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Review

Probiotics as a Therapeutic Strategy for Inflammatory Diseases: A Review on Mechanistic, Diagnostic, and Laboratory Perspectives

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Abstract

Introduction/Background: Inflammatory diseases are among the most common chronic health conditions and are frequently linked to microbial dysbiosis and immune system dysfunction. Although corticosteroids remain a standard therapeutic option, their long-term use is associated with serious adverse effects, highlighting the need for safer alternatives. Probiotics, live beneficial microorganisms, have emerged as promising therapeutic agents due to their anti-inflammatory, immunomodulatory, antimicrobial, and antioxidant properties. They can restore gut microbial balance, enhance epithelial barrier function, and modulate host immune responses through pathways involving cytokine regulation and short-chain fatty acid production.

Purpose/Objectives: This review provides a comprehensive overview of the molecular mechanisms through which probiotics mitigate inflammation, with particular attention to their role in modulating mucosal immunity, suppressing pro-inflammatory signaling, and enhancing intestinal integrity.

Findings: Clinical studies support the use of probiotics in managing a variety of inflammation-related conditions, including inflammatory bowel disease (IBD), respiratory tract infections, allergic responses, metabolic disorders, and neuroinflammation. However, strain specificity, formulation challenges, and host-related factors continue to influence clinical outcomes.

Conclusion: Overall, probiotics represent a promising, biologically based approach for managing inflammation and improving patient outcomes across a broad spectrum of chronic diseases. In addition, advanced diagnostic and laboratory techniques play a crucial role in elucidating the molecular and functional impacts of probiotics, enabling precise evaluation of their efficacy, strain-specific effects, and mechanisms of action in both experimental and clinical settings. Future research should focus on identifying the most effective probiotic strains, understanding host–microbe interactions, and conducting long-term studies to establish safety and efficacy.

Keywords: Probiotics; Inflammation; Inflammatory Diseases; Inflammatory Bowel Diseases (IBDs); Immune Modulation; Gut Microbiota, Mechanism of Action, Biomarkers, Microbiome, Therapeutic Strategy.

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1. Introduction

Inflammation is a physiological immune response to harmful stimuli such as pathogens, toxins, or damaged cells, playing a central role in tissue repair and defense (1-3). However, when dysregulated, it contributes to the pathogenesis of many chronic diseases, including ulcerative colitis, Crohn's disease

(4, 5), Alzheimer's and Parkinson's diseases (6), pancreatitis (7), asthma, COPD (8, 9), glomerulonephritis (10, 11), myocardial infarction (12, 13), and type 2 diabetes (14, 15). Despite variations in inflammatory mechanisms across diseases, common features include immune cell recruitment and increased expression of cytokines, chemokines, and inflammatory mediators.

Corticosteroids are among the most potent anti-inflammatory agents, exerting their effects by suppressing cytokine and chemokine production (16, 17). Nonetheless, some patients do not respond even to high doses, and long-term use is associated with significant adverse effects, including infections, metabolic disturbances, osteoporosis, and Cushing's syndrome (18, 19). These limitations have driven the search for alternative therapies with fewer side effects.

A promising strategy involves precision probiotic therapy (20). The gut microbiota is known to influence immune, neurological, and metabolic pathways, and its disruption (dysbiosis) is implicated in various inflammatory diseases (21-23). Probiotics, live non-pathogenic microorganisms, have shown antibacterial, antioxidant, and anti-inflammatory properties (24-28), contributing to improved gut health, immune modulation, and disease prevention (29-31). Specific bacterial strains may help alleviate symptoms in inflammatory disorders such as MS, RA, and IBD (32). This review explores the role of probiotics in treating inflammatory diseases and how probiotics can enhance their clinical efficacy.

2. Probiotics and Their Role in Inflammatory Diseases

2.1. Mechanisms of Probiotic Action

Probiotics can alter both local and systemic immune responses, preserve the integrity of the intestinal epithelial barrier, and enhance gut microbial function (33, 34). Probiotics may work directly with intestinal epithelial cells (IECs) and immune cells, or they may work through active metabolites like the short-chain fatty acid butyrate (35). According to a recent review, probiotics have a variety of different, heterogeneous, and strain-specific modes of action. For example, by controlling probiotic metabolism, boosting intestinal motility, attaching to and breaking down toxic compounds, and boosting host immunological function, *bifidobacteria* have been demonstrated to serve a preventative role in preserving a healthy intestinal microbiota (36, 37). *Streptococcus salivarius* also produces bacteriocin-like inhibitors, possesses immunomodulatory qualities (38), and has remarkable oral adhesion. *Bacillus coagulans* is utilized to support immunological and gastrointestinal health (38).

Probiotics exert their beneficial effects through a variety of mechanisms. These include the production of antimicrobial compounds, such as bacteriocins, that inhibit pathogenic microorganisms, as well as the restoration and stabilization of abnormal gut microbial communities in both adults and children. They also contribute to the synthesis of volatile fatty acids and enhance enzymatic activity, support mucin production for maintaining the integrity of the mucosal barrier, and promote cell adhesion and antagonistic interactions with harmful microbes. Furthermore, probiotics modulate the host immune system, interact with the brain–gut axis, and secrete bioactive proteins that influence host cellular processes (39, 40).

2.1.1. Antimicrobial Properties

One of the primary mechanisms by which probiotics exert their effects is through the production of antimicrobial compounds, particularly bacteriocins. Many strains of *Lactobacillus* and *Bifidobacterium* are capable of producing bacteriocins and other bioactive molecules, such as short-chain fatty acids, diacetyl, and hydrogen peroxide, which positively influence the gut microbiota. Bacteriocins derived from lactic acid bacteria (LAB), especially *Lactobacillus* species, the largest genus of LAB, are of particular interest due to their broad-spectrum antibacterial activity, stability under various pH and temperature conditions, and low toxicity (39). These bacteriocins often function through pore formation in bacterial membranes or inhibition of cell wall synthesis, exerting strong inhibitory effects even at nanomolar concentrations (41). However, not all probiotic strains produce antimicrobial compounds, and some may generate substances with a broad range of activity that could also affect beneficial bacteria (42). Probiotic strains have demonstrated inhibitory effects against a variety of pathogens, including *Rotavirus*, *Salmonella* spp., *Shigella* spp., *Helicobacter pylori*, *Listeria monocytogenes*, and *Escherichia coli* (43).

2.1.2. Colonization in Gut

One important element in the formation of the gut is probably the early colonization of the newborn gastrointestinal tract. The gut microbiota starts to build up prior to birth (44), and it enters the newborn gastrointestinal tract as soon as the fetal membrane ruptures. The intestinal flora stabilizes and turns into a unique fingerprint after two to three years of life (45). Through the ability of dendritic cells (DCs) to pierce the epithelium and directly acquire germs from the intestinal lumen, bacteria can move from the gastrointestinal tract to extra-digestive locations. After entering DCs or macrophages, circulating immune cells can carry germs across the circulation to different locations (40, 46). Because it hinders the mechanical removal of infections, bacterial adherence to host surfaces is a crucial component of host colonization (47). Probiotic treatment raises the generation of SCFAs, stool wetness, frequency, and volume in healthy persons (48).

Mucins, which are high-molecular-weight glycoproteins, are responsible for forming and maintaining the mucosal layer (49). Certain probiotic strains have been shown to modulate mucin expression, thereby indirectly regulating the gut immune system and altering the characteristics of the mucosal barrier (50). One of the ways probiotics inhibit pathogen colonization is through spatial adhesion competition, where beneficial microbes occupy binding sites on the epithelial surface, preventing pathogenic attachment (51).However, factors such as infection and nutrient deprivation can disrupt mucin production, weakening this barrier and contributing to the development of inflammatory bowel diseases.

2.1.3. Immunomodulation and Anti-Inflammation

There is growing interest in how the gut microbiota modulates host inflammation. Probiotics, particularly *Bifidobacterium* and *Lactobacillus* species, regulate immune responses by interacting with T cells, dendritic cells, and macrophages, promoting the release of anti-inflammatory cytokines such as IL-10, while suppressing proinflammatory markers like IL-6 and TNF-α (52, 53). Probiotics also reinforce the intestinal epithelial barrier, preventing the translocation of inflammatory agents and reducing systemic inflammation (54). In addition, by fermenting dietary fiber, they generate short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate, which nourish colonocytes and exhibit anti-inflammatory effects (55). By modulating the composition of the gut microbiota (enhancing beneficial bacteria and suppressing pathogens) probiotics help maintain microbial balance, thereby reducing intestinal inflammation (55, 56). Some strains can also directly inhibit pathogen adherence to the intestinal wall, further limiting inflammation (56).

The intestinal microbiota produces immunomodulatory and anti-inflammatory molecules that interact with epithelial cells, dendritic cells (DCs), macrophages, and lymphocytes to stimulate immune responses (39, 57). Probiotics influence immune regulation by modulating the expression of inflammatory mediators and proinflammatory cytokines in DCs of IBD patients (58). They also affect pathogen recognition by altering pattern recognition receptors (PRRs), such as TLRs and C-type lectins, on macrophages. As lymphocytes proliferate, probiotics contribute to increased IgA production and restore the Th1/Th2 balance (57, 59). Host–pathogen interactions shape T cell subsets, and disruption of the balance between T helper cells and regulatory T cells (Tregs) leads to impaired immunity (60). By promoting Treg formation and modulating immune responses, probiotics support intestinal immune homeostasis (61). Additionally, they enhance immune function through increased macrophage phagocytosis (62), elevated IgA levels (63), and a rise in natural killer (NK) cells.

2.1.4. Interaction Between Brain and Gut

The brain works in collaboration with the gut microbiota to process the vast array of chemical signals entering the gastrointestinal tract daily (64). Gut microbes communicate with the brain through multiple pathways, including the vagus nerve, the enteric nervous system, and the release of neurotransmitters, neuromodulators, proinflammatory cytokines, and short-chain fatty acids (SCFAs), all of which are neuroactive metabolites (64). Moreover, gut-brain signaling affects gene receptors involved in child development and may shape both short-term and long-term behavioral patterns (65).

2.1.5. Fatty Acid Synthesis and Enzymatic Activity

The enzymatic activity of probiotics in the colon plays a key role in their biological effects. *Lactobacillus* and *Bifidobacterium* species exhibit more than 20 types of enzymatic activity, with galactosidase being the most common. However, some enzymatic actions may be harmful; for example, intestinal bacterial β-glucuronidase can hydrolyze glucuronidated metabolites, generating toxic compounds that damage the intestinal epithelium (66). In contrast, beneficial effects are also mediated by postbiotics such as short-chain fatty acids (SCFAs), which regulate the adaptive immune system (67). *Lactobacillus casei* contributes to immune modulation through the enzymatic breakdown of casein into immunogenic peptides (68).

Probiotics also stimulate the production of secretory IgA (sIgA) by intestinal B cells, enhancing the mucosal immune barrier by facilitating its transport to the epithelial surface (39). In addition, probiotics influence bile acid metabolism, which affects cholesterol absorption. Many probiotic species, particularly those from the *Bacillus* genus, produce bile salt hydrolase (BSH), an enzyme involved in the initial deconjugation of bile salts (69). This BSH-mediated deconjugation is considered a key mechanism in the hypocholesterolemic effects of probiotics (69, 70).

2.1.6. Protein Secreted by Probiotics

Probiotic-secreted proteins play a critical role in maintaining host–microbe symbiosis. For instance, extracellular proteins from *Lactobacillus plantarum* BMCM12 have been shown to either strengthen the intestinal barrier or significantly reduce pathogenic microbial adhesion (39). Cell surface structures of *Lactobacilli* and other probiotics rely heavily on surface proteins such as LPxTG, which are hydrophilic, stable, adhesive, and highly permeable (71). Two well-characterized secreted proteins, p40 and p75, contribute to intestinal epithelial cell (IEC) homeostasis and protect tight junctions (TJs) from oxidative damage via a protein kinase C (PKC)-dependent mechanism (39). These proteins help preserve intestinal barrier integrity and have shown therapeutic potential in preventing epithelial injury and diseases such as colitis (67). Specifically, p40 has demonstrated protective effects in experimental colitis by reducing epithelial apoptosis, inflammation, and barrier dysfunction (72). Another promising protein, HM0539, also enhances the intestinal barrier, exhibits anti-inflammatory properties, and protects against bacterial infection, making it a potential candidate for the treatment of inflammatory bowel disease (67).

3. Probiotic-Based Therapeutic Approaches in Various Diseases

The function of probiotics in some inflammatory illnesses is covered in this section:

3.1. Application in Inflammatory Bowel Disease (IBD)

IBD symptoms can be alleviated by taking probiotics (39) because of their unique conditioning effects on the gut flora (73). By reducing the inflammatory response or changing the gut microbiota's makeup, they function as a regulatory mechanism (39). Five out of nine trials found that probiotics and conventional medication improved the condition of individuals with active ulcerative colitis. Probiotic therapy can be just as beneficial as conventional medication, according to three out of seven trials conducted on people with ulcerative colitis who were in remission (74). Additionally, probiotic administration may help IBD patients by lowering clinical symptoms, lowering serological inflammatory markers, and boosting beneficial gut flora, according to Xu et al (75).

3.2. Application in Allergies

By changing the bacterial balance in the stomach and affecting T cells, the probiotics in yogurt are believed to be useful in treating seasonal pollen allergies. In clinical trials, probiotic LAB bacteria, including *B. infantis*, *B. animalis*, *L. casei*, *L. paracasei*, *L. acidophilus*, *L. reuteri*, and *L. acidophilus*, have demonstrated a range of effects. It has been demonstrated that *L. rhamnosus* probiotic cultures raise the synthesis of anti-inflammatory chemicals, such as IL-8, while concurrently decreasing the synthesis of immunoglobulin IgE and histamine receptor 4 (76-78).

3.3. Application in Cancer

Consuming dairy products fermented with *lactobacilli* or *bifidobacteria* has been demonstrated in

certain epidemiological studies to lower the incidence of colorectal cancer. However, according to a number of metrics utilized in human studies (including fecal enzyme activity, fecal mutagenicity and genotoxicity, and immunological markers), there is proof that probiotics lower the risk of colorectal cancer (79).

3.4. Application in Infections of the Respiratory Tract

Certain probiotic strains have demonstrated protective effects against viral respiratory tract infections (RTIs) by adhering to epithelial surfaces, thereby forming a barrier that prevents pathogen colonization. These probiotics enhance barrier function, produce antimicrobial compounds, and modulate immune responses, particularly in children, by supporting both innate and adaptive immunity (38). Strains like *Lactobacillus rhamnosus*, *L. casei*, *L. gasseri*, *Bifidobacterium longum*, and *B. bifidum* have been effective in managing conditions such as otitis media, sinusitis, bronchitis, and pneumonia in the elderly. *L. rhamnosus GG (LGG)*, for instance, significantly reduces the duration of RTIs (80). In healthy children, *B. animalis* subsp. *lactic* Bi-07 and *L. acidophilus* NCFM have been shown to reduce fever, cough, and sneezing episodes, while in adults and elderly populations, *L. delbrueckii* subsp. *bulgaricus* OLL 1073R-1 lowers the risk of influenza and common cold (81). Additionally, a quadruplex oral tablet containing *B. infantis*, *L. acidophilus*, *E. faecalis*, and *B. cereus* not only increased bifidobacterial populations but also reduced RTI recurrence in children. Probiotic nasal sprays with *S. salivarius* 24SMB and *S. oralis* 89a have also proven effective in URTI prevention in pediatric patients (82), and *S. salivarius* has shown therapeutic potential in treating tonsillitis, otitis media, and pharyngitis (83).

3.5. Application in Infection with Helicobacter pylori

Several probiotic strains suppress *Helicobacter pylori* in vitro, which is linked to gastritis, stomach cancer, peptic ulcer disease, and lymphoma. Studies on humans have verified this inhibitory effect on *H. pylori*, which seems to be unrelated to bacterial survival (84).

3.6. Application in Autoimmune and Metabolic Inflammatory Disorders

Probiotics' effects on the immune system vary greatly depending on the strain. The immune system is affected differently by different bacterial strains. In a double-blind, placebo-controlled study, the effects of the probiotic bacterium *Lactobacillus delbrueckii* subsp2. *bulgaricus* 8481 were examined in senior volunteers (85). They discovered that the probiotic group had lower levels of IL-8 and higher levels of human beta-defensin 2 than the placebo group. Finamore et al. investigated how a probiotic combination of *Lactobacillus helveticus* Bar13 and B. longum Bar33 affected older people's immune systems. In comparison to the placebo group, this combination reduced the activity of memory T-lymphocytes and enhanced the activity of B-lymphocytes, regulatory T-lymphocytes, and natural killer (NK) cells. Thus, the findings clearly indicate that taking *L. delbrueckii* subsp. *bulgaricus* 8481 capsules greatly raised the percentage of NK cells in older adults, which could lead to the illness prevention and the preservation of a healthy lifestyle. Inverted $TCD4^+/TCD8^+$ ratio, a rise in the $CD8^+$ $CD28^{null}$ T cell subgroup, and CMV seropositivity are a number of factors that contribute to immune risk phenotype (86). Compared to the placebo group, elderly participants in the probiotic group exhibited a significant decrease in the $CD8^+$ T cell subset and an increase in the $CD4^+/CD8^+$ T cell ratio. Additionally, during the course of the trial, a noticeable decrease in the population of late differentiated memory and effector $CD8^+$ $CD28^{null}$ T cells was noted (87). In this study, older adults who took the probiotic capsules maintained the same CMV titer at baseline and at 6 months, whereas older adults who received the placebo capsules had a greater CMV titer at 6 months than at baseline. According to these findings, consuming probiotics may help stop CMV replication by limiting its dissemination and preventing additional viral reactivation through unknown mechanisms (88). Those taking the probiotic showed a significant rise in the $CD31^+$ marker in $CD4^+$ and $CD8^+$ T cells of $CD45RA^+$ phenotype. According to these findings, taking probiotics may activate the thymus gland. Additionally, it was reported that T cells from elderly participants in the probiotic group had a higher average TREC content, supporting an increase in naïve, less differentiated cells following probiotic consumption (89). The serum levels of the chemokine IL-8 decreased over a 6-month period in elderly individuals who took capsules containing *Lactobacillus delbrueckii* subsp. *bulgaricus* 8481.

Since IL-8 is a key mediator of the inflammatory response, this reduction may provide significant benefits to older adults (88).

3.7. Application in Neuroinflammation and Systemic Inflammation

Aging is associated with cognitive decline and an increased prevalence of neurodegenerative disorders, with emerging evidence suggesting a role for gut microbiota in these processes. Chronic constipation, more prevalent in the elderly due to age-related microbial shifts, has also been linked to impaired cognition (90, 91). Understanding the dynamic relationship between gut microbiota and aging is essential for developing effective interventions. Kim et al. reported that supplementation with *Bifidobacterium longum* BORI and *Bifidobacterium bifidum* BGN4 improved stress, mood, and cognitive flexibility in elderly participants, alongside increased serum levels of brain-derived neurotrophic factor (BDNF), indicating enhanced brain function. Similar benefits were observed after a 12-week probiotic intervention. Furthermore, a recent meta-analysis confirmed the positive impact of probiotic use in elderly patients undergoing antibiotic therapy (90, 92).

4. Encapsulation of Probiotics in Nanoparticles

Several recent studies have explored nanotechnology-based strategies to improve probiotic viability under gastrointestinal conditions. A study conducted in 2017 demonstrated that double-coated microcapsules using calcium alginate-chitosan and Eudragit S100 significantly enhanced the survival of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* in simulated gastric and intestinal fluids, compared to single-coated beads and free cells. The additional Eudragit layer improved bead stability in acidic environments, reducing bacterial loss (93). In 2021, another study encapsulated *L. acidophilus*, *L. rhamnosus*, *B. bifidum*, and *B. animalis* in electrospun nanofibrous mats made from corn starch and sodium alginate. This technique markedly improved probiotic survival in simulated digestive fluids compared to unencapsulated cells (94). Furthermore, a 2022 study suggested that combining probiotics with specific nanoparticles could enhance their long-term therapeutic efficacy against IBD. In DSS-induced colitis mouse models, nanoparticle-based probiotic delivery systems showed potential as effective in vivo treatments for ulcerative colitis (95).

Remarkably, oral administration of *Bacillus amyloliquefaciens* nanoparticles (BANPs) significantly mitigated body weight loss and spleen enlargement in DSS-induced colitis rats, compared to the untreated group, which exhibited up to 20% weight reduction and splenomegaly, an established marker of systemic inflammation (96, 97). BANP treatment also led to decreased serum C-reactive protein (CRP) levels, indicating reduced inflammatory activity (98). These effects are attributed to the polymeric nanoparticle formulation, which improves BA delivery to target tissues and enhances its cellular uptake (99). Inflammatory markers, including TNF-α, IL-1β, and IL-6, were highest in the DSS-only group, while BANP treatment markedly lowered IL-6 levels (by 65%) compared to DSS controls. Interestingly, while IL-10, an anti-inflammatory cytokine, was elevated in DSS-induced groups, the non-colitis group showed significantly higher IL-10 than all treated groups. Furthermore, TGF-β expression, which was suppressed in colitic rats, was restored in the BANP-treated group, suggesting its role in promoting mucosal healing (95).

Multi-strain probiotics (MSPs) have emerged as promising candidates for IBD treatment, and their encapsulation into nanomaterials (MSPNPs) may enhance their viability, resistance, and targeted delivery. A 2022 study using a DSS-induced colitis rat model evaluated the therapeutic potential of MSPNPs by assessing microbial composition, histopathology, inflammatory markers, tight-junction gene expression, and NLRP3 inflammasome signaling. Oral administration of MSPNPs significantly alleviated clinical symptoms, reduced fecal calprotectin, serum CRP, and colonic NO and MPO levels. Additionally, expression of NLRP3 downstream mediators, including caspase-1, IL-1β, and IL-18, was downregulated, correlating with decreased colonic inflammation. Compared to free MSPs, MSPNPs more effectively restored tight junction genes (e.g., occludin, ZO-1, MUC, FABP-2) and improved epithelial repair and microbiota composition (100). A 2024 study further supported the value of nanoparticle encapsulation by demonstrating enhanced anti-cancer activity of *Lacticaseibacillus paracasei* GMNL-133

(SGMNL-133) postbiotics in gastric cancer, due to protection from gastric acid and improved mucosal penetration. Encapsulated SGMNL-133 also significantly reduced tissue inflammation in vivo (101).

5. Experimental Assays for Evaluating Probiotic Effects

5.1. In Vitro Evaluation of Anti-inflammatory Activity

One of the most commonly used in vitro models is the RAW264.7 murine macrophage cell line, which provides a reliable system for evaluating the immunomodulatory effects of probiotics following lipopolysaccharide (LPS)-induced inflammation. For instance, Lu et al. conducted a comprehensive screening of 84 lactic acid bacteria (LAB) strains and identified *Limosilactobacillus reuteri* AUc2301 as a potent candidate that significantly suppressed the production of pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) and downregulated the expression of PGE2 and COX-2, partly via modulation of the NF-κB signaling pathway (102). Similarly, other studies have reported the ability of *Lactobacillus plantarum* and related strains to inhibit the activation of NF-κB and MAPK pathways in LPS-stimulated RAW264.7 cells, leading to reduced secretion of inflammatory mediators (103, 104). These cell-based assays are frequently coupled with ELISA and RT-qPCR to quantify cytokine levels and assess gene expression changes. Collectively, such laboratory techniques offer valuable mechanistic insights into how specific probiotic strains may exert protective effects in inflammatory conditions.

5.2. Measuring Cytokine Responses

Cytokine profiling through ELISA and quantitative real-time PCR (qPCR) is a widely adopted method to assess the anti-inflammatory potential of probiotics in both in vitro and in vivo settings. These assays enable precise quantification of key inflammatory mediators, such as TNF-α, IL-1β, IL-6, and the anti-inflammatory cytokine IL-10, following probiotic treatment. For example, *Limosilactobacillus reuteri* AUc2301 was shown to significantly downregulate pro-inflammatory cytokine expression in LPS-stimulated RAW264.7 macrophages, as measured by both ELISA and RT-qPCR techniques (102). In another study, *Lactobacillus plantarum* Z22 suppressed IL-6, TNF-α, and IL-1β expression at the transcriptional level, highlighting the utility of qPCR in evaluating gene-level regulation of inflammation-related targets (104). These findings demonstrate that ELISA and qPCR not only serve as robust analytical tools for quantifying cytokine shifts but also provide mechanistic insight into how probiotic strains may modulate host immune responses under inflammatory conditions.

5.3. Gut Barrier Integrity Assays

Maintaining the integrity of the intestinal epithelial barrier is a key mechanism through which probiotics exert protective effects in inflammatory conditions. Two widely used in vitro assays to evaluate gut barrier function are transepithelial electrical resistance (TEER) and FITC-dextran permeability tests. TEER measurements provide real-time, quantitative assessments of tight junction integrity, while FITC-dextran assays evaluate paracellular permeability by tracking the diffusion of fluorescent dextran molecules across epithelial monolayers. In a recent study, *Lactobacillus plantarum* postbiotics was shown to enhance TEER values and reduce FITC-dextran permeability in LPS-challenged Caco-2 cells, suggesting improved barrier function under inflammatory stress (105). Similarly, Liao et al. demonstrated that selected probiotic strains improved epithelial tight junction integrity and reduced permeability in an organoid model (106). These assays are often used alongside cytokine profiling and tight junction protein expression analyses to provide a comprehensive view of probiotic-mediated gut protection.

5.4. Flow Cytometry–Based Immune Profiling

Flow cytometry is a powerful tool for assessing the immunomodulatory effects of probiotics by enabling quantification of distinct immune cell subsets, such as regulatory T cells (Tregs) and Th17 cells, in both preclinical and clinical settings. In murine models, supplementation with *Weizmannia coagulans* BCG44 significantly reduced the proportion of pro-inflammatory $CD4^+IL\text{-}17A^+$ Th17 cells while increasing $CD4^+FoxP3^+$ Tregs in spleen tissue, highlighting a restored Th17/Treg balance via flow cytometric analysis (107). In a randomized controlled clinical trial involving ICU-admitted traumatic brain injury patients, a seven-strain probiotic cocktail led to

a substantial decrease in circulating Th17 cells and an increase in Tregs (as measured by $CD4^+CD25^+FoxP3^+$ markers), with a notably elevated Treg/Th17 ratio post-treatment (108). Collectively, these studies illustrate how flow cytometry-based profiling offers quantitative insights into the immunoregulatory potential of probiotic strains by mapping key T cell population dynamics under inflammatory stress.

5.5. Microbiota Analysis by 16S rRNA Sequencing

High-throughput 16S rRNA amplicon sequencing has become a cornerstone method for characterizing shifts in the gut microbial community following probiotic administration. In murine models, oral gavage with different probiotic strains, such as *Lactobacillus acidophilus*, *L. plantarum*, *Bacillus subtilis*, and *Enterococcus faecalis*, over a 14-day period resulted in significant alterations in the relative abundances of major bacterial phyla (Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria) and key genera including *Lactobacillus*, *Bacteroides*, and *Shigella*, as revealed by sequencing 16S rRNA from fecal samples (109). Moreover, in preterm infants, supplementation with multi-strain probiotics accelerated microbiota maturation and reduced inflammation, which was confirmed via longitudinal 16S profiling (110).

5.6. Assessment of Short-Chain Fatty Acid Production by HPLC/GC-MS

Quantifying probiotic-induced short-chain fatty acid (SCFA) production via chromatographic methods, such as high-performance liquid chromatography (HPLC-MS/MS) or gas chromatography–mass spectrometry (GC-MS), provides critical insights into the metabolites mediating anti-inflammatory effects. Calvigioni et al. developed an HPLC-MS/MS platform to precisely measure acetic acids, propionic acids, and butyric acids secreted by probiotic strains, demonstrating strain-specific variation in SCFA output and highlighting their potential as metabolic biomarkers (111). Moreover, Weng et al. introduced a high-throughput GC-MS method capable of quantifying multiple SCFAs and branched SCFAs simultaneously in fecal samples, enabling more comprehensive metabolic profiling (112). Together, these analytical techniques facilitate accurate quantification of SCFAs, enabling mechanistic linkage between specific probiotic strains, their metabolic outputs, and downstream modulatory effects on gut immunity and inflammation.

5.7. Histopathological Examination of Colon Tissue

Histopathological analysis of colon tissue provides crucial morphological evidence for the protective effects of probiotic intervention in inflammatory bowel disease models. In DSS-induced colitis mice, administration of *Lacticaseibacillus rhamnosus* G7 significantly ameliorated histological damage, as evidenced by reduced crypt loss, mucosal ulceration, and decreased inflammatory cell infiltration, confirmed by notably lower histopathology scores compared with DSS-only controls (113). Similarly, *Bacillus licheniformis* ZW3 treatment restored epithelial integrity, preserved goblet cell populations, reinstated tight junction proteins (ZO-1, occludin, MUC2), and significantly reduced neutrophil accumulation and MPO activity in colon sections (114). Additionally, novel candidate strains of *Bifidobacterium* and *Limosilactobacillus* demonstrated preserved colon length, lower disease activity index, and enhanced mucosal architecture with fewer crypt abnormalities and inflammatory infiltrates, as confirmed via H&E staining and histological scoring in DSS-colitis mice (115). Taken together, these studies underscore the value of histopathological assessment for confirming the tissue-level anti-inflammatory efficacy of probiotic treatment in preclinical colitis models.

5.8. Omics Approaches to Elucidate Probiotic Mechanisms

Integrated omics techniques, encompassing transcriptomics and metabolomics, offer unprecedented insights into how probiotics modulate host physiology and inflammatory pathways. High-throughput transcriptomic profiling, typically via RNA-sequencing, identifies differentially expressed host genes involved in immune regulation and epithelial defense following probiotic treatment. This is often complemented with untargeted metabolomic analyses (using NMR or MS-based platforms) to characterize changes in microbial and host-derived metabolites, including SCFAs and bile acids, which mediate anti-inflammatory effects. For example, multi-omics reviews have highlighted the synergistic power of combining metabolomic and transcriptomic

datasets to correlate probiotic-induced metabolic shifts with gene regulatory networks in host and microbial communities (116). Specific studies have demonstrated this approach in poultry and fish models, where Bacillus subtilis and LAB supplementation significantly altered lipid metabolic pathways, as evidenced by concordant transcriptome and metabolome changes (117, 118). These methodologies provide a systems-level perspective, linking probiotic interventions to molecular mechanisms underlying inflammation modulation and barrier integrity improvements.

6. Considerations for Safety and Biocompatibility of NPs

While nanoparticles have numerous biomedical applications, concerns about their toxicity and biocompatibility remain significant. Their cytotoxic properties are beneficial in targeting hyperproliferative diseases such as cancer and hypertrophic scars; however, achieving precise targeting remains challenging, and both local and systemic effects require further investigation (119). Damage induced by nanoparticles can affect entire organ systems by disrupting endogenous cells and biomolecules (120).

Many nanoparticles, including carbon-based, metal-based, and gold particles, have demonstrated cytotoxicity in vitro and in vivo (121, 122). For example, carbon nanotubes have been linked to mesothelioma, mimicking asbestos-like toxicity (123, 124). Similarly, silver and iron oxide nanoparticles exhibit toxicity mainly through the generation of reactive oxygen species (ROS) (120, 125), which can damage lipids, proteins, and DNA.

Nanoparticles also interact with proteins in biological fluids, potentially causing protein misfolding and aggregation, which may elicit immune responses. Moreover, they may disrupt cell membranes, leading to leakage of intracellular contents and compromised membrane integrity, a primary cause of cytotoxicity (120). Post-endocytosis, nanoparticles often accumulate in lysosomes, where they can interfere with normal lysosomal functions or attempt endosomal escape to exert therapeutic effects (126, 127). Organ-specific accumulation of nanoparticles is associated with greater tissue injury. Nonetheless, toxic nanoparticles may still be valuable in medicine when their damaging properties are used selectively against diseased tissues, eliminating the need for drug loading. This requires precise targeting mechanisms to avoid harming healthy tissues.

Surface modification strategies, such as coating with polymers, antibodies, or small molecules, can enhance nanoparticle targeting and reduce toxicity. For instance, iron/silica nanoparticles must be carefully engineered in terms of size, shape, and surface stability to ensure biocompatibility and functional specificity (128, 129).

7. Future Perspectives and Conclusion

Given how important the gut microbiota is to host health, altering its equilibrium can lead to a variety of illnesses, including inflammatory ones. Traditional treatments, namely steroids, are restricted due to significant side effects. As a result, the use of probiotics has gained popularity due to their role in reducing inflammatory responses. Nanomaterial encapsulation of probiotics has been found to be a potential technique for ensuring their stability and delivery to target areas. These carriers can penetrate the bloodstream and distribute probiotics precisely by enhancing their solubility, reducing the negative effects of standard medications and directing them to the exact place (130-133). However, there are worries regarding the compatibility and potential toxicity of nanomaterials, as some nanoparticles can disrupt intestinal microflora and metabolic processes, potentially causing inflammation or excessive depletion of beneficial microbes, thereby significantly impairing immunity.

As a result, further research is needed on nanoparticle toxicity, dosage requirements, and route of administration in order to limit side effects and employ them as a potential technique for illness treatment (134, 135).

Moreover, the application of advanced diagnostic and analytical techniques is crucial for monitoring the interactions between probiotics and nanomaterials, evaluating their biosafety, and elucidating their mechanistic effects within host systems.

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Conflict of Interest

The authors declare no conflict of interest.

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Authors' contributions

Conceptualization: M.H. and F.A.; Investigation: M.H., F.H., and Z.H; Writing (original draft preparation): M.H., F.H., and Z.H.; Writing (review and editing): F.A.; Supervision: F.A.; All authors approved the final draft.

Using AI Declaration

An AI language model (GPT-5 mini, OpenAI) was used for proofreading and improving language quality. It did not contribute to the scientific content, analysis, or conclusions.

References

1. Medzhitov R. Inflammation 2010: new adventures of an old flame. Cell. 2010;140(6):771-6.

2. Ferrero-Miliani L, Nielsen O, Andersen P, Girardin S. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. Clinical & Experimental Immunology. 2007;147(2):227-35.

3. Nathan C, Ding A. Nonresolving inflammation. Cell. 2010;140(6):871-82.

4. Cario E, Podolsky DK. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. Infection and immunity. 2000;68(12):7010-7.

5. Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. The Journal of clinical investigation. 2007;117(3):514-21.

6. Nelson PT, Soma LA, Lavi E. Microglia in diseases of the central nervous system. Annals of medicine. 2002;34(7):491-500.

7. Manohar M, Verma AK, Venkateshaiah SU, Sanders NL, Mishra A. Pathogenic mechanisms of pancreatitis. World journal of gastrointestinal pharmacology and therapeutics. 2017;8(1):10.

8. Leitch A, Duffin R, Haslett C, Rossi A. Relevance of granulocyte apoptosis to resolution of inflammation at the respiratory mucosa. Mucosal immunology. 2008;1(5):350-63.

9. Wong J, Magun BE, Wood LJ. Lung inflammation caused by inhaled toxicants: a review. International journal of chronic obstructive pulmonary disease. 2016:1391-401.

10. Ernandez T, Mayadas TN. The changing landscape of renal inflammation. Trends in molecular medicine. 2016;22(2):151-63.

11. Sanz AB, Sanchez-Nino MD, Ramos AM, Moreno JA, Santamaria B, Ruiz-Ortega M, et al. NF-κB in renal inflammation. Journal of the American Society of Nephrology. 2010;21(8):1254-62.

12. Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. Circulation. 1990;81(4):1161-72.

13. Opie LH, Commerford PJ, Gersh BJ, Pfeffer MA. Controversies in ventricular remodelling. The Lancet. 2006;367(9507):356-67.

14. Donath MY, Schumann DM, Faulenbach M, Ellingsgaard H, Perren A, Ehses JA. Islet inflammation in type 2 diabetes: from metabolic stress to therapy. Diabetes care. 2008;31(Supplement_2):S161-S4.

15. Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes research and clinical practice. 2014;105(2):141-50.

16. PJ B. How do corticosteroids work in asthma? Ann Intern Med. 2003;139:359-70.

17. Adcock IM. Glucocorticoids: new mechanisms and future agents. Current Allergy and Asthma Reports. 2003;3(3):249-57.

18. Adcock IM, Lane S. Corticosteroid-insensitive asthma: molecular mechanisms. Journal of Endocrinology. 2003;178(3):347-55.

19. Schäcke H, Döcke W-D, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. Pharmacology & therapeutics. 2002;96(1):23-43.

20. López AR, Barberis M. Metabolic modeling for probiotic and prebiotic production to treat inflammatory disorders. Chemical Engineering Journal. 2024;502:157852.

21. Robles-Alonso V, Guarner F. Progreso en el conocimiento de la microbiota intestinal humana. Nutricion hospitalaria. 2013;28(3):553-7.

22. Dinan TG, Cryan JF. Gut instincts: microbiota as a key regulator of brain development, ageing and neurodegeneration. The Journal of physiology. 2017;595(2):489-503.

23. Mori G, Morrison M, Blumenthal A. Microbiome-immune interactions in tuberculosis. PLoS pathogens. 2021;17(4):e1009377.

24. Santacroce L, Charitos IA, Bottalico L. A successful history: probiotics and their potential as antimicrobials. Expert review of anti-infective therapy. 2019;17(8):635-45.

25. Harsh B, Sangita J. Role of Probiotics in Human Health. Cureus. 2022;14(11).

26. Fijan S. Probiotics and their antimicrobial effect. MDPI; 2023. p. 528.

27. Cristofori F, Dargenio VN, Dargenio C, Miniello VL, Barone M, Francavilla R. Anti-inflammatory and immunomodulatory effects of probiotics in gut inflammation: a door to the body. Frontiers in immunology. 2021;12:578386.

28. Rwubuzizi R, Kim H, Holzapfel WH, Todorov SD. Beneficial, safety, and antioxidant properties of lactic acid bacteria: A next step in their evaluation as potential probiotics. Heliyon. 2023;9(4).

29. Kora AJ. Probiotics in the prevention and treatment of diarrheal disease. Probiotics in the Prevention and Management of Human Diseases. 2022:107-15.

30. Singh S, Singh M, Gaur S. Probiotics as multifaceted oral vaccines against colon cancer: A review. Frontiers in immunology. 2022;13:1002674.

31. Yadav MK, Kumari I, Singh B, Sharma KK, Tiwari SK. Probiotics, prebiotics and synbiotics: Safe options for next-generation therapeutics. Applied microbiology and biotechnology. 2022;106(2):505-21.

32. Chen Y, Cui W, Li X, Yang H. Interaction between commensal bacteria, immune response and the intestinal barrier in inflammatory bowel disease. Frontiers in Immunology. 2021;12:761981.

33. Dong Y, Li M, Yue X. Current research on probiotics and fermented products. Foods. 2024;13(9):1406.

34. Aspri M, Papademas P, Tsaltas D. Review on non-dairy probiotics and their use in non-dairy based products. Fermentation. 2020;6(1):30.

35. La Fata G, Weber P, Mohajeri MH. Probiotics and the gut immune system: indirect regulation. Probiotics and antimicrobial proteins. 2018;10:11-21.

36. Li M, Ding J, Stanton C, Ross RP, Zhao J, Yang B, Chen W. Bifidobacterium longum subsp. infantis FJSYZ1M3 ameliorates DSS-induced colitis by maintaining the intestinal barrier, regulating inflammatory cytokines, and modifying gut microbiota. Food & Function. 2023;14(1):354-68.

37. Bron PA, Kleerebezem M, Brummer R-J, Cani PD, Mercenier A, MacDonald TT, et al. Can probiotics modulate human disease by impacting intestinal barrier function? British Journal of Nutrition. 2017;117(1):93-107.

38. Zhang Y, Xu Y, Hu L, Wang X. Advancements related to probiotics for preventing and treating recurrent respiratory tract infections in children. Frontiers in Pediatrics. 2025;13:1508613.

39. Prajapati K, Bisani K, Prajapati H, Prajapati S, Agrawal D, Singh S, et al. Advances in probiotics research: mechanisms of action, health benefits, and limitations in applications. Systems Microbiology and Biomanufacturing. 2024;4(2):386-406.

40. Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A. Mechanisms of action of probiotics. Advances in nutrition. 2019;10:S49-S66.

41. Darbandi A, Asadi A, Mahdizade Ari M, Ohadi E, Talebi M, Halaj Zadeh M, et al. Bacteriocins: properties and potential use as antimicrobials. Journal of Clinical Laboratory Analysis. 2022;36(1):e24093.

42. Mokoena MP. Lactic acid bacteria and their bacteriocins: classification, biosynthesis and applications against uropathogens: a mini-review. Molecules. 2017;22(8):1255.

43. Das TK, Pradhan S, Chakrabarti S, Mondal KC, Ghosh K. Current status of probiotic and related health benefits. Applied Food Research. 2022;2(2):100185.

44. Hesla HM, Stenius F, Jäderlund L, Nelson R, Engstrand L, Alm J, Dicksved J. Impact of lifestyle on the gut microbiota of healthy infants and their mothers–the ALADDIN birth cohort. FEMS microbiology ecology. 2014;90(3):791-801.

45. EFSA Panel on Dietetic Products N, Allergies. Guidance on the scientific requirements for health claims related to the immune system, the gastrointestinal tract and defence against pathogenic microorganisms. EFSA Journal. 2016;14(1):4369.

46. Martín Ro, Langa S, Reviriego C, Jiménez E, Marín MaL, Olivares M, et al. The commensal microflora of human milk: new perspectives for food bacteriotherapy and probiotics. Trends in Food Science & Technology. 2004;15(3-4):121-7.

47. Szajewska H. What are the indications for using probiotics in children? Archives of disease in childhood. 2016;101(4):398-403.

48. Olivares M, Díaz-Ropero MP, Gómez N, Lara-Villoslada F, Sierra S, Maldonado JA, et al. Oral administration of two probiotic strains, Lactobacillus gasseri CECT5714 and Lactobacillus coryniformis CECT5711, enhances the intestinal function of healthy adults. International journal of food microbiology. 2006;107(2):104-11.

49. McGuire MK, McGuire MA. Human milk: mother nature's prototypical probiotic food? Advances in nutrition. 2015;6(1):112-23.

50. Iqbal Z, Ahmed S, Tabassum N, Bhattacharya R, Bose D. Role of probiotics in prevention and treatment of enteric infections: A comprehensive review. 3 Biotech. 2021;11(5):242.

51. Cai R, Cheng C, Chen J, Xu X, Ding C, Gu B. Interactions of commensal and pathogenic microorganisms with the mucus layer in the colon. Gut microbes. 2020;11(4):680-90.

52. Azad MAK, Sarker M, Wan D. Immunomodulatory effects of probiotics on cytokine profiles. BioMed research international. 2018;2018(1):8063647.

53. Plaza-Díaz J, Ruiz-Ojeda FJ, Vilchez-Padial LM, Gil A. Evidence of the anti-inflammatory effects of probiotics and synbiotics in intestinal chronic diseases. Nutrients. 2017;9(6):555.

54. Wang J, Ji H, Wang S, Liu H, Zhang W, Zhang D, Wang Y. Probiotic Lactobacillus plantarum promotes intestinal barrier function by strengthening the epithelium and modulating gut microbiota. Frontiers in microbiology. 2018;9:1953.

55. Ríos-Covián D, Ruas-Madiedo P, Margolles A, Gueimonde M, De Los Reyes-gavilán CG, Salazar N. Intestinal short chain fatty acids and their link with diet and human health. Frontiers in microbiology. 2016;7:185.

56. Flach J, Van Der Waal M, Kardinaal A, Schloesser J, Ruijschop R, Claassen E. Probiotic research priorities for the healthy adult population: A review on the health benefits of Lactobacillus rhamnosus GG and Bifidobacterium animalis subspecies lactis BB-12. Cogent Food & Agriculture. 2018;4(1):1452839.

57. Bunyavanich S, Shen N, Grishin A, Wood R, Burks W, Dawson P, et al. Early-life gut microbiome composition and milk allergy resolution. Journal of Allergy and Clinical Immunology. 2016;138(4):1122-30.

58. Ghavami SB, Yadegar A, Aghdaei HA, Sorrentino D, Farmani M, Mir AS, et al. Immunomodulation and generation of tolerogenic dendritic cells by probiotic bacteria in patients with inflammatory bowel disease. International journal of molecular sciences. 2020;21(17):6266.

59. Wendel U. Assessing viability and stress tolerance of probiotics—a review. Frontiers in Microbiology. 2022;12:818468.

60. Zhao Z, Xu S, Zhang W, Wu D, Yang G. Probiotic Escherichia coli NISSLE 1917 for inflammatory bowel disease applications. Food & Function. 2022;13(11):5914-24.

61. Mandel DR, Eichas K, Holmes J. Bacillus coagulans: a viable adjunct therapy for relieving symptoms of rheumatoid arthritis according to a randomized, controlled trial. BMC complementary and alternative medicine. 2010;10:1-7.

62. Aziz N, Bonavida B. Activation of natural killer cells by probiotics. Onco Therapeutics. 2016;7(1-2).

63. Raghuwanshi S, Misra S, Sharma R, Bisen P. Probiotics: nutritional therapeutic tool. J Probiotics Health. 2018;6:194.

64. Kim G-H, Shim J-O. Gut microbiota affects brain development and behavior. Clinical and Experimental Pediatrics. 2022;66(7):274.

65. Tillisch K, Labus J, Kilpatrick L, Jiang Z, Stains J, Ebrat B, et al. Consumption of fermented milk product with probiotic modulates brain activity. Gastroenterology. 2013;144(7):1394-401. e4.

66. Molska M, Reguła J. Potential mechanisms of probiotics action in the prevention and treatment of colorectal cancer. Nutrients. 2019;11(10):2453.

67. Bingöl FG, Ağagündüz D, Budán F. Probiotic Bacterium-Derived p40, p75, and HM0539 Proteins as Novel Postbiotics and Gut-Associated Immune System (GAIS) Modulation: Postbiotic-Gut-Health Axis. Microorganisms. 2024;13(1):23.

68. Kim D-H, Jin Y-H. Intestinal bacterial β-glucuronidase activity of patients with colon cancer. Archives of pharmacal research. 2001;24:564-7.

69. Pavlović N, Stankov K, Mikov M. Probiotics—interactions with bile acids and impact on cholesterol metabolism. Applied biochemistry and biotechnology. 2012;168:1880-95.

70. Kumar R, Grover S, Batish VK. Hypocholesterolaemic effect of dietary inclusion of two putative probiotic bile salt hydrolase-producing Lactobacillus plantarum strains in Sprague–Dawley rats. British Journal of Nutrition. 2011;105(4):561-73.

71. Ye Q, Lao L, Zhang A, Qin Y, Zong M, Pan D, et al. Multifunctional properties of the transmembrane LPxTG-motif protein derived from Limosilactobacillus reuteri SH-23. Journal of Dairy Science. 2023;106(12):8207-20.

72. Yan F, Cao H, Cover TL, Washington MK, Shi Y, Liu L, et al. Colon-specific delivery of a probiotic-derived soluble protein ameliorates intestinal inflammation in mice through an EGFR-dependent mechanism. The Journal of clinical investigation. 2011;121(6):2242-53.

73. Chen M, Feng Y, Liu W. Efficacy and safety of probiotics in the induction and maintenance of inflammatory bowel disease remission: a systematic review and meta-analysis. Annals of Palliative Medicine. 2021;10(11):118211829-.

74. Muller L, Lorentz A. Probiotics in the treatment of inflammatory bowel disease in adulthood: a systematic review. 2022.

75. Xu M, Zhang W, Lin B, Lei Y, Zhang Y, Zhang Y, et al. Efficacy of probiotic supplementation and impact on fecal microbiota in patients with inflammatory bowel disease: a systematic review and meta-analysis of randomized controlled trials. Nutrition Reviews. 2025;83(2):e65-e73.

76. Stavropoulou E, Bezirtzoglou E. Probiotics in medicine: a long debate. Frontiers in immunology. 2020;11:2192.

77. Lopez-Santamarina A, Gonzalez EG, Lamas A, Mondragon AdC, Regal P, Miranda JM. Probiotics as a

possible strategy for the prevention and treatment of allergies. A narrative review. Foods. 2021;10(4):701.

78. Mazziotta C, Tognon M, Martini F, Torreggiani E, Rotondo JC. Probiotics mechanism of action on immune cells and beneficial effects on human health. Cells. 2023;12(1):184.

79. Lamichhane P, Maiolini M, Alnafoosi O, Hussein S, Alnafoosi H, Umbela S, et al. Colorectal cancer and probiotics: are bugs really drugs? Cancers. 2020;12(5):1162.

80. Laursen RP, Hojsak I. Probiotics for respiratory tract infections in children attending day care centers—a systematic review. European journal of pediatrics. 2018;177:979-94.

81. Russo L, Danza M, Basile I, Soardo S, Capocasale G, Paparone S, et al. Use of a probiotic mixture containing Bifidobacterium animalis subsp. lactis BB-12 and Enterococcus faecium L3 as prophylaxis to reduce the incidence of acute gastroenteritis and upper respiratory tract infections in children. Minerva Pediatrics. 2020;73(3):222-9.

82. Andaloro C, Santagati M, Stefani S, La Mantia I. Bacteriotherapy with Streptococcus salivarius 24SMB and Streptococcus oralis 89a oral spray for children with recurrent streptococcal pharyngotonsillitis: a randomized placebo-controlled clinical study. European Archives of Oto-Rhino-Laryngology. 2019;276:879-87.

83. Peng X, Li Z, Pei Y, Zheng S, Liu J, Wang J, et al. Streptococcus salivarius K12 alleviates oral mucositis in patients undergoing radiotherapy for malignant head and neck tumors: a randomized controlled trial. Journal of Clinical Oncology. 2024;42(12):1426-35.

84. Roszczenko-Jasińska P, Wojtyś MI, Jagusztyn-Krynicka EK. Helicobacter pylori treatment in the post-antibiotics era—searching for new drug targets. Applied Microbiology and Biotechnology. 2020;104(23):9891-905.

85. Moro-García MA, Alonso-Arias R, López-Vázquez A, Suárez-García FM, Solano-Jaurrieta JJ, Baltar J, López-Larrea C. Relationship between functional ability in older people, immune system status, and intensity of response to CMV. Age. 2012;34:479-95.

86. Hadrup SR, Strindhall J, Køllgaard T, Seremet T, Johansson B, Pawelec G, Wikby A. Longitudinal studies of clonally expanded CD8 T cells reveal a repertoire shrinkage predicting mortality and an increased number of dysfunctional cytomegalovirus-specific T cells in the very elderly. The Journal of Immunology. 2006;176(4):2645-53.

87. Riha P, Rudd CE. CD28 co-signaling in the adaptive immune response. Self/nonself. 2010;1(3):231-40.

88. Moro-García M, Alonso-Arias R, Baltadjieva M, Benitez C, Barrial M, Ruisanchez E, et al. Oral supplementation with Lactobacillus delbrueckii subsp bulgaricus 8481 enhances systemic immunity in elderly subjects. Age (Dordr) 35: 1311-1326. 2013.

89. Ferrucci L, Corsi A, Lauretani F, Bandinelli S, Bartali B, Taub DD, et al. The origins of age-related proinflammatory state. Blood. 2005;105(6):2294-9.

90. Kim C-S, Cha J, Sim M, Jung S, Chun WY, Baik HW, Shin D-M. Probiotic supplementation improves cognitive function and mood with changes in gut microbiota in community-dwelling older adults: a randomized, double-blind, placebo-controlled, multicenter trial. The Journals of Gerontology: Series A. 2021;76(1):32-40.

91. Kadam O, Dalai S, Chauhan B, Guru RR, Mitra S, Raytekar N, et al. Nanobiotechnology Unveils the Power of Probiotics: A Comprehensive Review on the Synergistic Role of Probiotics and Advanced Nanotechnology in Enhancing Geriatric Health. Cureus. 2025;17(3).

92. Zhang L, Zeng X, Guo D, Zou Y, Gan H, Huang X. Early use of probiotics might prevent antibiotic-associated diarrhea in elderly (> 65 years): a systematic review and meta-analysis. BMC geriatrics. 2022;22(1):562.

93. Ansari F, Pourjafar H, Jodat V, Sahebi J, Ataei A. Effect of Eudragit S100 nanoparticles and alginate chitosan encapsulation on the viability of Lactobacillus acidophilus and Lactobacillus rhamnosus. AMB express. 2017;7:1-8.

94. Atraki R, Azizkhani M. Survival of probiotic bacteria nanoencapsulated within biopolymers in a simulated gastrointestinal model. Innovative Food Science & Emerging Technologies. 2021;72:102750.

95. Alkushi AG, Abdelfattah-Hassan A, Eldoumani H, Elazab ST, Mohamed SA, Metwally AS, et al. Probiotics-loaded nanoparticles attenuated colon inflammation, oxidative stress, and apoptosis in colitis. Scientific Reports. 2022;12(1):5116.

96. Kim JJ, Shajib MS, Manocha MM, Khan WI. Investigating intestinal inflammation in DSS-induced model of IBD. Journal of visualized experiments: JoVE. 2012(60):3678.

97. Akiyama S, Nesumi A, Maeda-Yamamoto M, Uehara M, Murakami A. Effects of anthocyanin-rich tea "Sunrouge" on dextran sodium sulfate-induced colitis in mice. BioFactors. 2012;38(3):226-33.

98. Zhang H-z, Li X-m, Gao F-p, Liu L-r, Zhou Z-m, Zhang Q-q. Preparation of folate-modified pullulan acetate nanoparticles for tumor-targeted drug delivery. Drug delivery. 2010;17(1):48-57.

99. Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nature biotechnology. 2015;33(9):941-51.

100. Alkushi AG, Elazab ST, Abdelfattah-Hassan A, Mahfouz H, Salem GA, Sheraiba NI, et al. Multi-

strain-probiotic-loaded nanoparticles reduced colon inflammation and orchestrated the expressions of tight junction, NLRP3 inflammasome and caspase-1 genes in DSS-induced colitis model. Pharmaceutics. 2022;14(6):1183.

101. Huang H-L, Lai C-H, Tsai W-H, Chen K-W, Peng S-L, Lin J-H, Lin Y-H. Nanoparticle-enhanced postbiotics: Revolutionizing cancer therapy through effective delivery. Life Sciences. 2024;337:122379.

102. Lu Y, Wu Y, Pan L, Wang J, Tang R, Deng F, et al. In Vitro Screening of Lactic Acid Bacteria with RAW264.7 Macrophages and the Immunoregulatory Mechanism. Processes [Internet]. 2024; 12(5).

103. Shi J, Li H, Liang S, Evivie SE, Huo G, Li B, Liu F. Selected lactobacilli strains inhibit inflammation in LPS-induced RAW264.7 macrophages by suppressing the TLR4-mediated NF-κB and MAPKs activation. Food Science and Technology. 2022;42.

104. Wang S, Nie Z, Zhu L, Wu Y, Wen Y, Deng F, Zhao L. Probiotic Characteristics and the Anti-Inflammatory Effects of Lactiplantibacillus plantarum Z22 Isolated from Naturally Fermented Vegetables. Microorganisms [Internet]. 2024; 12(11).

105. Zhang X, Li Y, Zhang C, Chi H, Liu C, Li A, Yu W. Postbiotics derived from Lactobacillus plantarum 1.0386 ameliorate lipopolysaccharide-induced tight junction injury via MicroRNA-200c-3p mediated activation of the MLCK-MLC pathway in Caco-2 cells. Food & Function. 2022;13(21):11008-20.

106. Liao J, Huang J, Li X, Kuang J, Li J, Wang W, Li J. Disclosing the benefits of multi-strain compounds and their health impact mechanisms utilizing intestinal biomimetic technology. Frontiers in Microbiology. 2025;Volume 16 - 2025.

107. Xu Y, Wang Y, Song T, Li X, Zhou H, Chaibou OZ, et al. Immune-enhancing effect of Weizmannia coagulans BCG44 and its supernatant on cyclophosphamide-induced immunosuppressed mice and RAW264.7 cells via the modulation of the gut microbiota. Food & Function. 2024;15(21):10679-97.

108. Abbaszadeh S, Yousefi M, Arefhosseini S, Mahmoodpoor A, Ebrahimi-Mameghani M. The Effect of Seven-Strain Probiotic Supplementation on Th17 and Treg in Patients with Severe Traumatic Brain Injury Admitted to ICU: A Randomized Controlled Clinical Trial2024.

109. Ren J, He F, Yu D, Xu H, Li N, Cao Z, Wen J. 16S rRNA Gene Amplicon Sequencing of Gut Microbiota Affected by Four Probiotic Strains in Mice. Veterinary Sciences [Internet]. 2023; 10(4).

110. Samara J, Moossavi S, Alshaikh B, Ortega VA, Pettersen VK, Ferdous T, et al. Supplementation with a probiotic mixture accelerates gut microbiome maturation and reduces intestinal inflammation in extremely preterm infants. Cell Host & Microbe. 2022;30(5):696-711.e5.

111. Calvigioni M, Bertolini A, Codini S, Mazzantini D, Panattoni A, Massimino M, et al. HPLC-MS-MS quantification of short-chain fatty acids actively secreted by probiotic strains. Frontiers in Microbiology. 2023;Volume 14 - 2023.

112. Ramos-Garcia V, Ten-Doménech I, Moreno-Giménez A, Campos-Berga L, Parra-Llorca A, Solaz-García Á, et al. GC-MS analysis of short chain fatty acids and branched chain amino acids in urine and faeces samples from newborns and lactating mothers. Clinica Chimica Acta. 2022;532:172-80.

113. Lao J, Chen M, Yan S, Gong H, Wen Z, Yong Y, et al. Lacticaseibacillus rhamnosus G7 alleviates DSS-induced ulcerative colitis by regulating the intestinal microbiota. BMC Microbiology. 2025;25(1):168.

114. Jia D, Li Y, Wang Y, Guo Y, Liu J, Zhao S, et al. Probiotic Bacillus licheniformis ZW3 Alleviates DSS-Induced Colitis and Enhances Gut Homeostasis. International Journal of Molecular Sciences [Internet]. 2024; 25(1).

115. Ahn K, Baek K-W, Yun K, Oh Y, Kim YS, Im E, et al. The effects of candidate probiotic strains on the gut environment in dextran sulfate sodium-induced colitis mouse. Scientific Reports. 2025;15(1):21103.

116. Kwoji ID, Aiyegoro OA, Okpeku M, Adeleke MA. 'Multi-omics' data integration: applications in probiotics studies. npj Science of Food. 2023;7(1):25.

117. Qin M, Ma C, Wang Z, Liang M, Sha Y, Liu J, et al. Integrated Transcriptome and Metabolomics Analysis Reveals That Probiotics and Tea Polyphenols Synergetically Regulate Lipid Metabolism in Laying Hens. Agriculture [Internet]. 2024; 14(11).

118. Chen M, Wang Z, He H, He W, Zhang Z, Sun S, Wang W. Multi-Omics Analysis Reveals the Regulatory Mechanism of Different Probiotics on Growth Performance and Intestinal Health of Salmo trutta (S. trutta). Microorganisms [Internet]. 2024; 12(7).

119. Arabiyat SA. Prospectus and Concerns of Immunomodulatory Nanotechnologies and Nanoparticles Biocompatibility and Toxicity. Nanotechnology Based Microbicides and Immune Stimulators: Springer; 2025. p. 165-89.

120. Wolfram J, Zhu M, Yang Y, Shen J, Gentile E, Paolino D, et al. Safety of nanoparticles in medicine. Current drug targets. 2015;16(14):1671-81.

121. Zhao Y, Xing G, Chai Z. Are carbon nanotubes safe? Nature nanotechnology. 2008;3(4):191-2.

122. Jia G, Wang H, Yan L, Wang X, Pei R, Yan T, et al. Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene.

Environmental science & technology. 2005;39(5):1378-83.

123. Donaldson K, Murphy FA, Duffin R, Poland CA. Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. Particle and fibre toxicology. 2010;7(1):5.

124. Poland CA, Duffin R, Kinloch I, Maynard A, Wallace WA, Seaton A, et al. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nature nanotechnology. 2008;3(7):423-8.

125. Wang B, Yin J-J, Zhou X, Kurash I, Chai Z, Zhao Y, Feng W. Physicochemical origin for free radical generation of iron oxide nanoparticles in biomicroenvironment: catalytic activities mediated by surface chemical states. The Journal of Physical Chemistry C. 2013;117(1):383-92.

126. Iversen T-G, Skotland T, Sandvig K. Endocytosis and intracellular transport of nanoparticles: Present knowledge and need for future studies. Nano today. 2011;6(2):176-85.

127. Ma X, Wu Y, Jin S, Tian Y, Zhang X, Zhao Y, et al. Gold nanoparticles induce autophagosome accumulation through size-dependent nanoparticle uptake and lysosome impairment. ACS nano. 2011;5(11):8629-39.

128. Yusuf A, Almotairy ARZ, Henidi H, Alshehri OY, Aldughaim MS. Nanoparticles as drug delivery systems: a review of the implication of nanoparticles' physicochemical properties on responses in biological systems. Polymers. 2023;15(7):1596.

129. Yan L, Zhao F, Li S, Hu Z, Zhao Y. Low-toxic and safe nanomaterials by surface-chemical design, carbon nanotubes, fullerenes, metallofullerenes, and graphenes. Nanoscale. 2011;3(2):362-82.

130. Gao J, Zong X, Yue X, Wang M, Lin L, Jin C, et al. Exploring the Properties of Silica Nanomaterials on Supporting Bimetallic PtZn Sites for Direct Propane Dehydrogenation. ACS Applied Nano Materials. 2024;7(7):7018-27.

131. Liang D, Liu C, Li J, Li Y, Li J, Tan M, Su W. Engineering probiotics-derived membrane vesicles for encapsulating fucoxanthin: evaluation of stability, bioavailability, and biosafety. Food & Function. 2023;14(8):3475-87.

132. Çanga EM, Dudak FC. Improved digestive stability of probiotics encapsulated within poly (vinyl alcohol)/cellulose acetate hybrid fibers. Carbohydrate polymers. 2021;264:117990.

133. Ye N, Zhao P, Ayue S, Qi S, Ye Y, He H, et al. Folic acid-modified lactoferrin nanoparticles coated with a laminarin layer loaded curcumin with dual-targeting for ulcerative colitis treatment. International Journal of Biological Macromolecules. 2023;232:123229.

134. Breisch ST, Zemlan FP, Hoebel BG. Hyperphagia and obesity following serotonin depletion by intraventricular p-chlorophenylalanine. Science. 1976;192(4237):382-5.

135. Chen A, Gong Y, Wu S, Du Y, Liu Z, Jiang Y, et al. Navigating a challenging path: precision disease treatment with tailored oral nano-armor-probiotics. Journal of Nanobiotechnology. 2025;23(1):72.