**Title: Nanotechnology Intervention to Alleviate Disease-Specific Dysbiotic Microbiome**

Suyash Arunrao Kathade suyash.kathade9@gmail.com

School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded. Maharashtra, India

Akib Nisar aakib.nisar@gmail.com

PharmaACE Analytics Pvt. Ltd., Pune, Maharashtra, India.

Mayur Arjun Aswani mayuraswani7@gmail.com

Interactive Research School for Health Affairs, Bharati Vidyapeeth (Deemed to be University), Pune, Maharashtra, India.

Corresponding author:

Dr. Suyash Arunrao Kathade

Address: School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded. Maharashtra, India (431603).

Phone: 9511267388

E-mail: suyash.kathade9@gmail.com

**Abstract**

Dysbiosis of the microbiome, characterized by an imbalance in the composition and functionality of microbial communities, has been linked to various diseases and disorders. The emerging field of nanotechnology offers promising strategies to address these dysbiotic conditions and restore microbial homeostasis. This abstract explores the application of nanotechnology interventions in alleviating disease-specific dysbiotic microbiomes. Nanoparticles in delivery systems provide unique opportunities for targeted and controlled delivery of therapeutic agents to the affected sites within the microbiome. Functionalized nanoparticles can be engineered to specifically interact with dysbiotic microbial populations, selectively delivering antimicrobial agents, probiotics, or prebiotics to restore a healthy microbial balance. Moreover, nanomaterials can be designed to enhance drug stability, solubility, and bioavailability, enabling precise modulation of microbial communities. Additionally, nanotechnology-based biosensors and diagnostic tools offer efficient and sensitive means for early detection and monitoring of dysbiosis. Miniaturized nanodevices can detect and quantify microbial markers, metabolites, and inflammatory biomarkers, enabling rapid and accurate diagnosis of dysbiotic conditions. These technologies can provide critical insights into disease progression, facilitate personalized treatment strategies, and monitor treatment efficacy. However, the translation of nanotechnology-based interventions for dysbiotic microbiomes into clinical applications requires addressing concerns related to safety, biocompatibility, and long-term effects. Comprehensive investigations of potential nanomaterial toxicity and environmental impacts are essential for ensuring the responsible development and deployment of nanotechnology in microbiome therapeutics. Further research and development efforts are necessary to advance these interventions benefiting patients suffering from dysbiotic microbiome-related conditions.

Keywords: Microbiome, Disease, Health, Probiotics, Immunomodulation.

**Introduction**

A human body contains about 100 trillion cells, and more than 1000 trillion bacterial cells are present in the human body which is 10 times more than human cells. The 30 thousand human genes present in a body are responsible for the expression of different characteristics that are 100 times more than microbial genes. These microorganisms present in our body are called microbiome and microorganisms present in our gut are specifically called the gut microbiome. These microbes are not passively present in our bodies but are extremely important. They digest our food, make vitamins, and also educate the immune system to keep bad microbes out. Scientific studies depicted that the specific microbial community modulation is responsible for the specific disease condition. Numerous investigations revealed a recurrent pattern of the microbiome that was unique to the condition; certain diseases were connected with over 50 genera, while others were found to have 10-15 genus-level modulations. Recent advancements in this field state that reverting to normal flora can overcome the disease by a therapeutic approach. The main problem is the lack of specificity in target-oriented modulation of the microbiota and metabolites. This limitation can be addressed using nanotechnology, as the usage of nanoparticles (NPs) in disease diagnosis and treatment has increased over the last few decades. Research on nanomedicine formulations for diagnostic and therapeutic purposes has produced a number of successful platforms, including those for integrated diagnosis, targeted drug delivery, and therapeutics. The development of nanoparticles with the proper sizes, morphologies, chemical compositions, and concentrations may be able to get around this fundamental barrier. Using nanoparticles as a delivery system for gut microbiota influences the route of biomarker detection and the route of the interaction of nanoparticles with target cells.

In recent studies on the microbiome and its impact on health is a topics of interest and the gut microbiome contributes to more than 90% part of the study. Trillions of microorganisms are present in our body majorly located inside the gut. Majorly there are two types of microbial community present in our body which is good and bad microorganisms. Good microorganisms are also called probiotic microorganisms [1,2]. Probiotics are live microorganisms when administrated in an adequate amount confers health benefits to the host [3,4]. Once established, these probiotic bacteria can exert their beneficial effect in many ways. Some reports showed the ability of probiotics that can produce vitamins, maintain gut pH, and modulate the host’s immune response. Moreover, they are well characterized for their ability to maintain the gut microflora, especially after an antibiotic course [2,5].

Imbalance in the gut flora, also known as dysbiosis is the decrease in the number of desirable microorganisms and an increase in the number of undesirable microorganisms in the gut [6]. Dysbiosis can lead to infections, poor nutrition, lack of nutrient absorption, etc. [7] as well as acute and chronic disorders such as Irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). Probiotics are generally regarded as living drugs with immunomodulatory, anti-carcinogenic, anti-allergic and anti-inflammatory effects [8]. Even though many reports are available regarding the inhibition of pathogenic microorganisms by probiotics, lack of specificity in target-oriented modulation of the microbiota and metabolites is the main problem. This limitation can be addressed using nanotechnology.

Nanotechnology is an applied scientific discipline and has diverse practical applications [9]. The term "nanotechnology" was initially used by the Japanese professor Norio Taniguchi [10]. A nanoparticle is a material with a diameter of 100 nm or less and is regarded as the fundamental unit of nanotechnology. Mycoplasma, the smallest microbe known to science, with a length of about 200 nm [11]. The Greek word "nano," which meaning "very small," is the basis of nanotechnology. Nanotechnology is a multidisciplinary science that combines several scientific fields including biotechnology, biology, chemistry, physics, medicine, pharmacy and engineering, etc. [12]. With nanotechnology intervention and interaction with microbiome to modulate hence alleviate dysbiosis. In this chapter we focus on the nanotechnology intervention to alleviate microbiome and addresses various diseases.

**Health benefits of probiotic microbiome**

The term “Probiotics” was derived from the Greek word for life [13]. Ellie Metchnikoff was the first researcher to propose the health benefits of probiotics, after observing the correlation between daily consumption of fermented food and health in Bulgarian populations. She explained that the microbiota present in fermented food plays a major role in maintaining a healthy gut environment. Recently, researchers have proven the ability of probiotics to control cholesterol in the blood as well as shown the link of probiotics in reducing heart disease, cancer and diabetes [14]. There is a proven link between the types of microflora present in the gut and the onset of disease. Evidence accumulated in the last decade clearly emphasizes probiotic intervention's importance for good microbiome health and clinical applications [15].

**Disease-specific microbiome and the role of nanotechnology**

Recent advances have been made in the understanding of probiotics and their beneficial and appropriate uses as therapeutic agents. It can be disease-specific probiotics which are stated by reported studies [16]. Change in the gut microbiome may be the centre point that can be responsible for various clinical conditions and maintaining the normal flora of the gut may be the best therapy to overcome [17]. Hundreds of studies are carried out on the association of the human microbiome and diseases and reported study states that the consistent pattern of the microbiome was found in specific diseases and can vary from disease to disease, some of the diseases associated with over 50 genera and some are 10-15 genus-level change [18].

According to WHO there are 35% of adults aged more than 20 and 400 million people were obese in 2008 and till 2015 it reaches 700 million found be obese research states that these changes are because by changes in eating habits, intake of abundant food and decrease in expenditure energy and because of high-fat sugar, and low fibre playing a key role in chronic diseases and metabolic syndrome such as obesity, diabetes, cardiovascular etc.

In a recent study, we come to know microbial ecosystems in obese and lean people are different, when obese lost weight then microflora revert back was observed [19]. Data also suggests that probiotics can modulate the markers of metabolic stress [20] and also help to decrease adiposity, fatty liver and glucose levels in different mice models. Manipulation of the microbial environment composition in the gut may be a novel method for the treatment of obesity. The gut microbiome plays important role in increasing body weight and insulin resistance which can be associated with increased energy harvest, increased blood LPS level and low-grade inflammation [21].

Modulation of gut microflora can be a potential target to treat obesity and diabetes, *Bifidobacterium* and *Lactobacillus* showed beneficial effects on obesity and diabetes. *Lactobacillus acidophilus* reported a decrease in insulin resistance and inflammatory markers [22]. The researcher found increased phyla Bacteroidetes as compared to Firmicutes in the diabetic condition which leads to decreased glucose tolerance which is a key problem of diabetes [23]. Lowering blood glucose by decreasing insulin resistance would be a possible way and it also lowers the hypertensive condition which is closely related to diabetes, Bifidobacterium was reported for levering insulin resistance [24]. A recent study indicates that dietary polyphenols contribute to maintaining gut microbial health stimulation of a good microbiome which is very low in diabetic patients polyphenols may reduce postprandial glucose response by increasing gut microbial health [25].

Changes in the gut microbiome depicted many diseases hypothesize associated with modulation of the specific microbial community in the specific disease condition, it includes metabolic disorders, inflammatory and autoimmune diseases, neurological conditions and cancer among others (Table. 1) but there is a lack of understanding precisely how microbial community and specific microbes with these community contribute to disease [26]. There are clinical indications for certain probiotic strains such as probiotics for necrotizing enterocolitis [27]. Antibiotic-associated diarrhoea and *H. pylori* infections defecation frequency infantile colic mild to moderate ulcerative colitis, IBS [28] Acute diarrhoea etc.

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| --- | --- |
| Probiotic culture | Effective against |
| *Lactobacillus acidophilus* | Maintain normal intestinal microbiota |
| *Lactobacillus paracasei* | Has antibacterial and anticandidal activity |
| *Lactobacillus rhamnosus* | Treat infectious diarrhoea  |
| *Lactic acid bacillus* | Alleviates intestinal bowel disease symptoms  |
| *Bifidobacterium lactis* | Eases ulcerative colitis |
| *Streptococcus faecalis* | Reduce typical symptoms of IBS |
| *Bacillus clausii spores* | Prevents side effects of *Helicobacter pylori* |
| *Saccharomyces boulardii* | Prevent antibiotic-associated diarrhoea |
| *Clostridium butyricum* | Effective against *Clostridium difficile* infection |
| *Bacillus mesentericus* | Decreases potentially pathogenic microorganisms |

Table no 1: List of reported disease-specific probiotics

Owing to the potential benefits, the nanotechnological intervention aims to develop more effective tools for the prevention and treatment of various diseases [29]. This could also offer solutions to long-lasting issues in medical research, such as poor drug solubility and a lack of target specificity for therapeutic compounds [30]. It has been shown that designing NPs with natural sources and lining them up into a methodical drug delivery mechanism is advantageous to gut microbiota. Curcumin and ginger-derived NPs are shown to improve absorption by gut microbiota, so that they can produce their respective effects [31,32]. Ginger NPs are made to contain microRNA that may help mice colitis, whereas studies have indicated that curcumin NPs inhibit the development of mouse colitis by increasing butyrate-producing bacteria and regulatory T cells (Tregs). Extracellular vesicles from other natural sources, such as milk, altered intestinal short chain fatty acids (SCFA) metabolites boosted gut immunity [33].

The ability to conceal NPs with natural cell membrane coating allows for their continued circulation and ultimate targeted administration. One of the most researched cell membrane-based nanocarriers for medication delivery to target tumour cells is red blood cell (RBC) membrane-coated NPs. Furthermore, inorganic NPs, particularly, such as silver, titanium dioxide, silicon dioxide and zinc oxide have been shown to affect gut microbiota through their interaction with the immune system. This alteration in the gut microbiota-immune axis is associated with many chronic diseases such as inflammatory bowel disease (IBD), diabetes and even colorectal cancer [34,35]. Silver NPs are used in hundreds of commercial products due to their anti-microbial properties and intentional and accidental uptake of silver NPs, which might affect the gut microbiome, are underestimated. Titanium dioxide nanoparticles, which are typically found in daily necessities, also alter the morphology and metabolism of the gut microbiota [36,37]. The detailed discerption for major diseases and the use of nanotechnology in targeted disease and gut microbiome is given below.

**Inflammation/Arthritis**

Probiotics are exhibit a direct effect on the gastrointestinal tract, these effects lead to an impact on immunity, via changes in inflammatory cytokines [38]. Inflammation associated with rheumatoid arthritis may be modulated by the use of probiotics. *Lactobacillus* GG has a potential character to reinforce mucosal barrier mechanisms in inflammation. Probiotics are known to increase phagocytosis and also help to increase anti-inflammatory cytokines like TNF [39]. Nanotechnology is proven for modulation of microbiome and induce the secretion of short chain fatty acids such as butyrate, propionate etc. which is reported to decrease inflammation [40].

Free radicals have been linked to a number of pathological diseases, including cancer, ageing, diabetes, atherosclerosis, Alzheimer's, cardiovascular diseases, and more. However, excessive free radical production causes oxidative damage, which in turn results in a number of chronic diseases. Because of their harmful toxicity in synthetic materials, the use of synthetic antioxidants is restricted. As a result, natural antioxidants are now the focus of research [41]. One of the key uses of the biologically created NPs is the search for green synthesised NPs for treating such free radical-related medical disorders.

**Lactose intolerance**

Lactose intolerance is the inability to digest milk sugar (lactose), or lactose digestive enzyme lactase. Consumption of lactose by those lacking lactase production in the small intestine can lead to lactose intolerance symptoms are gas, cramps, nausea, diarrhoea, abdominal pain and flatulence. Lactose intolerance can be cured by administrating probiotic bacteria. Probiotic microorganisms like *L. acidophilus* and *Bifidobacterial* reported improving lactose digestion [42].

**Vaginosis**

Microbiota is important to maintain vaginal health, vaginosis can cause by several different organisms, and in many cases. Lactobacilli predominate in the healthy vagina, and a lack of LAB or normal flora can lead to vaginosis. The Lactobacilli species and LAB can maintain the favourable pH in the vaginal tract and also produce bacteriocin, organic acid, hydrogen peroxide and other antimicrobial compounds to maintain healthy vaginal track Research suggests that Lactobacilli may help to control the incidence and duration of vaginal infections, but larger, controlled studies are needed [43].

**Diarrhoea**

Probiotics are widely used for diarrheal diseases. Major potential benefits are the prevention and treatment of acute viral and bacterial diarrhoea, as well as regulation of antibiotic-associated diarrhoea. Some particular strains, including *Lactobacillus GG, L. reuteri, Saccharomyces boulardii,* and *Bifidobacterium* these species are reported and effective against diarrhoea. Reported In vivo studies proved that saccharomyces boulardii is effective against antibiotic-associated diarrhoea [44].

**Elevated blood cholesterol**

Cholesterol is important to maintain body functions properly. Cholesterol plays an important role in the production of vitamins and hormones it acts as a precursor [45]. In the human body, cholesterol is important to various body functions and the body synthesizes and maintains the appropriate amount for smooth function. However, cholesterol considers a risk factor for heart and cardiovascular diseases [46]. Probiotics are well known for excess cholesterol reduction. Probiotics have considerable effects on lowering LDL and reducing cholesterol in the blood. Some studies reported that Lactobacillus and Bifidobacterium are effective to reduce cholesterol from blood serum [47]. The human microbiome plays important role in human metabolism, immunity and several diseases including coronary artery disease (CAD). However, intervention of nanotechnology along with gut microbiome enables the high efficacy and precision in therapeutic approach for CAD [48]

**Diabetes, gut microbiome and nanotechnology**

When dealing with a significant metabolic illness such as diabetes, maintaining a healthy microbiota composition in the gut is equally essential. Around the world, more than 380 million people are living with type 2 diabetes, and it is anticipated that this number will climb to more than 550 million by the year 2030 [49]. *Bifidobacterium, Bacteroides, Faecalibacterium, Akkermansia*, and *Roseburia* were found to be linked to type 2 diabetes in a bad way, while *Ruminococcus, Fusobacterium*, and *Blautia* were linked to type 2 diabetes in a good way [50]. Differences in the gut microbiome between people with and without type 2 diabetes may show how diet and other environmental factors affect insulin resistance and the development of type 2 diabetes. Multiple biological pathways by which gut bacteria contribute to metabolic illness and T2D have been addressed elsewhere in the recent past [51]. The gut microbiome can affect the host's insulin sensitivity, intestinal permeability, glucose and fat metabolism by interacting with food and habits [50]. The short chain fatty acids (SCFAs) work by activating the G proteins of the L-cells, which leads to the release of GLP-1 and peptide YY (PYY) to regulate glucose homeostasis. At the same time, the SCFAs also affect the intestinal barrier, up-regulate 5'-AMP-activated in muscle and liver tissues, and the protein kinase signalling pathway, all of which are linked to insulin resistance and inflammation [52].

Nanoparticles and protein bioconjugates have been studied for multiple biomedical applications. As per the study reported by Akib Nisar et al. (2022), the interaction and structural modifications of bovine serum albumin (BSA) with iron oxide nanoparticles (IONPs). The IONPs were green synthesized using *E. crassipes* leaf extract and characterized using transmission electron microscopy, energy dispersive X-ray analysis and X-ray diffraction. Native PAGE, HPLC, and FTIR analysis displayed a differential behaviour of IONPs with native and glycated BSA hence could be used against diabetes [53].

**Cancer**

Cancer emerges as a result of chronic inflammation due to various factors including microbiota. There is a drastic microbial change as seen in cancer patients that displays low microbial diversity with significant increase of pathogenic Proteobacteria and decrease in butyrate producing microbes such as Firmicutes and Actinobacteria when compared to healthy individual microbial profile [54,55]. These abrupt changes might trigger in pro-inflammatory opportunistic pathogens that could ultimately lead to tumour formation [56]. Use of anti-carcinogenic probiotic bacteria such as several species of *Bifidobacterium* and *Lactobacillus* have been reported [57]. Also, the approach of modulating and restoring microbiome through the use of prebiotics have also been reported [58]. These approaches for microbiome modulation, ultimately preventing or curing disease at a primary stage lack specificity in achieving the targeted modulation i.e. they were not originally developed to target tumour cells and hence does not have the ability to interact with tumour associated bacteria (TAB) [59,60].

In this context, nanotechnology seems a fruitful approach as it can (i) navigate complex microenvironment including microbiome and tumour microenvironment; (ii) specifically interfere with molecular pathways; and (iii) be functional at sites beyond the primary tumour e.g. metastases [61]. Since, the microbiome is patient unique ecosystem that dynamically changes in response to diet, drugs and so on, nanotechnology is capable of performing their targeted delivery and microbiome modulating functions even regulating metabolites that are known for carcinogenesis. Overall, the use of nanotechnology in microbiome modulation and anti-cancer applications is at nascent stage and further studies will be fruitful for exploiting the technology against cancer [61].

The intervention of nanotechnology with the microbiome modulates the microbial metabolites and can be designed to release chemotherapeutic agents upon interaction of microbiome. Nanotechnology was used to interrupt the chemical communication between microbial metabolites and the immune system to improve immunotherapy. Also known for its uses to enable stimuli-responsive drug release of a chemotherapeutic from a nanoparticle to facilitate the suppressing of tumours [62]. Nanotechnology for microbiome modulation plays a specific role, strategies to alter the microbiome composition towards protection from or treatment of cancer can include the addition of beneficial microbial species, deletion of cancer-causing microbial species or modulation of the existing commensal population to promote the proliferation of beneficial anti-cancer microbial species such as those that secrete the short-chain fatty acid [63].

**Antimicrobial probiotics and their mechanism of inhibition.**

There are many known antagonistic mechanisms of probiotic microorganisms, including alteration of the gut microbiota, competitive adhesion to the mucosa, epithelial reinforcement of the antimicrobial gut epithelial barrier, bacteriocins, adhesion, competitive exclusion, anti-inflammatory activity and immune system modulation to convey an advantage to the host [64]. Enhancement of epithelial barrier, Intestinal epithelial cells are in permanent contact with the diverse microbial community and epithelial integrity is essential to defend from pathogenic microorganisms [65]. Once the barrier function is disrupted bacterial food antigen can enter the submucosa and can induce an inflammatory response that leads to infectious diseases like IBD. Consumption of probiotic microorganisms which can maintain epithelial barrier and intestinal barrier function.

Studies show that enhancing the expression of genes involved in tight junction signaling may be the possible mechanism [66] Lactobacillus can modulate the regulation of several genes and junction proteins such as E-cadherin and b-catenin [67]. Mucin glycoproteins (mucins) are significant macromolecular constituents of epithelial mucus and have been used for health and diseases for a long time. However, certain problems associated with acquired resistance of microbes against antibiotics. Hence, researchers find an alternative to antibiotics as well as to minimize the risk of spreading such infectious diseases [40]. Thus, the great advancement in nanobiotechnology provides new tools to formulate innovative biologically originated NPs with antimicrobial potential [40].

Increase adherence to the intestinal mucosa

Adherence to the interaction between the probiotics and the host is also essential for the modulation of the immune system. Antagonism against pathogens intestinal epithelial cells (IECs) secretes mucin which is a complex glycoprotein mixture that can prevent the adhesion of pathogenic microorganisms because it presents lipids, free proteins, immunoglobulins and salt to prevent mucous gel adhesion [68] this interaction indicates possible competitive exclusion of pathogenic bacteria although mucous binding proteins (MBP) surface-associated proteins are present only on probiotic microorganisms. Probiotics such as *L. reuteri*, *L. fermentum*, *L. plantarum* are reported to induce MUC2 and MUC3 mucin to produce epithelial cells, which is responsible to inhibit adherence of enteropathogenic and *E.coli* [69]. Probiotics are bound to microbial binding sites and protect against invasion by pathogens. The establishment of a stable population or commercial microbiota will reduce nutrient availability for entering pathogenic micro-organisms and inhibit their colonization.

**Immunomodulatory Probiotics microbiome**

Probiotic microorganisms exert an immunomodulatory effect and interact with epithelial, dendritic cells (DCs) and with monocytes/macrophages and lymphocytes. It is studied that probiotics can interact with IECs and encounter DCs which have an important role in innate and adaptive immunity, through pattern recognition receptors (PPRs). Probiotics also improve the normal immune system by increasing the concentration of IgA producing plasma cells, improving phagocytosis as well as increasing the cell concentration of T-lymphocytes and natural killer cells. Some probiotics have been shown to increase phagocytosis or natural killer cell activity and interact directly with dendritic cells [70]. some are also upregulating the antibody secretion to improve defence against pathogenic microorganisms. Probiotics can increase the level of anti-inflammatory cytokines such as TNF [71].

In probiotics mainly LAB produces lactic acid and acetic acid as an end product of carbohydrate metabolism, and an increase in butyrate and other SCFA production [72] also by producing, bacteriocin contains antimicrobial proteins, peptides, antibiotic compounds etc. can be active against pathogenic microorganisms. After prebiotic consumption such as Galactooligosaccharides (GOS) consumption induce immunity by enhancing phagocytosis activity and natural killer cells and also maintaining Th1/Th2, although probiotics may show positive effects by enhancing non-specific (Innate) and antigen-specific (Adaptive) Immunity.

Engineered nanomaterials (ENMs) have been extensively used in a variety of industrial fields as well as in everyday life, raising questions about any potential negative effects. While ENMs do not typically appear to have negative effects on immunity or cause severe inflammation, it is less clear how these effects may manifest themselves indirectly. In particular, since the gut microbiota has been tightly associated with human health and immunity, it is possible that ingested ENMs could affect intestinal immunity indirectly by modulating the microbial community composition and functions [73].

In this viewpoint, some supporting data shows a potential relationship between ENM exposure, gut microbiota, and host immune response. According to some experimental studies, prolonged exposure to ENMs may alter the gut microbiota, which would affect the integrity of the intestinal epithelium and the degree of inflammation. Numerous microbiota-derived substances present in this microenvironment, including but not limited to SCFAs and lipopolysaccharide (LPS), may play a significant role in the ENM effect on intestinal immunity. As a result, upon ENM exposure, the gut microbiota is implicated as a critical regulator of the intestinal immunity. In order to evaluate ENM biocompatibility and immune-safety in the future, it is necessary to include gut microbiota analysis [73].

# Diet-Induced Microbiota Profile and Immunity

The human gastrointestinal tract (GI tract) is the site of focus where many kinds of reactions occur. However, recent discoveries have made it possible to answer the questions of how and why the GI tract is the focus of these reactions. The human GI tract lining consists of trillion cells of MOs such as bacteria, yeast, and archaea that form a complex microbial community called the gut microbiome. The gut microbiome plays a vital role in digestion, fermentation of complex dietary compounds which are indigestible to humans, protection from virulent pathogens, acting as producers of vitamins, neurotransmitters, maintaining human health by modulating host immunity, production of signalling molecules such as cytokines, maturation of immune system, etc­. Belkacem et al. reported that the administration of *Lactobacillus paracasei* and *L. Plantarum* in the GI tract modulated the immune system via regulating cytokine secretion and increasing immune cells in the lungs such as natural killer cells, macrophages and dendritic cells in influenza virus infection [74]. However, the balance of gut microbiota profile is of utmost importance as it plays a crucial role in maintaining human health throughout the life of an individual, and also, they are vital in providing the first line of defence. The gut microbiome seems to be very sensitive and does often change into several extrinsic and intrinsic factors such as genetics, dietary habits, age, geographic location, and ethnicity. Amongst the above-mentioned factors, dietary habit seems to affect the gut microbiome with a huge impact that is substantially observed in the research studies [75].

# Role of microbiome in Viral Infections

The human lungs have been adapted and improved the protection mechanisms from last hundreds of years to fight the invading infective viruses using the first line of defence system *viz.* mucus induction, continuous motion of cilia, nonspecific inhibitors for viral replications, secretion of Immunoglobulin A (IgA) in respiratory tract infections, etc. [76]. At the onset of a viral infection, a cascade starts that activates the body’s natural immune mechanism. Initially, Toll-like receptors (TLRs) mediate the antiviral immune responses by recognizing virus infection and activating the signalling pathway leading to the secretion of chemokines and cytokines such as interferons (IFN) type I. Chemokines activate the natural killer cells (NK cells) that result in disruption of viral RNA and stop replication. Furthermore, the dendritic cells (DCs) lead to an activation of CD4+ and CD8+ cells and develop antigen-specific T and B lymphocytes mediated immunity that works together to get rid of the invading infective stage [77]. Microflora other than the digestive system, particularly in the lungs is also established to fight the incurring viral infections by modifying and supporting the natural immune process called immunomodulation. MOs and their secreted metabolites interact with TLRs, IFN, DCs, and T regulatory lymphocytes along with other chemokines and cytokines which are responsible to induce host immunity [78]. Human microflora plays a key role to support innate and adaptive immunity whereas probiotics are proven to stimulate host immunity via immunomodulation of the immune system. These probiotic microbes translate the innate immunity and induce the acquired immunity that results in the stimulation of specific and non-specific immunity [79]

There are reports that probiotics such as *Bifidobacterium breve* shows anti-influenza effect by increasing the production of IgA, and IgG [23]. Hepatitis A and B were found to be reduced by *Lactobacillus acidophilus* and *Bifidobacterium bifidum* while *Thermophilus sp.* is known to work as an anti-herpetic agent [80]. Similarly, *Bifidobacterium lactis* and *Saccharomyces boulardii* can be used in antiviral therapy against Rotavirus [81]. A clinical study has reported that daily consumption of probiotics by HIV infected people showed improvement in CD4+ count [82]. It is also suggested that the consumption of probiotics like LAB and *Bifidobacteria* are found to reduce the risk of upper respiratory tract infections [83]. An animal study demonstrated that oral administration of probiotic strains like *Lactobacillus pentosus, L. casei, L. Plantarum, L. bulgaricus, L. rhamnosus, L. gasseri, L. Brevis,* and *B.breve* helped to suppress symptoms of virus infection [84].

## Anticipation of the Immunomodulatory Role of Probiotics in SARS-Cov-2 Infection

# The Lung Microbiome

A vast variety of microbial communities inhabits the human body that is found to be more prevalent on mucous membranes and play a vital role in various metabolic processes [85]. Historically, the lungs were thought to be sterile and free from any microbial contact, yet it is constantly exposed to microbiota through inhalation. Over the past decade, studies helped to understand how lungs and microbiota interact and exist together [86]. In comparison with gastrointestinal microbiota, lung microbiota hosts relatively lower microbial communities that range from 4.5 to 8.25 log CFU/ml as the lung hosts low nutrients than the intestinal tract [87]. Several studies have been conducted to explore the healthy lung microbiome that comprises two main phyla Bacteroidetes and Firmicutes [88]. However, other studies also postulated the dominance of phyla such as Proteobacteria, Actinobacteria, and Fusobacterium along with a relative abundance of Firmicutes and Bacteroidetes. A genus-level study by Erb-Downward *et al.* (2011) showed a dominance of *Pseudomonas, Streptococcus, Prevotella, Fusobacterium, Haemophilus,* and *Porphyromonas* in the lower respiratory tract of healthy individuals [87]. Others reported a lower abundance of genera*, Veillonella, and Leptotirchia,* while an ample amount of *Lactobacillus* and *Rothia* [89].

The healthy lung microbiome is sensitive to factors such as oxygen tension, blood flow, luminal pH, temperature, inflammation, allergen, and more precisely to the pathogenic MOs that may result in respiratory ailments and disorders [90]. The majority of respiratory infections are airborne and are caused by MOs that can travel and escape from mucosal and ciliary activity of epithelial cells present in the upper respiratory tract and adhere to the epithelial lining of the lower respiratory tract and profoundly multiple in lung alveoli. The result of infection would provoke immune-stimulating responses stimulating the respiratory microbiome to play a part in the prevention of respiratory infections.

Chronic obstructive pulmonary disease (COPD) is a group of respiratory diseases that are characterized by chronic obstruction of lung airflow which interferes with normal breathing. Many scientists have analyzed the lung microbiome of COPD patients and observed a lower bacterial diversity when compared to healthy populations [91]. At the genus level, the relative abundance of *Pseudomonas* was found which is one of the known opportunistic pathogens [92]. A similar study was stated by Huang *et al.* (2014) in COPD patients that observed enrichment of Proteobacteria *viz.* Moraxellaceae, Patuerellaceae, Pseudomonadaceae, and Enterobacteriaceae and concomitant reduction in the levels of Actinobacteria, Clostridia, and Bacteroidia [93]. ARI also show similar microbial signatures as that of COPD patient with an enriched microbiota of *Moraxella, Streptococcus,* and *Haemophilus* [94]. Pneumonia is characterized by flooding of fluid in the alveoli of lungs that contains enough nutrients and creates oxygen barrier conditions, hence impairing its clearance by ciliary action of epithelial cells and thereby facilitating the growth of the microbial community with the dominance of pathogen, progressing the disease [95]. Recent data suggest a reduction in the pulmonary microbial diversity and reduction in *Rothia*, *Lactobacillus,* and *Streptococcus* which increases the risk of pneumonia, predominantly in the nasal mucosal lining [89]. Additionally, patients with HIV in later stages showed dysbiosis in respiratory microbiota with an increase in Prevotella and Veillonella group amidst the treatment and this microbial signature persists for years [96].

Thus, it seems that a healthy lung microbiome responsible for the normal function of lungs, generally habitats the dominance of phyla such as Proteobacteria and Fusobacterium along with a relative abundance of Firmicutes and Bacteroidetes with a higher abundance of *Lactobacillus*. Phyla such as Proteobacteria and Fusobacterium are generally responsible to initiate a pro-inflammatory immune response that leads to the severity of the disease while on the other hand, *Lactobacillus* genera modulate the immune response by activation of Treg cells. These MOs are evidenced to play an important role in different respiratory diseases by creating an immunological barrier.

## The Gut-Lung Axis

The gastrointestinal microbiota can modulate lung microbiota majorly through the impact of microbial metabolites produced by the gut microbiome. Dysbiosis in the gut is found to be linked with various diseases and respiratory infections are one of them [97]. One study has reported a decrease in the density of *Bifidobacteria* while a simultaneous increase in *Clostridia* in the intestine is associated with asthma [98]. Another research showed that the influenza virus infection in the respiratory tract significantly increased the count of Enterobacteriaceae with a concomitant reduction in *Lactobacilli* as well as *Lactococcus* levels were seen in gut microbiota [99]. Furthermore, depletion in microbial diversity by antibiotics in the gut increased the infection rate of influenza virus infection in the lungs when studied in a mouse model. these findings corroborate that the gastrointestinal tract and lung are intensively linked organs that influence each other’s homeostasis.

Nanoparticles, gut microbes and SARS-CoV2

In the past twenty years, nanotechnology has been developed into a topic that applies to many subfields of study and may be utilised to produce nanoscale materials using a variety of processes, including chemical and physical processes. Nanoparticles have dimensions ranging from 1 to 100 nm and possess features that can be controlled precisely. These qualities are distinct from what the particles appear to be on a larger scale. This enables them to be employed in novel contexts [100]. Nanoparticles are used in many biomedical applications because of their unique properties. These include diagnostics, medical imaging, treatments, and medication delivery, all of which are being increasingly utilised in the management of SARS-CoV2 in the modern era. Based on what has been said, nanotechnology may be very important for quickly diagnosing COVID-19, keeping track of it, and coming up with effective ways to treat it, especially about how SARS-CoV2 affects the gut [101]. With arrays of nanomaterials, non-invasive breath tests can identify the presence of volatile organic compounds with the signatures of modulated microbiota and, therefore, the presence of SARS-CoV2 for quick diagnosis and monitoring [102,103]. On the other hand, a healthy gut is also important in SARS-CoV2 infections. Some studies point out the importance of good microbes in fighting this virus [104]. Nanotechnology can be used effectively to design smart drugs or functional foods that can be delivered locally in the gut. It can also be used to design smart functional foods [73,105]. These drugs and foods should go after bacterial strains that cause problems in the GI tract and improve its health by making the gut more resistant to pathogens and inflammatory chemicals and by laying the groundwork for developing disruptive treatments based on microbiome engineering [103]. We may one day be able to watch, traverse, and interact with the intricate ecology of the gut if we have the assistance of technologies that can function at the nanoscale level. This may assist us in locating a therapy or cure for COVID-19 as well as in maintaining control over SARS-CoV-2.

Immunomodulation and Anti-COVID mechanisms

Yet, no direct relation and study are available to justify the role of probiotics against SARS-CoV2 infections but many previous studies regarding probiotics and viral infections can be used to implement the possible mechanisms and their role. The pathogenesis of SARS-CoV and SARS-CoV2 relied on a common entry point by interacting with the ACE2 receptor present on epithelial cell surfaces in the lung and intestine. In the certain report of SARS-CoV2 infection, it has been postulated a dysbiotic condition caused by *Salmonella America*, a member of Enterobacteriaceae family was found to be abundant that increased the level of ACE2 receptors in the epithelial cells of the intestine resulting it to be more prone to get infected from these viruses [106]. The SARS-CoV2 virus has to surpass the immunologic barrier of respiratory tract epithelial to invade the cells through the ACE2 receptors whereas the probiotic microbes with commensal bacteria may help the immune system to reduce or inhibit this infection through immunomodulation.

Although, probiotics do not show a direct effect it creates an immunologic barrier by stimulating an immune response that supports the first line of defence of the body [107]. Generally, the probiotics interact with lung and intestinal epithelial as well as specialized cells (M cells) for immunoregulation through interaction with macrophages and dendritic cells which leads to activation of T and B lymphocytes. It may hamper the viral attachment by competitive inhibition via blocking the binding sites on the epithelial lining. The probiotics induce the upregulation of mucin-1 (MUC1) and mucin-2 (MUC2) which can also prevent the attachment of the virus to an epithelial cell and suppress replication. Finally, it also produces antimicrobial peptides and dehydrogenase and nuclease enzymes which can break down the viral nucleic acid, and also the co-aggregation of probiotics with viral particles interferes with the attachment of the virus to the epithelial cell line [108]. Probiotics also have a significant role in the induction of type 1 T helper (Th1) cell which is specific for antimicrobial/antiviral mediated immunity whereas IFN which is a glycoprotein and IgA are considered antiviral agents. One of the important molecules produced by probiotic MOs by breaking down the prebiotic compound is short-chain SCFA. It influences the immune system and induces pattern recognition receptors (PRR) by activating tumour necrosis factor- α (TNF-α). More precisely, probiotics like *Lactobacillus* and *Bifidobacterium* modulate the immune system by regulating the cytokines, increasing the production of IgA and IgG antibodies [109]. Specifically, the *Lactobacillus* species like *L. acidophilus, L. casei, L. rhamnosus, L. helveticus* are effective to enhance phagocytosis and improve the secretion of cytokines, immunoglobulin and plasma cells, as shown in a study, *L. casei* and *L. acidophilus* induced the interleukin (IL) such as IL-10 and CD4+ regulatory T (Treg) cells. Moreover, the administration of *L. Plantarum* and *L. reuteri* reduced inflammation while *L. rhamnosus* and *B. lactis* increased IFN-γ, IL-4, IL-10, and IL-6 in bronchoalveolar lavage [84]. Besides, probiotics can induce the level of Bcl2 (B cell lymphoma 2), which is responsible for the activation of cellular and humoral immunity leading to the activation and production of the cytokines along with Th1/Th2 expression.

Probiotics have also been studied for their influence on immune-related gene expression and activation of cytokines, depending on the contact-based mechanism. A study suggested that probiotics like *Lactobacillus* mediates the expression of TLR2 which stimulates TNF-α while *Bifidobacterium longum* mediated expression of IL-10 and IL-12 via a contact-based mechanism that resulted in the modulation of T helper cell response in the gut and lung [110].

The oral administration of 109 CFU of probiotics is known to be more effective that may exert long term homeostasis and immunomodulatory effect on the host. Oral administration of *Bifidobacterium bifidum* and *B. breve* have also been shown to increase humoral immune responses such as stimulation of IgA [111]. Thus, probiotics also show the possibility to use as a live vaccine for oral immunization. Moeini et al. (2011) used *L. acidophilus* as a live vehicle for oral immunization against chicken anaemia virus (CAV). The ACMA-binding domains present on the surface of *Lactococcus lactis* were used to display the viral protein 1 (VP1) CAV on *L. acidophilus* to immunize specific-pathogen-free chickens through the oral route. The immunization increased the levels of Th1 cytokines, such as IL-2, IL-12, and IFN-γ [112]. Furthermore, some studies have shown that probiotics can enhance the outcome of influenza virus infection when administered through the nasal pathway. The nasal administration of *Lactobacillus rhamnosus* strains CRL1505 and CRL1506 were able to improve respiratory antiviral defences and beneficially modulated the immune response by triggering the TLR3 and PRR (RIG-I, a retinoic acid-inducible gene I) against the respiratory syncytial virus (RSV) [113].

It has been now clear that probiotics are microbiota that works as a potential barrier in the case of any viral attack through immunomodulation as described earlier (Figure 1). It may act indirectly through competitive inhibition or directly via the interaction of immune cells by producing chemokines, and cytokines, and also be involved in other immunologic pathways. In light of this information, we can anticipate the possible role of these probiotics in the protection or reduction of the SARS-CoV2 infection. In this context, a model has been represented here showing the expected immunomodulatory role of probiotics along with prebiotics which may take place on the onset of SARS-CoV2 infection in a more or less similar way.



Figure no. 1 Anticipation of the Role of probiotics in immunomodulatory

**Conclusion**

Role of gut microbiome in our body is extensively hot topic of research. These microorganisms are extremely important for production of enzymes, vitamins, biomolecules and modulation of metabolic pathways and immune system. The main problem is the lack of specificity in target-oriented modulation of the microbiota and metabolites. This limitation can be addressed using nanotechnology. Research on nanomedicine formulations for diagnostic and therapeutic purposes has produced a number of successful platforms, including those for integrated diagnosis, targeted drug delivery, and therapeutics. Using nanoparticles as a delivery system for gut microbiota influences the route of biomarker detection and the route of the interaction of nanoparticles with target cells. In this chapter we discussed how different diseases are correlated with gut microbial profile and reverting dysbiosis can solve the problem with intervention of nanotechnology.

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