The Role of Pre- and Probiotics in the Treatment of Inflammatory Bowel Disease

John K. Triantafillidis*, Filippos Georgopoulos and Emmanuel Merikas

Department of Gastroenterology and Center for Inflammatory Bowel Disease, “Saint Panteleimon” General Hospital, Nica, Greece

Abstract

Many patients with inflammatory bowel disease use alternative therapy, mainly probiotics and synbiotics, to manage this intestinal condition. Despite widespread use of these natural therapies by patients, health care providers may be unfamiliar with probiotics as a treatment modality. This review describes the rationale for use of probiotics in patients with active or inactive inflammatory bowel disease, their mechanism(s) of action, and recent controlled clinical studies in which efficacy of probiotics, prebiotics and synbiotics in patients with active or inactive inflammatory bowel disease has been explored. Certain probiotics, particularly *E. coli Nissle* 1917 and a multi-agent mixture VSL#3, may benefit patients with UC or pouchitis, while *Lactobacillus rhamnosus* GG appears less useful. In general, probiotics show potential for therapeutic application mainly in pouchitis and to a lesser degree in UC, while their effects in maintenance therapy for CD have been much less promising. While there is suggestion of benefit when patients with ulcerative colitis use bacterial therapies small sample sizes and methodological weaknesses in study designs necessitate that additional studies must be conducted before probiotics and or synbiotics can be routinely recommended in clinical practice.

Introduction

The normal colonic microflora is intimately involved in the aetiology of inflammatory bowel disease (IBD). Both ulcerative colitis (UC) and Crohn’s disease (CD) are quite often refractile to conventional treatment thus requiring the use of alternative therapies based for example on the use of probiotics, prebiotics or combination of the two (synbiotics).

So far, most of the studies in this area have been performed using probiotics, and although an increasing interest on the use of synbiotics has been noticed recently, few randomised controlled trials have been conducted concerning both murine models of IBD and humans.

The available clinical and experimental data suggest that these functional foods can alter the composition of the colonic microbiota, and reduce inflammatory processes in the gut mucosa, thus having the potential to induce disease remission especially in patients suffering from UC.

In this review we will try to clarify the role of prebiotics, probiotics, and synbiotics on the clinical course of patients with IBD based on the results of the current literature.

Definitions

Probiotics are live microorganisms that when ingested in adequate quantities, exert a health benefit to the host. Inactivated bacteria or bacteria derived factors can also have probiotic properties, and thus might be considered as probiotics. The main probiotic preparations commercially available belong to a group of Gram-positive fermentative bacteria that are associated with the production of lactic acid from carbohydrates. Species of *Lactobacillus, Lactococcus, Bifidobacterium* and *Streptococcus thermophilus* are included in this group, being normal as well as important constituents of the human gastrointestinal microflora [1]. Potential probiotic roles probably share some other microbes including yeasts (*Saccharomyces boulardii, Saccharomyces cerevisiae*) and non-pathogenic strains of *E. coli* and *Bacillus* species.

Prebiotics are dietary substances (mostly consisting of non-starch polysaccharides and oligosaccharides not or poorly digested by human enzymes) that benefits the host by selectively stimulating the growth and activity of indigenous probiotic bacteria. According to the International Scientific Association for Probiotics and Prebiotics "a dietary probiotic is a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health [2]." The majority of prebiotics includes fibres and carbohydrates, such as resistant starch, wheat bran, and inulin. Their presence in the bowel lumen selectively enhances proliferation of certain probiotic bacteria, especially *Bifidobacteria* species. They also act as carbon and energy sources for bacteria growth in the large bowel, where they are fermented into short chain fatty acids, lactate, CO₂, and H₂ formation being energy sources for the gut mucosa especially of the left colon [3].

Synbiotics is a novel approach combining probiotics and prebiotics in an attempt to obtain synergistic effects of the two compounds by improvement of the probiotic colonisation or the metabolic effect.

Although the number of microorganisms that must be ingested to obtain a beneficial effect is largely unknown, a probiotic should contain several billion microorganisms to increase the chance of adequate gut colonization [4]. It must be stressed however, that probiotic benefits associated with one strain do not necessarily hold true for other.

Frequency of probiotic use by the patients with IBD

The most widely used probiotics are *Saccharomyces boulardii*

*Corresponding author: Prof John K. Triantafillidis, Associated Professor “Gr. T. Popa” University of Medicine and Pharmacy, Iasi, Romania. Head, Department of Gastroenterology and Center for Inflammatory Bowel Disease, "Saint Panteleimon" General Hospital, Nica, Greece, Tel: (Greece)-6944432917; Fax: (Greece)-2105810970; E-mail: jktrian@gmail.com

Received October 13, 2011; Accepted November 11, 2011; Published November 21, 2011


Copyright: © 2011 Triantafillidis JK et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited
and lactic acid bacteria, including *Lactobacillus* and *Bifidobacterium* spp [5]. *Lactobacillus johnsonii* formerly known as *Lactobacillus acidophilus* is a unique strain of bacteria having enhanced adherence properties to the intestinal epithelial layer, thus preventing colonization of the mucosa by potentially pathogenic bacteria. It harbors regulatory action on the mucosal immune system by sensitizing human intestinal epithelial cells to express Transforming Growth Factor (TGF), which may in turn control mucosal T-cell homeostasis [6].

It is a common sense that in many countries a large proportion of patients with IBD use probiotics to manage the intestinal disease. In a relevant case-control study it was described that significantly more IBD patients than controls had, at some time, used probiotics and those IBD patients had greater probiotic knowledge than controls [7]. Table 1 represents an example of the abundance of various pro- and prebiotic commercial formulas used in a Southern European country.

It seems certain that patients with IBD rely on nonclinical sources of information and often do not disclose probiotic use to healthcare professionals. Moreover, it is unclear how patients make decisions

<table>
<thead>
<tr>
<th>Number</th>
<th>Name</th>
<th>Probiotic</th>
<th>amount</th>
<th>Type</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Live-Bac</td>
<td>L. acidophilus</td>
<td></td>
<td>Tablets</td>
<td>Live bacteria up to several months after ingestion</td>
</tr>
<tr>
<td>2</td>
<td>Advanced 40+ Acidophilus Vegicaps</td>
<td>Acidophilus vegicaps L. bulgaricus L. paracasei B. lactis S. thermophilus</td>
<td>300 millions 300 millions 300 millions 300 millions</td>
<td>Capsules</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Advanced Acidophilus plus vegicaps L. acidophilus B. lactis</td>
<td>250 millions 250 millions</td>
<td>Capsules</td>
<td>Advanced formula.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Multiacidophillus</td>
<td>L. acidophilus B. Lactis S. thermophilus L. Bulgaricus</td>
<td>2.1 billions 2.1 billions 550 millions 250 millions</td>
<td>Powder</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Advanced Multi-Billion Dophilus L. acidophilus B. bifidum L. bulgaricus</td>
<td>333 billions 333 millions 333 millions</td>
<td>Capsules</td>
<td>Complex of citrus pectin and cellulose (4mg)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Ultra-probiotics vegetableian</td>
<td>L. acidophilus B. lactis</td>
<td>40 billions</td>
<td>Capsules</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>VSL</td>
<td>Bifidobacterium breve, longum and infantis L. acidophilus L. plantarum L. paracasei L. bulgaricus Streptococcus thermophilus</td>
<td>450 billions</td>
<td>Sachets</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Energie probiotiques</td>
<td>L. acidophilus</td>
<td>4 probiotics 3 billions</td>
<td>Capsules</td>
<td>It contains also inulin (prebiotic)</td>
</tr>
<tr>
<td>9</td>
<td>Probiotics</td>
<td>B. bifidum B. longum L. acidophilus</td>
<td>?</td>
<td>Capsules</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Acidophilus plus L. acidophilus L. casei casei L. casei rhamnosus</td>
<td>2 billions</td>
<td>Capsules</td>
<td>It contains also maltodextine</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Zenbis</td>
<td>Lactobacillus plantarum</td>
<td>10 billions</td>
<td>Sachets</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Ultra-leur Sacharomuces boulardi</td>
<td>L. acidophilus</td>
<td>1 billion</td>
<td>Capsules</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Bio colon Sacharomuces Hansen</td>
<td>L. acidophilus</td>
<td>10 billions</td>
<td>Sachets</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Bion-3</td>
<td>L. gasseri B. bifidum B. longum</td>
<td>12 vitamins 12 trace elements</td>
<td>Tablets</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Beneflora</td>
<td>L. acidophilus B. species S. thermophilus L. casei L. acidophilus L. bulgaricus</td>
<td>6 probiotics and 1 mix of natural fibers</td>
<td>Powder</td>
<td>It contains also prebiotics (Maltodextrin, Fructo-oligosaccharids of chicory, Picum satinium fibres)</td>
</tr>
<tr>
<td>16</td>
<td>Symbiotic 2000 Probiotics: Pediococcus pentosaceus, Leuconostoc mesenteroides, L. paracasei ssp. paracasei; L. plantarum Prebiotics Inulin, oat bran, pectin, Resistant starch</td>
<td>Cocktail containing 4 probiotic species [10^11 cfu/each]: and 4 prebiotics.</td>
<td>Sachets</td>
<td>Available also as Symbiotic 2000 “Forte” containing triple amount of pre- and probiotics than the the simple form.</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Probiotics and synbiotics available in the market of a South European country (Greece).
about probiotics and what role they expect their gastroenterologists to play regarding the use of probiotics. Despite the widespread use of these natural products by patients, health care providers are largely unfamiliar with probiotics as a potential treatment modality. A recent study suggests that patients with IBD will look to gastroenterologists and other clinicians as trustworthy advisors regarding the utility of probiotics as an alternative or supplement to pharmaceutical drugs. Therefore, gastroenterologists who care for patients with IBD should be prepared to discuss the potential benefits and risks of probiotics and assist patients in making informed decisions about their use [8].

**Mechanism of action**

Probiotics exert their beneficial effect via various and rather complicated mechanisms which seem to be unique for each strain. The activity of a given strain depends on a number of other factors, such as the presence of bacteria in the intestine and the kind of the disease in which the strain is being used [9].

The biological effects of probiotics can be categorised as follows (see also table 2).

**Antimicrobial effects**: The antimicrobial effects of probiotics are succeeded via [10]

- Production of inhibitory substances through modification of pH and production of bacteriocins, defensins, deconjugated bile acids, organic acids, and H2O2,
- Induction of heat shock proteins and endogenous antimicrobial peptides (mainly defensins) via activation of NF-κB, MAPK, and JNK. Since defensins are implicated in the pathogenesis of IBD, increased expression by probiotics provides a possible mechanism for clinical efficacy seen in certain IBD patients,
- Blocking of the sites of adhesion, because probiotics act as a competitive exclusion to bacterial adhesion sites thus impeding invasion by pathogenic bacteria [11,12],
- Competition for essential nutrients by consuming nutrients that otherwise would be utilized by potentially harmful microorganisms and,
- Degradation of toxin receptor via inhibition of toxin expression in pathogens, such as in *Clostridium difficile*.

**Promotion of gut integrity this multi-function includes the following parameters:**
- Enhancement of epithelial barrier function,
- Stabilization of tight junctions,
- Induction of mucin gene expression and up-regulation of mucus production [13],
- Enhancement of epithelial cell glycosylation,
- Stimulation of intestinal epithelial cell proliferation, intestinal mucin production, excretion of pancreatic enzymes and intestinal motility, and decrease epithelial cell apoptosis.

**Modulation of host immune responses**: The modulation of the host immune responses seems to be the most important action of probiotics. It has been shown that probiotics can actively interfere with regulatory and pro-inflammatory signalling pathways resulting in a reduction in Th-1 proinflammatory response and a greater T-regulatory anti-inflammatory response. Therefore, probiotics exert their immunomodulatory effects through enhancement of antibody production and natural killer cell activity, modulation of dendritic cell phenotype and function, modulation of NF-κB and AP-1 pathways, modulation of apoptosis, induction of regulatory T-cells and PPAR-g, alteration of cytokines release, influence on the innate immune function including c-Jun NH2-terminal kinase, and inhibition of proteasome activity [14].

**Specific probiotics’ action**

*LactoBacillus species*: It has been shown that *Lactobacillus* species inhibit NF-κB nuclear translocation, blockage of IkB degradation, inhibit production of IL-6 (L. casei), up-regulate intestinal MUC3 and MUC5 mRNA expression, inhibit apoptosis of intestinal epithelial cells (*L. GG*), induce COX-2 expression (*L. rhamnosus*) decreases the plasma and lymphocyte content of proinflammatory cytokines in patients with UC and decrease translocation of commensal bacteria (*L. plantarum*, *L. GG*) [15].

Hegazy et al described recently that administration of *Lactobacillus delbruekii* and *Lactobacillus fermentum* for 8 weeks significantly ameliorated the inflammation by decreasing the colonic concentration of IL-6, expression of TNF-α and NF-κB p65, leukocyte recruitment, and the level of fecal calprotectin compared to sulfasalazine and the control group [16]. Recently it was demonstrated that the anti-inflammatory effect of certain lactobacilli is via NOD2-mediated signalling, thus the inconsistent clinical results of lactobacilli use in patients with CD might be related to a relative deficiency of NOD2 [17].

The effect of *Lactobacillus* casei DG, on colonic-associated microbiota, mucosal cytokine balance, and toll-like receptor expression was evaluated in 26 patients with mild left-side UC under 5-ASA treatment were randomly allocated to receive oral *L. casei* DG, rectal *L. casei* DG, and oral 5-aminosalicylic acid alone, for an 8-week treatment period. 5-ASA alone or in combination with oral *L. casei* DG failed

**Table 2**: Mechanisms of action of probiotics [7].

<table>
<thead>
<tr>
<th>Antimicrobial effect</th>
<th>Restoration of gut integrity</th>
<th>Modification of the host immune response</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Decreased colonization and invasion by pathogenic organisms</td>
<td>• Restoration of intestinal permeability [21].</td>
<td>• Reduction of proinflammatory cytokine content on plasma and lymphocytes [12].</td>
</tr>
<tr>
<td>• Modification of pH</td>
<td>• Up-regulation of T-bet/band enhancement of mucosal barrier function with up-regulation of tight junction molecules [20].</td>
<td>• Decrease in the colonic concentration of IL-6, TNF-α and NF-κB p65, leukocyte recruitment, and decrease in colonic MPO activity [16].</td>
</tr>
<tr>
<td>• Production of inhibitory substances</td>
<td>• Block of adhesion sites</td>
<td>• Expansion of mucosal regulatory cells [22]</td>
</tr>
</tbody>
</table>
to affect colonic flora and toll-like receptor expression in a significant manner, but when coupled with rectally administered L. casei DG, it modified colonic microbiota by increasing Lactobacillus spp. and reducing Enterobacteriaceae as well as reducing Toll-like receptor-4 and IL-1β mRNA levels and increasing mucosal IL-10 [18].

**Bifidobacterium species**: Bifidobacterium species suppress the growth of Bacteroides vulgatus, increase IL-10 secretion by mesenteric lymph nodes, and reduce malonperoxidase activity, tissue contents of immunoglobulin, and TNF-α production. Bifidobacterium longum has also been shown to inhibit NF-κB activation in lamina propria mononuclear cells and down-regulate inflammatory cytokine secretion from inflamed tissues of patients with active UC [19]. Steed et al found that administration of Bifidobacterium longum and Synergy 1 in patients with active UC significantly reduced the expression of TNF-α after 3 months’ treatment [20]. Takeda et al suggested that Bifidobacterium longum could exert their beneficial effect in patients with UC by up-regulation of T-betand enhancement of mucosal barrier function and subsequently up-regulation of tight junction molecules [21].

**Escherichia coli**: Escherichia coli down-regulates the expansion of newly recruited T cells into the mucosa, regulates intestinal inflammation via TLR-2 and TLR-4, and restore the disrupted epithelial barrier in the colonic epithelial cell line T84 [22].

**Saccharomyces boulardii**: Saccharomyces boulardii decreases the infiltration of T-helper 1 cells into the mucosa, by blocking NF-κB and through IL-8 down-regulation, modulates the activity of the mitogen-activated protein kinases ERK1/2 and p38 and activates the expression of PPAR-γ, suppresses bacterial overgrowth, release protease cleaving Clostridium difficile toxin A, and stimulates the antibody production against toxin A [23].

Finally, Clostridium butyricum is able to produce high amounts of short chain fatty acids. Saccharomyces boulardii added to baseline therapy improved intestinal permeability in patients with CD, even though complete normalization can not be achieved [24].

**VSL#3**: The probiotic mixture, VSL#3, was described to increase interleukin-10 production as well as to down-regulate the Intereukin-12p40 production by dendritic cells in vitro [25]. Pronio et al found that 2 sachets of VSL#3 once daily administered in patients with ileal pouch anal anastomosis, influence regulatory T cells by increasing the percentage of mucosal CD4+CD25(high) and CD4+ LAP-positive cells compared with baseline values [26].

**Specific probiotic strains**: On the other hand, mechanistic studies have also been performed in humans to elucidate the mode of action of specific probiotic strains. Lerea-Barajoa et al [27] examined the effect of yogurt supplemented with L. rhamnosus GR-1 and L. reuteri RC-14 on T-regulatory cells, cytokines in T cells, monocytes, dendritic cells, and fecal and serum cytokine concentrations. The proportion of T-regulatory cells increased significantly in IBD patients both before and after treatment, but no significant difference was observed in controls. The basal proportion of TNFα-1/IL-12-1/1 monocytes and myeloid dendritic cells decreased in both subject groups, but only in stimulated cells of patients with IBD. Probiotic treatment significantly decreased serum IL-12 concentration in both controls and IBD patients, and also decreased serum TNF-α concentration in healthy patients.

**Efficacy of probiotics in IBD**

Several barriers exist to advocating broad use of probiotics in clinical practice, not least of which is the considerable heterogeneity in the experimental designs with respect to species and strains of probiotics and the various animal models utilized.

It must be stressed that the results of some clinical trials examining the role of probiotics in UC, CD, and pouchitis are inconsistent, and that large, well-designed trials are lacking. An additional factor pertains to issues of quality control such as the determination whether a commercially available probiotic actually contains the live organisms it purports to contain.

However, based on the encouraging results in animal models some investigators have pursued clinical studies looking on the therapeutic effects of the administration of probiotics in UC patients [28]. Promising results have been obtained in some studies concerning especially VSL#3.

Results of published studies regarding the use of probiotics in either active or inactive UC CD and pouchitis are subsequently analyzed.

**Efficacy of probiotics in patients with active UC**: So far 8 studies have been looked at the efficacy of various probiotics on patients with active UC (Table 3).

Guslandi et al [29] investigated the efficacy of the non-pathogenic yeast Saccharomyces boulardii in 25 patients with active UC patients of mild to moderate severity, and unsuitable for steroid therapy, in an open-label trial. Patients received additional treatment with S. boulardii 250 mg three times daily for 4 weeks during maintenance treatment with mesalazine. A significant reduction in UC disease severity index scores was observed, and 71% achieved endoscopic remission.

VSL#3 was added for 6 weeks to current treatment (mesalazine, corticosteroids, 6-MP, or azathioprine) in 34 ambulatory patients with active UC, who had failed to respond to conventional therapy. The presence of VSL#3 species confirmed by DNA sequencing of 16S rRNA meaning that bacteria incorporated in the probiotic reached the target site in amounts that could be detected. VSL#3 addition led to either remission or response in 77% of patients [30]. Again, VSL#3 achieved significantly greater reduction in UC activity index scores (UCDAI) and individual symptoms at weeks 6 and 12 in patients with UC compared with the placebo group [31].

In a more recent study it was reported that the decrease in UCDAI of 50% or more was significantly higher in the VSL#3 group than in the placebo group (63.1 vs 40.8). Moreover, significant improvement with VSL#3 in the UCDAI score and the degree of rectal bleeding was noticed. Remission rate was significantly higher in the VSL#3 group than in the placebo group (47.7% vs 32.4%) [32]. The results of the previously mentioned 3 studies suggest that VSL#3 supplementation seems to be safe and able to reduce UCDAI score in patients with relapsing mild-to-moderate UC who are under treatment with mesalazine and/or immunosuppressants.

Positive results have also been reported from the use of BIO-THREE tablet formulation (9 tablets daily for a period of 4 weeks) in 20 patients with mild to moderate distal UC. This probiotic combination of Streptococcus faecalis T-110, Clostridium butyricum TO-A, and Bacillus mesentericus TO-A led to remission of disease in 45% of patients, response in 10%, no response in 40%, and worsening in only 5%. Fecal samples revealed an increase in bifidobacteria, although no bifidobacterium were administered within the probiotic supplement [33]. This improvement in intestinal microflora probably represents a consequence of the treatment altering the microbial environment perhaps by removal of competing pathogens.
<table>
<thead>
<tr>
<th>Author / Journal</th>
<th>No of pts</th>
<th>Probiotic/ treatment</th>
<th>Primary end-point</th>
<th>Results</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Guslandi M et al Eur J Gastroenterol Hepatol. 2003;15: 697-6.</td>
<td>25</td>
<td>Additional treatment with S. boulardii 250 mg X 3 id for 4 weeks during maintenance treatment with mesalazine</td>
<td>Rachmilewitz's clinical activity index. Therapeutic success if final score &lt;6.</td>
<td>Clinical remission. 17/24 (71%) (endoscopic confirmation)</td>
<td>S. boulardii can be effective in the treatment of UC.</td>
</tr>
<tr>
<td>2 Bibiloni R, et al. Am J Gastroenterol-rol. 2005;100: 1539-46</td>
<td>34 pts not responding to conventional therapy No control group</td>
<td>VSL#3, 3,600 billion bacteria/d in two divided doses for 6 wk.</td>
<td>Ulcerative colitis disease activity index (UCDAI). Endoscopic assessment. (ITT analysis) Remission: (UCDAI &lt; or = 2): 53% (n = 18). Response (decrease in UCDAI &gt; or = 3, but final score &gt; or =3) 24% (n = 8), no response 9% (n = 3); worsening: 9% (n = 3)</td>
<td>Induction of remission/response rate of 77% with VSL#3 with no adverse events.</td>
<td></td>
</tr>
<tr>
<td>3 Sood A et al. Clin Gastroenterol Hepatol. 2005;7:1202-1209.</td>
<td>183 pts (102 vs 81)</td>
<td>VSL#3, 3.6 x 10(12) CFU VSL#3 (n = 77) or placebo (n = 70), twice daily for 12 weeks.</td>
<td>50% decrease in the ulcerative colitis Disease Activity Index at 6 weeks</td>
<td>VSL#3 group: 25 pts (32.5%) achieved a 50% reduction in UCDAI Placebo group: 7 pts (10%). Week 12: 33 pts (42.9%) vs 11 pts (15.7%); Greater decreases in UCDAI and individual symptoms in the VSL#3 vs placebo</td>
<td>VSL#3 is effective in achieving clinical response and remission in mild-to-moderate active UC.</td>
</tr>
<tr>
<td>4 Tursi A, et al. Am J Gastroenterol-rol. 2010;105: 2218-27.</td>
<td>144 pts</td>
<td>8 weeks’ treatment with VSL#3 (3,600 billion CFU/day) (71 pts) or placebo (73 pts)</td>
<td>(65 and 64 pts respectively, completed the study) Reduction in ulcerative colitis disease activity index</td>
<td>Higher decrease in UCDAI scores in the VSL#3 group than in the placebo group (63.1 vs. 40.8; per protocol P=0.010, intention to treat P=0.031). Higher remission in the VSL#3 group than in the placebo (47.7% vs. 32.4%)</td>
<td>VSL#3 supplementation is able to reduce UCDAI in patients with relapsing mild-to-moderate UC who are under treatment with 5-ASA and/or immunosuppressants.</td>
</tr>
<tr>
<td>5 Tsuda Y, et al Scand J Gastroenterol. 2010;42: 1306-11</td>
<td>20</td>
<td>9 BIO-THREE tablets per day for 4 weeks</td>
<td>UCDAI scores before and after administration of BIO-THREE. Fecal microflora was analyzed before and after probiotics administration</td>
<td>Remission (UCDAI score &lt; or =2): 45% (9/20 pts) Response: 10% (2/20); No response: 40% (8/20); Worsening: 5% (1/20); T-RFLP analysis: Increase in bifidobacteria.</td>
<td>BIO-THREE tablets, is safe and efficacious in active UC.</td>
</tr>
<tr>
<td>6 Hegazy SK et al. World J Gastroenterol. 2010;16:4154-51</td>
<td>30</td>
<td>sulfasalazine 2400 mg/d vs sulfasalazine 2400 mg/d with Lactobacillus delbrueckii and Lactobacillus fermentum</td>
<td>Colonic activity of myeloperoxidase, colonic content of interleukin, fecal calprotectin and expression of NF-kB p65 and TNF-α in colonic tissue</td>
<td>Significant decrease of all inflammatory indices after 8 week treatment</td>
<td>Oral supplementation with probiotics could be helpful in maintaining remission and preventing relapse of UC.</td>
</tr>
<tr>
<td>7 Matthès H, et al. BMC Complement Altern Med. 2010;10:13</td>
<td>90</td>
<td>2 wks treatment (once daily) with either 40, 20, or 10 ml enemas (n = 24, 23, 23) containing 106B EcN/ml or placebo (n = 20).</td>
<td>Clinical DAI &lt;or = 2 within that time</td>
<td>Significant correlation of per-protocol responder rates (p = 0.0446, 2-sided).</td>
<td>Efficacy of rectal EcN application is significant in PP analysis. It points to EcN as a well tolerated treatment.</td>
</tr>
</tbody>
</table>

Table 3: Clinical trials on the efficacy of probiotics in active ulcerative colitis.
Thirty patients with mild to moderate UC were randomly classified into two groups namely sulfasalazine group, who received sulfasalazine 2400 mg/d; and probiotic group, who received sulfasalazine 2400 mg/d with probiotic. The patients were investigated before and after 8 wk of treatment with probiotic containing Lactobacillus delbrueckii and Lactobacillus fermentum. The use of probiotic for 8 wk significantly ameliorated the inflammation by decreasing the colonic concentration of IL-6, expression of TNF-α and NF-kappaB p65, leukocyte recruitment, and the level of fecal calprotectin compared to sulfasalazine group and the control group thus leading to the conclusion that oral supplementation with probiotics could be helpful in maintaining remission and preventing relapse of UC [16].

Induction of remission in patients with active distal UC by E. coli Nissei (EcN) administered in the form of enemas was investigated in a recent clinical trial. Patients were assigned to treatment with 40, 20, or 10 mL enemas containing 10E8 EcN/mL or placebo once a day for 2 weeks. In the intention-to-treat analysis the number of responders was not significantly higher in the EcN group than in the placebo group, although the efficacy of rectal EcN was significant in the per-protocol analysis [34]. The results support EcN as a well-tolerated alternative treatment in moderately active distal UC.

Twenty-six patients with mild left-sided UC were randomly allocated to one of three groups for an 8-week treatment period: the first group of 7 patients received oral 5-ASA alone, the second group of 8 patients received oral 5-ASA plus oral L. casei DG, and the third group of 11 patients received oral 5-ASA and rectal L. casei DG. 5-ASA alone or together with oral L. casei DG failed to significantly affect colonic flora and TLR expression, but when coupled with rectally administered L. casei DG, it modified colonic microbiota by increasing Lactobacillus spp. and reducing Enterobacteriaceae. It also significantly reduced TLR-4 and IL-1β mRNA levels and significantly increased mucosal IL-10. Manipulation of mucosal microbiota by L. casei DG and its effects on the mucosal immune system seem to be required to mediate the beneficial activities of probiotics in UC patients [18].

In a prospective, randomized, double-blind, placebo-controlled study, 48 healthy volunteers took EcN in a run-in phase for 17 days (5-50 x 10⁹ viable bacteria od). If stool samples became positive for EcN, volunteers received combination treatment with EcN plus either mesalazine (1500 mg twice a day) or placebo for 1 week. Fecal samples were further tested for EcN in 2- to 3-day intervals until a maximum of 48 weeks after treatment. During run-in, viable EcN were detected in 45 of the 48 volunteers (94%). From days 1 to 7 of combination treatment (n=40), the number of EcN-positive volunteers varied between 70% and 80% in the mesalazine group and between 85% and 95% in the placebo group. Differences between the groups were not significant. At treatment discontinuation, 16/20 volunteers in the mesalazine group and 15/20 volunteers in the placebo group were EcN positive, whereas this figure dropped continuously up to week 12 after discontinuation [35]. It seems that the combination of EcN and mesalazine has no significant effect on the survival of EcN in healthy volunteers.

In conclusion it seems that probiotics and especially VSL#3 could induce remission in patients with mild or moderately active UC. All the available clinical studies described favourable results either as sole or complementary agents to mesalazine treatment. The total number of patients included in these studies (552) is satisfactory. However, despite these favourable results we suggest that large multicenter studies using large number of patients, with different kinds of probiotics and in large doses are needed in order to confirm the so far described results. Until then, probiotics could be aided to the classical treatment at least on patients with mild or moderately active UC.

Probiotics as maintenance treatment in ulcerative colitis: So far 5 studies including 682 patients have been contacted regarding the role of probiotics in the maintenance treatment of patients with UC (Table 4).

Rembacken et al [36] investigated whether the administration of a non-pathogenic strain of E. coli Nissle 1917 was as effective as mesalazine in preventing relapse of UC as well as whether the addition of E. coli to standard medical therapy increased the rate of remission of active UC. The results revealed no significant differences as 75% of patients in the mesalazine group achieved remission compared with 68% in the E. coli group and in the mesalazine group, 73% of patients relapsed compared with 67% in the E. coli group.

In a double-blind study Kruis et al [37] tested the efficacy to maintain remission of the probiotic preparation Escherichia coli Nissle 1917 on a total of 327 patients. The per protocol analysis revealed relapses in 40/110 (36.4%) patients in the E. coli Nissle 1917 group and 38/112 (33.9%) in the mesalazine group so establishing this probiotic therapy as equivalent to mesalazine.

A study using VSL#3 provided much more cutting edge results [38]. For 1 year, 20 patients with UC in remission and intolerant to mesalazine treatment received 3g of pure VSL#3 bacteria, equivalent to 1200 billion LAB, twice daily. Four of the patients relapsed after 3, 5, 5, and 7 months; 1 was lost to follow-up and the remaining 15 were in remission after 12 months. VSL#3 was able to colonize the intestine, and it could be useful in maintaining the remission in UC patients intolerant or allergic to 5-ASA.

Similar to the above mentioned results were reported in another open-label trial of Lactobacillus GG as maintenance treatment in 187 UC patients with quiescent disease [39]. Patients were randomized to receive L. GG, mesalazine or L. GG plus mesalazine. Overall analysis showed no difference in relapse rate at 6 and 12 months among the three treatment groups. However, the treatment with L. GG appeared to be more effective than standard treatment with mesalazine in prolonging the relapse-free time.

A recently published study [40] investigated the effect of treatment with Lactobacillus acidophilus, La-5, and Bifidobacterium animalis subsp. lactis BB-12 (Probio-Tec AB-25) to maintain remission in patients with UC. Thirty-two patients with left-sided UC were entered in a double-blind placebo-controlled study to Probio-Tec AB-25 (20 patients) or placebo (12 patients) for 52 weeks. The results revealed no significant differences, as 3 patients (25%) in the Probio-Tec AB-25 group and 1 patient (8%) in the placebo group maintained remission after 1 year of treatment. In this trial no significant clinical benefit of Probio-Tec AB-25 could be demonstrated in comparison with placebo for maintaining remission in patients with UC.

In conclusion the 5 available clinical trials aiming to evaluate the probiotic efficacy for maintaining remission of UC have produced rather conflicting results. Trial results comparing Escherichia coli Nissle 1917 to mesalazine have reported equivalent rates of UC relapse. Treatment with Lactobacillus rhamnosus GG strain alone or in combination with mesalazine resulted in a nonsignificant odds ratio decrease for relapse and a significant increase in time to relapse compared to treatment with mesalazine alone. Probiotics were well tolerated, with adverse event rates similar between treatments.
VSL#3 had an episode of acute pouchitis compared with 8/20 (40%) (n=20) or placebo (n=20) immediately after ileostomy closure for 1 month. Patients who underwent ileal pouch-anal anastomosis for UC to prevent or minimize pouchitis [42]. VSL#3 increased significantly from baseline levels only in the VSL#3 group, compared with 20 (100%) in the placebo group.

Table 4: Clinical trials on the efficacy of probiotics in maintaining remission in ulcerative colitis.

<table>
<thead>
<tr>
<th>Author / Journal</th>
<th>No of pts</th>
<th>Probiotic</th>
<th>Primary end-point</th>
<th>Results</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rembacken BJ, et al. Lancet. 1999;354(9179): 635-9</td>
<td>116</td>
<td>Randomization: 59 to mesalazine &amp; 57 to E. coli. Follow-up: 12 months</td>
<td>Clinical assessment for relapse</td>
<td>mesalazine group: 44/59 (75%) vs E. coli group 39/57 (68%).</td>
<td>Non-pathogenic E. coli has an equivalent effect to mesalazine in maintaining remission of UC</td>
</tr>
<tr>
<td>Kruis W, et al. Gut. 2004;53: 1617-23</td>
<td>327 (162 vs 165)</td>
<td>E. coli Nissle 1917 200 mg once daily (n = 162) vs mesalazine 500 mg three times daily (n = 165). Study duration: 12 months.</td>
<td>Prevention of relapses</td>
<td>(PPA) Relapse rate 40/110 (36.4%) pts in the E. coli Nissle 1917 group vs 38/112 (33.9%) in the mesalazine group (p = 0.003).</td>
<td>Maintaining remission of UC with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine</td>
</tr>
<tr>
<td>Venturi et al. Aliment Pharmacol Ther. 1999;13(8): 1103-8</td>
<td>20 UC pts intolerant or allergic to 5-ASA.</td>
<td>VSL#3, [fx10(11) cells/g of 3 strains of bifidobacteria, 4 strains of lactobacilli and 1 strain of Streptococcus salivarius ssp. Thermophilus]. Two doses of 3 g were administered o.d. for 12 months.</td>
<td>Faecal samples for stool culture at the beginning of the trial and after 10, 20, 40, 60, 75, 90 days, 12 months and at 15 days after the end of the treatment.</td>
<td>Faecal concentrations of Streptococcus salivarius ssp. thermophilus, lactobacilli and Bifidobacteria increased significantly in all patients, from the 20th day of treatment and remained stable throughout the study.</td>
<td>This probiotic preparation is able to colonize the intestine, and suggest that it may be useful in maintaining the remission in ulcerative colitis patients intolerant or allergic to 5-ASA.</td>
</tr>
<tr>
<td>Zocco MA et al. Aliment Pharmacol Ther. 2006;23:1567-74</td>
<td>187 (65 vs 60)</td>
<td>Randomization: Lactobacillus GG 18 x 10(9) viable bacteria/d (65 pts), vs mesalazine 2.4g/d (60 pts) vs Lactobacillus GG + mesalazine (62 pts).</td>
<td>Sustained remission.</td>
<td>No difference in relapse rate at 6 and 12 months among the three treatment groups. Treatment with Lactobacillus GG was more effective than mesalazine in prolonging the relapse-free time.</td>
<td>Lactobacillus GG seems to be effective and safe for maintaining remission in pts with UC</td>
</tr>
<tr>
<td>Wildt S, et al. J Crohns Colitis. 2011;5: 115-21.</td>
<td>32 (20 vs 12)</td>
<td>Lactobacillus acidophilus La-5 and Bifidobacterium animalis subsp. lactis BB-12</td>
<td>maintenance of remission</td>
<td>No significant differences between pts and those receiving placebo</td>
<td>No significant clinical benefit compared to placebo</td>
</tr>
</tbody>
</table>

[41] The number of studies dealing with probiotics as maintenance treatment in UC patients is limited. Thus, questions remain regarding optimal probiotic, dosing, specific patient populations, and placement in therapy. As all authors emphasize, large, randomized, controlled trials are needed to be conducted before probiotics can be routinely recommended for maintaining remission of UC.

Probiotics in the treatment of pouchitis: Pouchitis is a common and troublesome condition in patients operated on with ileal-pouch-anal-anastomosis (IPAA). So far 5 studies have been conducted concerning the role of probiotics in the treatment of pouchitis. The results are shown in table 5 and analyzed subsequently.

In the first trial 40 patients in clinical and endoscopic remission were randomized to receive either VSL#3, 6 g/day, or an identical placebo for 9 months. Significantly lower number of patients relapsed (3 patients, 15%) in the VSL#3 group, compared with 20 (100%) in the placebo group. Fecal concentration of lactobacilli, Bifidobacteria, and S. thermophilus increased significantly from baseline levels only in the VSL#3-treated group. The authors suggest that oral administration of this probiotic preparation is effective in preventing flare-ups of chronic pouchitis [42].

The same group of investigators randomized 40 consecutive patients who underwent ileal pouch-anal-anastomosis for UC to receive either VSL#3 (1 packet containing 900 billion bacteria/day) (n=20) or placebo (n=20) immediately after ileostomy closure for 1 year. Significantly smaller number of patients (2/20, 10%) treated with VSL#3 had an episode of acute pouchitis compared with 8/20 (40%) treated with placebo. VSL#3 produced also significant improvement in IBD Questionnaire score, compared with placebo [43]. The results suggest that treatment with VSL#3 could be effective in the prevention of the onset of acute pouchitis and improves quality of life of patients with IPAA.

Finally, in a subsequent study from the same group of investigators, 23 patients with mild pouchitis were treated with VSL#3, 2 sachets b.i.d. (3,600 billion bacteria/day) for 4 weeks. Patients in remission after treatment were treated with VSL#3, 1 sachet b.i.d. (1,800 billion bacteria), as maintenance treatment for six months. The quality of life was assessed with the IBD Questionnaire. They found that 16/23 patients (69%) were in remission after treatment. The median total pouchitis Disease Activity Index score was significantly reduced of the onset of acute pouchitis and improves quality of life of patients with IPAA.
Laake et al administered 500ml of a fermented milk product containing live lactobacilli (La-5) and Bifidobacteria (Bb-12) was given daily for 4 weeks to 51 UC patients and six UC patients operated on for IRA [45]. They found that number of lactobacilli and Bifidobacteriae increased significantly during intervention in the UC patients operated on with IPAA and remained significantly increased one week after intervention. Involuntary defecation, leakage, abdominal cramps and the need for napkins (category I), faecal number and consistency (category II) and mucus and urge to evacuate stools (category III) were significantly decreased during intervention in the UC/IPAA patients.

Kühbacher et al [46] investigated the mucosa associated pouch microbiota before and after therapy with VSL#3. Patients who developed pouchitis while treated with placebo had low bacterial and high fungal diversity. Bacterial diversity was increased and fungal diversity was significantly reduced in patients in remission maintained on with IPAA and remained significantly increased one week after intervention. Involuntary defecation, leakage, abdominal cramps and the need for napkins (category I), faecal number and consistency (category II) and mucus and urge to evacuate stools (category III) were significantly decreased during intervention in the UC/IPAA patients. Probiotic therapy with VSL#3 increases the total number of intestinal bacterial cells as well as the richness and diversity of the bacterial microbiota, especially the anaerobic flora. Restoration of the integrity of a “protective” intestinal mucosa related microbiota could therefore be a potential mechanism of probiotic bacteria in inflammatory barrier diseases of the lower gastrointestinal tract.

In conclusion, the available controlled trials have demonstrated that probiotics are effective in maintenance of remission in pouchitis patients, although 3 out of 5 available studies have been published by the same group of investigators, and the number of patients included in the 5 studies does not exceed the 225 in total. The benefit observed in patients with UC compared to CD could be attributed to fact that UC is a Th2-type inflammation whereas CD is a Th1/Th17-type inflammation and the former may be more amenable than the latter to the enhancing effects of probiotics on the regulatory T cells. In addition, UC pathology is centered on epithelial cells and thus may respond better to the restorative effects of probiotics on epithelial cells.

### Efficacy of probiotics in Crohn’s disease

So far, 7 studies including 316 patients in total have been published regarding the usefulness of probiotics as agents either inducing or maintaining remission after surgery in patients with CD. The results of these studies are shown in table 6 and analyzed subsequently.

Promising results came early in 1997 from a pilot study [47] in which the non-pathogenic strain Escherichia coli Nissle 1917 (serotype 06:K5:H1) was tested for efficacy and tolerance in maintaining remission in patients with colonic CD. Twenty-eight patients were randomized to either a preparation of Escherichia coli strain Nissle 1917 (n=16) or placebo (n=12) for 1 year. From the E. coli group, 33.3% of patients had a relapse within the 1 year treatment period compared with 63.6% in the placebo group, while from patients who had stopped taking prednisolone before the relapse; only 30% of the E. coli but 70% of the placebo patients experienced a relapse. Although none of the results in these studies are shown in table 6 and analyzed subsequently.

<table>
<thead>
<tr>
<th>Author / Journal</th>
<th>No of pts</th>
<th>Probiotic/ treatment</th>
<th>Primary end-point</th>
<th>Results</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gionchetti P, et al. Gastroenterology.</td>
<td>40 (20 vs 20)</td>
<td>Randomization: VSL#3, 6 g/day, or identical placebo for 9 months.</td>
<td>Assessment: Clinically every month endoscopically and histologically every 2 months</td>
<td>Relapse: 3/20 (15%) in the VSL#3 group vs 20 (100%) in the placebo group (P &lt; 0.001).</td>
<td>Oral administration of VSL#3 is effective in preventing flare-ups of chronic pouchitis.</td>
</tr>
<tr>
<td>1</td>
<td>Gionchetti P, et al. Gastroenterology.</td>
<td>40 (20 vs 20)</td>
<td>Randomization: VSL#3 (900 billion bacteria/day) or placebo immediately after ileostomy closure for 1 year.</td>
<td>Patients were assessed clinically, endoscopically, and histologically after 1, 3, 6, 9, and 12 months.</td>
<td>Relapse: 2/20 (10%) in the VSL#3 group vs 8/20 (40%) in the placebo group (P &lt; 0.05).</td>
</tr>
<tr>
<td>2</td>
<td>Gionchetti P, et al. Dis Colon Rectum. 2007;50:2075-82.</td>
<td>23</td>
<td>VSL#3, 2 sachets b.i.d. (3,600 billion bacteria/day) for 4 weeks. After remission, pts were given VSL#3, 1 sachet b.i.d. (1,800 billion) for 6 months.</td>
<td>Symptomatic, endoscopic, and histological evaluations before and after treatment according to PDAI.</td>
<td>16/23 (69%) were in remission after treatment. The median total PDAI scores before and after therapy were 10 and 4 respectively (P &lt; 0.01).</td>
</tr>
<tr>
<td>3</td>
<td>Pronio A, et al. Inflamm Bowel Dis. 2008;14:662-8.</td>
<td>31</td>
<td>Randomization: 2 sachets of VSL#3 once daily or no treatment for 12 months.</td>
<td>PDAI was evaluated at baseline and after 3, 6, and 12 months.</td>
<td>VSL#3-treated patients showed a significant reduction in PDAI score.</td>
</tr>
<tr>
<td>4</td>
<td>Laake KO et al. Scand J Gastroenterol. 2005;40:43-51.</td>
<td>51 UC pts operated on with IPAA</td>
<td>500 ml of fermented milk product containing live lactobacilli and bifidobacteriae daily for 4 weeks.</td>
<td>Endoscopic evaluation before, during and after intervention. Symptomatology was examined using diary cards before and on the last day of intervention.</td>
<td>Involuntary defecation, leakage, abdominal cramps and the need for napkins, faecal number and consistency and mucus and urge to evacuate stools were significantly decreased during intervention.</td>
</tr>
</tbody>
</table>

### Table 5: Clinical trials investigating the efficacy of probiotics on patients with pouchitis.
were statistically significant because of the small number of patients, the study does suggest that the application of *E. coli* Nissle 1917 reduced the risk of relapse and minimized the need for corticosteroids. This is the only study to date conducted with these bacteria in CD patients.

The efficacy and safety of the probiotic preparation VSL#3 was evaluated in relation to the prevention of post-operative recurrence of CD [48]. Forty patients were randomized to receive either rifaximin for 3 months followed by VSL#3 for 9 months (n=20) or mesalazine for 12 months (n=20). At the end of treatment 4 patients [20%] in the antibiotic/probiotic group had a severe endoscopic recurrence versus 8 patients [40%] in the mesalazine group. The results suggest that the combination of a non-absorbable antibiotic with a highly concentrated probiotic preparation in the prevention of severe endoscopic recurrence of CD after surgical resection seems to offer benefit.

Table 6: Efficacy of probiotics in patients with Crohn’s disease (induction of remission or postoperative prophylaxis).

<table>
<thead>
<tr>
<th>Author / Journal</th>
<th>No of pts</th>
<th>Probiotic / treatment</th>
<th>Primary end-point</th>
<th>Results</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Malchow HA</td>
<td>J Clin Gastroenterol 1997; 25:653-8.</td>
<td>28</td>
<td>Non-pathogenic strain <em>Escherichia coli</em> Nissle 1917</td>
<td>Appearance of relapse and reduction in the need for corticosteroids</td>
<td>Relapse: <em>E. coli</em> group, 33.3% compared with 63.6% in the placebo group (No significant)</td>
</tr>
<tr>
<td>3 Gislandi M, et al.</td>
<td>Dig Dis Sci 2000;45:1462-4</td>
<td>32</td>
<td>Treatment for six months with either mesalamine 1 g X3/d or mesalamine 1 g X2/d plus Saccharomyces boulardii 1 g daily.</td>
<td>Clinical relapse (CDAI)</td>
<td>Clinical relapses were observed in 37.5% of patients receiving mesalamine alone and in 6.25% of patients in the group of mesalamine plus the probiotic agent.</td>
</tr>
<tr>
<td>4 Prantera C, et al.</td>
<td>Gut 2002;51:405-9.</td>
<td>37 (15 vs 17)</td>
<td>Patients operated on CD were randomly allocated to receive Lactobacillus rhamnosus strain GG [12 billion cfu] or placebo, for one year.</td>
<td>Ileocolonoscopy at the end of the trial or at the onset of symptoms. Endoscopic recurrence</td>
<td>9/15 patients in clinical remission on Lactobacillus [80%] had endoscopic recurrence compared with 6/17 (35.3%) on placebo. (No Significant)</td>
</tr>
<tr>
<td>5 Schultz M et al.</td>
<td>BMC Gastroenterol. 2004 Mar 15;4.</td>
<td>11 pts, active CD</td>
<td>a double-blind, placebo-controlled trial. Pts receive either Lactobacillus rhamnosus strain GG [2× 10^9 cfu/day] or placebo for six months.</td>
<td>Sustained remission, at the 6 months follow-up visit.</td>
<td>The median time to relapse was 16±4 weeks in the L. GG group and 12±3 weeks in the placebo group (NS). No significant difference in either the rate of inducing or sustaining remission for 6 months between the 2 groups.</td>
</tr>
<tr>
<td>6 Marteau P et al.</td>
<td>Gut 2006;55:842-7.</td>
<td>98 (48 vs 48)</td>
<td>Lactobacillus johnsonii L1 LA1 Randomization: 2 packets per day of lyophilised LA1 (2 x 10^9 cfu) or placebo for six months; no other treatment.</td>
<td>Endoscopic recurrence at six months</td>
<td>Endoscopic recurrence was observed in 30/47 (64%) in the placebo group and in 21/43 (49%) in the LA1 group (p = 0.15).</td>
</tr>
<tr>
<td>7 Van Goossen A, et al.</td>
<td>Inflamm Bowel Dis. 2003;13: 135-42.</td>
<td>70 (34 vs 36)</td>
<td>Treatment with either Lactobacillus johnsonii, LA1, (1010 colony-forming units, CFU) (n = 34) or placebo (n = 36) for 12 weeks.</td>
<td>Endoscopic recurrence at 12 weeks</td>
<td>Mean endoscopic score was not significantly different between the two treatment groups at 3 months, and neither was the percentage of severe recurrence nor the clinical relapse rate.</td>
</tr>
</tbody>
</table>

or identical placebo, for one year was investigated in a clinical trial concerning 37 patients operated on for CD [51]. Clinical recurrence was ascertained in 3 (16.6%) patients, who received *Lactobacillus* and in 2 (10.5%) who received placebo. Nine of 15 patients in clinical remission on *Lactobacillus* (60%) had endoscopic recurrence compared with 6/17 (35.3%) on placebo. There were no significant differences in the severity of the lesions between the two groups. In this study, the statistical insignificance of the results could relate to the small number of patients in each arm of the study.

A double-blind, placebo-controlled trial randomized 11 patients with active CD to receive either Lactobacillus rhamnosus strain GG [2 × 10^9 cfu/day] or placebo for 6 months and no other treatment. Endoscopic recurrence was noticed in 10% of the patients in the placebo group compared to 0% in the probiotic group. The median time to relapse did not differ in the two groups. There was no significant difference in the rate of inducing or sustaining remission for 6 months.

Another randomised double-blind, placebo-controlled study using *Lactobacillus johnsonii LA1* suggested a modest improvement in recurrence rates after surgical resection of the diseased bowel [53]. Ninety-eight patients, who had undergone surgical resection earlier in the month, were randomized to receive lyophilised LA1 or placebo for six months and no other treatment. Endoscopic recurrence was noticed in 49% versus 64% in the placebo group.

In a more recent study 70 CD patients were enrolled prior to elective ileo-caecal resection and randomly assigned after surgery to daily treatment with either *L. johnsonii* (10^9 cfu) or placebo for 12 weeks. The mean endoscopic score was not significantly different between the two treatment groups at 3 months, and neither was the percentage of severe recurrence nor the clinical relapse rate [54].

In conclusion, it seems that probiotics offer no benefit in patients with CD in either inducing remission or sustaining remission. However, more studies with a large number of patients are needed in order to definitely consider probiotics as an ineffective treatment in patients with CD.

**Efficacy of prebiotics and synbiotics in Inflammatory Bowel Disease**

So far 9 studies including 317 patients have been conducted aiming to investigate the efficacy of various preparations of symbiotics and prebiotics in patients with either UC or CD (Table 7).

Furrie et al [55] enrolled 18 patients with active UC in a 4-week double-blinded randomised controlled trial. The test group, comprising nine patients, was given a synbiotic formula consisting of 2g of oligofructose-enriched inulin [Synergy 1] and capsules containing 2 × 10^11 freeze-dried *Bifidobacterium longum* per day, while the placebo group was receivied the same capsules. The results showed that Bifidobacterial numbers on the rectal mucosa increased 42-fold in patients given the symbiotic compared to a 5.8-fold increase in the control group. Moreover symbiotic treatment was accompanied by marked reductions in TNF-α and IL-1α in mucosal tissue while mRNA levels for human β defensins 2, 3, and 4, were also found to be significantly reduced. Sigmodoscopy scores improved, while histopathology showed both, marked reductions in inflammatory infiltration and increased regeneration of normal epithelium in the symbiotic compared to placebo group. Although this study demonstrated the favourable results of short-term symbiotic treatment in active UC the number of patients was small to draw firm conclusions.

In an open-label trial [56], 12 UC patients received 4.5g of the prebiotic Bifidogenic growth stimulator (a prebiotic preparation produced by *Propionibacterium freudenreichii* isolated from Swiss cheese) daily for 4 wk while the baseline anti-inflammatory therapy was continued. Clinical activity index scores decreased significantly from 7.4 to 4.7, and endoscopy scores from 4.4 to 2.8. Fecal butyrate excretion was increased significantly. Oral Bifidogenic growth stimulator may represent a non-toxic way to treat UC patients.

Fujimori et al [57] randomized 120 outpatients with UC into three groups of 40 patients each for probiotic, prebiotic, or symbiotic therapy. The probiotic group received one daily capsule consisting of *Bifidobacterium longum* 2 x 10^9 colony-forming units and the prebiotic group received daily 8.0 g doses of psyllium. The symbiotic group underwent both treatments. Total IBQ scores improved within groups by the end of the trial (probiotics 162 to 169, NS; prebiotics 174 to 182, NS; symbiotics 168 to 176, P=0.03). Individual scores improved as follows: probiotics, emotional function (P=0.03); prebiotics, bowel function (P=0.04); and symbiotics, systemic and social functions (P=0.008 and P=0.02). CRP decreased significantly only with symbiotic therapy (from 0.59 to 0.14 mg/dL, P=0.04). Patients with UC on symbiotic therapy experienced greater quality-of-life changes than patients on probiotic or prebiotic treatment. It seems that symbiotic therapy may have a synergistic effect in the treatment of UC.

The same group of authors investigated the effect of synbiotic therapy, consisting of probiotics (*Bifidobacterium* and *Lactobacillus* 75 billion colony forming units [CFU] daily) and prebiotics (psyllium 9.9 g daily) in 10 outpatients with active CD [58]. The duration of the trial was 13.0 +/- 4.5 months. By the end of therapy, 7 patients had improved clinical symptoms following symbiotic therapy. Both CDAI and IOIBD scores were significantly reduced after therapy (255-136, P=0.009; 3.5-2.1, P=0.03, respectively). Six patients had a complete response, one had a partial response, and three were non-responders. Two patients were able to discontinue their prednisolone therapy, while four patients decreased their intake. Although this study showed that high doses of synbiotics can be safely and effectively used for the treatment of active CD, the small number of patients and the lack of control group weaknesses the power of the results.

The Symbiotic 2000 regimen comprising four lactic acid bacteria (*L. raffinolactis, L. paracasei* sus *paracasei* 19, *L. plantarum* 2362 and *Pediococcus pentosaceus, each 10^9*) and four fermentable fibers (*β-glucans, inulin, pectin, and resistant starch, each 2.5g*), has been tried in two controlled trials, in the first of which, reported only as an abstract, 63 patients, after an initial treatment with infliximab, were randomized to receive either Symbiotic 2000 or placebo daily. The results showed that the median time to relapse did not differ significantly between the two groups (9.8 versus 10.1 months) [59].

The second was a randomised, multicenter, placebo-controlled study. Synbiotic was given postoperatively, once daily in 20 patients for 24 months, while 10 subjects served as controls. The results showed that the synbiotic preparation had no effect on endoscopic or
clinical relapse rates in both groups [60]. The authors speculated that increasing the numbers of patients and the quantity of the probiotics might be more effective in preventing recurrence of the disease. It must also be noted that Symbiotic 2000 was originally designed for critically ill patients, and the bacteria were chosen for their ability to survive in the upper gastrointestinal tract rather than their anti-inflammatory

Table 7: Efficacy of synbiotics and prebiotics in patients with IBD.
characteristics.

A recently published randomized, double-blind placebo-controlled trial was conducted in 35 patients with active CD, using a symbiotic comprising *Bifidobacterium* longum and Synergy 1. Significant improvements in clinical outcomes occurred with symbiotic consumption, with reductions in both CDAI (P=0.020) and histological scores (P=0.018). The symbiotic had little effect on mucosal IL-18, INF-γ and IL-1β; however, significant reductions occurred in TNF-α expression in symbiotic patients at 3 months. Mucosal bifidobacteria proliferated in symbiotic patients [61]. The study results support the assumption that symbiotic consumption might improve clinical symptoms in patients with active CD.

Two other studies investigated the influence of probiotics on patients with IBD. Lindsay et al. [62] conducted an open-label study on ten patients with moderately active ileo-colonic CD in order to assess the effect of fructo-oligosaccharides on fecal and mucosal *Bifidobacteria*. Fructo-oligosaccharides was found to induce a significant reduction in the Harvey Bradshaw index, while there was also a significant increase in fecal *Bifidobacteria* concentration and in both, the percentage of IL-10 positive dendritic cells and the percentage of dendritic cells expressing toll-like receptors, suggesting fructo-oligosaccharides treatment as promising therapeutic strategy.

Promising results have also been obtained from a small prospective, randomized, placebo controlled pilot trial of the benefits of the prebiotic inulin alone in 19 patients with a mild to moderate relapse of UC [63]. Patients being on treatment with mesalazine (3g/day) were randomly allocated to receive either oligofructose-enriched inulin (12g/day) or placebo (12g/day of maltodextrin) for 2 weeks. The test product was Synergy 1 which consists of a selected combination of long-inulin chains together with the shorter oligo-fructose chains [oligofructose-enriched inulin], both obtained from the chicory root. Inflammation score decreased in both groups, reaching statistical significance at day 14 (P<0.05). At day 7, an early significant reduction of calprotectin was observed in the group receiving oligofructose-enriched inulin but not in the placebo group.

In conclusion although synbiotics could be of benefit in inducing remission mainly in patients with active UC, both the small number of studies conducted so far and the small number of patients included in these studies, do not allow their regular use in patients with either UC or CD. However the potential benefit of these agents must be further investigated in the near future bearing in mind the high cost of the established therapeutic strategies.

**Conclusion**

Over the last decade, the possibility of treating UC and CD by means of agents that are inexpensive, easy to administer, safe, and have no side-effects has motivated numerous studies testing the therapeutic potential of probiotics, prebiotics and synbiotics both in murine models and in humans with IBD.

There is growing experimental and clinical evidence that probiotics could prevent the activation of inflammatory processes, although the clinical trials to date generally have not supported a rationale for their widespread use, with the exception of active UC.

More specifically, the results of the previously analyzed trials using probiotics and synbiotics in IBD can be summarized as follows: no superiority of any probiotic was clearly evident; certain probiotics, particularly *E. coli Nissle 1917* and a multi-agent mixture VSL#3, may be benefit patients with UC or pouchitis, while *Lactobacillus rhamnosus GG* appears less useful. In general, probiotics show potential for therapeutic application mainly in pouchitis and to a lesser degree in UC, while their effects in maintenance therapy for CD have been much less promising.

We must emphasize the fact that the bacteria used in many studies were not selected on the basis of demonstrable immune-modulating properties. Instead, many probiotics have been employed on the basis of availability, their adherence properties, or their survivability in the upper gastrointestinal tract. Additionally, the small number of patients, the different disease state and manifestation and the insufficient dosage of probiotics used are all explanations for the inefficacy in most studies.

To achieve optimal results however, future work should focus on specific combinations of probiotics and prebiotics whose immunoregulatory properties are well understood and which target specific immune disorders in the gut. Well-designed, randomized and placebo-controlled trials with uniform criteria and with larger patient cohorts, taking into account all subtypes within the groups both in terms of disease spectrum and individual microflora differences, are necessary to clarify the efficacy of probiotic therapy and optimize results.

**Acknowledgment**

The authors wish to thank Dr Eleni Triantafillidi (Pharmacist, University of Athens, Greece) for her valuable help during the preparation of the manuscript.

**References**


28. Triantafillidis JK, Merikas E, Georgopoulos F, Merikas E


