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Review Article

Probiotics: Modulator of Human Health and Potential Fighter against Coronavirus Pandemic

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Abstract

Digestive tract of human is rich in bacterial microflora which not only protects the intestine from pathogenic microorganisms but also provide beneficial substances e.g., Vitamin B12 and lactic acid which are helpful in metabolism. In some individuals, microflora could not develop properly which result in recurrent infections, suppressed immune system, disturbed gut-brain axis leading to neural disorders in some severe cases. Probiotics are the microorganisms which impart health benefits if administered in appropriate amount. They have potential to improve gut-brain barrier thus reducing the chances of nervous system disorders like anxiety and depression. Pro-apoptotic and anti-proliferative activity of probiotics has also demonstrated on human gastric and colorectal cancerous cells. Modulation of probiotics can be helpful to prevent colorectal cancer which has ranked to be third major types of cancers prevalent in developing countries. SARS CoV-2, the responsible factor of current Coronavirus pandemic requires Angiotensin Converting Enzyme 2 (ACE-2) as receptor for infection which is digestion related enzyme of human enterocytes. Expression of viral receptors can be elevated in invasive bacterial infections such as Salmonella enterica infecting small intestine, however healthy gut with proper dose of probiotics can help prevent such infections and indirectly the chances of Coronavirus infection by reducing the expression of ACE-2. Interleukin-17 associated endoplasmic reticulum stress induced in Coronavirus infection can also be reduced by some *Bifidobacterium* strains which have inhibitory effects against IL-17. Other probiotic microorganisms include *Saccharomyces* spp., *Lactobacillus* spp., *Enterococcus* spp. and *Streptococcus* spp. which are added in different food products like yogurt, curd, cheese and processed meat to stimulate metabolism.

Keywords: Gut-Brain Axis; Probiotics; SARS CoV-2; Interleukin-17

Introduction

Human digestive tract is populated with variety of bacterial species known as intestinal microbiota that establish a symbiotic relationship with host. These bacterial species play an important role in food digestion, gut immune system development, resistance to colonization of gut with pathogenic microorganism, and production of essential vitamins and short chain fatty acids [1,2]. The number of microorganisms that harbor digestive tract have been estimated to be 10^{14} in number with bacterial cell count ~10 higher than number of human cells [4,5]. Intestinal microbiota is distributed in gastrointestinal tract along three main parts: 1) stomach contains < 10^{12} cfu/mL microbes with streptococci and lactobacilli more common, 2) ileum contains 10^{2}-10^{3} cfu/mL with *Klebsiella*, *E. coli*, *Enterococcus*, and *Bacteroides* most frequent, 3) large intestine

with largest microbial population of 10^{10}-10^{12} cfu/mL bacteria [3].

A wide range of beneficial properties are provided to host by gut microbiota. Butyrate, propionate and acetate are main short chain fatty acids present in gut that are carbohydrate generating metabolites and absorbed by epithelial cells to be involved in cellular processes regulation [6]. Bacteria present in gut have carbohydrate-active enzymes which enable them to ferment these carbohydrate generating metabolites [7]. Microbiota of gastrointestinal tract is also crucial for production of essential vitamins [8]. Vitamin B12 is primarily produces by lactic acid bacteria (LAB) [9]. *Bifidobacteria* are responsible for production of folate involved in metabolic processes of host [10]. Development of intestinal mucosal and systemic immune system is also mediated by gut microbiota. Thus, an altered gut microbial composition can result in disruption of such important beneficial mechanisms, a condition called dysbiosis, [11] which can be cured by probiotic supplements. "Probiotics are referred to as micro-organisms that, when administered in appropriate amounts, provide health benefits to host" [12]. An ideal probiotic should be of human origin, safe, does not contain any vector responsible for transfer of toxicity or pathogenicity factors, or resistance to antibiotics, and also have survival capacity in intestinal conditions (enzymes, acid pH, bile salt etc). Probiotic should have beneficial health effects on human by stimulating immune system and exhibiting antagonistic effects against pathogens [13,14]. Results from first investigation about lactic acid producing bacteria and health effects they exert on human suggested that ingestion of lactic acid bacteria have improved host health. Lactic acid bacteria are heterogeneous micro-organism group present in human gut and are introduced via fermented food ingestion like yogurt, fermented milk products, fermented cured, meat byproducts and various types of cheese. *Bifidobacterium*, *Saccharomyces boulardii*, *Lactobacillus, Escherichia coli* (Nissle, 1917), and *Enterococcus* strains are widely used probiotic bacteria but some *Streptococcus, Lactococcus, Pediococcus* and *Leuconostoc* strains are also used [13]. Probiotics being able to alter disturbed microbiota and restore microbial diversity are current strategy to treat dysbiosis [13,15] figure 1.

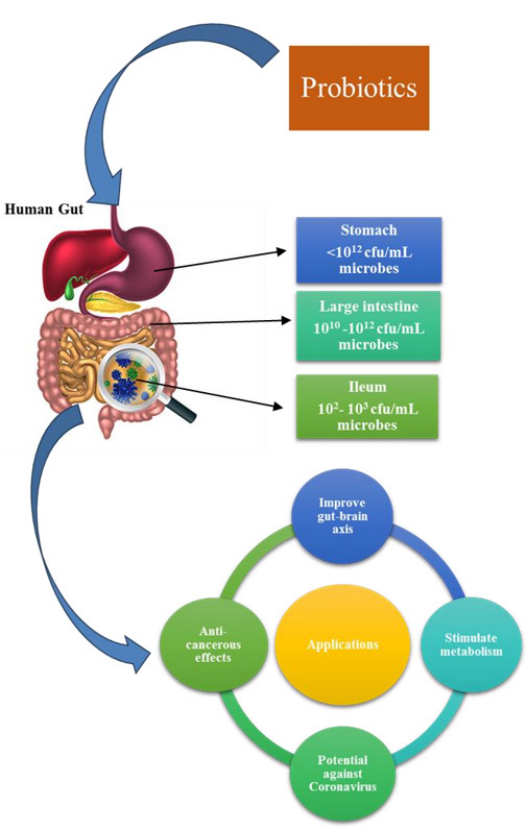

Figure 1: Application of probiotic to improve human health.

Potential of Probiotics to improve gut-brain barrier

Gut microbiota has risen as topic of research interest in recent years. Studies have revealed how variations in gut microbiota composition can influence normal physiology of human and result in diseases. Gut microbiota also influence brain functioning and behavior by communicating with central nervous system (CNS) through endocrine, neural and immune pathways [16]. Several studies have shown that healthy gut functions can be linked to normal CNS function [17-20]. Sherwin E., *et al*. has also realized the fact that microorganisms residing in our gut form complex communication networks called microbiome-gut-brain axis [21]. Gut-brain axis is bidirectional and consists of neural pathways (vagus, enteric nervous system, spinal and sympathetic nerves) and humoral pathways (hormones, cytokines and neuropeptides) [22]. Gut releases neurotransmitters, hormones and immunological factors that transmit signals to brain directly or through autonomic neurons [23].

The concept is being supported that disruption of gut microbiota composition can contribute in several diseases including disorders of CNS [16]. Recent studies have focused on effects of varia-

tions in microbiome on several disorders of central nervous system including depression disorders, anxiety, autism and schizophrenia [24,25]. Role of gut microbiota in regulation of mood, anxiety, pain and cognition have been well demonstrated by studies in animals exposed to probiotic bacteria, pathogenic bacterial infections and in germ-free animals. Imbalance gut microbiota has also been related to autism, obesity and multiple sclerosis. Mechanism involved in effecting gut-brain signaling by microbiota may involve immune activation, alteration in microbial composition and tryptophan metabolism, vagus nerve signaling, and production of microbial neuroactive metabolites [16].

Therapeutic interventions are coming forefront to treat microbiota imbalance or disturbance in gut and diminish its effects on gut-brain axis [23]. Balance of gut microflora can be maintained by recommending probiotic ingestion as preventive measure to enhance well-being. Certain patient populations have been identified from research that may benefit from probiotics in certain disorders and illness [26]. Research has been carried out on probiotics use (both as adjunct to common prescribed medication and standalone) to treat depression and anxiety [23]. Understanding the mechanism of gutbrain axis and how disturbance of gut microbiota can affect brain, approaches to use microbial base therapeutics (probiotics) to maintain microbiota balance and prevent central nervous system disorders can be developed [16] (Figure 2).

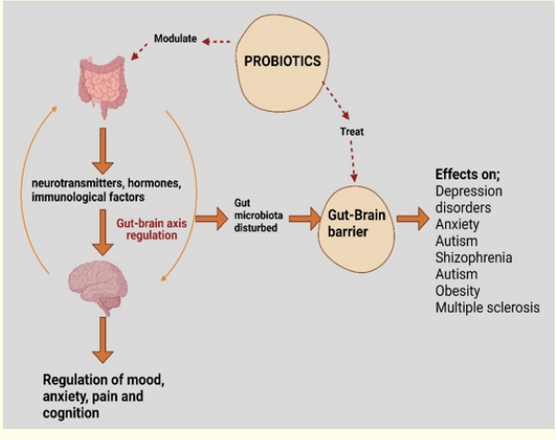

Figure 2: Gut-Brain axis and its modulation through probiotics.

Anti-cancerous effects of probiotics

Pro-apoptotic and anti-proliferative activities of probiotics have been demonstrated by research on human cancer cells with most common studies on gastric cancer cells and colonic cancer cells [27]. Gastric cancer is fourth most common and deadly cancer type and cause second highest rate of cancer related deaths throughout the world [28,29]. Approximately, 880,000 people were diagnosed with gastric cancer in 2000 and about 650,000 people died [30,31]. In United States, colorectal cancer (CRC) is third most deadly type of cancer and prevailing in other developing countries, despite of advanced technology and awareness, due to occupational hazards and varying diet habits [32]. According to centre for disease control and prevention (CDC), about 141,000 Americans were diagnosed with colorectal cancer [33]. Colorectal cancer is series of sequences caused by activation, mutation and deletion of tumor suppressor gene and onco-genes [34] and mostly influenced by diet, dietary habits, tobacco consumption and physical activity [31]. Gut microbiota composition have been shown to be associated with cancer development by recent evidences. Modulation of gut microbiota by probiotics could be beneficial to prevent colorectal and other gut cancers [35] (Figure 3).

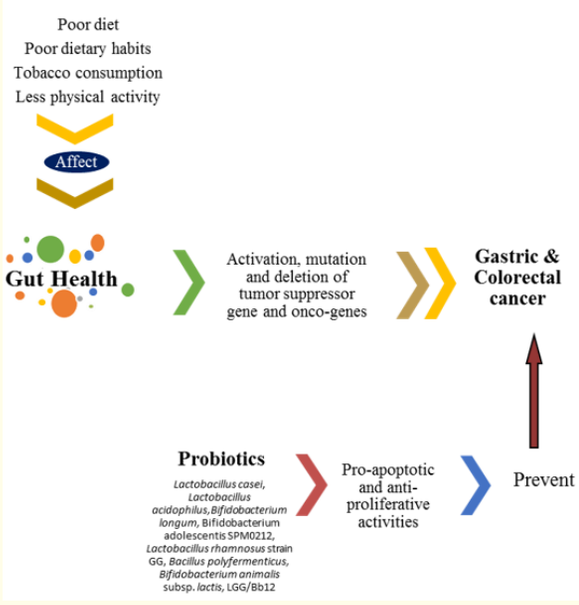

Figure 3: Role of probiotics to prevent gastric and colorectal cancers.

Significant anti-tumor activities have been shown in some cancer lines by cytoplasmic fractions of *Lactobacillus casei*, *Lactobacillus acidophilus* and *Bifidobacterium longum* [27]. *Bifidobacterium adolescentis* SPM0212, a probiotic product, has been demonstrated to show anti-proliferative activities in human colon cancer cell lines SW-480, HT-29 and Coca-2 [36]. *Lactobacillus rhamnosus* strain GG has also exhibited antiproliferative activities in human colonic and gastric cancer cells [37-39]. Antitumor activities against cancer cells have also exhibited by other probiotic products including *Lactobacillus acidophilus* 606, *Bacillus polyfermenticus, Bifidobacterium animalis* subsp. *lactis*, and LGG/Bb12 [40]. A study by Cousin., *et al*. demonstrated that cytotoxicity of camptothecin, a chemotherapeutic agent against gastric cancer, was enhanced by *Propionibacterium freudenreichii* containing fermented milk [41].

Probiotics have shown inhibitory effects against cell invasion during *in vitro* studies. Some probiotic products have seen to be effective against breast, myeloid and cervix cancer cells. Since, pro-apoptotic effects of probiotics on cancer cells have been indicated by various *in vitro* studies, they might be used as adjuvant treatment during chemotherapy [40].

Potential of probiotics against corona virus

Corona viruses are major pathogens of human and other vertebrates having potential to infect respiratory, hepatic, gastrointestinal and central nervous system of human birds, livestock, mouse, bats and other animals. Corona viruses are part of Coronavirinae subfamily in Coronaviridae family of Nidovirales order and this subfamily has alphacoronavirus, beta coronavirus, gamma coronavirus, and delta coronavirus genera. Transmission of emerging corona viruses from animal to human and human to human have been demonstrated from 2002/2003 outbreak of severe acute respiratory syndrome (SARS) and outbreak of Middle East Respiratory Syndrome (MERS) in 2012. Since December 2019, an outbreak of pneumonia in Wuhan has pulling attention worldwide. Deep sequencing and etiological investigation has investigated the causative agent of pneumonia as a novel coronavirus [42]. Virus, presently known as SARCCoV-2, has found to be closely related to two SARC-like bat-derived coronaviruses named bat-SL-CoVZXC21 and bat-SL-CoVZC45 and transmit from human-to-human through droplets or direct contact. Fever is most common symptom in patients suffering from COVID-19 (pneumonia from SARC-CoV-2) followed by cough [43]. At present, there is no availability of specific anti-viral therapy against COVID-19, and only supportive treatment is being used [44]. Currently, primary intervention being used is to prevent the SARC-CoV-2 spread by controlling infection [43].

COVID-19 pandemic is associated with respiratory distress as well as to the signs and symptoms of gastrointestinal track. Viral effects on gastrointestinal system indicate that immunological processes are also important in illness [45]. Expression profile of receptors of coronaviruses can help to understand how and where coronavirus infect human body. According to the study, receptors of corona virus are digestion related enzymes of human enterocytes including angiotensin converting enzyme 2-ACE2 (for SARS-CoV and SARS-CoV-2). Receptors are highly expressed in enterocyte of epithelial cells, stromal cells, glial cells and mononuclear phagocytes in different tissues.

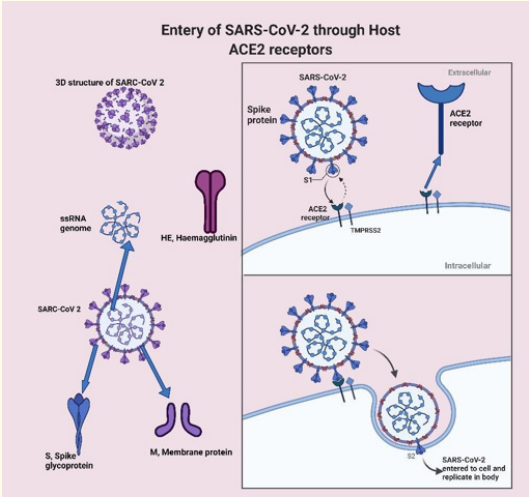

Figure 4: Virus transmission and spread in body through receptors.

Expression of viral receptors can be elevated in invasive bacterial presence. In *Salmonella enterica* infected small intestine, number of enterocytes with high expression of receptors for coronavirus was increased leading to easy accessibility of virus to enterocyte [47]. Gut microbiota has ability of immune response modulation but during infection of coronavirus, proportion of probiotics like Lactobacillus, Bifidobacterium, and Eubacterium was reduced significantly [46]. Thus, a new approach of treatment model by immune modulation with probiotics can be promising [45]. Probiotics are also in use to treat diarrhea and enteritis [47]. Probiotic bacteria regulate initial immune stimulating response and promote host defense system against certain viral infection [49]. Probiotics were widely used in Wuhan for SARSCoV-2 infected patients with diarrhea [47].

Interleukin-17 (IL-17) is pro-carcinogenic and pro-inflammatory cytokine having important role in adaptive immune response and also induces endoplasmic reticulum (ER) stress and autophagy. Inflammation and autophagy related to ER stress have been prevented by blocking IL-17. Inflammatory bowel disease also has an important role of IL-17. Effect of coronavirus relationship with gastrointestinal system has also likely to show through interleukin-17. Some *Bifidobacterium* strains have inhibitory effects against IL-17. In patients infected with coronavirus especially those with gas-

trointestinal symptoms, endoscopic administration of single high-dose of reliable strain of *Bifidobacterium* (BB-12, Infantis) or use of lipo-polysaccharide membranes of these strains in development of vaccine can be considered as a good preventive and therapeutic approach. *Bifidobacterium animalis* has found to have potential to prevent coronavirus replication by reducing autophagy related to endoplasmic reticulum (ER) stress [45].

According to experimental study, transmissible gastroenteritis (TGE) caused by coronavirus, induced severe diarrhea leading to death in piglets. Probiotic *Lactobacillus plantarum* have shown protective effects experimentally and supplementation showed dose dependent protection of viability of infected cells. Cells' pretreatment with probiotic products reduced proliferation of virus and improve inflammation [48]. *Lactobacillus acidophilus* secretes EPS polysaccharides that exhibited the inhibitory effect on transmissible gastroenteritis infection caused by virus and also improved IL-8, IL-6 and IFN-ϫ levels. *L. acidophilus* has found to induce indirect immune stimulation by reducing ROS (reactive oxygen species) that can depress immune response and also exhibit effectiveness against upper respiratory tract infections [49,50]. Viral growth and penetration inside cell is inhibited by *Lactobacillus* [51].

According to a study conducted after first SARS-CoV outbreak, immunization with recombinant *Lactobacillus casei* with surface expressed S protein of SARS associated coronavirus was found to be effective in eliciting protective anti-viral immune response. To develop immune response against virus, *Lactobacillus casei* was transformed using PgsA (poly- ϫ-glutamic acid synthetase A) protein of *Bacillus subtilis* as anchoring matrix. PgsA was combined with SA and SB segment of S (spike) protein to form recombinant fusion protein that was expressed successfully in *L. casei.* High level of mucosal IgA, and serum immunoglobulin IgG was observed after nasal and oral administration of recombinant *L. casei.* These antibodies were found to exhibit neutralizing activity against SARS (severe acute respiratory syndrome) viruses. This recombinant *Lactobacillus casei* vaccine can be promising candidate as vaccine against SARS coronavirus infection [52]. New approaches of probiotic bacteria can have promising effects in terms of treatment and vaccination model [45].

Conclusion

It is concluded that probiotics can be used as human health modulators by imparting beneficial effects as gut microflora, boosting our immune system and gut brain axis, having anti-cancerous activity and improving metabolism. Healthy gut flora supported by probiotics can render reduced availability of ACE-2 receptors necessary for establishment of COVID-19 infection in susceptible individuals. Further investigations can establish relationship of probiotics with reduced concurrent infections including COVID19, a drastic pandemic of current era.

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