REVIEW

The Potential Use of Probiotics in Coronavirus Diseases (COVID-19)

Herbal Medicine Research Centre
Institute for Medical Research
National Institutes of Health
Ministry of Health Malaysia

Date of Report: 31st Mac 2020

Report Written By:	**Reviewed By:**
1. Terence Tan Yew Chin 2. Suganthi a/p Jeyabalan 3. Dr Murizal B. Zainol 4. Lim Xin Yi 5. Norfarahana Bt. Japri 6. Nur Salsabeela Bt. Mohd Rahim 7. Nurmaziah Bt. Mohammad Shafie	Dr Ami Fazlin Syed Mohamed Head of Centre

Herbal Medicine Research Centre (HMRC)
Institute for Medical Research
Level 5, Block C6,
National Institutes of Health Complex
No 1, Jalan Setia Murni U13/52, Section U13
40170 Setia Alam, Selangor

Disclosure

The authors of this review have no competing interest in this subject.

Disclaimer

This review is essentially a brief report, prepared on an urgent basis, to reflect the highest level of evidence available regarding the subject at this specific time. The conclusion draws on restricted reviews from analysis of pertinent literature, on expert opinion and/or regulatory status where appropriate. All efforts have been made to ensure all relevant published material has been reviewed but this document may still not fully reflect all scientific research available. Additionally, other relevant scientific findings may have been reported since completion of this review.

TABLE OF CONTENT

Executive Summary

Title

The Potential Use of Probiotics in Coronavirus Disease (COVID-19)

Objective

The objective of this report is to assess current available evidence on the potential of probiotics in COVID-19 management based on the following:

- Efficacy: focusing on probiotics' reported properties of (1) antiviral, (2) modulation of immune response, and (3) role as other supportive therapy or management of disease related complications; and their respective potential mechanism(s) of actions.
- Safety of probiotics

Methodology

Electronic databases were searched using pre-determined terminologies such as 'probiotics', 'antiviral', immunomodulatory', 'immune response', 'mechanism of action', and 'safety'. All clinical and preclinical studies (both *in vitro* and *in vivo*) related to safety and efficacy or effectiveness of probiotics in treating viral diseases were included.

Results

Based on literature search, evidence which consists of human trials, *in vivo* and *in vitro* studies showed antiviral effects of probiotics, as well as immunomodulatory effects contributing towards indirect antiviral effects. However, most efficacy studies investigated influenza and upper respiratory infections, which were not specific towards SARS-CoV-2 virus. Many factors can influence efficacy, including the type of probiotic strain (*Lactobacillus* spp. as the most studied species), mechanism of action (twelve possible mechanisms), and dosage. A recent study on current management of COVID-19 in Zhejiang, China reported probiotics being used as supportive treatment for intestinal microbial dysbiosis complications caused by SARS-CoV-2 virus.

Conclusion

Based on literature search, probiotics showed potential antiviral and immunomodulatory effects, though not specific towards SARS-CoV-2 virus. Further investigation is required to explore these potentials in COVID-19 management. Although probiotics are considered to be generally safe for human consumption, special safety considerations must be given to the critically-ill such as intensive care unit (ICU) patients, where there were reports on increased risk of bacteraemia. It is important that pathogenicity and infectivity, intrinsic properties, as well as virulence factors related to toxicity and metabolic activity of the microorganisms be clearly studied.

Full report on probiotics potential in COVID-19 management

Introduction

Probiotics are microorganisms associated with beneficial effects for human and animal health. Increased interest and research on probiotics have reported several potential useful properties including anti-pathogenicity, anti-diabetic, anti-obesity, anti-inflammatory, anti-cancer, anti-allergic, and angiogenic effects, as well as benefits on the brain and central nervous system. (1)

In general, the microbes that are commonly used as probiotics can be broadly categorised into two categories including: (2)

- Bacteria - common species (i) *Lactobacillus*: *acidophilus*, *sporogenes*, *plantarum*, *rhamnosum*, *delbrueck*, *reuteri*, *fermentum*, *lactus*, *cellobiosus*, *brevis*, *casei*, *farciminis*, *paracasei*, *gasseri*, *crispatus*; (ii) *Bifidobacterium*: *bifidum*, *infantis*, *adolescentis*, *longum*, *thermophilum*, *breve*, *lactis*, *animalis*; (iii) *Streptococcus*: *lactis*, *cremoris*, *alivarius*, *intermedius*, *thermophilis*, *diacetylactis*; (iv) *Leuconostoc mesenteroides*; (v) *Pediococcus*; (vi) *Propionibacterium*; (vii) *Bacillus*; (viii) *Enterococcus*; (ix) *Enterococcus faecium*;

- Yeast and molds - *Saccharomyces* cerevisiae, *Saccharomyces bourlardii*, Aspergillus niger, *Aspergillus oryzue*, *Candida pintolopesii*, *Saccaromyces boulardii*.

During these challenging times of COVID-19 pandemic, a company, TT Organics, have offered and requested that their probiotic formulation, EM solution, be considered for testing against the SARS-CoV-2 virus, by the Ministry of Health. The company proposed that the grease and oil removing properties of their formulation as beneficial in breaking the chain of virus transmission.

Objective

The objective of this report is to assess current available evidence on the potential of Probiotics in COVID-19 management based on the following:

- Efficacy: focusing on probiotics' reported properties of (1) antiviral, (2) modulation of immune response, and (3) role as other supportive therapy or management of disease related complications; and their respective potential mechanism(s) of actions
- Safety of probiotics

Methodology

Searching

Electronic databases were searched using pre-determined terminologies such as 'probiotics', 'antiviral', 'immunomodulatory', 'immune response', 'mechanism of action' and 'safety'. Any peer-reviewed journals found were included which covers PubMed, Ovid Medline(R), EBM Reviews-Cochrane Central Register of Controlled Trials, EBM Reviews-Cochrane database of systematic reviews. The articles included in the search strategy were limited to those which were published within the year 2001 to March 2020. All clinical and preclinical studies (both *in vitro* and *in vivo)* that evaluated probiotics in any antiviral, modulation of immune response, and safety studies were included.

Selection

All published articles related to safety and efficacy or effectiveness of probiotics in treating viral diseases were included.

Results & Discussion

Efficacy

Antiviral effects

- A review study that evaluated the clinical efficacy of 12 different strains of various major lactic acid bacteria for infectious diseases reported the probiotic strain *Lactococcus lactis* JCM 5805 to demonstrate anti-influenza activity, potentially via direct stimulation of plasmacytoid dendritic cells through toll like receptor (TLR)-9, and hence promoting viral control. However, more detailed research is required as most human trials included in this review focused on common cold symptoms instead of specific viral disease activities (3).

- A systematic review with meta-analysis concluded that probiotic consumption appears to be a feasible way to decrease incidence of respiratory tract infections in children. However, the optimal probiotic strains, dosing, administration form, time of intervention, and long-term follow-up should be considered in future clinical trials (4).

- A systematic review among patients with human immunodeficiency virus (HIV) infection showed that the *Lactobacilli* species in combination with other genera may have clinically meaningful effects among those with poor immunological response despite being on antiretroviral. However, the effects of probiotics on inflammatory markers and immunological outcomes (e.g. CD4 counts, activation markers) needs to be further investigated with longer duration, particularly in the context of antiretroviral use (5).

- As numerous strains of probiotics have reported various antiviral efficacy, a summary table depicting each strain studied with their corresponding antiviral activity can be found in Appendix 1: Data Extraction Table for Antiviral Effects of Probiotics.

Immunomodulatory effects

- An *in vivo* study showed that orally administered *L. rhamnosus* M21 activated the humoral and cellular immune responses, conferring increased resistance to the host against influenza virus infection. (6)

- A meta-analysis showed that concomitant prebiotics or probiotics supplementation with influenza vaccination may hold great promise for improving vaccine efficacy. However, high heterogeneity was observed and further studies are warranted. (7)

- A review study summarized the effects of probiotics in respiratory virus infections in cell models, in animal models, and in humans. Few clinical studies on specific viruses-caused respiratory tract infections showed promising data demonstrating that specific probiotics are able to shorten the duration or reduce the risk of respiratory infections. The probiotics may exert antiviral effects directly in probiotic-virus interaction or via stimulation of the immune system. 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. (8)

Other benefits in COVID-19 related complications

- A summary on the management of current COVID-19 in Zhejiang, China highlighted the effectiveness of their treatment strategy for clinical practice which increased cure rate and reduced mortality. Some patients with COVID-19 had intestinal microbial dysbiosis with decreased probiotics such as *Lactobacillus* and *Bifidobacterium*. Thus nutritional support and application of prebiotics or probiotics were suggested to regulate the balance of intestinal microbiota and reduce the risk of secondary infection due to bacterial translocation. (9)

Possible mechanism of probiotics' antiviral effect

The following mechanisms have been reported for probiotics against other viruses, but are yet to be evaluated against the SARS-CoV-2 virus; and hence can be considered to be included in future research: (8)

- Bind directly to virus and inhibit virus attachment to the host cell receptor
- Adhesion of probiotics on the epithelial surface may block viral attachment by steric hindrance, cover receptor sites in a nonspecific manner, or by competing for specific carbohydrate receptors
- Induce mucosal regeneration; intestinal mucins may bind to viruses, and inhibit their adherence to epithelial cells and inhibit virus replication
- Antimicrobial activity against pathogens by producing antimicrobial substances
- Induction of low-grade nitric oxide (NO) production and dehydrogenase production may have antiviral activities
- Modulation of immune response through epithelial cells
- Modulation and activation of immune responses through macrophages and dendritic cells
- Modulation and activation T-lymphocyte responses
    - Upon activation, CD8+ T lymphocytes differentiate into cytotoxic T lymphocytes (CTLs) which destroy virus infected cells
    - CD4+ T lymphocytes differentiate into Th1 & Th2 cells
    - T-helper cell types 1 activates phagocytes which promotes virus killing
    - Th2- cells induces 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 infection site
- Secretory antibodies neutralizes the virus

Safety

- Strains of the *Lactobacillus* and *Bifidobacterium* genera are usually regarded as safe from the basis of long-term human use. (14)

- Members of other genera such as *Bacillus licheniformis* have also been investigated to be used as probiotics. However, it should not be concluded that all members belonging to the *Bacillus* genus can be used as probiotics. This is because there are some strains from the *Bacillus* genus that are associated with diseases such as *Bacillus cereus*, which can cause food-borne illnesses. (15-16)

- Pathogenicity and infectivity, intrinsic properties as well as virulence factors related to toxicity and metabolic activity of the microorganisms are factors that need to be addressed during the safety assessment process of probiotics. (17)

- Special safety concerns of probiotic use in the critically-ill should also be given attention. Although consumption of probiotics is generally safe, there are case reports of possibility of bacteremia and fungemia associated with administration of *Lactobacillus* probiotics and *Saccharomyces* species in critically-ill patients. The risk of infection may also be related to improper handling of the probiotics, as there are concerns of cross-contamination after administering oral probiotics (via feeding tube), on to the hand of healthcare workers, and then translocation via central catheters into the patient's systemic circulation. (18)

- There are also recent emerging clinical evidence of genomic evolution and mutation after consumption of probiotics among intensive care unit (ICU) patients, which may further increase the risk of bacteraemia. (19)

Conclusion

Documentations provided by the company were mostly focused on agriculture use of probiotics while claims of human efficacy were based on patient and customer testimonials. Therefore, it is clear that the available evidence is still preliminary and more research is needed to provide scientific data for objective assessment of probiotics' potential in COVID-19 management. Although probiotics are generally considered to be safe for human consumption, special safety considerations must be given to the critically-ill such as intensive care unit (ICU) patients, where there were reports on increased risk of bacteraemia. It is important that pathogenicity and infectivity, intrinsic properties, as well as virulence factors related to toxicity and metabolic activity of the microorganisms be clearly studied.

Reference

1. George KR, Patra JK, Gouda S, Park Y, Shin, HS, Das G. Benefaction of probiotics for human health: A review. J Food Drug Anal. 2018;26(3):927-939.
2. Amara AA, Shibl A. Role of probiotics in health improvement, infection control and disease treatment and management. Saudi Pharm J. 2015;23(2):107-114.
3. Kanauchi O, Andoh A, AbuBakar S, Yamamoto N. Probiotics and paraprobiotics in viral infection: Clinical application and effects on the innate and acquired immune systems. Curr Pharm Des. 2018;24(6):710-717.
4. Wang Y, Li X, Ge T, et al. Probiotics for prevention and treatment of respiratory tract infections in children: A systematic review and meta-analysis of randomized controlled trials. Medicine (Baltimore). 2016;95(31):e4509.
5. Carter GM, Esmaeili A, Shah H, et al. Probiotics in human immunodeficiency virus infection: A systematic review and evidence synthesis of benefits and risks. Open Forum Infect Dis. 2016;4(4):ofw164.
6. Song JA, Kim HJ, Hong SK et al. Oral intake of *Lactobacillus rhamnosus* M21 enhances the survival rate of mice lethally infected with influenza virus. J Microbiol Immunol. 2016; 49(1):16-23.
7. Yeh TL, Shih PC, Liu SJ, et al. The influence of prebiotic or probiotic supplementation on antibody titers after influenza vaccination: A systematic review and meta-analysis of randomized controlled trials. Drug Des Devel Ther. 2018;12:217-230.
8. Lehtoranta L, Pitkäranta A, Korpela R. Probiotics in respiratory virus infections. Eur J Clin Microbiol Infect Dis. 2014;33(8):1289-1302.
9. Xu K, Cai H, Shen Y, et al. Management of corona virus disease-19 (COVID-19): The Zhejiang experience. J Zheijiang Univ. Med Sci. 2020;49(1):0.
10. Ang LYE, Too HKI, Tan EL, et al. Antiviral activity of *Lactobacillus reuteri Protectis* against Coxsackievirus A and Enterovirus 71 infection in human skeletal muscle and colon cell lines. Virol J. 2016;13:111.
11. Lehtoranta L, Kalima K, He L, et al. Specific probiotics and virological findings in symptomatic conscripts attending military service in Finland. J Clin Virol. 2014;60(3):276- 281.
12. Abdelhamid AG, El-Masry SS, El-Dougdoug NK. Probiotic *Lactobacillus* and *Bifidobacterium* strains possess safety characteristics, antiviral activities and host adherence factors revealed by genome mining. EPMA J. 2019;10(4):337-350.
13. Bae JY, Kim JI, Park S, et al. Effects of *Lactobacillus plantarum* and *Leuconostoc mesenteroides* probiotics on human seasonal and avian Influenza viruses. J Microbiol Biotechnol. 2018;28(6):893-901.
14. Shi LH, Balakrishnan K, Thiagarajah K, Mohd Ismail NI, Yin OS. Beneficial properties of probiotics. Trop Life Sci Res. 2016;27(2):73-90.

15. European Food Safety Authority (EFSA). Opinion of the scientific committee on introduction of a qualified presumption of safety (QPS) approach for assessment of selected microorganisms referred to EFSA. The EFSA Journal. 2007;5(12):1-16.
16. Leuschner RGK, Robinson TP, Hugas M, et al. Qualified presumption of safety (QPS): A generic risk assessment approach for biological agents notified to the European Food Safety Authority (EFSA) Trends in Food Science and Technology. 2010;21(9):425-435.
17. Ishibashi N, Yamazaki S. Probiotics and safety. Am J Clin Nutr. 2001;73(2 Suppl):465S-470S.
18. Vitko HA, Troxell JJ. Probiotics in the critical care unit: Fad, fact, or fiction? J Emerg Crit Care Med. 2018;2:95.
19. Yelin I, Flett KB, Merakou C, et al. Genomic and epidemiological evidence of bacterial transmission from probiotic capsules to blood in ICU patients. Nat Med. 2019;25:1728-1732.

Appendix 1

DATA EXTRACTION TABLE FOR ANTIVIRAL EFFECTS OF PROBIOTICS

No	Probiotic organism	Experimental system	Type of Virus	Major findings	References
1	*Lactobacillus casei* (Yakult)	Human trials (review)	Upper respiratory tract infection Epstein–Barr virus (EBV) Cytomegalovirus (CMV)	Reduced plasma CMV and EBV antibody titers	Kanauchi et., 2018 (3)
	Enterococcus faecalis FK-23	Human trials (review)	Hepatitis C virus	Significant decrease of alanine aminotransferase. No significant change in viral load	
	Bifidobacterium animalis (Bb12)	Human trials (review)	Polio- and rota-virus in infants	Bb12 significantly increased fecal anti-poliovirus specific IgA, and increased anti-rotavirus specific IgA.	
	Lactococcus lactis JCM5805	Human trials (review)	Virus for common cold	*L. lactis* JCM 5805 activated plasmacytoid dendritic cells (pDC) which is responsible for regulation of antiviral immune responses among peripheral blood mononuclear cells (PBMC) and significantly reduced	

			the risk of morbidity from the common cold	
		Influenza virus	Significant decrease in the cumulative incidence days of ‘cough’ and ‘feverishness’. Significant increase in interferon (IFN)-α-inducible antiviral factor, interferon-stimulated gene 15 (ISG15)	
		Influenza virus	Significant decrease in the cumulative incidence days of ‘sore throat’ and ‘cough’. Significant increase in IFN-α mRNA in PBMC	
		Human influenza A virus (H1N1)	Significantly increased pDCs activation and increased mRNA expression of ISG15. Significant decrease in the cumulative incidence days of cold-like symptoms	

			Influenza virus	Significant increase in secretory IgA in saliva Significant prevention of decrease in phagocytic activity of neutrophil during common cold season	
2	*Lactobacillus rhamnosus; Lactobacillus* GG; *L. helveticus; L. acidophilus; L. fermentum; L. casei; L. bulgaricus; Bifidobacterium infantis; B. lactis; B. animalis* subsp. *lactis*; *Streptococcus thermophilus.*	Human trials (systematic review)	Respiratory tract infection viruses in children	Probiotic consumption appears to be a feasible way to decrease the incidence of respiratory tract infections in children	Wang et al, 2016 (4)
3	*Lactobacillus rhamnosus; L. reuteri; L. plantarum; L. sporogens*; *L. paracasei* subsp *paracasei*; *Bifidobacterium lactis; B. bifidum; Pediococcus pentosaceus; Leuconostoc mesenteroides*; *Saccharomyces boulardii; Streptococcus thermophilus; Bacillus coagulans..*	Human trials (systematic review)	Human Immunodeficiency Virus (HIV)	Probiotics appear to exert some positive influence on clinical symptoms, a moderate improvement on CD4 count, and limited effects on markers of translocation	Carter et al., 2016 (5)
4	*Lactobacillus rhamnosus* M21	In vivo (mice)	Influenza virus	*L. rhamnosus* M21 activates humoral as well as cellular immune responses, conferring increased resistance to the host against influenza virus infection	Song et al, 2016 (6)

5	*Lactobacillus fermentum*, *L. casei*, *L. rhamnosus*, *L. case*i, *L. plantarum*, *L. paracasei*, *Bifidobacterium longum*.	Human trials (systematic review and meta-analysis)	2 A strains (A/H1N1 and A/H3N2) and 1 B strain (Victoria or Yamagata lineages)	Supplementation with prebiotics or probiotics may enhance the influenza hemag-glutination inhibition antibody titers in all A/H1N1, A/H3N2, and B strains (20%, 19.5%, and 13.6% increases, respectively).	Yeh et al., 2018 (7)
6	*Lactobacillus* spp., *Bofidobacterium* spp., *Propionibacterium* spp.	Human trials (review)	Respiratory viruses	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.	Lehroranta et al., 2014 (8)
7	*Lactobacillus* & *Bifidobacterium*	Review	Corona virus disease-19 (COVID-19)	The condition of some patients with COVID-19 showed intestinal microbial dysbiosis with decreased probiotics such as *Lactobacillus* and *Bifidobacterium*. Thus nutritional support and application of prebiotics or probiotics were suggested to regulate the balance of intestinal microbiota and reduce the risk of secondary infection due to bacterial translocation.	Xu et al., 2020 (9)

8	*Lactobacillus reuteri* Protectis & *Lactobacillus casei* Shirota	In vitro	Coxsackieviruses and Enterovirus 71 (EV71), the main agents responsible for hand, foot, and mouth disease (HFMD)	*L. reuteri* Protectis displays a significant dose-dependent antiviral activity against several strains of Coxsackievirus type A in which likely achieved through direct physical interaction between bacteria and virus particles, which impairs virus entry into its mammalian host cell.	Ang et al., 2016 (10)
9	*Lactobacillus rhamnosus* & *Bifidobacterium animalis* ssp. lactis	Human trials	Respiratory viruses	RCT study showed that *Lactobacillus rhamnosus* GG and *Bofidobacterium animalis* ssp. lactis BB-12 did not reduce viral occurrence in symptomatic conscripts. However, probiotics decreased the presence of picornaviruses after 3 months, which may imply that probiotics play a role against viruses causing common cold.	Lehroranta et al., 2014 (11)
10	*Lactobacillus acidophilus*, *L. helveticus*, *L. plantarum* ss. *plantarum*, *L. rhamnosus*, *Bifidobacterium longum*, *B. bifidum*.	In vitro (baby hamster kidney (BHK) cells and primary chicken embryo fibroblast (CEF) cell culture)	Newcastle disease (ND) and infectious bursal disease (IBD) viruses	In vitro study showed that safety privileges, antiviral activities, and genomically encoded host interaction factors confirmed probiotic features of the six probiotic strains and their potential in promoting human health.	Abdelhamid et al, 2019 (12)

11	*Lactobacillus plantarum* & *Leuconostoc mesenteroides*	In vitro (human cell) and In vivo (mice)	Human H1N1 and avian influenza H7N9 viruses	In vitro study showed that *Lactobacillus plantarum* (Lp) and *Leuconostoc mesenteroides* (Lm) strains significantly restrained viral replication in mouse lungs. In vivo study showed that the Lp and Lm strains also exhibited their beneficial effects by increasing the mean days and rates of survival of the infected mice.	Bae et al, 2018 (13)

Summary of evidence: Based on the 11 references extracted, most of the human trials have shown the potential of *Lactobacillius* spp and *Bifidobacterium* spp to be effective in influenza virus and respiratory tract infection, not specific towards SARS-CoV-2. Only one reference on management of COVID-19 in China showed benefit of probiotics towards patients with complications of intestinal microbial dysbiosis due to COVID-19 but in a form of supportive treatment.