Review Article

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A Revitalising Approach towards Aging: Unveiling the Potential Use of Probiotics for Wellness in Elderly Populations

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Abstract:

Probiotics have emerged as a potential alternative for treating various diseases affecting general population. Mounting data suggests that probiotics can be beneficial for older individuals, who often have weakened immune systems and are more susceptible to illnesses. Dysbiosis commonly occurs in elderly individuals, which is implicated as a major reason for various diseases. The effectiveness of probiotics is influenced by the specific strain and the dosage administered. Extensive studies have shown how probiotics affect prevalent issues, such as functional bowel diseases and other gastrointestinal disorders. Probiotics exert their effects by synthesizing different microbial peptides, such as bacteriocins, antibiotics, lipopolysaccharides, short-chain fatty acids, butyric acid, propionic acid and other specific secretory metabolites. The common probiotics that are found to benefit the health of elderly populations include *Lactobacillus*, *Bifidobacterium lactis, Bacillus subtilis*, *Bifidobacterium longum and Bifidobacterium breve* Further research is necessary to delve into the mechanism of action for both existing and novel probiotic strains for use in combating or aiding in diseases. Genetically, modified probiotics can be potentially used in the future to deliver immunity-restoring genes and as oral mode of vaccination in humans. Fecal microbiota transplantation has been gaining interest, used to restore gut conditions, and has also been linked to alleviating neurodegenerative diseases. The focus of this review was to present the available evidence on the immunomodulatory effects of probiotics in the elderly population; aiming to gain a better understanding of their mechanism of action and to assess the progress made in utilizing probiotics as therapeutic interventions.

Keywords: bacteriocins, disease management, dysbiosis, gut homeostasis, immunomodulatory, immunosenescence, microbiota therapy, probiotics

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Introduction

Probiotics are living microorganisms that, when appropriately administered, can confer health benefits to the host[1]. Probiotics are administered in the form of capsules, tablets, powder, drinks and fermented foods, such as yogurt[2]. For a strain of bacteria to be called a probiotic, it has to confer specific properties, such as resistance to gastric acid and bile acids, bile salt hydrolase activity, ability to adhere to mucus or epithelial cells, exhibit antimicrobial properties and potentially reduce the infection of pathogenic bacteria in the gut[3]. No specific mode of action is clearly understood; however, probiotics are known to exhibit competitive exclusion by reducing the luminal pH and competing for nutrition and resources, majorly by producing bacteriocins or specific metabolites[4]. The commonly used probiotics include: Lactobacilli, such as *Lactobacillus acidophilus*, *L. paracasei*, *L. casei*, *L. rhamnosus*, *L. plantarum*, *L. delbrueckii* subsp. *bulgaricus*, *L. brevis*, *L. johnsonii*; and *Bifidobacteirum* such as *Bifidobacterium infantis*, *B. breve*, *B. adolescentis*, *B. animalis* subsp *animalis*, *B. animalis* subsp *lactis*, *B. bifidum* and *B. longum*[3].

Various species of Lactic acid bacteria (LAB), such as *Lactobacillus*, *Lactococcus*, *Bifidobacterium* and *Leuconostoc*, are prominent exopolysaccharide (EPS) producing probiotics[5]. The health benefit of LAB can be conferred to its production of EPS that are claimed to have immunostimulatory activity, antitumor potential and cardiovascular effects[6]. Probiotic EPS can be supplemented in diseases, such as inflammatory bowel diseases, autoimmune diseases, gastric ulcers and cardiovascular disease, as they have the ability to survive in the antagonistic conditions of the gut and also act against antibiotics, which suppresses the immune responses and affects the gut microflora if ingested regularly[5].

Aging is the functional deterioration of the body due to a multifactorial process that occurs in the body caused by DNA damage, resulting in immunological alterations[7]. As individuals age the bacterial metabolites produced are also are altered, such as short-chain fatty acids (SCFA), acetate, butyrate, lactate and succinate. SCFAs play a major role in preventing gastrointestinal diseases, such as metabolic syndromes and bowel disorders.

Antibiotic resistance is a leading global problem in medicine and cures. Antibiotic resistance has led to many problems, such as antibiotic-associated diarrhea and gut dysbiosis, which leads to an imbalance in the microflora. *Lactobacillus*, a gram-positive bacteria, has a wide range of applications as a probiotic. Previously, different strains of Lactobacillus have been observed to have deliberate, beneficial effects like inhibition of pathogenic bacteria, such as *Helicobacter* and *Salmonella*, alleviation of gastrointestinal inflammation, antibiotic-associated diarrhea and boosting of the immune system, *L. paracasei* has also been observed to have potential anti-colon cancer properties[8-9]. Probiotics produce specific metabolites, such as conjugated linoleic acids, which are proven to prevent the differentiation of cancer cells[10]. Metabolites, like butyrate in colon cancer cells, were found to inhibit cell growth and differentiation through their action on the inhibition of histone deacetylases[11].

Different probiotic strains can treat divergent diseases based on their mode, mechanism of action and dosage. *Bifidobacterium* and *Faecalibacterium prausnitzii* both exhibit anti-inflammatory responses and are seen in lower numbers in diabetic individuals[12,13]. Probiotics *Lactobacillus casei, Lactobacillus acidophilus* has been observed to lower blood glucose level and alleviates diabetes-related symptoms, such as dyslipidemia, glucose tolerance, hyperglycemia and oxidative stress in trials on rats[14,15]. Probiotics potentially modulate the toll-like receptors (TLR) and proteoglycan recognition proteins in enterocytes leading to activation of Th1 responses, which can supress Th2 responses in allergic disorders. Allergies are associated to a shift in Th1/Th2 cytokine towards a Th2 response causing the release of interleukin-4, interleukin-5, interleukin-13 and IgE production involved in

allergic reactions[16,17]. *L. plantarum* was shown to increase IL-10 concentration, indicating the anti-inflammatory effects, ameliorate inappropriate inflammation and induce tolerance[18]. Additionally, the *Bacteroides fragilis strain ZY-312* has been previously found to be beneficial in antibiotic-associated diarrhoea (AAD) in mouse model studies through epithelial cell proliferation and differentiation[19].

As individuals age, there is a decline in liver volume and blood flow as well as which liver regeneration is slower. The liver has an indirect connection with gut microbiotia. When the liver is under stressed conditions, due to various diseases, the intestinal microbiota undergo dysbiosis, which accelerates the liver fibrosis and cirrhosis process by increasing inflammatory responses. Animal model-based studies have suggested that aging leads to a decline in the drug-metabolizing ability of the liver, decreased microsomal content of cytochrome p-450 and decreased alcohol dehydrogenase activity[20-22]. Non-alcoholic fatty liver disease is predominantly a disease affecting the middle aged to older people, and is characterized with excess fat deposition in the liver, advanced fibrosis and can also lead to hepatocellular cancer[23]. Studies have shown that some strains of probiotics can improve liver conditions by bringing about changes in the gut, via decreasing the production of endotoxins and toxic substances, such as ethanol, phenol, and indoles, which have the tendency to damage the liver. They help downregulate NF-κ B and reduce the production of pro-inflammatory factors, such as TNF-α, IL-6 and IFN-γ. Commonly targeted probiotics include *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei*, *Lactobacillus bulgaricus, Bifidobacterium berve, Bifidobacterium longum* and *Bifidobacterium infanti;* however, most of the experiments have been conducted on animal models and the data on human clinical trials is insufficient[24].

Probiotics regulate host bile salt metabolism through a series of enzymatic reactions and exhibit bile salt hydrolase activity. Any disruption in bile salt pools can lead to dysbiosis, which is common in elderly populations. The microbiota express, farnesoid X receptor (FXR), a bile acid-activated regulatory element and G-protein-coupled bile acid receptor (TGR5), and any imbalance in the gut can affect the probability of acquiring liver[25]. The Probiotic strains *Bifidobacterium* and *Lactobacillus* bacteria have shown bile salt deconjugation activity and high bile salt hydrolase (BSH) activity[26]. Several probiotic strains have a cholesterol-lowering activity, such as *Lactobacillus acidophilus, L. bulgaricus* FTCC 0411, *L. reuteri* NCIMB 30242 and *L. delbrueckii ssp. bulgaricus* A13[27-31].

Certain strains of probiotics, which confer beneficial effects, can also be pathogenic to a few. Studies have found that Bacteroides can cause endotoxicity in host individuals with diabetes or surgical patients, as these strains have lipopolysaccharides that can activate TLR-2 and TLR-4 signaling[32]. Probiotics can also be used as adjuvants to antibiotic therapies to increase the effectiveness of the treatment. Previously Beausoleil et al, employed $50x10^9$ CFU units of *L acidophilus* CL1285 and *L casei* on elderly patients with gastrointestinal disorders and found that the response to treatment in the probiotic-administered group was better than the placebo group. This review summarizes the various findings and evidence about probiotics' use and potential therapeutic applications on the elderly and older populations[33].

Sources and secretory metabolites of probiotics

Different strains of the same probiotic can have different effects on the human body, and a single dose cannot be generalized to be effective for all strains[34]. The common source of probiotic isolation is dairy, and dairy-related products, such as *Lactobacillus* and *Bifidobacteria,* which are obtained from fermented milk. The probiotic bacterial strain *L. plantarum* was previously isolated from Italian, Argentinean, and Bulgarian cheeses[35]. Human

breast milk has also been recognized as a rich source of probiotics, such as *Bifidobacterium breve*, *Bifidobacterium bifidum*, *Bifidobacterium adolescentis*, *Lactobacillus gasseri*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, and *Lactobacillus salivarius,* which confers to infant's enhanced development, increased immunity and a healthy gut[36]. In an experiment, Li et al, isolated probiotic *B. velezensis* from the faeces of healthy domestic yak, *L. plantarum* from beef cattle faeces and *L. salivarius* from swine to test its performance in improvement of intestinal health in mice[37]. Fermented food products are also sources for the isolation of several probiotics, such as Kimchi (Korean dish) containing several vegetable mixtures, and fermented olives have been used to isolate *L. brevis*, *L. plantarum*; *L. brevis,* and *L. Lactis* from Bamboo shoots; and *L. rhamnosus* from fermented cabbage[38]. The animal gut also harbors several naturally beneficial probiotics, which can be isolated from the faeces of the organism. Handajani et al, isolated *Limosilactobacillus fermentum* from tempeh, a traditional Indonesian food made of fermented soyabeans, to study the effect of probiotics on an elderly population with cognitive impairment[39] (Table 1).

The diverse synthesis of antimicrobial peptides by probiotics called: bacteriocins, helps it compete with the gut pathogens and also modulates the insensitive bacterial species in the Gastrointestinal (GI) tract[40]. Bacteriocins can impart immunity by releasing cell lysing compounds that cause viral lysis, Lantibiotics, which are class I bacteriocins, inhibit bacterial cell wall biosynthesis by binding to carrier molecules of peptidoglycan monomes required for building the cell wall; called the Lipid II, and also use this lipid molecule as a docking molecule to initiate pore formation in the bacterial membrane. Certain bacteriocins (class IIc) can directly interact with the bacterial membrane without any receptor molecules[41].

Microbes colonize the body soon after birth and act to strengthen the gut epithelium. The imbalance in gut microbes, due to several factors in aged individuals,

Table 1 Different sources of isolation of probiotics strains

Source	Probiotic strains	References
Milk	*Lactobacillus*	39
Breast milk	*Bifidobacterium breve*, *Bifidobacterium bifidum*, *Bifidobacterium adolescentis*, *Lactobacillus gasseri*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, and *Lactobacillus salivarius*	
Cheese	*L. plantarum*	42
Butter milk	*Lactococcus lactis*, *Lactobacillus bulgaricus*	52–53
Whey beverage	*Bifidobacterium lactis*, *Lactobacillus acidophilus*	52–54
Kimchi, olives (fermented food)	*L. brevis*, *L. plantarum*; *L. brevis,*	43
Bamboo shoots	*L. Lactis*	39
Fermented cabbage	*L. rhamnosus*	42
Tempeh	*Limosilactobacillus fermentum*	43
Fermented drink Neera (coconut)	*L. brevis*	55
Kefir (acidic–alcoholic fermented milk product)	*L. kefiranofaciens, Lactobacillus acidophilus, L. delbrueckii ssp., L. plantarum*	56
Fruits (guava, green apples, corn, oranges)	*Lactiplantibacillus plantarum, Leuconostoc pseudomesenteroides, Weissella confusa , Leuconostoc mesenteroides*	57
Faeces (Yak and Cattle)	*B. velezensis*, *L. plantarum*	37

causes dysbiosis, alleviating human diseases, such as an observed increased fat diet in individuals suffering from type II diabetes, which has been associated with increased concentrations of lipopolysaccharides and decreased numbers of *Bifidobacterium* in the gut causing metabolic endotoxemia[42,43]. These microbes secrete several metabolites by metabolizing the undigested substrate of the human gut such as SCFAs, including acetic acid, butyric acid, propionic acid and proteins. SCFAs play an essential role in regulating the pH, and increasing the absorption of ions such as magnesium, iron and calcium, and aid in the metabolism of glucose and proteins in the liver. They also help in maintaining gut integrity, via mucus production as well as the function in immunomodulation, appetite regulation and overall homeostasis[44,45]. Butyrate, mainly produced by *Bacteriodetes* and *Firmicutes,* regulates epithelial cell proliferation, differentiation, anti-inflammatory responses, apoptotic pathways in addition to exerting its effect on gut T-regulatory cells (Treg cells). Probiotics secrete glycoproteins, which exert its effects on gut physiology, and may be involved in immunomodulation of the host tissue, surface recognition and molecular cross-talking where components of one signal pathway affects the other; signalling cascades. Probiotic *L. plantarum* has been identified to secrete proteins, such as PL23: known as muramidase and PL24; known as GAPDH. Muramidase surface proteins cause hydrolysis of the peptidoglycan layer of bacteria and are involved in cell wall turnover, cell separation and division. GAPDH is a cytoplasmic protein found to bind to human colonic mucin and blood antigens[46]. *L. rhamnosus* GG (LGG) secretes proteins p40 and p75, which promote intestinal epithelial cell homeostasis via pathways and involving 3 main mechanisms. These soluble proteins transactivate the epidermal growth factor receptor, which upregulates expression of a proliferation-inducing ligand (APRIL), that in turn promotes the production of Ig-A immunoglobulin and impairs the cytokine induced apoptosis in small intestinal epithelial cells of mouse models. Intestinal bacteria trigger T-cell dependent B-cell class switching for IgA production via expressing signalling cytokines, such as APRIL[47]. The activation of EGFR by p40 ameliorated apoptosis of colon intestinal cells and chronic inflammation in oxazolone-induced colitis[48]. P70 and p40 can also stimulate the epithelial cells to produce heat stress shock proteins Hsp72 and Hsp25 that protects the tight junctions, and activate the signalling pathway Akt-pathway in phosphatidylinositol 3-kinase-dependent manner, which increases the proliferation and survival of the guts epithelial lining[48,49]. Fermented soya milk containing probiotic *L. rhamnosus s*howed hypotensive properties, higher antioxidative properties and blood pressure-lowering effects potentially through the production of metabolites, such as aglycone isoflavones[50]. Bacteria also produces antimicrobial proteins; ribosomally synthesized: called bacteriocins, which have a wide range of applications, such as anti-viral, and anti-microbial, and act synergistically with food-grade substances[51]. Certain probiotic strains like the *lactobacillus* could inhibit enterohemorrhagic *E. coli* strains in mice by producing bacteriocins[45].

Dysbiosis in the elderly and diseased population

Aging is associated with various changes in the guts microbiome composition. Aging is often accompanied by dysbiosis, resulting in a significant shift in the ratio of bacterial colonial phyla, or influencing the colonization of new bacterial groups that often leads to imbalance promoting for the occurrence of various diseases. An overrepresentation of pathobionts, such as Enterobacteriaceae, and a loss in the beneficial microflora can also characterize dysbiosis. The elderly have been found to have lower populations of *Firmicutes,* majorly *Clostridium IXVa*, *Faecalibacterium prausnitzi* and *Actinobacteria*[58]. Dysbiosis is associated with aberrations of the gut integrity and enhanced effect of pro-inflammatory cytokines, all such factors eventually can be potent causes of pathogenesis and progress of various other metabolic diseases, which are seen to be more prevalent

in old individuals, such as insulin resistance, adiposity, cardio-vascular diseases, atherosclerosis[59].

A pro-inflammatory state caused because of the expansion of leakage of microbial contents leads to an inflammatory condition that impairs the nervous system[60]. Due to the reduction of beneficial commensals, the expression of different metabolites, like the lipid metabolites and SCFA, such as acetate, butyrate, and propionate, get reduced. Acetate, after absorption, increases cholesterol synthesis; while propionate is known to inhibit cholesterol synthesis after being metabolized by the liver. These SCFA together help eradicate pathogenic bacteria by lowering the pH of the gut[61]. The loss of this metabolic activity collectively leads to dysbiosis in adults. Gram-negative bacteria, such as *Enterobacteriaceae,* produce lipopolysaccharides that act as bacterial endotoxins released in the gut, leading to endotoxemia[62]. In a study on rat models, probiotics *Bifidobacterium breve and Lactobacillus fermentum* prevented dysbiosis and reduced endotoxemia mainly by acting on the Tregs[63]. The SCFA, besides providing energy within the colon, also regulates the signaling pathway and plays a part in the regulation of glucose/insulin regulation through their interaction with G-protein receptors, such as GPR41 and GPR43. Probiotics also act as histone deacetylase inhibitors and indirectly act on gene regulation inducing anti-inflammatory responses. The accumulation of pro-inflammatory commensals also leads to age-related morbidities, systemic inflammation and may cause premature death[64]. Decreased microbial diversity due to aging also leads to a decrease in the secretion of major cofactors and the functioning of DNA repair genes, which caused enhanced muscle wasting in animal mice models[64]. The concept that probiotics can have beneficial effects on the health and immunity of elderly individuals has been validated by various previous studies.

A study on 161 elderly subjects over the age of 65 years was carried out to correlate the functional leakage of gut microbiome and the associated health risk through fecal microbiome screening. The results showed that *Bacteroides* dominated 68% of the individuals gut; averaging 57% across the subjects; whereas, Phylum *Firmicutes* had an average of 40% abundance. There also occurred varied proportions of disease-associated genera of bacteria including: *Proteobacteria, Actinobacteria*, and *Faecalibacteria, which* was observed in different individuals. The effects of antibiotics on microbial composition were also observed as a significant reason for causing an increase in Bacteroidetes: Firmicutes ratio and a relative decrease in *Firmicutes.* The data showed lower diversity of microbiota in elderly individuals compared to young adults[65]. Eloe-Fadrosh et al., investigated as to whether *Lactobacillus rhamnosus* (LGG) could alter the transcriptional response of gut microbiota. The trial was conducted on 12 individuals that were administered 10^{10} CFU twice a day of LGG. Metagenomic analysis and 16S rRNA profiling of the gut gene content revealed that *Bifidobacterium* and major butyrate-producing *Roseburia* and *Eubacterium* expression increased through the consumption of probiotic LGG, which suggested an increased interaction between the microbiota and the host epithelium[66]. An animal model study conducted on Kyoto rats (WKY) and hypertensive rats (SHR), to study the effect of probiotics *Lactobacillus fermentum* (LC40) and *Bifidobacterium breve* (BFM), showed that the rats treated with probiotics prevented dysbiosis through lowering the *Firmicutes/Bacteroidetes* ratio and increased butyrate-producing bacteria; resulting in a lower incidence of endotoxemia[67].

Fecal microbiota transplantation (FMT) therapy has recently gained much interest in re-establishing eubiosis in patients suffering from gut-microbial imbalances, aiming to enhance the patient's health. FMT introduces fecal suspension from a healthy donor to a diseased individual's GI to normalize the composition and benefit the patient. FMT is a successful therapy for treating *Clostridium difficile* infection and other gastrointestinal diseases, such as inflammatory bowel disease[68]. Additionally, synbiotics

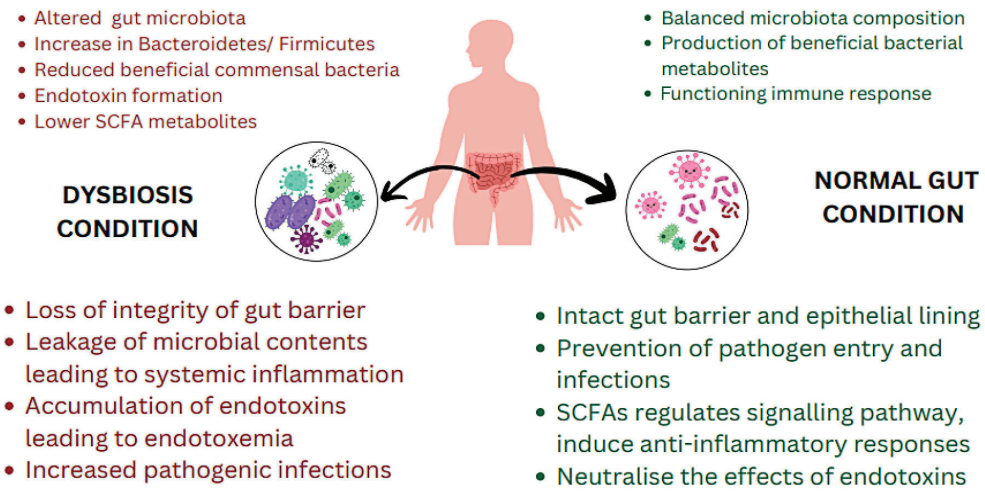
have also been use in probiotic therapy as of late. This is a combination of two or more probiotics, or bacterial strains that improve the growth of beneficial commensals and also stimulate the proliferation of native bacterial strains in the gut. The synbiotics exert their effect by modulating the bacterial metabolite secretions and exhibiting anticarcinogenic effects by inactivating cancerogenic substances[69]. Based on the data available, further analysis is required to potentially use probiotics as a therapy to restore the dysbiosis in the gut and alleviate the symptoms caused because of it.

Probiotics and nutritional therapy for older population

The interplay of gut microbiome in aging

The age related decline, termed as: immunosene scence, is majorly attributed to the significant shift in the composition of the gut microbiome and the deterioration of its functions. Disability, mortality, and age-related disorders that come with aging are accomplice to the imbalance in the gut microbiome structure and dysbiosis. The continuously changing abode of the gut, makes the microbiome able to respond dynamically by changing its bacterial structural build-up and its functions[70].

It was found that elderly individuals contain different gut microbiome associated with several factors, such as lifestyle changes, altered morphology, slower physiological processes, a weakened immune system, deterioration of functioning of various organs and glands, medications and hospitalization. The intestinal microbiota is crucial in expanding and developing gut mucosal and systemic immune systems. A reduction in microbiota diversity is speculated to have an increase in age-related frailty. Studies on the aging intestine of Drosophila suggested a stage of increased immune dysfunction, inflammatory condition, commensal dysbiosis and over-proliferation of intestinal stem cells[71]. Maintaining a state of equilibrium in the gut microbiome can help limit such response and improve overall immune health. A recent study on the mice model showed that in aged mice, imbalanced gut conditions lead to increased gut permeability. This further leads to leakage of microbial metabolites and causes systemic inflammation.



SCFA=short-chain fatty acid

Figure 1 Dysbiosis vs. Normal Gut Flora: a schematic representation of gut conditions

Dysbiosis associated with aging disrupts the balance between pro-inflammatory and anti-inflammatory molecules, leading to a state of inflammation described as chronic and a non-resolving inflammatory state. This increases the exposure of cell inflammatory mediators to tumor necrosis factor- alpha (TNFα), interleukins and C-reactive proteins, which are common in aged individuals[70]. These inflammatory mediators cause age-related morbidities, such as insulin resistance, cardiovascular diseases and a decline in the activity of CNS and mortality.

Several studies and trials have shown that *Lactobacillus* spp. and *Bifidobacterium* spp. can control oral microorganisms; including cariogenic *streptococci*. Several mechanisms of action have been suggested; for example: allowing probiotics to interact directly with dental plaque colonies by disrupting the plaque biofilm through competitive binding to host tissues and for nutrients. Lactic acid bacteria produce antimicrobial compounds, such as hydrogen peroxide, bacteriocins, organic acids and anti-adhesion molecules, which help in inhibition of the growth of other oral bacteria. Indirect actions of probiotics in the oral cavity include modulation of the immune system via interaction with immunocompetent cells. This enhances mucin production, up-regulating host defence peptides and promoting wound healing and angiogenesis[72-73.] *Lactobacillus rhamnosus* GG was found to alter the microbial balance in the oral cavity and reduce the overgrowth of pathogens, such as *Candida,* which are found to increase as aging due to impaired immunity. In the mice model experiment, *Lactobacilli* was seen to shorten the duration of *Candida* colonization in the oral cavity by inducing the production of IL-4 and IFN-γ in lymph nodes and nitric oxide saliva[74,76].

Immunosenescence also contributes to altered bacterial composition leading to bowel diseases as a result of increased intestinal permeability, as there is increased interleukin-6 secretion and elevated claudin-2 levels[77]. Microbiota also affects the gut's immune-modulatory status, which is associated with increased TNF, IL-6 and IL-8[78]. Due to reduced microbial diversity in elderly individuals there is also a reduction in microbiota-related metabolites, such as SCFAs such as butyrate, and propionate, which may be associated with age-related irregular bowel transit, reduced appetite, cognitive decline, hypertension, arthritis, sarcopenia and so forth. This may also increase the susceptibility to Inflammatory bowel disease (IBD) as well as Crohn's disease, due to an increase in pathobionts into the intestinal mucosa. The decline in bacterial metabolites can affect the neuro-immuno axis activation by affecting processes, such as gluconeogenesis, which may also be linked with neurodegenerative diseases[59].

Immuno-modulatory effect of gut microbiome in elderly population

Aging is accompanied by immunological deterioration, which especially affects the immune cells and the cell-mediated immunity due to a decrease in proliferation of the lymphocyte and interleukin-2 production. During aging there is an increased accumulation of reactive oxygen species and an increase in the proinflammatory cytokines[79]. In an experiment conducted by Chiang et al., a group of 50 healthy volunteers, aged 41-81 years old, were given low-fat milk containing 25g/ 200ml of *B. Lactis.* The study was conducted to understand the immune enhancement by supplementing probiotic *Bifidobacterium lactis* HN019. It was observed that groups treated with *B. Lactis* had significant changes in the polymorphonuclear cells phagocytosis and the NK cell's tumor killing activity. The activity was seen higher in individuals with more exposure to the probiotic bacteria having a longer duration of consumption. The consumption of probiotic Lactic acid bacteria increases the body combat ability against infections, which is particularly more relevant in aging individuals that are more prevalent to infections because of decreased immunity[80]. Lefevre, et al, conducted a study on 100 individuals, aged 60-74

years, to analyse the effect of *Bacillus subtilis* consumption on immunity and resistance to common infectious diseases. The subjects consumed $2x10^9$ *B. subtilis* spores per day for 10 days intermittently, with an 18 day break interval, which was then repeated 4 times during the course of the study. The study showed that consumption of probiotics significantly increased the fecal and salivary secretory of IgA antibody concentrations compared to the placebo group. A post-study analysis also showed a decreased frequency of respiratory infections in the probiotic-supplemented group[81].

The bacteria in the gut continuously interact with the intestinal mucosa. Probiotics show various initiating interactions with the intestinal enterocytes that produce immunomodulatory molecules, such as the production of chemokines and cytokines. It was observed that certain bacterial strains, like *Lactobacillus sakei,* induced interleukine production, such as IL-1β, IL-8 and tumor necrosis factor-α. In contrast, another strain *L. johnsonii* stimulates TNF-β in an in-vitro experiment on the expression of anti-inflammatory molecules in a strain-dependent manner[82]. Another study by Galdeano and Perdigon proved that probiotics, or their metabolic products, persist in close association with gut-associated lymphoid tissue. They carried out fluorescence labeling of bacteria Lactobacilli and traced its presence in Peyer's patches of mice, which were made to feed on these tagged bacteria. Fluorescence was detected in the immune cells of Peyer's patches, crypt and lymph nodes in the small intestine[83]. The presence of these bacteria or their metabolic product persistence was time-relative and specific to initiate a specific response. In another clinical trial Akatsu et al., the effect of probiotic *Bifidobacterium longum* BB536 on elderly patients, fed by tube feeding, was assessed for its impact on the determination of immune function and intestinal microbiota composition. The study was conducted for 12 weeks on 45 elderly patients (mean age 81.7 years), who were supplemented with BB536 powder containing $5x10^{10}$ CFU/ 2 g daily twice for 12 weeks, while the placebo group were administered 2 g of dextrin powder. After 4 weeks of the administration period, all the patients were given an influenza vaccination. The results showed that the total *B longum* number increased significantly in the probiotic group compared to the placebo group, and the activity of the natural killer cells also decreased significantly in the placebo group, but not in the probiotic-administered group. The data indicated that long-term ingestion of *Bifidobacterium longum* BB536 could have a beneficial immune modulation effect in elderly patients[84].

Probiotics also exerts their effects on natural killer (NK) cells; experimentally, *L. rhamnosus* and *B. Lactis* were seen to increase the cytotoxic potential of NK cells effectively. This ability decreased after the probiotic consumption was stopped. It was observed that a combination of *L. casei* coupled with dextran enhanced the activity of NK cells. This property of probiotics is linked to the production of IL-12 from the intestinal epithelial cells; an essential cytokine for NK cell activity[85]. In a randomized placebo-controlled single-blind crossover study conducted by[86] 30 healthy older volunteers, aged 55-74 years, were assessed for the immunomodulatory effect of *Lactobacillus casei* Shirota. The subjects were given 2x65 ml of Yakult light drink containing $6.5x10^6$ CFU/bottle. The volunteers showed increased activity of NK cells during the probiotic drink intervention period, while their phagocytosis, mucosal immunoglobulin A production also was seen to be improved. Many other physiological model experiments demonstrated that probiotic colonised guts increased the secretions of various Interleukins, natural killer cells and other immune cells through its immune-modulatory effects. Probiotics can also stimulate IgA production by B-cells and help in humoral immunity by binding to the antigens and limiting infections to the gut epithelium[87] (Table 2).

Probiotics are also involved in maintaining bone health. Osteoporosis has been linked to inflammatory

bowel diseases and the result of intestinal inflammation. In an experiment treating healthy mice with *Lactobacillus reuteri* it was seen to enhance intestinal TNFα levels and enhance bone density. Additionally, it was seen to increase trabecular bone parameters, such as mineral density, bone volume fraction and trabecular thickness in the distal femur metaphyseal region and lumbar vertebrae. However, no such effects were seen in experiments in female mice[88]. In animal models, probiotics have been shown to prevent bone loss by modulating bone resorption formation by osteoclasts and osteoblast cells. They are found to interfere with the concentration of 25-hydroxyvitamin D and in calcium intake, helping to decrease bone loss in elderly and post-menopausal women[89]. Probiotic *Lactobacillus reuteri* on oral supplementation, with bile salt hydrolase, actively increases circulating 25- hydroxyvitamin D levels in hypercholesterolemic adult men and women between aged 20-75 years old[90].

Table 2 Probiotics for elderly: dosage and disease response

Probiotics	Dosage	Effects	Source
Biotics *Bifidobacterium breve and Lactobacillus fermentum*	-	Reduction in endotoxemia and regulate T-cells	79
Lactobacillus rhamnosus GG	10^{10} CFU twice a day	Alters transcriptional responses in gut	82
Bifidobacterium Lactis	25g/200 ml	Increased activity of Natural killer cells and phagocytosis	80
Lactobacillus casei Shirota	2x65 ml of yakult light drink containing $6.5x10^6$ CFU/bottle	Increased activity of natural killer cells, enhanced phaocytosis and musical immunoglobulin A production	81
Bifidobacterium longum **BB536**	$5x10^{10}$ CFU/2 g daily twice for 12 weeks	Enhanced natural killer cell activity, increased number of *B. longum* micro-flora in gut	84
Bacillus subtilis CU_1	$2x10^9$ spores/day	Increased the secretory of IgA antibody, decreased common infections	81
lactobacillus; *L. plantarum, L. paracasei, L. bulgaricus, L. acidophilus*, Bifidobacterium, *B. breve*, *B. longum*, *B. infantis*, and *Streptococcus thermophiles*	$4.9x10^{11}$ lyophilized bacteria/ sachet	Lower incidence of diarrhoea, increased level of serum albumin and prealbumin	95
Bifidobacterium longum	10^9 CFU/day	Higher levels of TNF-α, lower serum IL-1o levels, influences the immune inflammatory response	94
L. johnsonii La1	Fermented milk containing 10^9 CFU for 12 weeks	Improvement in immune response, increase in serum cholinesterase levels and nutritional status, lower level of infection	96
Lactobacillus acidophilus CL1285® and *Lactobacillus casei* LBC80R®	Capsule contained $50x10^9$ CFU	Lower incidence of antibiotic- associated diarrhoea	102
A. muciniphila	$5×10^8$/0.5 ml–$5x10^6$/0.5 ml	Reducing hepatic glycogen levels, resist gluco/ lipotoxicity	19
Pediacoccus pentoseceus, Leuconostoc mesenteroides, Lactobacillus paracasei and *Lactobacillus plantarum*	10^{10} CFU/sachet	Significantly decrease pathogenic bacterial outgrowth, decreases minimal hepatic encephalopathy in cirrhotic individuals	93

CFU=colony forming unit

The use of probiotics for functional bowel disease in an elderly population

Gastrointestinal microbiota and the probiotics have metabolic activity, which helps convert non-digested carbohydrates into shorter fatty acid chains. Many bowel diseases are linked to the presence and diversity of the gut microbiome. Irritable bowel syndrome is characterized by symptoms, such as abdominal pain associated with disordered defecation or bowel habits. The disease at severe levels may lead patients to undergo a hysterectomy, appendectomy or cystoscopy. IBS is denoted as a biopsychosocial disorder; wherein, the majority of three interacting mechanisms are altered: the psychosocial factors, altered motility, leading to bowel dysfunction and increased sensitivity of the intestine walls[91]. Studies have investigated the microbiota associated with the intestine in patients with IBS. Meta-analysis has indicated that probiotics have beneficial effect on abdominal pain and stomach bloating[92].

Inflammatory bowel disease can be described as: ulcerative coitis with continuous inflammation and sores occurring along the large intestine and Corhn's disease; wherein, the inflammation is discontinuous. This leads to diarrhea, internal bleeding in the rectal and abdominal walls. It has been found that *Faecalibacterium prausnitzii* has anti-inflammatory properties, and is naturally found to be in lesser numbers in individuals suffering from IBD[93]. Therapeutic use of probiotics has arisen from the findings that the microbiota has a major role in the predisposition of this disease. Different bacterial strain modules affect different intercellular signaling pathways through different pattern recognition receptors. They mediate their actions by manipulating the intestinal mucosal lining as well as the enzymes and secretions they release. Currently, five different probiotic mechanisms are being focussed on for therapeutics of IBD: 1) the probiotics competitively act on the receptors by preventing the microbial pathogens from acting on them, 2) through their immunomodulatory effect and stimulating the gut-associated lymphoid patches to act on these pathogens, 3) release of antimicrobial factors from the Probiotics, such as lactic and acetic acid, bacteriocins, 4) enhancing the role of the mucosal barrier through probiotics and 5) inducing T-cell apoptosis. Ouwehand et al., conducted a double-blind placebo-controlled trial on 209 elderly individuals to study the influence of *Bifidobacterium* microbiota on the immunity of the elderly, and its association with the level of cytokine regulation. The intervention group (n=56) received an oat-based drink; consisting of 10^9 CFU/day of *Bifidobacterium longum (B. longum)* 2C, the placebo group was supplemented with the non-probiotic product, while the control group were given a commercial oat drink. The study showed that *B. longum* was the most detected probiotic in the gut of subjects (94.2 %), followed by *B. adolescentis* (63.5%). The control group had the lowest levels of IL-10 during the course of the study (6 months) compared to the placebo and intervention group. The tumor necrosis factor (TNF-α) was seen at higher levels in the intervention group. The serum IL-10 levels were significantly lower in individuals colonised with *B. longum* (2.5 pg mL^{-1}), and a similar trend was observed in case *B. animalis.* The results indicated that *Bifidobacterium* may influence the inflammatory response in the elderly correlating to the increased serum level of transforming growth factor (TGF-β1)[94].

A study was conducted on 243 elderly patients with bowel problems and diarrhea to test the impact of the probiotics on prevention of problems with bowel movement and infection. The patients were assigned into two groups. The study group (n=129) received $4.9x10^{11}$ viable lyophilized bacteria containing four strains of lactobacillus: *L. plantarum, L. paracasei, L. bulgaricus, L. acidophilus* , *Bifidobacterium*; *B. breve*, *B. longum*, *B. infantis*, and *Streptococcus thermophiles* and the placebo group (n=114). The results showed that the incidence of diarrhea was significantly

lower in the study group compared to the placebo group. The serum albumin, prealbumin and level of protein was also significantly increased in the treatment group among patients aged lower than 80 years of age[95].

In another study, conducted by Fukushima, et.al., 24 elderly patients (above the age of 70 years) were given probiotics fermented milk to test the effects of probiotic *L. johnsonii* La1 on infection incidence and nutritional status. The subjects in the experimental group were administered fermented milk containing 10^9 CFU and non-probiotic strain S*treptococcus thermophilus* at 10^8 CFU/90 g for 12 weeks. It was observed that the overall immune response improved in addition to the infection duration being reduced after administering fermented milk containing the *L. johnsonii* La1 probiotic. There was also an increase in serum cholinesterase levels, a marker for liver function seen in the probiotic-administered group[96].

Probiotics in prevention of diarrhoea in an elderly population

Diarrhoea is characterised by loose, watery stools, possibly from frequent bowel movements associated with bacterial infections, viruses in the intestine, malabsorption of food and certain toxins released through food intake. At severe times, it is accompanied by symptoms, such as vomiting, abdominal pain, weight loss, and fatigue. If the condition lasts for more than 2 days it is called: acute diarrhoea. It can be self-treated by replacing the lost fluids and electrolytes through electrolyte replacement drinks or intravenous therapy. Certain probiotics, such as *Lactobacillus GG Saccharomyces boulardii* and a combination of *B. lactis and S. thermophilus* have been tested in preventing ADD[97]. A study conducted[98] on 135 patients (mean age 74 years), having antibiotic unrelated diarrhoea, were treated with probiotic yoghurt drink containing *Lactobacillus casei* ($1x10^8$ CFU/ml, *S thermophilus* (1.0×10^8 CFU/ml), and *L bulgaricus* (1.0×10^7 CFU/ml). the participants drank 97 ml of the drink twice before meals. A significant reduction in the incidence of *C. difficile was* associated with diarrhoea and antibiotic-associated diarrhoea in the probiotic group.

Antibiotic-associated diarrhoea (AAD) is an intestinal complication commonly found in older adults of age 65 years and above, due to antibiotic-lead disturbances causing altered microbiota in older patients[99,100]. Castagliuolo et. al. reviewed that *Saccharomyces boulardii* may have unique properties to secrete a particular protease that hydrolyses Toxin A produced by the *C. difficile,* which is the major pathogen involved in AAD and it induces an anti-inflammatory response by secreting IgA antibodies specific for *C. difficile* toxin in the colon[101].

Another study, conducted by Gao et.al on 236 adult patients, for the efficiency of probiotic supplements on AAD, included the samples being divided into 3 groups, all having a differing dosages of probiotics. Group 1 received 1 tablet, group 2 received 2 tablets per day and a third was a placebo group. Each capsule contained $50x10^9$ CFU of *Lactobacillus acidophilus* and *Lactobacillus casei;* with the exception of the placebo group. They observed a dosage response relationship; wherein, a higher dose of probiotics resulted in a lower incidence of AAD. The patients treated with the capsules containing the probiotics reported a shorter duration of AAD[102].

Probiotics in hepatic diseases in an elderly population

Hepatic encephalopathy (HE) is characterised by chronic liver disease: cirrhosis, which causes impaired brain functioning and neurological disorders. A broad spectrum of neurological dysfunction can be observed in patients suffering from HE. The pathogenesis of HE is not specific, but mostly multifactorial. The ammonia produced in the body is considered to be a vital factor for the pathogenesis of HE. There is an observed loss of ammonia production and clearance in patients with HE. Several toxin accumulation

and antibiotic therapies are among the other reasons that can possibly cause HE. Probiotic administration can have beneficial effects on the treatment of minimal HE. In action, probiotics can help treating HE by decreasing urease activity, decreasing ammonium absorption and intestinal permeability together, so as to decrease the total ammonia in the blood portal. In normal human beings, ammonia converts to urea, which is excreted by the kidneys; however, in patients with HE there is an excess of urease enzyme activity of the gut microbiome. The target of these therapies is to block this urease enzyme activity. Probiotics can also dissolve toxins and convert them, so as to produce less toxic products, thereby reducing inflammation and oxidative stress[103].

Ultrastructural changes in the hepatic sinusoidal endothelium and deposition of lipofuscin and multinucleated cells in the hepatic parenchyma generally occur when individuals age. Hepatic malfunctioning can occur due to the bacterial endotoxins released from the gut microflora. Endotoxemia is reconsidered to cause liver damage[104]. Endotoxemia occurs when the death of the bacteria release endotoxins into the bloodstream. Probiotics can be administered to increase the number of beneficial bacteria and decrease the toxic effect of endotoxemia in older individuals[105].

A research experiment was conducted to screen for minimal HE and its potential treatment with synbiotics. Patients with cirrhotic liver disease and minimal HE were randomized to receive oral supplementation of symbiotic preparation, containing freeze-dried *Pediacoccus pentoseceus, Leuconostoc mesenteroides Lactobacillus paracasei* and *Lactobacillus plantarum* (10^{10} CFU per sachet). The patients were divided into 3 groups (aged grouping of 55 years and above) and were administered the supplements for 30 days. The cirrhotic patients with minimal HE significantly decreased the outgrowth of *E.coli* and *staphylococcus spp.* compared to healthy controls. There was a 50% reversal in minimal HE in patients and a significant reduction in endotoxemia[106]. A similar result was observed in a study conducted on intestinal lymphocytes of an experimental mice model with cirrhosis; wherein, the administration of *Bifidobacterium pseudocatenulatum* decreased expression of pro-inflammatory chemokines receptors; like CCR6, which are upregulated in patients with chronic liver diseases[107].

Primary biliary cirrhosis, wherein the bile ducts are destroyed, is a prevalent disease in middle-aged women and individuals over 65 in 30%-40% of cases[108]. The Kupffer cells are subset of macrophages present in the liver cells, which act as the primary line of defence against pathogens regulating the liver immune homeostasis. TLR expressed in the liver sense the endotoxins of gut derived pathogens and contribute to macrophage activation. The increased susceptibility to LPS may lead to lipotoxicity and alleviate liver injury[68]. In Zhang et al., they conducted a study experiment on a mice model and showed that *A. muciniphila* can resist gluco/lipotoxicity by reducing hepatic glycogen levels, and could potentially be used as an ideal probiotic[19].

The intestinal microbiota regulates the reactive oxidative species produced in the body. Oxidative stress damages the liver cells causing lipofuscins bodies, which are cross-linked under degradable protein aggregates that accumulate in the liver cells. Lipofuscins caused increased generation of reactive oxidative species and decreased cell survivability[109].

Safety and regulation

The world health organization and food and agriculture organization (FAO) of the United Nations have permitted the acceptable use of certain living organisms or Probiotics that confer potential health benefits to individuals. As stated by FAO, probiotics should be able to not only survive the digestive tract, but also be able to proliferate

and be resistant to gastric juices and intestinal conditions. For the use of probiotics in humans, there should be in-vitro tests that predict the ability of the probiotic to function. The preliminary strain selection of probiotics includes: investigating its functional features and effects on resistance to gastric acidity and bile toxicity, stimulation of the host immune system, adhesion to the gut epithelium and its capability to hydrolysed host metabolites, synthesis of antimicrobial substances and it's resistant to antibiotics. Certain bacterial strains may produce extreme antimicrobial resistance, which can be considered potent to the gut as it could disrupt the normal microbial balance. Antibiotic resistance genes have been documented in *Lactobacillus* strains isolated from wine, cheese, poultry, feces and fermented food items. The presence of such genes can be analyzed by conducting whole genome sequencing and standard polymerase chain reaction (PCR) protocols[110,111].

Lactobacilli strains, which are the most commonly administered probiotics, have been recorded to cause bacteremia and liver abscess in 82-year-old women suffering from diabetes mellitus, hypertension and renal disease. However, such cases are rare, and only 7 reported incidences have been found previously[112]. Probiotic intake has also been linked to many infectious complications. Fungemia and sepsis are found to be the most occurring infectious complications caused by probiotics. Fungemia was reported most frequently associated with S*accharomyces* and *S. boulardii;* a commonly used probiotic for treating gastroenteritis, which is considered nearlyidentical to the *S. cerevisiae strain* and can have potential disease-causing abilities[113]. *Lactobacilli*, which are rarely pathogenic, have shown a possible case of *L. rhamnosus* bacteremia in a 50-year-old male individual causing infectious endocarditis[114]. Another case of bacteremia caused by *L. rhamnosus* was also recorded in an adult patient suffering from severe, active ulcerative colitis, while receiving treatment along with a probiotic formulation[115.]

Generally higher doses for adults of more than 10 billion CFUs daily are associated with higher study outcomes[116]. However, it is the primary priority of the probiotic manufacturer to ensure the safety standard of probiotic supplements it produces. Probiotics' safe use and dosage are established through documentation, experiments or historical data from previous studies[117]. The supplier must guarantee a stringent quality of probiotic supplements and contamination-free composition. Failure of such safety may lead to toxic effects and immunological implications[61]. A broader comprehension of the probiotic interaction with microbial, genetic and environmental factors within humans is required to address more microbiota associated conditions. Genetically modified probiotics can be used as a supply of epitopes for improving natural immunity and restoring antigen-specific tolerance as well as oral vaccine delivery. The cell surface components can be targeted as a potential strategy to treat inflammatory intestinal disorders using probiotic bacteria. Fecal microbiota transplantation, which is the transfer of intestinal microbiota from faeces of healthy donor to the GI tract of diseased patients, is growing in popularity. It is mostly used to treat diseases caused by pathogenic or conditionally pathogenic microbes in the gut, and there are increasing studies to apply this therapy in diseases, such as Cronh's disease, multiple sclerosis and neurodegenerative diseases, such as Parkinson's and Alzheimer's disease[118]. Fecal microbiota transfer is also seen to enhance insulin sensitivity in patients with metabolic syndrome; however, future interventions are required to define the vital functionalities of probiotics[119].

Conclusion

In the aged population, the interplay of gut plays a crucial role in overall health of an individual. The relationship between aging, microbiome imbalance and the role of probiotics in restoring the well-being of age individuals is of great interest. Aging can cause a disruption in the composition of bacteria, due to immunosenescence leading

to dysbiosis, systemic and local inflammation as well as more age-related morbidities. Probiotics can be isolated from traditional sources, such as milk and dairy related products, fermented products and non-conventional sources, such as fruits, vegetables, and juices.

When administered in appropriate dosages, probiotics have been proven to be beneficial to the elderly population in combating various diseases, such as diarrhea, functional bowel diseases and hepatic diseases through their immunomodulatory effects. Probiotics enhance the activity of immune cells, such as NK cells, increase phagocytosis and stimulate the activity of signaling molecules. Probiotics, such as interleukins. *Lactobacillus plantarum, Bifidobacterium longum, Lactobacillus acidophilus, Bifidobacterium animalis, L. acidophilus* and *P. pentosaceus,* are some of the commonly used probiotics having beneficial effects on the host when administered in appropriate dosages. This review gives an insight into the major and recent findings in the use of probiotics in elderly and adult populations In additionally to the potential therapeutic activities of previously identified probiotics that could be more extensively studied and used in future interventions as an alternative to antibiotic therapies or as a supplement to benefit the overall health of a diseased individual.

Conflict of interest

The authors declare that they have no conflicts of interest.

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