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Role of prebiotics, probiotics, and synbiotics in management of inflammatory bowel disease: Current perspectives

Roy S *et al*. Prebiotics, probiotics and synbiotics in IBD

Abstract

Experimental evidence supports the fact that changes in the bowel microflora due to environmental or dietary factors have been investigated as implicating factors in the etiopathogenesis of inflammatory bowel disease (IBD). The amassing knowledge that the inhabited microbiome regulates the gut physiology and immune functions in IBD, has led researchers to explore the effectiveness of prebiotics, probiotics, and synbiotics in treating IBD. This therapeutic approach focuses on restoring the dynamic balance between the microflora and host defense mechanisms in the intestinal mucosa to prevent the onset and persistence of intestinal inflammation. Numerous microbial strains and carbohydrate blends, along with their combinations have been examined in experimental colitis models and clinical trials, and the results indicated that it can be an attractive therapeutic strategy for the suppression of inflammation, remission induction, and relapse prevention in IBD with minimal side effects. Several mechanisms of action of probiotics (for *e.g., Lactobacillus* species, and *Bifidobacterium* species) have been reported such as suppression of pathogen growth by releasing certain antimicrobial mediators (lactic and hydrogen peroxide, acetic acid, and bacteriocins), immunomodulation and initiation of an immune response, enhancement of barrier activity, and suppression of human T-cell proliferation. Prebiotics such as lactulose, lactosucrose, oligofructose, and inulin have been found to induce the growth of certain types of host microflora, resulting in an enriched enteric function. These non-digestible food dietary components have been reported to exert anti-inflammatory effects by inhibiting the expression of tumor necrosis factor (TNF)-α-related cytokines while augmenting interleukin (IL)-10 levels. Although pro-and prebiotics has established their efficacy in healthy subjects, a better understanding of the luminal ecosystem is required to determine which specific bacterial strain or combination of probiotics and prebiotics would prove to be the ideal treatment for IBD. Clinical trials, however, have given some conflicting results, requiring the necessity to cite the more profound clinical effect of these treatments on IBD remission and prevention. The purpose of this review article is to provide the most comprehensive and updated review on the utility of prebiotics,

probiotics, and synbiotics in the management of active Crohn's disease and ulcerative colitis/pouchitis.

Key Words: Ulcerative colitis; Crohn's disease; Pouchitis; Dysbiosis; Microbiota; Inflammation

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Core Tip: Current treatments for inflammatory bowel disease (IBD), such as corticosteroids and immunosuppressants, have potential adverse effects, and a significant proportion of patients dependent on these treatments are exposed to these associated long-term side effects. The discovery of novel and efficacious therapeutic strategies is a worldwide goal of IBD research, and probiotics, prebiotics, and synbiotics can offer viable solutions. These products offer a novel strategy to deliver beneficial components into the gut and emerge as promising new treatments for IBD, as intestinal dysbiosis has been reported as a major cause of his IBD. The review highlights the current state and action mechanism of these microbial therapies along with various studies that have reported their effectiveness in restoring balance in the gastrointestinal microbiota and thus eventually reducing intestinal inflammation.

INTRODUCTION

Inflammatory bowel disease (IBD) is an idiopathic disease resulting from a debilitating immunological response of the body to the host's gastrointestinal (GI) microflora (mainly colon and duodenum), that is triggered by several host factors, such as genetic makeup, environmental factors like smoking, stress, certain medication as well as physiological factors. IBD classified as Crohn's disease (CD) and ulcerative disease (UC), was previously anticipated to be triggered by adaptive immune responses, however, the recent research findings suggest the prominent role of the innate immune

system in instigating an imbalance and disparity between the beneficial microbiome and commensal microflora harboring in the human gut[1,2]. This imbalance, known as dysbiosis (Figure 1), leads to an aggravated inflammatory response, causing IBD. Alterations in the microbiome affect host homeostatic systems and interactions with luminal stimuli, which can ultimately lead to uncontrolled inflammation in the intestinal mucosa, leading to IBD[3,4]. This suggests that the human gut microbiome is beginning to be recognized for its important role and potential therapeutic solution for IBD. A better comprehensive understanding of the synergy between host genetics, external environmental factors and gut microbiome has opened new paradigms for seeking alternative effective therapies[5].

Gut microbiota - intestinal epithelial cell crosstalk

The human gut harbors 100 trillion varieties of microbial organisms, including viruses, bacteria, protozoa, as well as fungi that make up the microbiota or microbial flora[6]. Based on culture-independent molecular methods, more than one thousand species of bacteria live in the GI tract (GIT)[7,8]. The gut microbiota plays a central role in maintaining the integrity of the intestinal epithelium, leading to intestinal epithelial cell (IEC) turnover, apoptosis, and preserving the expression and function of tight junctions (TJs)[9,10]. TJs amid adjacent IECs is an additional significant factor involved in epithelial integrity and intestinal permeability. Several factors associated with reformed bacterial equilibrium in the intestinal ecosystem possess detrimental effects on the regulation of TJ expressions and functions, leading to the direct contact of pathogenic organisms on the intestinal epithelium, and augmentation of pro-inflammatory mediators release as well as oxidative stress[11]. Immunologically, through the generation of microbe-associated molecular patterns (MAMPs), the gut microbiota interacts with both adaptive and innate immune systems. The MAMPS induces various effects, such as the production of antimicrobial peptides, and tissue healing or repair, eventually contributing to intestinal immunomodulation[12]. Pathogen-associated molecular patterns are recognized by pattern recognition receptor-bearing cells [toll-like receptors

(TLRs) and Nod-like receptors] of the innate immune system and many epithelial cells. Various substances synthesized by commensal bacteria, such as short-chain fatty acids (SCFAs), flagellin, lipopolysaccharide (LPS), and sphingolipids, regulate the switch from B cells to immunoglobulin A (IgA)-producing plasma cells and promote the conversion of T cells to T helper 17 (Th17) cells. This in turn induces the differentiation and proliferation of regulatory T cells (Treg)[13,14]. Metabolically, the microbiota ferment carbohydrates and indigestible oligosaccharides to synthesize SCFAs such as propionate, butyrate, and acetate, that are abundant energy sources in the intestinal epithelial tissues[15]. Furthermore, the gut microbiome stops the evolution of potentially infective bacteria by antagonizing receptors and nutrients, releasing antimicrobial factors, and conferring resistance to colonization[16,17].

ROLE OF DYSBIOSIS IN IBD PATHOPHYSIOLOGY

Both commensal and pathogenic bacteria are dispersed all over the mucous layer in a healthy gut, but as the IEC is intact, it, therefore, causes resistance to any pathogenic invasion. However, in IBD patients, perturbation of the microbiota instigated by numerous factors leads to a disproportion in the composition of gut microbiota favoring pathogens and dysbiosis[18]. Dysbiosis refers to unfavorable alterations in the composition and physiology of the gut microbiota that steadily and eventually modify the host-microbiota interaction as well as the host immune system[19]. Abnormal microbiota deprives enterocytes of the necessary energy supply through reformed production of SCFAs, sphingolipids, and flagellin, thus promoting defective regenerative responses and increased apoptosis, disrupting TJ integrity, and dysregulating the mucosal immune response. Activated mucosal macrophages lead to amplified production of reactive oxygen species (ROS) and cytokines that further damage the intestinal epithelium and facilitate bacterial transmigration processes, perpetuating this vicious cycle of intestinal inflammation[20].

Dysbiosis and altered gut microbiota in patients with IBD

Both quantitative and qualitative fluctuations in gut microbiota composition are reported in IBD patients (Tables 1 and 2). The transition from dominant' commensal' microbes to potentially harmful 'pathogenic' microbes is now well documented for IBD. A decline in bacteria having anti-inflammatory potential (*e.g., Faecalibacterium prausnitzii*) and growth in bacteria having inflammatory potential [*e.g., Escherichia coli* (*E. coli*)] is commonly observed in IBD patients compared to healthy subjects[21-23]. Although fungi account for only 0.02%-0.03% of the gut microbiota composition, there has been emerging evidence supporting the role of enteric fungi in the pathogenesis of IBD[24]. In IBD, IEC integrity is lost due to persistent inflammation associated with disruption of the TJ occludin and zona occludens (ZO)-1. Pathogens such as fungi and bacteria can therefore penetrate the mucosal barrier and activate lamina propria TLRs, Dectin-1, and CARD9, leading to a more severe inflammatory phenotype[25].

Dysbiosis and disruption of epithelial barrier integrity

Human studies and experimental IBD models show that persistent inflammation combined with TJs disruption leads to IEC integrity damage. This encourages the escape of luminal pathogens through the epithelial barrier and stimulation of caspase-recruiting domains, which eventually leads to a profound inflammatory response[26]. The key strategies for accomplishing the ideal equilibrium between tolerance and immune response are bacterial recognition by TJs and the underlying gut-associated lymphoid tissue (GALT)[27]. The faulty activation of GALT on gut microbiota components has also been implicated in IBD pathogenesis, leading to the development of intestinal inflammation[28].

Dysbiosis and oxidative stress

The induction of ROS associated with gut dysbiosis plays an important role in IBD. The gut microbiota can directly generate ROS during inflammation or can produce other enzymes involved in endogenous ROS generation such as nitric oxide synthase (NOS) by macrophage activation that promotes DNA damage. Oxidative stress caused by the

disruption of pro-oxidant molecules and antioxidant defenses leads to cellular damage including abnormal membrane function, protein aggregation, and DNA damage[29,30]. Oxidative stress promotes early inflammatory responses through positive feedback, increasing ROS generation and resulting in tissue damage[31].

PROBIOTICS, PREBIOTICS, AND SYNBIOTICS AS THERAPEUTIC STRATEGY

Standard clinical treatment of IBD consists of agents that modulate the inflammatory pattern of the GIT, including mesalamine, azathioprine, anti-tumor necrosis factors (TNFs), and glucocorticoids. However, these drugs often appear to have serious side effects, and some patients require higher doses throughout the course of treatment[32]. A significant proportion of patients with IBD either initially do not respond to treatment or lose response over time[33]. The inability to perform surgical procedures due to the physical extent or misalignment of the lesion also poses significant challenges to the management of IBD[34]. Various new drugs targeting cytokines, adhesion molecules, or tyrosine kinases are presently in clinical trials[35]. Gut microbiota modulation has emerged as an attractive new therapeutic approach for IBD, and gut microbiota-targeted/based therapies have been intensively investigated with varying degrees of success. Although the exact etiology of IBD remains unknown, the critical role of the gut microbiota in the development and persistence of IBD highlights the importance of microbiota-host interactions in health and disease[36]. Recent advances in assessing the therapeutic potential of microbiota in the treatment of IBD support the reconstitution of microbial resident populations by administration of appropriate microbes. The gut microbiota influences the host by modulating physiological, pathophysiological, and immunological processes. Experimental animal studies along with clinical data have confirmed the influence of the gut microbiome in ameliorating inflammation, highlighting its potential as a therapeutic strategy for treating inflammatory diseases. Numerous therapeutic strategies have been developed to modify and remodel the gut microbiome for the treatment of other GI diseases, including IBD. Prebiotics, probiotics

(PROs), synbiotics, and fecal microbiota transplantation (FMT) are currently considered to be the most common treatments[37].

The use of PRO organisms to alter the composition of the gut microbiota and restore resistance to bacterial antigens in the host microbiota has been expansively studied. The initiation of regulatory and effector immune responses at the intestinal mucosa can also be modified by diet-related variations in the composition of the gut microbiota. Therefore, alteration of the composition of the gut microbiome with fermented milk products, prebiotics, and PROs may improve the host health, and reduce the severity or symptoms of the disease. It is being researched as a promising prophylactic and therapeutic tool against gut inflammation by supporting conventional treatment. This new therapeutic approach is supported by the ability of the intestinal microbiota to regenerate itself completely and also by the multifactorial mechanism of the disease[38].

PROS

PROs are specific live microorganisms, when consumed in appropriate amounts, are beneficial to the health of the host. Specific strains must be strictly identified at the genus, species, and strain level, and specific strains must be registered in international cultural collections. Generalizations regarding the validity of whole species or genera are therefore likely to be misleading. PRO therapy involves the targeted introduction of beneficial microorganisms into the intestinal flora. This causes many beneficial bacteria to compete for nutrients and starve harmful bacteria. PROs participate in many positive health-promoting activities in human physiology, including the maintenance of a healthy gut[39]. The most common strains currently available as PROs and possessing beneficial health effects are *Enterococcus faecium, Bifidobacterium, Bacillus, Saccharomyces boulardii* (*S. boulardii*), *Lactobacillus strains*, and *Pediococcus*. Molecular mechanisms for the beneficial effects of these PROs include (Figure 4): (1) Production of butyrate, IgA, and SCFA formation and stimulatory signaling proteins; (2) Reduced secretion of pro-inflammatory cytokines; (3) Increased mucin-2 expression; (4) Increased autophagy; and (5) Augmented upregulation of defensins. Although PROs have shown promise both

preclinically and clinically, the theoretical risks have been explained in several case reports, clinical trial results, and experimental models. These include systemic infections, adverse metabolic activity, overstimulation of the immune system in susceptible individuals, gene transfer, and GI side effects[40].

Desirable properties of PROs

A microbial strain must meet several specific characteristics to be considered as a PRO. These standards are divided into safety, performance, and technical aspects. Criteria also depend on the specific purpose of the strain and on the location where it expresses certain traits. For safety reasons, PRO strains must be of human origin and isolated from the GIT of healthy hosts. They must have generally considered safe (GRAS) status, be non-pathogenic, and have no prior association with diseases such as infective endocarditis and GIT disorders. PRO strains should not deconjugate bile salts and should not carry antibiotic-resistance genes that could be transmitted to pathogens. Hosts must be tolerant to PROs and strain should not induce any immune reaction in the host. Even in immunocompromised individuals, the strain itself, its fermentation products, or its cellular components should be non-toxic, non-pathogenic, non-allergenic, and non-carcinogenic. It must have antimutagenic and anticarcinogenic properties and not promote inflammation in individuals[41].

In terms of efficiency, in order to survive the GIT, a sufficient number must endure and be able to attach to the intestinal mucosal surface. They should have antagonistic activity against pathogens that adhere to mucosal surfaces such as *Clostridium difficile Salmonella*, and *Listeria*. PRO strains are believed to stimulate an immune response, thereby positively affecting the host. PROs must endure the environmental conditions at their target site of action and proliferate there. They must be able to adhere to and colonize the lining of IECs, to establish themselves in the colon[42]. They must withstand the stresses during product processing and storage. In particular, the organism must be able to withstand harsh environmental conditions such as gastric and bile acids, and digestive enzymes of the stomach and small intestine. Additionally, technical aspects

should be considered before choosing a PRO strain. The strain should be capable of being prepared on a large scale and should multiply rapidly, with good viability and stability during storage. They must be metabolically active within the GIT and biologically active against their identified targets[43].

PROs' role in IBD

Only those organisms that adhere to the intestinal epithelium provide the beneficial effects of the ingested PRO bacteria. The existence and adherence of the PROs to the intestinal mucous membrane shape up a robust natural biological barrier for several pathogens. Adhesion is therefore considered to be the first step toward colonization. Adhesion to intestinal epithelia can be specific, including adhesion of bacteria and receptor molecules on the IECs, or non-specific, based on physicochemical factors.

PROs interconnect with IECs and various cell groups involved in both innate and adaptive immune responses through pattern- recognition receptors. They can improve intestinal barrier function and reduce intestinal permeability of gut microbes and other antigens. For example, several *Lactobacillus* strains can upregulate the expression of MUC3 gene, which results in increased mucus production by intestinal goblet cells. Numerous PRO strains can stimulate the production and secretion of various antimicrobial peptides such as phospholipase lysozyme, defensins, or lactoferrin by IECs[44,45].

PROs may also induce the production of various antibacterial components such as bacteriocins, hydroperoxides, and lactic acid. As living organisms, PROs should reach the intestine and should be capable of maintaining homeostasis. Their utmost significant mechanisms of action are reliant on the strain and may include microbiota modulation, improvement in the barrier function, and immunomodulation through the direct action of PRO bacteria on different immune and epithelial cell types. They can also block binding sites on IECs and upregulate TJ molecules in the mucosal barrier. They can induce toxin-receptor degradation, pH changes, and competition for essential nutrients and directly decrease epithelial cell apoptosis. Thus, the role of PROs in IBD is

mainly focused on: (1) Antagonizing pathogenic microorganisms; (2) Restoring biodiversity within the microbiota; (3) Stimulating epithelial proliferation to achieve mucosal healing; (4) Modulating intestinal permeability; (5) Improving mucus production in terms of abundance and composition; and (6) Mediating both anti-inflammatory and anti-fibrotic effects[46].

Common PRO microorganisms

Many microorganisms are currently used as PROs. However, the most commonly used are bacteria of the genus *Lactobacillus*, which are regarded as the first and largest group of microorganisms to be considered as PROs and *Bifidobacterium*. These bacteria are indigenous to the human GIT and are known to possess no harmful action. Species of *Lactobacilli* include *L. curvatus*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactobacillus brevis*, *Lactobacillus reuteri*, *Lactobacillus delbrueckii*, *Lactobacillus bulgaricus*, *Lactobacillus johnsonii*, *Lactobacillus casei*, *Lactobacillus cellobiosus*, *Lactobacillus gasseri* and *Lactobacillus plantarum*. The most recognized *Bifidobacteria* species used are *Bifidobacterium animalis* subsplactis formerly *Bifidobacterium lactis*, *Bifidobacterium breve* (*B. breve*), and *Bifidobacterium longum*. Lactic acid bacteria from genera such as *Lactococcus*, *Streptococcus*, *Leuconostoc*, *Propionibacterium*, *Enterococcus*, and *Pediococcus* are also regarded as PROs now. Other non-related microbes used include bacteria such as non-pathogenic *E. coli* Nissle 1917 and *Saccharomyces cereviciae*, *Clostridion butyricum*, *S. boulardii*, *Aspergillus oryzae*, and some spore-forming bacilli[47].

PREBIOTICS

Prebiotics are non-digestible food ingredients that selectively stimulate the growth of beneficial bacteria or promote the activity of a limited number of health-promoting bacteria. However, prebiotics can also help to improve the existence and effectiveness of ingested PRO bacteria. According to the most recent definition, “a prebiot ic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the GI microbiota that confers benefits upon host well-being and

health". Now, the International Scientific Association of PROs and Prebiotics has introduced a new definition of prebiotics as "a substrate which is selectively fermented by the gut microflora and bestows health benefits to the host". This new definition states that non-carbohydrate constituents are also considered prebiotics and their applications are not constrained to the GIT only[48].

Desirable properties of prebiotics

To classify a substance as a prebiotic, several criteria must be met: Safety, resistance to digestion in the upper GIT, organoleptic properties, stability, fermentability in the colon, and intestinal benefits along with their ability to encourage the evolution of beneficial bacteria in the gut. For a product to be regarded as a prebiotic, it must fulfil the following conditions: (1) Stimulate the growth and activity of selected strains of bacteria that have a beneficial effect on health; (2) Decrease the pH of the intestinal contents; (3) Resistant to hydrolysis and the action of GI enzymes; (4) Unabsorbed in the upper GIT; (5) Act as a medium for colonic beneficial microorganisms; (6) Stable in the product processing process; and (7) Promote the growth of PROs[49,50].

Prebiotics' role in IBD

Prebiotics aid as nutrient sources for the PROs to help in their growth, proliferation, and increase in the intestinal flora. Prebiotics are not digested and remain in the gut lumen and serve as a substrate for many bacteria. Prebiotics are fermented in the colon by commensal bacteria to SCFAs and lactic acid, thereby lowering the luminal pH and thus stimulating the proliferation of beneficial microorganisms such as *Lactobacillus*, *Firmicutes* (F), and *Bifidobacteria* while obstructing the growth and activity of pathogenic organisms such as *Bacteroidetes* and *Clostridium difficile*. This ultimately corrects the imbalance in the intestinal microbiota. These beneficial effects are primarily due to the type and amount of prebiotics used in the diet and the density of *Bifidobacteria* in the host gut[51]. Additionally, the prebiotics has a positive effect in preventing IBD by modulating flora-tropic functions in providing other health benefits[52].

Common prebiotic examples

The majority of the prebiotics belong to carbohydrates under oligosaccharide subsets. The most common prebiotics known for their beneficial effects would be galactooligosaccharides (GOS), oligosaccharides, and fructooligosaccharides (FOS). Other examples include pectins, arabinose and galacturonic acid[53]. Fruits and vegetables such as asparagus, bananas, barley, chicory, garlic, honey, Jerusalem artichokes, onions, sugar beets, rye, soybeans, wheat, tomatoes, human and cow milk, peas, and beans are the natural sources of prebiotics. Seaweed and microalgae have also been reported. Human milk is known to contain much more GOS than cow's milk. Oligosaccharides can be found in amounts of up to 12 g/L, making them the third bulkiest component of human milk. Intake of foods containing prebiotic oligosaccharides is not sufficient to modulate intestinal flora, as they are present in these foods at low concentrations. Therefore, prebiotics are produced on a large industrial scale from raw materials such as lactose, sucrose, and starch[54].

Examples of non-digestible dietary oligosaccharides include carbohydrates such as oligofructose, inulin, Xylooligosaccharides (XOS), gentio-oligosaccharides, isomalto-oligosaccharides, lactulose, transgalacto-oligosaccharides, soybean-oligosaccharides, and polysaccharides as starch and pectins are considered to be effective prebiotics[55]. Some herbs can also have a prebiotic effect on the host such as *Piper nigrum*, *Zingiber officinale*, and *Ocimum sanctum*. The latter two exhibited a higher growth of *Lactobacillus* and *Bifidobacterium* and have a greater prebiotic potential as compared to the most commonly used prebiotic, FOS. *Piper nigrum* has shown similar prebiotic actions as that of FOS. These herbs are utilized for regulating the gut microbiota which eventually prevents inflammatory disorders[56].

SYNBIOTICS

Combinations of PROs and prebiotics are viewed as promising new approaches and provide an opportunity to explore their potential and efficacy in human IBD. When

PROs and prebiotics are combined in a product to achieve synergistic actions, they are commonly referred to as synbiotics. Many examples have demonstrated that prebiotics appears to be more efficacious when used along with a PRO as a part of the synbiotic combination. The term synbiotic refers to synergism where the prebiotic component is selectively favoured by the live PRO organism. The synbiotic combination is intended to enhance the *in vivo* survival and activity of proven PROs to promote or enhance the beneficial properties of both products. However, recently the term 'synbiotics' has been re-defined as preparations favoring synergism, where the PROs metabolize the complemented prebiotics to induce specific rebalancing of the dysbiotic gut and host health[57]. Synergistic PROs and prebiotics stimulate selective microbial growth or activate specific metabolism *via* gut flora. The presence of the readily fermentable substrate should enhance the survival of the PRO. The prebiotic component should also protect the PRO from gastric acidity and proteolysis, possibly through steric hindrance and coating of the PRO. Therefore, it is important to select specific substrate and microbial combinations in synbiotic products that can enhance beneficial effects compared to products containing PROs or prebiotics alone[58].

Synbiotics' role in IBD

Synbiotics have been suggested to help reduce the metabolic activity of unwanted microorganisms, decrease the harmful microbiota, such as *Clostridium* and *Enterobacterium* as well as increase the beneficial strains such as *Lactobacilli* and *Bifidobacteria*. It also prevents bacterial translocation by restoring the barrier function of the intestinal epithelium by upregulating mucus production and inducing host immunomodulatory activity. An *in vitro* study showed that synbiotics exert anti-inflammatory effects and some demonstrate antiproliferative properties[59].

Examples of synbiotics

The most typical used prebiotics are oligosaccharides like FOS, GOS, XOS, and inulin while the most commonly used PROs in synbiotic preparations include *Bifidobacteria*

spp., *Lacbobacilli*, *B. coagulans*, *S. boulardii*. Synbiotic supplements available include a combination of *Bifidobacteria lactobacilli* with either FOS or inulin or combinations of *Lactobacillus* and inulin or *Bifidobacteria* with FOS. *Lactobacilli*/Lactitol, and *Bifidobacteria*/GOS combinations have also been tried as synbiotics in addition to *Bifidobacteria*/FOS[60].

MECHANISM OF ACTION OF PROS, PREBIOTICS, AND SYNBIOTICS

PROs

PROs, prebiotics, or synbiotics can achieve therapeutic effects in IBD through various mechanisms. They influence the composition of the gut microbiome and modify the metabolic properties of the microbiota. The mechanisms of action of PROs include competitive actions with commensal and pathogenic bacteria and effects on epithelial function and immune responses. By augmenting the production of SCFA, they can lower the pH of the intestinal environment, thereby inhibiting the growth of potentially pathogenic microorganisms. They influence the metabolites and biodiversity of the microbiota. Competitive exclusion of bacterial substrates, space occupation, and increased mucosal IgA are the main mechanisms that explain these modifications. Some PROs enhance the integrity of the mucosal barrier thereby normalizing intestinal permeability. All these factors make them interesting candidates for the treatment of intestinal inflammation. Several mechanisms have been proposed to explain the effects of PROs[61]. The effects of PROs vary and depend on type and dose as well as on their interaction with the host in different ways. Some exhibit direct antibacterial action *via* the production of substances such as bacteriocins, hydroperoxides, lactic acid, and defensins. Others exhibit non-immunological action such as competing with pathogens for nutrients, increasing mucus production, changing intestinal pH, by promoting the formation of TJs, or enhancing tissue repair processes, thereby reducing intestinal mucosal permeability. Finally, PROs can also modulate the immunological response (immunoglobulin production, pro-inflammatory cytokine production) by releasing cell wall fragments or DNA in the intestinal lumen.

Extensive literature confirms the anti-inflammatory effects of PROs, which reflect the immune tolerance that exists between the host and its microbiota. PROs can directly or indirectly modify the immune system response in the gut, reflecting their distinct immunoregulatory properties. Thus, PROs can be subdivided into two broad groups depending upon their effect on the immune system: One demonstrating immunostimulant activities and the other exhibiting anti-inflammatory actions. Initial experiments with a PRO strain, *Bifidobacterium longum*, showed a potential anti-inflammatory action attributed to the significant decline in the level of proinflammatory mediators, simultaneously preserving the level of anti-inflammatory cytokines. Furthermore, the strain could also reduce C-reactive protein (CPR) levels in patients with UC. Consequent research confirmed its ability to counteract the other pathogens and its actions with other PRO strains to produce a potent anti-inflammatory response[62-64]. Another strain *Faecalibacterium prausnitzii* has been reported to be protective against IBD in humans, by inducing IL-10 in murine dendritic cells and humans and eventually preventing the development of chronic inflammation as well as suppressing the disease symptoms.

Some PRO strains can induce the maturation of intestinal dendritic cells, an important part of antigen presentation and immune regulation, and prolong their survival. Some PROs work by enhancing Treg responses, which are antigen-specific T cells involved in preventing autoimmunity and maintaining tolerance to harmless antigens, including the gut commensal microbiota[36]. They also regulate the overactivation of the nuclear factor kappa light chain enhancer of activated B cells (NFκB) pathway, reduce the production and secretion of pro-inflammatory cytokines [such as IL-8, TNF-α, interferon gamma (IFN-γ)], and induce the production of anti-inflammatory cytokines such as IL-10 and transforming growth factor (TGF)β. Some studies have shown that PROs amend the mucosal immune system through a process mediated by TLRs, promoting the differentiation of TH1 cells, thereby increasing antibody production, and phagocytes[44]. It has been shown to increase natural killer cell activity and induces T-cell apoptosis, simultaneously increasing intestinal anti-

inflammatory activity while down-regulating pro-inflammatory cytokines. There may be other mechanisms of PRO effects that are yet to be proven. Given that the etiology of each type of IBD is different and that the mechanism of action of PROs is strain-specific and quite versatile, it is expected that different PROs will be effective for each type and stage of the disease[62,65].

PROs improve gut barrier physiology by promoting the protein synthesis that are key components of TJs, inhibiting IEC apoptosis, and increasing the mucus layer. PROs constructively alter the microbiota composition by constraining the growth of potentially pathogenic bacteria through the production of various substances such as bacteriocins. In addition, butyrate inactivates the intracellular transcription factor NFκB pathway, thus weakening the synthesis of inflammatory cytokines, thus demonstrating to act directly as an anti-inflammatory agent[66]. The various other strains can also produce antibacterial substances such as hydrogen sulfide, hydrogen peroxide, and lactic acid, which are again useful in displacing deleterious microbes from the luminal-mucosal interface. PROs create a more acidic environment that is toxic to inflammatory bacteria and help promotes the dominance of beneficial *Lactobacillus* and *Bifidobacterium* species while decreasing the pathogenic fungal diversity. It also increases the production of fatty acids with anti-inflammatory and anti-carcinogenic properties. Additionally, PRO effects on physiological processes such as visceral sensation may alleviate symptoms of IBD, and effects on the central nervous system may positively influence comorbidities such as depression[67,68].

Prebiotics

Prebiotics can be described as "non-digestible food ingredients stimulating the growth of a certain number of bacteria in the colon, which can improve the host's health". Prebiotics enter the large intestine intact and get fermented by the residing bacteria. As prebiotics penetrate through the intestinal lumen, they bind with water thereby increasing the volume of intestinal contents. Prebiotics exert beneficial effects on IBD through multiple mechanisms of action. Primarily they accelerate the selective

proliferation of native bacteria of the gut microbiota. These ingredients provide a better breeding space for beneficial microorganisms due to their loose structure and large surface area and at the same time inhibit the growth of pathogens[69]. Secondly, they increase the production of SCFAs such as acetate, butyrate, and propionate. These SCFA are formed during the fermentation of prebiotics and play a very vital role in the proper functioning of the intestine. They accelerate the regeneration and healing process of the IEC; augment mucus production; maintain the correct pH in the intestine. They also inhibit the attachment of pathogenic microbes to enterocytes. Acetate is commonly used as a cellular fuel for building muscle and colonic tissue. Butyrate exhibit various beneficial effects on the host, such as improving metabolism, modulating the host's immune system, and promoting anti-inflammatory actions, therefore receives special attention[70].

Therefore, prebiotic consumption has been shown to boost host immune function, reduce infection rates, enhance colonic integrity, and downregulate allergic reactions. They prominently alter both, colonocyte morphology and physiology and consequently repair the inflamed intestinal epithelium in IBD patients. Prebiotics also have been shown to accelerate intestinal barrier integrity by modulating the immunological responses by declining the activation of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-17. They also hold a beneficial role in stimulating epidermal growth factor receptor that aids in maintaining intestinal homeostasis, further suppressing the inflammatory cytokines, and NFκB. All these actions lead to the reduction of colonic inflammation in IBD[71].

However, these effects are not directly imposed by ingesting prebiotics. It was suggested that the benefits of prebiotics are achieved indirectly. Prebiotics improves the mucosal barrier *via* encouraging the PROs growth that can upregulate epithelial defense mechanisms[60].

Synbiotics

Synbiotics aims to enhance the persistence, activity, and survival of established PROs *in vivo*, thereby promoting the advantageous properties of both products. Synbiotics were developed to overcome the potential survival difficulties of PROs, especially during passage through the upper GIT. Therefore, the use of synbiotics is said to contribute to more effective engraftment of PROs into the colon and promote the proliferation of PRO strains.

In vitro studies specify that synbiotics exert primarily anti-inflammatory actions along with some antiproliferative activities[63]. The literature on synbiotics is difficult to interpret, as it is often impossible to distinguish whether the desired therapeutic benefits are attributable to prebiotics, PROs, or synergistic interactions between them. Various studies have provided robust preliminary evidence that synbiotic administration to IBD patients results in beneficial therapeutic effects. In one study, prebiotic Synergy 1 in combination with *Bifidobacterium longum* improved sigmoidoscopy scores and reduced b-defensins, TNF-α, and IL-1α in biopsy samples from UC patients[72]. In another study, patients who received *Bifidobacterium longum* and prebiotic Synergy 1 (with FOS/inulin blend) combination revealed a significant histological improvement in comparison to the placebo group. Synbiotics significantly reduced the TNF-α expression and thereby reported the potential beneficial effect of synbiotics in the management of IBD. Combinations of synbiotics may exert beneficial impacts on the intestinal mucosa[71]. Therefore, evaluating the role of synbiotics as an alternative form of IBD treatment should be considered[73].

PROS IN IBD

Various literature searches indicate that IBD patients have a particular interest in using complementary and alternative medicine. This is often due to fear of side effects and lack of efficacy of treatment with conventional drugs. IBD patients have also shown great interest in the potential treatment with PROs. Research on the use of PROs in the treatment of IBD has been conducted since 1997[74]. A 50% increase in PRO use has been reported in IBD patients. This is due to the belief that PROs are safe and beneficial as

adjunctive therapy for IBD patients in both exacerbation and remission. Despite the moderately huge data reports on the use of PROs in IBD, the possibility to draw firm conclusions is significantly limited. This may be due to the small number of patients in the study groups, large differences in intervention types, or lack of standardization in study methods. There are also few published clinical studies on the effects of PROs on inflammatory changes examined by GI endoscopy in IBD patients. However, the potential use of well-selected commensal microbial species with protective effects on the intestinal mucosa and modulation of immune responses offers hope for new treatment options for patients with IBD. There are many reports on the efficacy of PROs in treating functional abdominal pain, so the use of PROs in the management of patients with overlapping dysfunction and IBD may be of great benefit[75]. The evidence for the anti-inflammatory effects of PROs comes from three types of research: *In vitro* studies using cell lines, animal models, and clinical studies. Because PROs are used at different types, doses, frequencies, and durations of administration, the effects of PRO administration vary in both animal models and clinical studies[76]. In general, either single strains or consortia of PRO strains have been used for PRO therapy in both animal and human trials[77]. Various preclinical studies have been conducted to explore the efficiency of PROs in the IBD are summarized in Table 3 and the clinical interventions are summarized in Table 4.

Effectiveness of PROs in the animal models of colitis

Eradication of ROS by antioxidant enzymes such as catalase from the inflammatory site may efficiently restrain IBD pathogenesis. A genetically engineered PRO *E. coli* Nissle 1917 (EcN), which acts by overexpressing the catalase and superoxide dismutase, was evaluated for the treatment of intestinal inflammation in a mouse IBD model induced by dextran sodium sulfate (DSS), 2,4,6-trinitrobenzene sulfonic acid (TNBS) and oxazolone. The PRO bioavailability in the GIT was increased by the application of chitosan and sodium alginate effective biofilms. It effectively relieved inflammation and repaired epithelial barriers in the colon and restored the expression of TJ-associated

proteins. It also regulated the gut microbial flora and augmented the abundance of vital microbes that helped in the maintenance of intestinal homeostasis such as *Lachnospiraceae*_NK4A136 and *Odoribacter*. Thus, this study laid a foundation for the development of living therapeutic proteins using PROs to treat intestinal-related diseases[78].

With remarkable advances in genetic engineering, scientists have recently developed bacterial/PRO strains that are genetically engineered to function as 'gut biosensors' that can help to detect inflammatory markers or 'resident cell factories' of therapeutic molecules that will act as biotherapeutic drugs to improve the drug delivery at mucosal surfaces. Most technological approaches have focused on the transfection of plasmids encoding immunomodulatory cytokines, reporter substrates or anti-inflammatory mediators[79].

Wang *et al*[80] showed that an engineered EcN discharging the immunoregulatory protein Sj16 isolated from the helminth *Schistosoma japonicum* alleviated the DSS-induced colitis in mice by modifying the gut microbiota. The immunoregulatory protein exhibited a protective effect against colitis through its action on the peroxisome proliferator-activated receptor-alpha (PPAR-α) receptor, reestablishing populations of the *Ruminococcaceae* family, thereby augmenting intestinal butyrate levels. Trefoil factors (TFF) and anti-TNF-α nanobodies have been constitutively expressed in *Lactococcus lactis*. These therapeutic compounds were tested in DSS-induced colitis in mice. TFFs peptides anti-TNF-α nanobodies exhibited prominent protective and repairing effects on the intestinal epithelium.

Zhang *et al*[81] established a constitutively expressing IL-35 *E. coli* as a novel oral delivery system with immunosuppressive acctions, facilitated by regulatory Tregs and B cells. The IL-35-producing *E. coli* demonstrated a decrease in the inflammatory response in a mice model of colitis by downregulating Th17 cells. Another research group followed a different approach. They have examined a light-responsive EcN-secreting IL-10 in DSS-treated mice. After oral administration, the tissue-permeable near-infrared light was converted to blue light by up-conversion microgels for

activating the recombinant EcN in the gut and to permit the controlled IL-10 release in mice. It was reported that treatment with IL-10-secreting EcN suppressed the colonic inflammatory symptoms and shielded the intestinal mucosa from DSS-mediated cell damage.

Lactobacillus paracasei has been genetically engineered with the human N-acylphosphatidylethanolamine-specific phospholipase D gene and, when potentiated with an ultra-low exogenous dose of exogenous palmitate, it selectively induced palmitoylethanolamide in the GIT. PEA exerted potent anti-inflammatory effects, and it had showed improvement in inflammation in animal models of colitis[82]. Liu *et al*[83] designed an engineered *Bifidobacterium longum R0175* (*B. longum*) that expresses the antioxidant enzyme manganese superoxide dismutase. The PRO helped to improve colitis symptoms by attenuating the ROS-mediated oxidative stress and constraining endothelial cell activation. Following the treatment, attenuation in TNF-α, IL levels, as well as a complete improvement in the macroscopic and microscopic inflammatory markers was observed. The treatment also showed to regulate the expression of adhesion molecules and leukocyte-endothelial interactions[83]. Yet another study reported the beneficial effect of genetically modified *B. longum* that expresses αmelanocyte-stimulating hormone in a DSS model of colitis. The PRO exhibited significant anti-inflammatory properties by suppressing the release of proinflammatory cytokines such as ILs, TNF-α, and NO, while increasing the anti-inflammatory cytokine (IL-10) release[84].

Feng *et al*[85] explored the ameliorating effects of pasteurized PRO fermented milk on DSS-induced IBD in rats and found that intragastric gavage of milk prominently declined the disease activity index (DAI) scores and alleviated the colon tissue damage. The improvement was ascribed to the anti-inflammatory effect of the PRO by decreasing TNF-α and IL-6 levels. Furthermore, pasteurized PROs significantly increased the density of beneficial bacteria while simultaneously reducing inflammatory pathogens. There was significant enrichment in biosynthesis pathways of

amino acids, nucleotides, and vitamins. The pasteurized PRO fermented milk alleviated IBD by reducing the inflammatory response and restoring the gut microbiota.

Javed *et al*[86] showed that *Bifidobacterium infantis* had beneficial effects in alleviating TNBS-induced colitis. Supplementation with *Bifidobacterium infantis* demonstrated significantly less damage to mucosal cyto-structures and reduced the colitis symptoms. This demonstrates the importance of PROs in protecting the goblet cells and epithelial cell layers. In a mouse model of TNBS colitis, *Bifidobacterium bifidum* supplementation appeared to attenuate disease progression in terms of colonic edema, gross lesions, and histologic values, as well as prevented weight loss[86]. Based on another research group, *Bifidobacterium bifidum* supplementation significantly increased IL-10 levels and decreased IL-1β levels in colonic sections, confirming anti-inflammatory effects. These results seem to support the regulatory properties of *Bifidobacterium infantis* and *Bifidobacterium bifidum* to reduce inflammation and clinical signs of colitis[87].

In IBD, a decrease in F abundance and an increase in the *Bacteroidetes* (B) bacteria are found to be associated with the disease progression. Certain PRO strains can be used to treat IBD by modulating intestinal homeostasis. A decreased F/B ratio has been found in many cases of IBD. The common PROs with anti-inflammatory effects in IBD are bacteria of the phylum *F*, but only a few are able to effect the F/B ratio. Early administration of *L. reuteri* DSM 17938 to C57BL/6J mice improved the abundance of *F* and diminished the abundance of *Bacteroidetes* comparatively, thereby altering gut microbial homeostasis. PROs further augmented the proportion of FoxP3+ Treg in the mouse gut and decreased the quantity in enteric lymph nodes, thus indicating that *L. reuteri* DSM 17938 assists in the trafficking of Treg from lymph nodes to the intestine[88].

L. plantarum is one of the most frequently used PROs for IBD. Its mechanism for the prevention and treatment of IBD is diverse. Live and dead *L. plantarum* AN1 administered to an IBD mouse model *via* drinking water exhibited intestinal regulatory and anti-inflammatory properties. In the IBD mouse model, both live and heat-killed bacteria increased the frequency of the F and decreased the frequency of Bacteroidetes. A combination of two diverse PROs (*Bifidobacterium bifidum* WBIN03 and *L. plantarum*

ZDY2013) diminished the UC in mice by altering the microbiota and reducing oxidative stress as well as inflammation. The combination upregulated antioxidant factors and downregulated TNF-α in UC mice. A PRO blend augmented the frequency of *F* and reduced the frequency of *Bacteroidetes*[89].

Lactobacillus fermentum KBL374 and *L. fermentum* KBL375 PROs blend transformed microbiota in mice with colitis. The co-administration of the *L. fermentum* KBL374 and KBL375 amended gut dysbiosis and ameliorated colitis by decreasing the pro-inflammatory cytokine levels, and augmented anti-inflammatory cytokines. These PROs mechanism of action includes balancing the F/B ratio, epithelial cell barrier improvement and altering cytokine secretion[90].

Many reports are confirming that the positive effects of PROs can only be achieved by consuming a mixture of different strains. Numerous data reports the utilization of multi-strain PRO preparations. The VSL#3 is well known for its efficiency in IBD[91,92]. VSL#3 is a commercial PRO blend composed of 8 bacterial strains. Four lactobacillus strains (*Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus delbrueckii subsp. bulgaricus*), three Bifidobacterium strains (*B. breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*), and a streptococcal strain (*Streptococcus salivarius subsp. thermophilus*). Several studies have demonstrated the effect of VSL#3 on DSS-induced colitis[93]. One study showed that VSL#3 (0.5 mL/d) reduced gut bacterial diversity associated with tissue injury. Conjugated linoleic acid was locally produced by VSL#3, which suppressed colitis by targeting myeloid cell PPARγ in the colon. Moreover, the anti-inflammatory effect remains the most important therapeutic mechanism of VSL#3 in DSS-induced rat colitis[94]. Both live and heat-killed VSL#3 decreased the expression of IL-23, IL-6, STAT3, and phosphorylated STAT3 (P-STAT3) in colonic tissue, thus reducing DSS-induced colitis in rats. The expression of inflammation-related mediators such as iNOS, NFκB was also inhibited by VSL#3[91].

In DSS-induced murine colitis, VSL#3 up-regulated the fibroblast growth factor-1, TGF-β, and vascular endothelial growth factor-A, while it suppressed the production of macrophage inflammatory protein-2 and pro-inflammatory chemokines KC in MUC2-

deficient mice. Moreover, myeloid differentiation marker 88 and TLR9 signaling also played important role in arbitrating the anti-inflammatory effects of VSL#3. VSL#3 significantly improved the various quantifying parameters of DSS-induced colitis[91]. However, in one study, VSL#3 (0.2 mL/d, 4×10^9 CFU of lyophilized VSL#3 used for 14 d) failed to cure DSS-induced murine colitis or strengthen the mucus barrier. VSL#3 was found to educe histological inflammation but did not alter the inflammatory properties of the colon. Furthermore, the increase in *Bifidobacteria* was not sufficient to repair the intestinal barrier impairment[95]. The dose, route of administration, and course of treatment of VSL#3 also influence results to some extent.

Various experimental studies have concluded that VSL#3 normally acts on four components of the intestinal barrier: Biological, chemical, mechanical, and immune barriers. Regarding biological barriers, VSL#3 can increase the abundance of commensal gut bacteria and reduce the abundance of fungi. Regarding chemical barriers, VSL#3 can increase MUC2, MUC3, and MUC5AC gene expression and regulate mucus secretion. Regarding the mechanical barrier, VSL#3 can augment ZO-1 and occludin while attenuating claudin-2 to improve the function of TJs proteins. About the immune barrier, VSL#3 can inhibit the pro-inflammatory NFκB pathway while upregulating PPARα signalling. An appropriate dose of VSL#3 can induce the maturation of dendrite cells, and VSL#3 can inhibit IFN-inducible protein-10 in IEC and the LPS-induced expression of chemokines (CXCL9, CXCL10, CCL2, CCL7, and CCL8) by inhibiting STAT-1 phosphorylation[91].

A research group led by Hrdý *et al*[96] demonstrated that PRO strains affect host cells in different ways. The mechanism of action of *Bifidobacterium animalis* species *lactis* B15764 and *Lactobacillus reuteri* Lr5454 were determined in mouse models of TNBS-induced colitis. Both strains exert beneficial effects on the host as expressed by body weight, gross indices of inflammation (Wallace score) and histopathological analysis (Ameho score), and lipocalin-2 levels in feces[96].

Another published report showed that PRO strains can have different immunomodulatory properties. These differences can be attributed to experimental

conditions such as animal models (rat, mouse), the colitis inducer chosen (DSS or TNBS), colitis severity (dose-dependent), and differences between bacterial strains. It is worth stating that microbiota composition and environmental factors also modify bacterial properties[97]. All of the above studies led to the inference that even a simplified preclinical model of colitis, which skips the genetic and external environmental influences, requires a broad and multidisciplinary approach.

Clinical study of PROs among IBD patients

Several trials have reported the therapeutic action of the most common PRO cocktail of proven efficacy, VSL#3 in adults with mild-to-moderate UC. In two clinical studies, VSL#3 was able to reduce DAI scores and significantly reduce clinical UC symptoms compared to the placebo. One study showed that 42.9% of patients treated with VSL#3 achieved remission in comparison to the placebo patients (15.7% remission only)[91]. Furthermore, VSL#3 and conventional drugs appear to have a synergistic effect. Although the mechanism is unknown, it is suggested that VSL#3 could enhance the anti-inflammatory effects of 5-aminosalicylic acid (5-ASA), inhibit free radical production, and suppress leukotriene and IL-1 production. A study also showed that combination therapy with VSL#3 and low-dose balsalazide was more effecacious than mesalazine or balsalazide alone in achieving remission of UC. Longer treatment with VSL#3 may result in greater improvement[110]. Furthermore, in an open-label study, treatment with VSL#3 resulted in remission and improvement in 77% of patients with active mild-to-moderate disease UC. Two bacterial components *B. infantis* VSL#3 and *S. salivarius* subspecies *thermophilus* contributed a significant role in inducing remission by reaching the intestinal site of the disease. The effect of alkaline sphingomyelinase was also examined in 15 UC patients treated with VSL#3 for 5 wk and the outcome showed that VSL#3 upregulated mucosal alkaline sphingomyelinase activity and improved UC[111,112].

VSL#3 has also demonstrated valuable effects in children having IBD. A study showe that VSL#3 was operative in maintaining remission and reducing relapses in children

with active UC. Apart from its role in maintaining remission, VSL#3 therapy also resulted in disease remission in children with mild to moderate acute UC as reported by another pilot study[104].

Jia *et al*[113] performed a meta-analysis of remission, relapse, and complication rates between EcN 1917 and mesalazine. The results demonstrated that there were no significant differences in the EcN1917 group or mesalazine-treated patients and were safe and well-tolerated. In summary, EcN 1917 has a comparable efficacy to mesalazine in terms of remission induction. This PRO could be considered as an alternative for patients with IBD. Tamaki *et al*[114] reported that treatment with *Bifidobacterium longum* in patients with mild to moderate UC, prominently reduced DAI score and decreased rectal bleeding, as well as showed that they also achieved clinical remission. Treatment with PROs and commonly used anti-inflammatory drugs together appears to be a more effective solution than treatment with PROs alone. Palumbo *et al*[115] combined mesalazine with a PRO mixture (*Lactobacillus salivarius, Lactobacillus acidophilus*, and *Bifidobacterium bifidum* strain). The combination showed beneficial effects among UC patients. The dual-treatment group demonstrated shorter recovery time, lower disease activity, and showed better endoscopic images. Another research group found that oral administration of *Bifidobacterium infantis* suppressed the CRP and TNF-α levels in both GI inflammatory diseases but did not specifically affect UC disease[116].

Angiogenesis presents a state of chronic inflammation in the intestine that progresses to IBD. One of the studies demonstrated that PRO yeast could help in preventing angiogenesis thereby reducing inflammation. However, another study reported that PRO treatment using PRO yeast *S. boulardii* was not found to be efficacious in clinical trials. Also in one study, *S. boulardii* and VSL#3 in combination with conventional therapy in mild to moderate UC (involving 244 patients) showed no improvement in remission rates. However, only modest benefits were obtained in terms of reduction in disease activity[117]. EcN, *S. boulardii*, *B. breve,* and *Bifidobacterium bifidum* strains Yakult has demonstrated efficacy and safety similar to standard 5-ASA in maintaining remission in patients with mild to moderate UC based on histology, endoscopy, or

quality of life. Presently, only four clinical trials have reported the use of *S. boulardii* as a PRO therapy for IBD, with three reports of efficacy. PRO therapy using PRO yeast such as *S. boulardii* could be a probable treatment for clinical trials, but the validation of reported efficacy in animal models requires multiple placebo trials[117,118].

An RCT evaluated the ability of VSL#3 to prevent the endoscopic recurrence of CD in humans after surgery. In this study, 10% of patients in the early VSL#3 group (VSL#3 administered throughout 365 d) had no severe lesions on day 90, but severe lesions developed on day 365 compared to the 26% of patients in the late VSL#3 group (administration of VSL#3 from day 90 to day 365) The finding suggested that VSL#3 exposure time was closely related to its therapeutic efficacy. However, DAI and IBD questionnaire (IBDQ) scores were similar in the two groups[104].

Few clinical studies have also shown that VSL#3 can prevent or maintain remission in chronic pouchitis. It was stated that after ileal pouch-anal anastomosis for UC, in the VSL#3 group 10% of patients had an onset of acute pouchitis when compared to the 40% of patients in placebo group. VSL#3-treated patients (17 patients, 85%) maintained antibiotic-induced pouchitis remission compared to placebo-treated patients (1 patient, 6%). The recommended level was an A rating. Additionally, an open-label study revealed that high doses of VSL#3 (3.6×10^{12} bacteria/d) were effective in treating mild active pouchitis. In this study, nearly 70% of patients achieved complete remission after their treatment with VSL#3. The recommended level was a C rating. It was suggested that the recommended concentration of VSL#3 for inducing remission of pouchitis is not as good as the concentration recommended for preventing and maintaining remission of pouchitis. VSL#3 may also improve patients' quality of life by significantly improving IBDQ scores. A potential mechanism of VSL#3 can be mediated by enhancing IBF. Although VSL#3 was effective in treating chronic pouchitis, an open-label study found that most patients with antibiotic-dependent pouchitis were refractory to long-term treatment with VSL#3, mainly due to recurrent symptoms[104].

One of the most frequently asked questions about PRO therapy is effective dosage. Several PRO supplements available commercially comprise one to 10 billion CFU per

dose. This dose is usually suggested in PRO therapy because viable cells must pass through the adverse conditions of the GIT and be present in sufficient numbers to exert PRO effects. Thus, a range of 10^6 to 10^{11} CFU of PROs was commonly used in all animal studies and human trials. In both preclinical and clinical studies, PROs were administered at least once a day and up to three times a day[119]. However, the total duration of PRO therapy varies between studies. The minimal duration would be seven days signifying that PROs must be consumed for atleast one year uninterruptedly to detect their efficiency. Although PROs are known as GRASS and the efficacy of various doses and types has been demonstrated in human volunteers, animal model studies are important for investigations to understand the chronological pathogenesis of IBD[119,120].

PREBIOTICS IN IBD

Since IBD is an idiopathic disease, whose pathophysiology relies on exacerbated inflammation and dysbiosis, it is possible that the imbalance can be corrected by improving the microbiome. Numerous studies have demonstrated the role of prebiotics on the intestinal flora and demonstrated that the use of prebiotics can enhance the metabolic function of the intestinal flora. Various studies reported that prebiotics diminishes the inflammatory cytokines, such as IL-1α, IL-1β, IL-6, IL-12, TNF-α, IFN-γ, and improve the natural intestinal barrier by increasing the mucinous layer and TJs between epithelial cells[129]. Their ability to decrease pathological bacteria in the gut and provide commensal bacteria with substrates capable of being metabolized into substances that contribute to the production and secretion of anti-inflammatory cytokines make them interesting candidates for various researchers working in IBD management. Surprisingly, more research has been done preclinically and a low number of significant prebiotic-associated human clinical trials are reported. The restricted research studies number is a foremost drawback and not adequately suuficient to support the use of prebiotics to treat IBD. Most prebiotics used in animal studies were polysaccharides derived from grapes, mushrooms such as *Ganoderma*

lucidum, and herbs. In contrast, most human clinical trials described the usage of FOS for prebiotic treatment of IBD[13,129,130].

Prebiotic effectiveness in animal model of colitis

The use of prebiotics has shown promise in the management of colitis and is also widely used in animal models (Table 5). Various prebiotic preparations have been tested in animal models of colitis. Inulin shows promise as a dietary supplement for many diseases, including IBD. It may be an interesting addition to standard IBD treatments because it is readily available, economical, and has no major toxic effects[131]. The prebiotic effects of inulin have been studied in DSS-induced distal colitis in a rat model histologically resembling human UC. Daily administration of inulin by the oral route increases the number of natural *lactobacilli* in the lumen of the cecum and also lowers colonic pH. In rats with DSS-induced colitis, mucosal inflammation and histological injury scores are reduced by oral administration of inulin. Furthermore, inulin-fed rats showed a lower degree of mucosal damage and less severe crypt damage compared to controls. Treatment with orally administered inulin showed similarly beneficial effects, regardless of whether treatment was provided before or during the DSS exposure[132,133].

A nutritional combination of inulin and oligofructose at 5 g/kg body weight reduces intestinal inflammation in transgenic rats. An HLA-B27 transgenic rat model was used in this research study to assess the mechanism of action of prebiotics in chronic colitis. The research outcome reported the beneficial effects of inulin and PROs in transgenic rats. An increase in *Bifidobacteria* and *Lactobacilli* was observed in the gut along with a decrease in pro-inflammatory cytokines and an increase in the growth factor-β that alters immune regulation. Taken together, these results suggest that combination therapy with different prebiotics may be more effective thanmonotherapy due to the fact that each drug has specific biological properties[133].

Pectic polysaccharides (PPS) are thought to be essential carbohydrates available to the gut microbiota, playing a dominant role in maintaining intestinal balance and are more

potent than some conventional prebiotics. It also show excellent efficacy in alleviating IBD. PPS is an indigestible polysaccharide because humans lack PPS-degrading enzymes. Therefore, most PPS (usually over 80%) remains intact as it passes through the stomach and small intestine. Additionally, PPS can stimulate the growth of beneficial bacteria, such as *Lactobacillus*, *Bacteroides*, and *Bifidobacterium*, fully meeting the condition of a "stimulating PRO".

Various precise mechanisms of pectin glycans have been described. Some aberrant PPS have shown specific immunological capabilities compared to the PPS on the market. For example, sweet cherry PPS significantly induces the expression of NO and some immune proteins such as IL-6 and IL-10. Moreover, PPS from silver linden flowers enhances immunity in mice by inducing ROS and NO and suppressing iNOS[134]. One study shows that PPS from *Gentiana crassicaulis* can enhance host immunity in terms of immune complement fixation[25].

Lactulose is reported to reduce inflammation and instigate the growth of lactic acid bacteria in IL-10 knockout mice while administration of inulin and germinated barley foodstuff (GBF) reduced DSS-induced colitis in rats. It has been shown to increase the luminal concentration of SCFA, as well as increase the density of *Lactobacillus* and *Bifidobacterium*[60]. Experiments on FOS yielded conflicting results. One research group reported that FOS alleviated TNBS-induced colitis in rats while another group stated that the FOS does not revealed any benefit DSS rat model of colitis[54,60].

Recently, mushrooms and the associated polysaccharides have been identified as promising sources of prebiotic fiber. Their polysaccharides have advantageous impressions on the microbiome. β-glucans (BGs) are the most prominent fungal polysaccharides. Mushrooms consist of diverse polysaccharides with prebiotic potential similar to BGs, including α-glucans, chitin, mannans, xylans, and galatians[31]. Xie *et al*[135] stated that the prebiotics *Ganoderma lucidum* polysaccharide enhance SCFA-producing bacteria and augments SCFA production and suppresses DAI prominently. The study also described a decrease in infective microbiota such as *Shigella* and *Escherichia* in the rat model.

The effect of neoagarotetraose (NT), a hydrolytic product of agar by β-agarase, was evaluated in the DSS-induced murine model. The data show that NT intake improved intestinal integrity and inflammation scores. NT reversed the density of *Proteobacteria* from the DSS-induced increased levels. It significantly augmented the profusion of *Verrucomicrobia*. Moreover, NT significantly increased the profusion of *Akkermansia* and *Lactobacillus* and decreased the abundance of *Sutterella*. It also significantly regulated relevant gut metabolites, predominantly those linked to histidine, polyamine, and tocopherol metabolism. Together, the findings provided novel insights into the mechanisms by which NT modulated the gut microbiome and metabolome and should facilitate the development of NT as a potent prebiotic for colitis management[136].

Reduced clinical signs and increased MUC-3 expression were observed in rats that were nourished with goat's milk oligosaccharides as compared to the DSS-induced colitis rats. In trinitrobenzene sulfonates that provoke colitis rats, the colonic inflammation and necrotic lesions are also reduced by goat's milk oligosaccharides as compared with control rats[133].

Though, it is not necessary that all findings using prebiotics shows a positive effect. One group reported oligofructose to be worthless in fixing DSS evoked intestinal inflammation in rats, and another group found the same inefficaciousness of GOS in TNBS- induced intestinal inflammation rats[60,72].

Clinical study of prebiotic among IBD patients

Although there are few human studies using prebiotics, some new evidence suggests that the prebiotic treatment holds promise. After colectomy for UC inulin showed a positive effect in the management of chronic pouchitis. A small, open-label study in 10 patients with active CD showed that the 21-d oral administration of 15 g of oligofructose and inulin significantly reduced the disease status[145]. In a UC patients, *Plantago ovata* (psyllium) outperformed placebo in reducing symptom severity and significantly increasing fecal concentrations of *Bifidobacteria*. Psyllium seeds formerly stimulated SCFA production, when tested as maintenance therapy for the UC patient in

remission in an open randomized study. In this, patients received mesalamine (500 mg/d thrice daily for 1 year), psyllium seed alone (10 g twice daily), or a combination of both at the same doses. Remission were comparable in all groups, and a substantial augmentation in fecal butyrate concentration was detected after the administration of *Plantago ovata* seeds[146,147].

GBF consists of an extract rich in glutamine and hemicellulose. In small pilot and placebo-controlled studies, its use was evaluated in UC patients having mild to moderate disease severity. GBF significantly increased fecal levels of *Bifidobacteria* and reduced clinical and endoscopic activities at a doses of 25-30 mg/d. Comparable outcomes were described by 24-wk open-label study. A combination of 15 g/d oligofructoses and inulin was investigated on ten patients with active CD in a small open-label study. A substantial decrease in DAI accompanied by a substantial increase in mucosal *Bifidobacteria* was observed. Interestingly, prebiotics amplified colonic dendritic cells expressing IL-10, TLR-2, and 4, signifying the mechanism of the prebiotics on the mucosal innate immune response.

Wilson *et al*[148] explored the effects of prebiotic GOS supplementation on colonic inflammation in 17 patients with active UC. Patients reported improved stool consistency, decreased incidence and severity of loose stools, and decreased urgency of defecation after administration of GOS at 2.8 g/d for 6 wk. The proportion of *Bifidobacterium* and *Christensenelaceae* increased only in patients with low disease activity, suggesting that prebiotic effects may depend on disease activity. A controlled study is required to validate these observations to essentially determine if the GOS prebiotic is a useful adjunct therapy in active UC[148].

The effect of enteral inulin on ileal pouch function was assessed by examining epithelial gene expression, cell turnover, and mucosal morphology. The authors found that enteric supplementation with 24 g/d inulin increased butyrate production, reduced inflammation-related factors, decreased secondary bile acids, and significantly reduced endoscopic and histological DAI scores[149].

SYNBIOTICS IN IBD

Synbiotics not only improve the survival of beneficial microorganisms added to food and feed but are also used to stimulate the growth of certain natural bacterial strains present in the GIT. It should be mentioned that the health effects of synbiotics are probably related to individual combinations of PROs and prebiotics. Given a large number of possible combinations, the application of synbiotics to modulate the human gut microbiota appears promising. Symbiotic products combining *Lactobacillus* or *Bifidobacterium* bacteria with FOS seem to be the most popular[155].

Effectiveness of synbiotic in animal models of colitis

Recently, several preclinical studies have shown that the use of PROs and prebiotics as a synbiotic combination alleviates intestinal inflammation more than either PROs or prebiotics alone. It has been suggested that it is effective in preventing abnormalities in the intestinal flora induced by DSS and TNBS. The effects of formulated prebiotic mixtures, PRO mixtures, and synbiotics were investigated in the colitis model induced by DSS in mice. Results in Synbiotic-treated colitis mice showed the preservation of colonic histological architecture and mucin production, upregulation of occludin expression, and diminished cell infiltration[156,157]. A significant decrease in plasma IL-6 levels was observed after treatment. Treatment also modified gut microbiome, improved colonic integrity, upregulated anti-inflammatory cytokines, and suppressed inflammation markers, possibly through inhibition of IL-6/STAT3 signaling. In addition, synbiotic-treated mice displayed the highest levels of anti-inflammatory mediator IL-10 among the treatment groups in colitis mice. Among the treatments, synbiotics showed the most pronounced effect, indicating the highest potential for prevention and treatment of IBD[156].

Kangwan *et al*[158] demonstrated a protective effect of *L. pentosus* A14-6, CMY46 against DSS-induced intestinal inflammation. A14-6 and CMY46 are the novel strain of *L. pentosus* isolated from tea leaves (Miang) in Northern Thailand. The anti-inflammatory actions of *L. pentosus* CMY46 combined with GOS and *L. pentosus* A14-6

combined with XOS and were explored in C57BL/6 mice for 21 d. Synbiotics ameliorated DSS-induced colitis by preserving weight loss, reducing DAI, restoring colon length, and suppressing histopathological damage. Moreover, synbiotics enhanced intestinal barrier integrity and reduced colonic inflammation. Further, synbiotics possessed excellent anti-inflammatory and immunomodulatory activities, as evidenced by decreased inflammatory mediator expressions of TNF-α, IL-1β, IL-6, and cyclooxygenase-2 (COX-2) in the colon. Symbiotic CMY46 in combination with GOS markedly increased IL-10 expression. These results suggest that synbiotics isolated from Mian are more effective than sulfasalazine. These outcomes recommended that synbiotics had more efficiency than sulfasalazine. Therefore, they may represent new potential natural active ingredients against colonic inflammation[158].

The efficacy of *Bacillus amyloliquefaciens* enriched camel milk (BEY) was evaluated in TNBS-induced colitis mice models. Results showed that BEY treatment attenuated the proinflammatory cytokines (IL-1β, IL-6, IL-8, and TNF-α) and myeloperoxidase levels. In addition, the protein markers such as phosphatase and tensin homolog, NFκB, COX-2, proliferation nuclear antigen, and occludin were substantially downregulated by BEY treatment. The BEY alleviated the colitis symptoms[159].

Bacillus coagulans FCYS01 spores in combination with chitooligosaccharides (COS) were evaluated for the possible ameliorating effects on DSS-induced colitis in mice. In comparison to the DSS group, the supplement significantly modulated the levels of CPR and cytokines IL-4, IL-6, IL-8, and IL-10. It significantly restored the TJ proteins and mucin protein expressions, thereby promoting the recovery of the intestinal barrier Additionally, these dietary supplements improve SCFA production by modulating the composition of the gut microbiota and enhancing SCFA-producing bacteria. In conclusion, synbiotics mitigated the inflammatory status of the experimental UC model and showed better therapeutic efficacy than individual *B. coagulans* or COSs[160].

In another study, supplementation with synbiotics could substantially ameliorate the disease activity in DSS-induced acute colitis mice. The synbiotic significantly preserved the epithelial TJ proteins at colon, signifying the shielding of the intestinal barrier. The

pro-inflammatory cytokines was reduced while augmentation of anti-inflammatory cytokines was mediated by the symbiotic treatment. The synbiotic used in the study was composed of 8 PRO strains, including *Bifidobacterium animal*, *Lactobacillus paracasei*, *Bifidobacterium lactis*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *B. breve*, *Lactobacillus fermentum*, and *Streptococcus thermophilus* along with FOS[161].

In another study, a synbiotic consisting of *Lactobacillus fermentum* HFY06 and arabinoxylan showed that the synbiotic can prevent and treat DSS-induced colitis. The results exhibited their synergistic effect by inhibiting the activation of the NFκB signaling pathway, upregulating the mRNA expression of NFκB inhibitor-α, downregulating mRNA expressions of NFκB-p65, inhibiting the cytokines TNF-α, inducible NOS, and COX-2, and exerted anti- colitis effects[162].

Synbiotic supplement with PRO *Bacillus coagulans* spores and prebiotic green banana resistant starch ameliorated intestinal inflammation in the murine IBD model induced by DSS. A considerable efficacy of synbiotic supplementation was highlighted as it reduced the colitic manifestations and its severity. A significant anti-inflammatory effect was produced by suppressing abnormal immunological responses and colonic damage induced by DSS. Synbiotic accounted for about 29% increase in IL-10 levels and about 37% suppression of CPR along with about 40% IL-1β suppression compared to that of the DSS-control. The combination also improved SCFA production. Together, they can help modulate the parameters of inflammation and reduce the severity of the disease. The synbiotic supplementation amended the complete inflammatory condition *via* synergistic actions[163].

Clinical study of synbiotic among IBD patients

The effects of daily supplementation of total gut repair (TGR) on microbial community composition and activity were investigated in the short-term-Quad-M-SHIME model inoculated with gut microbiota from two individual IBD donors. TGR comprises of PROs, prebiotics along with combination of amino acids, immunoglobins and flavonoids. TGR supplementation increased SCFA production, increased beneficial

bacterial density, decreased inflammation, and damage to the intestinal barrier from endotoxin exposure. Intestinal barrier function was improved compared to controls, and levels of the anti-inflammatory molecules IL-6 and IL-10 were elevated. TGR supplementation daily promoted changes in the gut microbiota of IBD patients[170].

The efficacy of synbiotic therapy has been evaluated in UC patients in a clinical study. Patients received a synbiotic formulation consisting of prebiotic FOS along with 6 PRO strains. The results showed a significant decrease in inflammation and improved disease status[126]. The double-blind randomized, placebo clinical trial study by Liang *et al*[46] confirmed the efficacy of a synbiotic formulation in suppressing IBD symptoms. The synbiotic consisted of FOS and *L. acidophilus*, *B. bifidum*; *B. longum*; *B. lactis*; and *L. rhamnosus*. Rectal pain, bloating, incomplete bowel movements and diarrhea sensations were significantly improved in patients compared to placebo[31].

A recent randomized, double-blind, controlled trial observed synbiotic use in 18 subjects with functional UC. The treatment included the prebiotics inulin and oligofructose. Sigmoidoscopy inflammation scores were reduced in the synbiotic group compared to the placebo group. The TNF and IL-1α levels in the intestine were also reduced. Furthermore, rectal cultures showed greater epithelial regeneration and reduced inflammation in synbiotic-treated subjects. A tiny, open-labeled trial of 10 active CD subjects, 21 d of 15 g oligofructose and inulin oral administration also showed a substantial lowering of the disease symptoms[171].

Since it has been shown that obesity-induced gut microbiota aggravation can exacerbate IBD symptoms, BG from the Schizophyllum commune, a PRO, and a synbiotics containing both BG and PRO (SYN) may improve symptoms of obesity-related colitis. BG and PRO protected intestinal TJs, but did not modulated the inflammatory markers (*i.e.*, IL-6 and TNF-α) infiltration. In contrast, SYN displayed more prominent actions in attenuating colonic inflammation. SYN treatment group supported the growth of both indigenous and supplemented bacteria while maintaining bacterial diversity, thereby improving the obesity-associated colitis symptoms[172].

SAFETY ASPECTS OF PRO, PREBIOTIC, AND SYNBIOTIC PRODUCTS

The Centers for Disease Control and Prevention advises that over-the-counter prebiotics and PROs are generally safe for use by healthy people. The ignoring the importance of dose and strain specificity is a concern today. PROs manufactured as dietary supplements rather than pharmaceuticals are not subject to regulatory review because they are not required to support claims about the safety or efficacy of food or dietary supplements. This is one of the main reasons for the insufficient or nonexistent information on the efficacy and safety of most marketed products. The centuries-old use of PRO bacteria in health and disease is the best evidence of their safety. However, insufficient data, particularly when a novel PRO strain is a candidate to be placed on the market for commercialization, randomized studies conduction to assess the safety of PRO strains is the essence. PRO is characterized by a generally safe profile but should be used with caution in certain population groups such as pregnant women, neonates born prematurely, or with immune deficiency. A World Health Organization working group proposed several criteria that are to be considered in order to define strains with GRAS status: Resistance of PRO strains to antibiotics; evaluation of metabolic properties (lactate production, bile deconjugation) monitoring of side effects in clinical trials, epidemiological studies on the occurrence of side effects after commercial approval, identification of all substances excreted by strains that are toxic to mammals, and determination of the hemolytic capacity of strains[175].

Risks and side effects

No serious side effects of PRO interventions in IBD patients have been reported. Inadequate immunological stimulation, genes transfer, systemic infections, and fatal metabolic activities have been detected in certain individuals receiving PRO supplementation. A mild dry cough has been reported in one UC patient with *B. longum* 536 supplementation. Septicemia, and certain cased of endocarditis, have been associated with certain PRO strains, like *L. acidophilus, L. rhamnosus, Bacillus subtilis, S. boulardii, L. Casey,* and *B. breve.* Administration of *Lactobacillus rhamnosus* in 64-year-old

UC patient who was treated with prednisone, caused bacteremia due to bacterial transfer from intestinal lumen to the blood. PRO interventions have been reported to induce an inflammatory response in the small intestinal region, leading to D-lactic acidosis[176]. Possible gene transfer between gut bacteria, overstimulation of both innate and adaptive immune systems, and adverse effects on the GIT has also been reported. GIT distress, such as bloating is the most common side effect encountered. The complications in certain patient populations, especially those with compromised immune systems are accelerated by *S. boulardii* and *Lactobacillus GG* administration. The report and doctors argued that PRO use depends on a person's birth, age, and health status and that it is important to inform users of the unpleasant effects of PROs. Pregnant women, new-borns, and the elderly are at increased risk of potential PRO infections due to their weakened immune systems. Several *Lactobacillus* strains are inherently resistant to vancomycin, raising concerns about the potential transmission of such resistance to more pathogenic organisms in the intestinal environment. Fermentation of FOS in the colon produces hydrogen and carbon dioxide, causing severe discomfort. Excessive intake of prebiotics, especially oligosaccharides such as FOS and GOS, causes discomfort such as gas and bloating in the abdomen, causing marked bloating[44]. PROs are considered safe, but it is important to remember that side effects such as bacterial transfer and sepsis may occur in people with weakened immune systems. However, PROs benefits appears to outshine the potential side effects in IBD patients[177,178].

STUDY LIMITATIONS

Significant heterogeneity between studies jeopardizes the interpretation of the current literature on PROs and prebiotics in IBD. Choice of prebiotic or PRO studied, the trial design, their doses, and the outcome were also reported to vary. Study populations varied, with some studies included active disease patients while others working on the remission maintenance persuaded by conventional therapy, antibiotics, or surgery. Most studies enrolled small numbers of patients, limiting their statistical power, which

is especially important given the high placebo response rates observed in IBD clinical trials. Finally, no studies provided information on patient diet, which may have a significant impact on the effectiveness of microbial therapy. The exact mechanisms of action have not yet been elucidated. The understanding of the mechanisms responsible for the beneficial effects of PROs, PROs, and synbiotics is rather superficial[130].

It is not clear whether colonic colonization by PRO species is required. This is because the few studies signifying therapeutic efficacy have not confirmed such colonization. Further research is needed to explore whether prebiotics act through an increase in the number of bacteria recognized by immune cells or through the effects of SCFAs on the mucosa[121]. Inadequate evidence on PRO dosages essential for specific clinical effects has amplified the necessity for molecular description of PROs to establish health claims. Evidence for immunological mechanisms of PROs is still limited. Evaluation of interactions between cocktail of PRO strains in formulations such as #VSL-3 have not been considered and yet to be investigated. Clinical trials and validation studies planned with larger sample sizes require an understanding of the interactions between the microbiota, host, and prebiotic components[179]. Due to the very limited published literature in the field of manufacturing processes and subsequent formulation, much needs to be done to improve strain viability during formulation and storage[77].

OBSTACLES, CHALLENGES, AND FUTURE PROSPECTS

The main barrier with the pre-, pro-and synbiotics is the "difficulty in demonstrating clinical efficacy". The situation is complicated by the different levels of evidence essential to support health claims from country to country. The Food and Drug Administration now states that active ingredients, including PROs, taken to cure, alleviate, treat, diagnose, or prevent disease must be classified as pharmaceuticals and go through the same approval process as new drugs. Eventually, high-quality human intervention studies are needed to substantiate health claims on products[155].

PRO supplements vary widely in composition, dosage, as well as in host interactions, and these should be specifically considered before recommending their use.

Remarkably, several prebiotic and synbiotic products contain a slight amount of prebiotic ingredients (per serving), that may be too small to produce any health benefits. Lower doses are used in part to avoid unwanted GI discomfort, but possibly also for cost reasons[155]. Developing clinically effective synbiotic combinations is a major challenge and must meet several requirements. It is generally expected that the minimum effective dose of each component must be determined[180].

Maintaining PRO bacteria viability is a foremost marketing and technical challenge in PRO applications. A basic prerequisite for PROs is that the product comprises a adequate number of microorganisms by the expiration date. Therefore, PROs should cover precise strains and maintain a specific number of viable cells to provide a health benefit to the host. Many viable cultures die during final product manufacturing, storage, transportation, and passage through the gut. As a result, the majority die before consumers can reap the health benefits. Market research has also shown that even before the expiry date, product show much lower count. Therefore, the shelf life of PROs cannot be accurately predicted. As a result, the industry has to struggle alot to substantiate the label's claims[181].

Also, for optimal effectiveness, PROs must remain viable after contact with stomach acid, bile, and digestive enzymes to cross the upper GIT. This is a basic property that many products have not tested. Microorganisms may die while passing through the upper intestinal tract to the colon and thus may not be able to colonize the colon. Therefore, they must withstand the gastric acid and bile salts encountered during transit through the GIT. Their GIT survival depends on strain- and species-specific resistance to low pH values of gastric juice (pH values between 1.3 and 3.0) and bile salts in the small intestine[182]. PRO bacteria only work if they find the right environmental conditions and are protected from the stress (extreme temperatures, high pressure, shear forces, *etc.*).

Furthermore, several PROs already marketed are not clinically studied for their claimed effectiveness in IBD. Although it is tolerated well and generally safe, hypothetical concerns about its use in immunocompromised patients with altered

mucosal barriers persists. Long-term maintenance studies of IBD are needed because they need to be used repeatedly or indefinitely to sustain effects.

There are various reasons why synergistic effects between PROs and prebiotics are seldom observed *in vivo*. Some synbiotics used in previous studies were not rationally designed, but were formulated considering the arbitrary criteria like cost, marketing implications, shelf life. Even when screening of synbiotic combinations was performed *in vitro* or *in situ*, the methods ignored the environmental factors that influence PRO strains *in vivo*. Also, competition for the prebiotic substrate between the PRO strain and members of the gut microbiota was not considered. Identifying prebiotics that specifically and selectively boost the PRO strain of interest can be challenging[183].

Clinically effecacious synbiotic development remains a challenge and must meet numerous requirements. It is generally expected that the minimum effective dose of each component must be determined. Including adequate controls in synbiotic studies is particularly challenging. Prebiotic-only and PRO-only controls must be included, in addition to standard control, for checking the synergistic or additive actions. Justification on how the PROs and prebiotics were selected and combined should be included[184].

FMT IN IBD

For patients with metabolic syndromes linked with gut dysbiosis, FMT is an evolving microbial therapy. The technique involves the transfer of healthy fecal microbe population to patients with metabolic conditions. FMT's technical approach involves oral capsules, nasogastric or nasojejunal tubes, and enemas that are utilized for restoring a healthy GI microbiome[185]. FMT samples are carefully chosen from healthy donors who have undergone a standard screening procedure for avoiding the risk of transmission of unknown pathogens from donor to recipient. Donors are usually evaluated for their historical backgrounds such as health profiles, family history of autoimmune reactions, metabolic disorders, transfusion details, or any previous surgery. Other donor data include travel history, food intake, particularly alcohol and

drugs, and sexual behavior. After selecting an appropriate donor, their stool and blood samples are tested for the presence of pathogens. Extensive support and education are provided to the patients undergoing FMT prior to the treatment. No fecal substances such be present in the colon. Patients may even consume loperamide prior to infusion to make sure that transplanted feces stays there for at least 4 h. The recipient is not allowed to consume antibiotics 48 h prior to infusion[186].

There are several methods of transferring fecal material to the recipients. Presently, fecal material is administered *via* the upper GIT or lower GIT route, or as oral capsules. For patients who are suffering from an inflamed colon, FMT is performed *via* nasogastric tube, esophagogastroduodenoscopy, nasojejunal tube, or upper GI route *via* nasoduodenal tube. Lower GI route FMT can be accomplished by retention enema or by colonoscopy. While colonoscopy aids in the successful recolonization of all the parts of the colon with favorable microflora, retention enemas are only restricted to the distal colon. Retention enemas are however much cheaper and less invasive than colonoscopy. For most reported treatments, FMT patients receive an average relative dose of 25 g *via* the upper GI route compared to an average of 90 g *via* colonoscopy[187]. In an RCT, colonoscopy using a 152 g stool sample reported a 90% success rate in preventing recurrent infections. Another research group reported that consuming 17 g of frozen and thawed or fresh FMT showed 60% efficacy using the retention enema method. The fecal capsules can also aid in restoring ecosystem integrity and overcoming microbial loss in the GI environment.

Recently, a study reported that FMT in IBD patients showed a response rate of 53.8% and a complete response rate of 37%. Furthermore, it has been reported that FMT is a more practical treatment with safe and beneficial results for the treatment of active UC patients. Pooled results exhibited that FMT treatment might improve clinical and endoscopic rates of active UC. FMT also significantly alters the microbiota composition of UC patients as compared with control groups[188]. In UC patients, Tian *et al*[189] assessed the *Bacteroidetes* proportion that exhibited a steady rising trend after FMT. *Prevotella* and *Proteus* were also prominently augmented as compared to healthy

control. On other hand, it was found that the populations of *Klebsiella* and *Streptococcus*, which are pathogenic bacteria decreased significantly after FMT treatment. Reducing the abundance of *Prevotella* while increasing the proportion of *Klebsiella* and *Streptococcus* was a key factor in the development of UC. Unfortunately, several studies have stated conflicting results, with FMT therapy failing to ameliorate the disease severity and restore the gut microbiota.

In this regard, several studies have found that the efficacy of FMT for treating IBD is unpredictable. Therefore, it is still unclear whether FMT fits into the therapeutic paradigm. Despite reports of significant positive taxonomic changes in the GIT in patients diagnosed with FMT, observations remain conflicting and its functional and metabolic effects are not well documented. For UC, FMT may be a promising treatment, but for CD or pouchitis, very limited information have been available to draw good conclusions[190,191].

CONCLUSION

Human gut microbiota plays an important role in the initiation, progression, maintenance, and exacerbation of IBD. Unfortunately, research conducted so far regarding the specific core microbiome established in both the healthy condition and the etiology of IBD is still unclear. PROs, prebiotics, and synbiotic therapies represent an effective toolkit to restore and modulate the gut microbiota, eventually leading to a potential cure for IBD as dysbiosis play a prominent role in the disease pathogenesis. PROs are reported to influence gut microbiota, gut barrier function, and immune responses, offering an alternative approach to IBD prevention and treatment. An inadequate knowledge and information about the molecular mechanisms involved in IBD pathogenesis seems to complicate the issue. Researchers have attempted to explicate the underlying mechanisms of PRO action, in order to search for an effective PRO treatment for IBD. Comprehensive understanding the mechanisms of crosstalk interactions between microbiota and host tissues is another future challenge. This contributes to the conceptualization of successful new beneficial strategies based on

distinct pathogenic progressions, etiologies, and expectable responses. Although PROs and prebiotics have been studied in many animal models and clinical trials of intestinal inflammation and offer health benefits, the individual efficacy of each PRO strain and its administration remains uncertain. Large, rigorously designed, high-quality human studies need to be evaluated to examine dosage, duration of use, formulations containing one or more strains, and PROs, prebiotics, antibiotics as well as simultaneous use of substances. Furthermore, the potential anti-inflammatory effects mediated by new PRO/synbiotic treatments must outweigh the risks and are accepted by the population. However, important clinical outcomes reported are still less in number and insufficient to effectively predict future trends. Therefore, a clear and focused multidisciplinary clinical trial needs to be developed for understanding the impact of PRO, prebiotic, and synbiotic therapies on IBD.

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