal dysbiosis is prevalent in COVID-19 patients. Indeed, this is important considering the emerging role of the mycobiome and fungal dysbiosis as a contributor to immune dysregulation in infectious diseases [97] immune system development [98].

Host transcriptomics show that severe COVID-19 patients that develop secondary bacterial infections such as ventilator-associated pneumonia (VAP) may have respiratory microbiome disturbances and impaired antibacterial immune defenses before infection onset. Which begs the question whether point-of-care prognostic tools may be developed to predict VAP prior to occurrence [99]. Indeed, one study has shown sampling on arrival to either the Emergency Department or COVID-19 unit of one hospital was predictive of the need for respiratory support. An oropharyngeal microbiome denoted by a reduced abundance of *Prevotella salivae* and metabolic pathways associated with mycolic acid and liposaccharide biosynthesis appeared to be of prognostic value [100]. Severe COVID-19 patients also experience acute respiratory distress syndrome (ARDS) [101], which induces the production of pro-inflammatory cytokines such as TNF-a, IL-10, IL-7, IL-2, MCP1 and others that can cause extensive organ damage [102]. Studies prior to the pandemic have shown that certain gut microbiome colonization patterns are associated with these same inflammatory markers [103] indicating a potential role of the gut microbiome in ARDS.

Gut dysbiosis appears to be a common clinical feature of COVID-19 patients [104] and has been correlated with IL-18 detection in fecal material, which is unique to COVID-19 patients [105]. COVID-19 patients also have 3-fold increase in fungal diversity and pathogens from the genus *Aspergillus* in the gut. More severe COVID-19 symptoms were associated with patients who had high abundance of more than two *Aspergillus* pathogens. Despite nasopharyngeal clearance of SARS-CoV-2 these fungi were found to be present in the feces, hinting to persistent colonization of fungal pathogens and a potential threat to the host in the longer term [106]. The same study found that there was an increase in the opportunistic pathogens over beneficial commensals, which is in accordance with previous studies [34].

It is also important to recognize that pre-existing conditions play a role in COVID-19 outcomes [24]. Inflammatory diseases such as inflammatory bowel disease (IBD), colon cancer, and even diseases such as rheumatoid arthritis and liver cirrhosis have been associated with microbial translocation along the oral–gut axis [107]. This implies that microbes from either the gut or lung may have the capacity to translocate to each other or different organs that may affect COVID-19 outcomes and may have long-term health implications.

4. Investigation of probiotics for COVID-19 mitigation in clinical trials

The popularity of probiotics has increased over the last two decades, and they have been investigated during the pandemic due to the putative health benefits [108,109]. Several authors have speculated that prebiotics/probiotics may prevent and/or treat COVID-19 [16,86,110–115] via modulation of the gut microbiota and the gut-lung axis. Previous studies have shown a beneficial effect of probiotic intake against some respiratory infections [116–118], potentially through immunomodulatory effects [2,3] and anti-inflammatory effects [119,120]. Probiotics may even enhance the antibody response generated by immunization [121–124]. To date, there have been few clinical trials published (Table 1) investigating the potential therapeutic role of probiotics in relation to COVID-19. However, there are over 25 registered clinical trials that aim to investigate the biological and therapeutic roles of probiotic administration on COVID-19 management (Table 2).

Various individual species and formulations of probiotics are thought to affect immune function [120,125], which may also affect COVID-19 outcomes, which is reflected in the combinations of probiotics administered to patients in both Table 1 and in Table 2. In general, the majority of probiotics utilized consist of lactic acid bacteria (LAB) including *Lactobacillus* and *Bifidobacterium*. LAB have long been touted as potential immune modulators and as such are the obvious choice to investigate.

Of the limited published trials to date (Table 1), the main findings as a result of probiotic administration appears to be a more rapid improvement of symptoms, reduced fatigue, and potentially resolution of gastrointestinal issues. Gutiérrez-Castrellón, Gandara-Martí [126] observed more rapid recovery ($p < 0.0001$) and shortening of symptom duration following administration of a probiotic (n = 147; *Lactiplantibacillus plantarum* KABP022, KABP023, and KAPB033, and *Pediococcus acidilactici* KABP021) versus placebo (n = 146) in a 30-day intervention in a blinded, randomized, placebo-controlled trial. Zhang, Han (129) retrospectively analyzed the efficacy of a probiotic (*Bifidobacterium, Lactobacillus,* and *Enterococcus* - detailed breakdown unavailable) administered to 196 COVID-19 patients receiving standard of care (SOC) versus a matched cohort of patients receiving SOC alone (n = 179). They noted an improved time to clinical improvement ($p < 0.022$),

Table 1
Published studies investigating the administration of probiotics and their effects on SARS-CoV-2 transmission, infection, COVID-19, and related post sequelae.

Reference	Country	Aim	Study Design	Outcomes	Strains
Gutiérrez-Castrellón, Gandara-Martí [126] (Preprint)	Mexico	To assess the efficacy and safety of probiotics in symptomatic adult COVID-19 outpatients	Single center, quadruple blinded, randomized placebo-controlled trial Treatment: Probiotic formulation 30-day intervention Placebo n = 146 Treatment n = 147	Disease recovery (remission) was more rapid in the probiotics group ($p < 0.0001$) Shortening of symptom duration No hospitalizations/deaths occurred during the intervention No adverse events reported	*Lactiplantibacillus plantarum*KABP022, KABP023, and KAPB033, *Pediococcus acidilactici*(KABP021)
Rathi, Jadhav [127]	India	To assess the efficacy and safety of probiotics and a systemic enzyme complex in adults with fatigue induced by post sequelae of previously confirmed COVID-19	Multicenter, double blinded, randomized placebo-controlled trial Treatment: Probiotic formulation and an enzyme supplement (containing serrapeptase, bromelain, amylase, lysozyme, peptidase, glucoamylase, catalase, papain, lactoferrin) 14-day intervention Placebo n = 100 Treatment n = 100	Fatigue was resolved in 91% of participants versus placebo 15% by day 14 ($p < 0.001$) Improved mental and physical fatigue scores in the treatment group versus the control arm No adverse events reported	*Bacillus coagulans* LBSC (DSM 17654), *Bacillus subtilis* PLSSC (ATCC SD 7280), and *Bacillus clausii* 088 A E (MCC 0538).
Dehghan, Mente [128]	Russia	To assess the efficacy and safety of probiotics for the treatment of hospitalized COVID-19 patients	Single center, open-label, randomized placebo-controlled trial Treatment: Probiotic formulation Placebo n = 101 Treatment n = 99	In patients who had diarrhea, it was resolved earlier in the treatment group versus the control group ($p < 0.049$) Hospital-acquired diarrhea occurred less frequently in the treatment group than the control group in patients who received a single antibiotic ($p < 0.023$), but not in those who had more than one antibiotic administered No significant changes reported for mortality, disease duration, mechanical ventilation, oxygen support, ICU admission, inflammatory markers, or markers of liver injury	*Lacticaseibacillus rhamnosus*PDV 1705, *Bifidobacterium bifidum* PDV 0903, *Bifidobacterium longum* subsp. *infantis* PDV 1911, and *Bifidobacterium longum*subsp. *longum* PDV 2301
Zhang, Han [129]	China	To retrospectively assess the efficacy of probiotic treatment in COVID-19 patients	Open label propensity-score matched retrospective cohort Jan −Apr 2020 Treatment: Probiotic formulation in conjunction with SOC SOC n = 179 Treatment and SOC n = 196	Reduced time to clinical improvement in treatment group ($p = 0.022$) Reduced hospital stay length in treatment group ($p = 0.009$) Shorter time to viral RNA clearance in throat swabs of treatment group ($p < 0.001$) No significant changes in blood counts, D-dimer, inflammatory biomarkers, liver enzymes, CD4+ or CD8+ T cell counts.	*Bifidobacterium, Lactobacillus,* and *Enterococcus.* Detailed breakdown unavailable.
Li, Cheng [130]	China	To retrospectively assess the efficacy of probiotic treatment of severe COVID-19 patients	Single-center retrospective cohort study Feb 2020 Treatment: Probiotic formulation in conjunction with SOC Mean treatment duration 12.9 days SOC n = 188 Treatment and SOC n = 123	Moderately reduced number of in-inpatient days in the treatment group ($p < 0.001$) Shorter time to viral RNA clearance in throat swabs of treatment group ($p < 0.001$) Probiotic groups had longer duration of antibiotic treatment ($p = 0.001$) and No significant difference of mortality and non-significant changes in CD4+ or CD8+ T cells and inflammatory markers reported	Oral *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Dung enterococcus,* and *Bacillus cereus* or Oral *Bifidobacterium longum, Lactobacillus bulgaricus,* and *Streptococcus thermophiles* or Oral *Enterococcus faecium* and*Bacillus subtilis*

Abbreviations: ICU = Intensive care unit; SOC = Standard of care.

Table 2
A summary of some of the randomized controlled trials registered in ClinicalTrials.gov underway or recruiting that are investigating the application of probiotics for the prevention and/or treatment of COVID-19.

Clinical Trial Identifier	Date Posted	Study Title	Location	Study Design	Intervention	Outcomes Measured	Status
NCT04366180	28-Apr-20	Evaluation of the probiotic *Lactobacillus coryniformis K8* on COVID-19 prevention in healthcare workers	Granada, Spain	Randomized parallel assignment	*Lactobacillus Coryniformis* K8	Incidence of infection by PCR or antigen test	Completed
NCT04390477	15-May-20	Study to evaluate the effect of a probiotic in COVID-19	Alicante, Spain	Randomized parallel assignment	Not disclosed	Percentage of patients admitted to ICU	Completed
NCT04399252	22-May-20	Effect of *Lactobacillus* on the microbiome of household contacts exposed to COVID-19	North Carolina, United States	Randomized parallel assignment	*Lactobacillus rhamnosus* GG	Incidence of COVID symptoms during the study period	Completed
NCT04517422	18-Aug-20	Efficacy of *L. plantarum* and *P. acidilactici* in adults with SARS-CoV-2 and COVID-19	Mexico City, Mexico	Randomized parallel assignment	*Lactobacillus plantarum* 30,292,7484 & 7485, & *Pediococcusacidilactici*	Severity progression, ICU duration & mortality ratio	Completed
NCT04621071	9-Nov-20	Efficacy of probiotics in reducing duration and symptoms of COVID-19 (PROVID-19)	Quebec, Canada	Randomized parallel assignment	Not disclosed	Duration before symptoms disappear	Completed
NCT04666116	14-Dec-20	Changes in viral load in COVID-19 after probiotics	Valencia, Spain	Randomized parallel assignment	*Bifidobacterium longum*, *Bifidobacterium animalis subsp. lactis* and *Lactobacillus rhamnosus*	Impact on viral load in COVID-19 infected patients	Recruiting
NCT04730284	29-Jan-21	Evaluation of a synbiotic formula in patient with COVID-19	Hong Kong	Single group assignment	Not disclosed	Changes in the gut microbiome measured at week 5	Recruiting
NCT04734886	2-Feb-21	The effect of probiotic supplementation on SARS-CoV-2 antibody response after COVID-19	Sweden	Randomized parallel assignment	*Lactobacillus reuteri*	Specific antibodies in serum associated with SARS-CoV-2	Completed
NCT04756466	16-Feb-21	Effect of the consumption of a *Lactobacillus* strain on the incidence of COVID-19 in the elderly	La Coruña, Spain	Randomized parallel assignment	*Lactobacillus*	Incidence of infection by PCR or antigen test	Active, not recruiting
NCT04854941	22-Apr-21	Efficacy of probiotics in the treatment of hospitalized patients with novel coronavirus infection	Moscow, Russia	Randomized parallel assignment	*Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Bifidobacterium longum Subsp. infantis &Bifidobacterium longum*	Mortality of patients during hospitalization	Completed
NCT04877704	7-May-21	Symprove (probiotic) as an add-on to COVID-19 management	London, United Kingdom	Randomized single group assignment	Multi -Strain Probiotic (Symprove)	Length of hospital stay	Not yet recruiting
NCT04884776	13-May-21	Modulation of gut microbiota to enhance health and immunity	Hong Kong	Randomized parallel assignment	Probiotics blend (3 Bifidobacteria)	Percentage of patients achieving restoration of gut dysbiosis	Recruiting
NCT04907877	1-Jun-21	Bifido- and Lactobacilli in symptomatic adult COVID-19 outpatients (ProCOVID)	Warsaw, Poland	Randomized parallel assignment	Mixture of bifido- and lactobacteria	Global symptom score	Not yet recruiting
NCT04937556	24-Jun-21	Evaluation of a probiotic supplementation in the immune response of participants with COVID-19 (coronavirus disease). (PROVID)	Madrid, Spain	Randomized parallel assignment	*Lactobacillus salivarius*	Concentration of specific antibodies related to SARS-CoV-2 virus	Recruiting
NCT04979065	27-Jul-21	Nutrition, immunity, and COVID-19 in obese people (NICO)	Jakarta, Indonesia	Phase 1: Cross-sectional Phase 2: Double-blind, randomized, placebo-controlled trial with two arms of intervention	Probiotics (Strain not disclosed) +Vitamin D	Zonulin & Vit D level, gut microbiome, inflammation markers, body mass index (BMI)	Not yet recruiting

(*continued on next page*)

Table 2 (*continued*)

Clinical Trial Identifier	Date Posted	Study Title	Location	Study Design	Intervention	Outcomes Measured	Status
NCT05043376	14-Sep-21	Study to investigate the treatment benefits of probiotic *Streptococcus salivarius* K12 for mild-to-moderate COVID-19	Lahore, Pakistan	Randomized parallel assignment	*Streptococcus Salivarius K12*	Patients with clinical improvements	Recruiting
NCT05080244	15-Oct-21	WHO COVID-19 - evaluation of the efficacy of probiotics to reduce the occurrence of long COVID (PROVID-LD)	Quebec, Canada	Randomized parallel assignment	Not disclosed	COVID-19 symptoms, anxiety, functioning difficulties 90 days after the diagnosis	Recruiting

reduced hospital stay length ($p = 0.009$), and shorter time to RNA clearance measured via throat swabs ($p < 0.001$) in the treatment group. In accordance with these findings, Li, Cheng (130) reported a moderate reduction of in-patients days ($p < 0.001$) and shorter time to viral RNA clearance in throat swabs ($p < 0.001$) in COVID-19 patients receiving various probiotic formulations (n = 123) versus SOC (n = 188) in another retrospective study. In a preprint, Rathi, Jadhav [127] also reported improvements in patients receiving probiotics and an enzyme supplement. Indeed, 91/100 COVID-19 patients that received a probiotic (*Bacillus coagulans*, *Bacillus subtilis*, and *Bacillus clausii*) reported less physical and mental fatigue versus 15/100 placebo patients by day 14 of treatment ($p < 0.001$). However, it is not known whether these effects are a result of the probiotic or enzyme treatment or a combination of both. These studies together indicate that there may be a modest benefit of probiotic supplementation on time to patient recovery. However, blinded, randomized, placebo-controlled clinical trials are required to confirm these findings. In another trial that have been published (Table 1) [131], gastrointestinal symptoms appeared to improve ($p < 0.049$) and less frequent hospital-acquired diarrhea occurred ($p < 0.023$) in COVID-19 patients that received a probiotic (n = 99) versus those that received a placebo (n = 101) in a randomized, placebo-controlled trial. However, the aforementioned study by Li, Cheng (130) found that those who had received a probiotic (albeit a different formulation) required a longer duration of antibiotic treatment to recover from diarrhea.

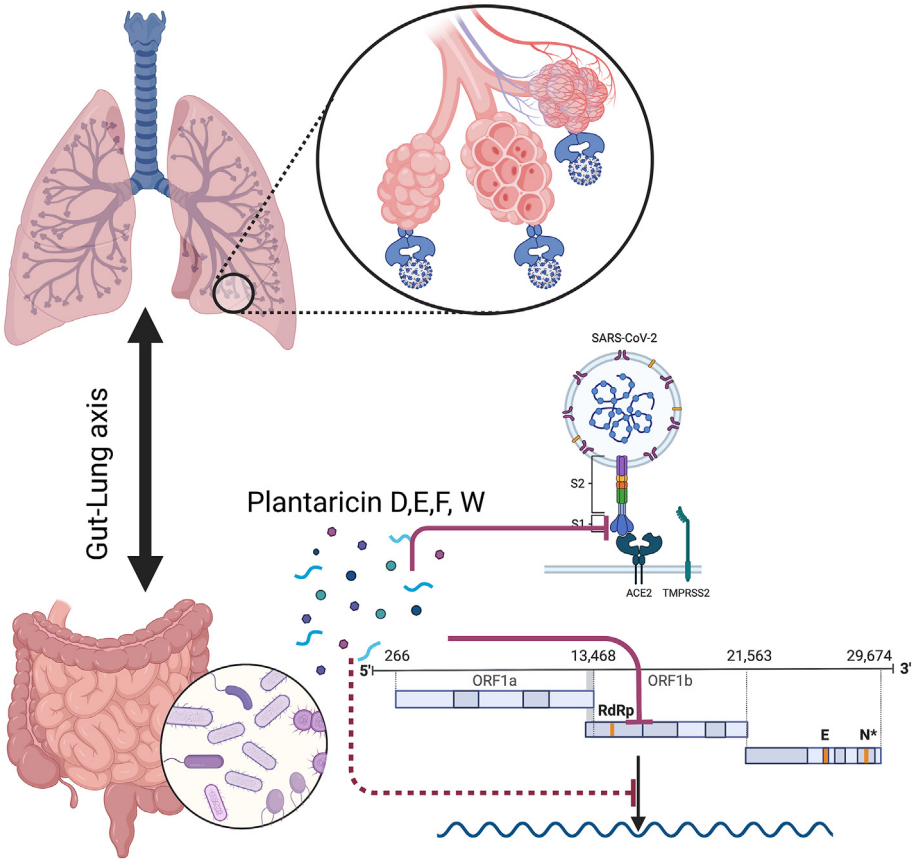

Fig. 1. Bacteriocins such as plantaricin secreted by probiotic bacteria such as *L. plantarum* are predicted to be involved in inhibiting SARS-CoV-2 entry and replication. Molecular docking studies have projected that the plantaricin construct may obstruct the entry of the virus by targeting S protein or bind to the RNA-dependent RNA polymerases (RdRP) thereby impeding transcription of the genome.

5. Proposed mechanisms of action for probiotics in COVID-19

The studies discussed in this review indicate that there may be some benefits to probiotic administration but considering the current pandemic and the progress made on existing therapeutics [132] it is not known whether probiotics may impact patient outcomes. Despite the many potential mechanisms already discussed in this review, a clearer understanding of the implicated mechanisms of action of probiotics to alleviate COVID-19 symptoms are not yet studied in detail. However, a few published studies speculate and/or provide insight into the role of bioactive compounds produced by probiotic bacteria in COVID-19 management as depicted in Fig. 1. Apart from the mechanisms of anti-inflammatory action, immune modulation, and modulation of the microbiome previously discussed, probiotics may also inhibit the binding of SARS-CoV-2 to its receptor via bacteriocins. Bacteriocins are antimicrobial peptides or proteins synthesized by Gram-positive and Gram-negative bacteria commonly found in fermented foods and currently under investigation as potential food additives and antimicrobial therapeutic agents [133,134]. An *in-silico* study by Anwar et al. [135] used protein modeling to propose a mechanism of action for metabolic products of *Lactobacillus plantarum* in COVID-19 management. Using a molecular dynamics simulation model, the study suggested that Plantaricin (Pln) W, D, and JLA-9- compounds inhibit the entry of SARS-CoV-2 by blocking ACE2 receptors and virus transcription by targeting the S protein and blocking RNA polymerase (RdRp). Additionally, *in vitro* study by Rather, Choi (136) showed reduction in SARS CoV-2 viral titers post treatment with cell free supernatant from *L. plantarum*- Probio-88. Furthermore, molecular docking by Rather et al. predicted that PlnE and PlnF from *L. plantarum* could inhibit the SARS CoV-2 replication by binding at either ssRNA or ATP binding sites of helicase. Molecular docking studies have also indicated the potential of pediocin PA-1, salivaricin P, and salivaricin B to bind with the SARS-CoV-2 spike protein receptor-binding domain, thereby inhibiting viral entry into host cells [137]. Considering endogenous microbial peptides such as cathelicidin are under investigation for their effects on SARS-CoV-2 infection [82], further studies of administration of antimicrobial peptides or probiotics are warranted.

6. Future perspectives on probiotics and COVID-19

Moving forward, there are several factors trialists should consider in relation to the gut microbiome, probiotics, COVID-19. For instance, it is important to consider the intervention duration while studying the effects of dietary strategies, treatments or interventions on the gut microbiota because the gut microbiota responds rapidly to short-term dietary alterations, however, long-term dietary interventions may only maintain microbiota changes if the intervention becomes habitual, due to the fact that ceasing the intervention can cause the microbiota composition to revert back to pre-intervention composition [138]. This concept explains the “responder/non-responder” phenomenon, which is based on the changes of an individual's gut microbiota in response to prebiotic interventions [139,140].

The utility of prebiotics and synbiotics has also largely been neglected. Prebiotics are fibers including various oligosaccharides provided by foods or supplements that promote the growth or activity of healthy microorganisms in the gut. On the other hand, synbiotics are a mixture of both probiotics and prebiotics that can be ingested via foods or supplements with intended health benefits [141]. To date, the beneficial effects of prebiotic, probiotic, and synbiotics on the host's antiviral response have mostly focused on the intestinal tract with limited evidence on their ability to modulate responses in distant respiratory mucosal sites.

Another notable issue is that the ongoing clinical trials have used bacterial formulations capable of eliciting specific anti-inflammatory and antiviral activities [142,143]. However, studies have shown that not all probiotics are created equal, nor do they elicit the same immune benefits. For example, *L. plantarum* CRL1506 stimulates antiviral intestinal immunity, however, is incapable of modulating respiratory antiviral immune response [144,145]. Therefore, results between trials are not always directly transferable or interpretable to other bacterial preparations and there may be considerable heterogeneity between trial outcomes. Depending on the delivery mechanisms, which is generally oral consumption, some probiotics may be prone to degradation by the digestive system, hence why novel methods of probiotic delivery are being assessed [146]. Indeed, next-generation probiotics, which are probiotic formulations more akin to pharmaceutical-grade supplements, are also being investigated for various conditions, but have yet to be fully explored for COVID-19 [86,146].

Additionally, while inferring the results of clinical trials (Table 1, Table 2) it is important to be aware of confounding factors such as various age groups with different immune system statuses, the season of the year, doses of probiotics, nutritional status, other supplements being consumed, etc. Therefore, a more targeted approach may be warranted, whereby a patients gut microbiota profile is sequenced at a bacterial strain level to fully address the role of the microbiota and their interactions between diet and extrinsic stressors against SARS-CoV-2 infection. This level of diagnostic capacity could potentially lead to personalized treatments or formulations of probiotics or next-generation probiotics in the future.

An important question is if these probiotics, probiotics, and synbiotics can be used for preventive or therapeutic interventions? None of the published studies have reported using these immunobiotics for the management of COVID-19. Considering the fact that people with co-morbidities are at higher risk of worse outcomes post SARS-CoV-2 exposure, these bacterial formulations should be considered to strengthen the immune response of healthy and at-risk populations rather than a therapeutic measure. People with comorbidities, old age, nightshift, and jetlag are more susceptible to disease progression owing to disrupted circadian rhythms and vice-versa [147–149]. Recent studies have shown that host circadian rhythms influence the outcomes of respiratory infections [150,151]. One cannot ignore a tripartite crosstalk between circadian rhythm, commensals and viral infections while considering microbial formulations as a supplement for COVID-19 management [152], especially since patients in intensive care units suffer from circadian rest/activity misalignment [153], which may affect the microbiome and lead to severe disease outcomes.

Probiotics are not a panacea for the treatment of COVID-19, but their potential modulation of the gut microbiome may beneficially or negatively affect the efficacy and bioavailability of some drugs, including those that were on trial for treatment of COVID-19. It is important to consider the role of the microbiome and its interactions with drug metabolism and pharmacokinetics [154,155]. Some drugs are metabolized by the microbiome and therefore their efficacy and bioavailability may be affected [156]. Some drugs negatively affect the microbiome [157]. For example, tocilizumab, an anti-IL-6 monoclonal antibody used to treat ARDS in COVID-19 that may affect the microbiome. Individuals with diverticular disease, a disease known to alter the gut microbiome, are at higher risk of lower intestinal perforation when medicated with tocilizumab [158]. Indeed, IL-6 deficient mice also have thinning of the mucus layers and impaired gut-epithelial barrier function [159]. This indicates that COVID-19 patients treated with tocilizumab may impair gut-epithelial barrier function via IL-6 depletion. Therefore, these patients may be candidates to receive probiotic treatments to

ameliorate dysbiosis induced by both COVID-19 and resulting treatments. In fact, it is largely unknown what effects other medications targeting COVID-19 may have on microbiota, thus further research is required.

The efficacy and the safety of probiotics has been contentious in the literature. For example, a case report on a immunocompromised patient showed development of fungemia as a result of treatment of antibiotic-associated pseudomembranous colitis with the probiotic *Saccharomyces cerevisiae* var. *Boulardii* [160]. Another report presented a clinically significant bacteremia in immunocompromised patients who received *Bacillus clausii* [161]. A clinical study conducted at Boston Hospital showed alarming results of developing *Lactobacillus* bacteremia in ICU patients who received *Lactobacillus rhamnosus* strain GG (LGG) probiotics [162]. Moreover, Kothari et al. have written an exhaustive review on deleterious effects of administration of probiotics that were not only limited to localized and opportunistic infections but also resulted in sepsis, ischemia, infective endocarditis, and horizontal transfer of antibiotic resistant genes [163]. At the time of writing, there is no direct evidence that probiotics or their metabolites or bioactive compounds attenuate the complex symptoms related to COVID-19.

Probiotics also come in various compositions and so it is likely that multiple mechanisms contribute to some of the observed benefits of their administration against respiratory infections and COVID-19. However, determining the most effective composition and concentration will be important if probiotics are to be used in COVID-19 treatment. Formulations may also need to be adjusted depending on the symptoms. Indeed, a formulation that may be beneficial for one patient could be detrimental to another. Therefore, considerably more research is required.

Among the limited studies published to date, there appears to be some promising but mixed results for the use of probiotics in hospitalized COVID-19 patients. Indeed, many of the trials that have been registered (Table 2) are further investigating the potential therapeutic benefits of co-administering probiotics with SOC. Until these findings have been published, it is not possible to provide evidence-based recommendations for the administration of probiotics to patients and therefore, we conclude that probiotics have not yet been shown to be substantially effective against COVID-19 to warrant inclusion in COVID-19 treatment plans. However, complementary use of probiotics as an adjuvant treatment may be used cautiously for the enhancement of antiviral defenses and prevention of secondary microbial infections as was previously proposed by the Chinese health authorities [164]. Further research is required to discern the role of the microbiome in the gut-lung axis and the role of probiotics in the prevention and treatment of SARS-CoV-2 and COVID-19 and the modulation of the gut and lung microbiomes.

Author contributions

Conceptualization, R.L.; writing—original draft preparation, S.B., A.N., and R.L.; writing—review and editing, S.B., A.B., and R.L.; Visualization, A.N; project administration and supervision, R.L. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors have no conflicts of interest to declare. This study did not receive funding.

Acknowledgments

A.N. and R.L. would like to thank the Children's Hospital of Philadelphia, the Institute for Translational Medicine and Therapeutics, and the Perelman School of Medicine for their continued support. Figure created using Biorender.

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