Probiotics in the Management of Inflammatory Bowel Disease: A Systematic Review of Intervention Studies in Adult Patients

SYSTEMATIC REVIEW

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Probiotics in the Management of Inflammatory Bowel Disease

A Systematic Review of Intervention Studies in Adult Patients

Daisy Jonkers,[1] *John Penders*,[2] *Ad Masclee*[1] and *Marieke Pierik*[1]

1 Division of Gastroenterology-Hepatology, Research School Nutrim, Maastricht University Medical Centre, Maastricht, the Netherlands
2 Department of Medical Microbiology, Research School Nutrim, Maastricht University Medical Centre, Maastricht, the Netherlands

Abstract

Introduction: Mounting evidence suggests an important role for the intestinal microbiota in the chronic mucosal inflammation that occurs in inflammatory bowel disease (IBD), and novel molecular approaches have further identified a dysbiosis in these patients. Several mechanisms of action of probiotic products that may interfere with possible aetiological factors in IBD have been postulated.
Objective: Our objective was to discuss the rationale for probiotics in IBD and to systematically review clinical intervention studies with probiotics in the management of IBD in adults.
Methods: A systematic search was performed in PubMed up to 1 October 2011, using defined keywords. Only full-text papers in the English language addressing clinical outcomes in adult patients were included. The 41 eligible studies were categorized on disease type (ulcerative colitis [UC] with/without an ileo-anal pouch and Crohn's disease [CD]) and disease activity. Pooled odds ratios were only calculated per probiotic for a specific patient group when more than one randomized controlled trial was available.
Results: Well designed randomized controlled trials supporting the application of probiotics in the management of IBD are still limited. Meta-analyses could only be performed for a limited number of studies revealing overall risk ratios of 2.70 (95% CI 0.47, 15.33) for inducing remission in active UC with Bifido-fermented milk versus placebo or no additive treatment (n = 2); 1.88 (95% CI 0.96, 3.67) for inducing remission in active UC with VSL#3 versus placebo (n = 2); 1.08 (95% CI 0.86, 1.37) for preventing relapses in inactive UC with *Escherichia coli* Nissle 1917 versus standard treatment (n = 3); 0.17 (95% CI 0.09, 0.33) for preventing relapses in inactive UC/ileo-anal pouch anastomosis (IPAA) patients with VSL#3 versus placebo; 1.21 (95% CI 0.57, 2.57) for preventing endoscopic recurrences in inactive CD with *Lactobacillus rhamnosus* GG versus placebo (n = 2); and 0.93 (95% CI 0.63, 1.38) for preventing endoscopic recurrences in inactive CD with *Lactobacillus johnsonii* versus placebo (n = 2).

Conclusion: Further well designed studies based on intention-to-treat analyses by several independent research groups are still warranted to support the promising results for *E. coli* Nissle in inactive UC and the multispecies product VSL#3 in active UC and inactive pouch patients. So far, no evidence is available to support the use of probiotics in CD. Future studies should focus on specific disease subtypes and disease location. Further insight into the aetiology of IBD and the mechanisms of probiotic strains will aid in selecting probiotic strains for specific disease entities and disease locations.

1. Introduction

The recent introduction of phylogenetic molecular approaches targeting the small subunit (SSU) ribosomal RNA gene of bacteria has resulted in a major breakthrough in knowledge on the composition of the intestinal microbiota. This complex ecosystem develops after birth, when our sterile gastrointestinal tract (GIT) becomes rapidly colonized by successive waves of micro-organisms until a dense microbial population, comprising $10^{13–14}$ bacteria and hundreds of different species, stabilizes at about time of weaning. The density and diversity increases from the stomach towards the colon. The individual faecal microbiota has been found to be relatively stable over time, but differs between subjects.[1,2] Despite the high inter-individual diversity, the human gut microbiota is dominated by the phyla Firmicutes and Bacteroidetes[2-4] and contains a core microbiome with shared functionality.[5] A recent publication in *Nature*[6] revealed that the intestinal microbial composition of humans segregates into three distinct clusters, designated as 'enterotypes'. Independent of ethnic background, gender, age and body mass index (BMI), the intestinal microbiota of each individual can be mapped to one of these three ecosystems. These enterotypes are driven by enrichment of the genera *Bacteroides*, *Prevotella* and *Ruminococcus*, respectively. Furthermore, this study demonstrated that abundant molecular functions were not necessarily provided by abundant species, indicating that both compositional and functional analyses of the intestinal microbiota are important.

Lessons from germ-free animals illustrate that commensal micro-organisms are necessary for the development and maturation of the intestinal immune system and the epithelium.[7] Furthermore, the intestinal microbiota contributes to the defence against pathogens by the mechanism of colonization resistance and has a high metabolic activity. Saccharolytic bacterial fermentation of non-digestible carbohydrates, occurring mostly in the proximal colon, is especially important because of the production of short-chain fatty acids (SCFAs, i.e. acetate, propionate and butyrate). Butyrate is a major energy source for intestinal epithelial cells, affects cell proliferation, differentiation, mucus secretion and barrier function, and has anti-inflammatory and anti-oxidative potential.[8]

The intestinal microbiota is increasingly recognized as involved in a wide range of not only gastrointestinal but also systemic disease entities, including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), obesity and the metabolic syndrome.[9,10] In the present review we address the role of the intestinal microbiota and the rationale for interventions with probiotics in IBD. A systematic overview is performed of clinical intervention studies with probiotics for the management of IBD in adults.

2. The Intestinal Microbiota in Inflammatory Bowel Disease (IBD)

IBD is a group of chronic relapsing inflammatory disorders of the GIT and primarily comprises ulcerative colitis (UC) and Crohn's disease (CD). In the US, approximately 1.5 million people suffer from IBD. The incidences vary between 6 and 8 per 100 000 for CD and between 9 and 12

per 100 000 for UC.[11] IBD is an invalidating disease with a peak onset in subjects between 15 and 30 years of age. Ethnic and familial clustering suggested a genetic background of IBD that was confirmed with the identification of the first CD gene *CARD15*.[12,13] However, geographic variation and the worldwide increase in incidence in line with Western lifestyles, as well as migration studies, also indicate that environmental factors may play a major role.[11,14,15] IBD is generally acknowledged to have a complex multi-factorial aetiology that results in a heterogenic clinical presentation. Although UC and CD are both characterized by the clinical symptoms abdominal pain, diarrhoea, rectal bleeding and weight loss, they are different pathological entities, both with various disease phenotypes. UC is characterized by continuous superficial mucosal inflammation restricted to the colon, spreading out from the distal part. CD is most frequent in the ileum and the colon, but can affect any part of the GIT. The inflammation is discontinuous, and often transmural, and therefore CD can be complicated by strictures and fistulas. Furthermore, CD is associated with intestinal granulomas, which are not typical for UC. Extra-intestinal disease manifestations of the skin, the liver, the eyes or the joints are present in up to 40% of CD and UC patients.

The first evidence for a role of the intestinal microbiota in IBD came from a variety of animal colitis models, which did not develop inflammation when raised under germ-free conditions.[15] In IBD patients, the relevance of intestinal bacteria is supported by findings that intestinal inflammation is often present in anatomical areas with high bacterial numbers,[16,17] the mucosal bacterial counts are higher in IBD patients than in healthy controls[17,18] and diversion of the faecal stream proximal to the inflamed mucosa resulted in a decreased disease activity in CD patients.[19-22] Furthermore, a recent meta-analysis, including ten and nine randomized controlled trials (RCTs), showed a significant benefit of antibiotic over placebo treatment for inducing remission in CD (risk ratio [RR] 0.85, 95% CI 0.73, 0.99) and UC (RR 0.64, 95% CI 0.43, 0.96), respectively.[23]

Apart from inconsistent findings on the aetiological role of *Mycobacterium paratuberculosis*, *Listeria monocytogenis* and paramyxoviruses,[24] no specific micro-organism has yet been identified as a possible causal factor, but a dysbiosis of the commensal intestinal microbiota has been reported in IBD. Using, for example, fluorescent *in situ* hybridization (FISH), quantitative polymerase chain reaction (PCR) and pyrosequencing approaches, the faecal microbiota was found to be less diverse[25-27] and to have a different composition in IBD compared with controls as well as in UC versus CD patients.[4,28,29] Also in mucosal specimens, the diversity was found to be lower,[4,30,31] but total bacterial numbers were increased[32,33] and the overall composition was different in IBD compared with controls.[4,30,31] In general, fewer Firmicutes were found,[28,34,35] as well as a reduced diversity within this phylum.[3] Low bacterial counts of *Faecalibacterium prausnitzii*, a major representative of the *Clostridium leptum* group within the phylum Firmicutes, were found to be associated with active IBD[36] and with CD,[29] which is of interest because of its butyrate-generating and anti-inflammatory capacity. The diversity of the Bacteroidetes phylum was also found to be reduced in IBD,[3] but data on their bacterial counts were inconsistent.[29,34,35] Furthermore, less bifidobacteria[37-39] and an increase of Enterobacteraceae, especially increased numbers of adherent *Escherichia coli*, are demonstrated by several groups.[35,39,40] With regard to the metabolic activity of the intestinal microbiota, decreased faecal concentrations of butyrate have been reported in UC,[4,41] and a significant decrease of the transcriptional activity of the mucosa-associated microbiota has been found in both IBD groups compared with healthy volunteers.[34]

A few groups studied the intestinal microbiota in relation to disease activity. Although not confirmed in all studies, different bacterial numbers were found comparing active with inactive UC or CD patients.[29,36,39] A different composition and less diversity was also found comparing inflamed with non-inflamed mucosal samples within patients,[29,35,42] but so far no bacterial species or clusters have been identified as associated with inflammatory status. In line with the heterogeneity in disease phenotypes, both the

faecal- and mucosa-associated microbiota have been found to differ between subjects with ileal versus colon predominant CD.[25,43] This observation was recently confirmed by Frank et al.,[30] who further found an altered microbiota composition by disease genotype based on the presence of *NOD2* and/or *ATG16L1* gene mutations.

2.1 Loss of Oral Tolerance to Commensal Bacteria

Apart from a dysbiosis, patients with IBD are characterized by an exaggerated response to commensal bacteria, which is thought to be an important factor driving the intestinal inflammation. Although the exact mechanisms involved in loss of oral tolerance in IBD remain enigmatic, an increased mucosal infiltration of activated CD4+ lymphocytes, dysfunctional dendritic cells, dysregulated macrophage-induced immune responses and abnormalities in regulatory pathways have been demonstrated.[44,45] The subsets of CD4+ T cells involved in either UC or CD seem to be different. In CD, an increased production of T helper (T_h)-1 cytokines and the T_h17 cytokine interleukin (IL)-17 has been observed,[7,45,46] whereas in UC patients, a preferential expression of T_h2 cytokines IL-4 and IL-5 as well as an increase in IL-17 has been reported.[44,45,47] Reduced numbers of regulatory T cells, producing the anti-inflammatory cytokines IL-10 and/or transforming growth factor (TGF)-β, are found in both UC and CD.[7,44] Host defense against commensal bacteria could be further diminished by an aberrant expression of or response to Toll-like receptors (TLR);[45,48,49] a decreased production of antimicrobial peptides, mucous and secretory immunoglubulin A (sIgA); deficiencies in inflammasomes being sensors of damage-associated molecular patterns leading to inflammation and apoptosis and a disruption of intercellular tight junctions as found in (subgroups of) IBD patients.[44,50,51]

The genetic susceptibility observed in IBD will contribute to the loss of oral tolerance. Genome-wide association studies have identified 99 susceptibility loci, of which 71 are associated with CD, 47 with UC and 28 with both CD and UC.[52] Many of them encode proteins involved in innate or adaptive immunity. Genes involved in IL-10 signalling and in the IL-23/T_h17 pathway are examples of associations with both UC and CD,[13,52] whereas mutations in the intracellular pattern recognition *NOD2/CARD15* and in the autophagy genes *ATG16L1* and *IRGM* are associated with an increased risk of developing CD.[13,44] These associations can further differ between disease phenotypes, as Wehkamp and colleagues[53,54] found decreased messenger RNA (mRNA) levels of human α-defensin 5 in CD patients with ileal disease, which were associated with the presence of mutations in the *NOD2* gene.

3. Rationale for Probiotics in IBD

Both UC and CD are associated with a significant burden for patients as well as for the healthcare system. Treatment is based on medical therapy and surgical interventions. In the US, approximately one-third of the estimated $US63 billion for IBD-associated costs are spent on medication.[11,55] For induction of remission or maintenance, 5-aminosalicylic acid (5-ASA) drugs, corticosteroids, budesonide, thiopurines, methotrexate and tumour necrosis factor (TNF)-α blocking agents are used. They are associated with a wide range of possible severe side effects, such as leukopenia, liver function abnormalities, interstitial nephritis and pancreatitis.[11,55] With the exception of mesalazine, all the drugs used reach their effect by non-specific suppression of the immune system and therefore patients can develop (opportunistic) infections. Furthermore, an increased risk of developing lymphomas has been suggested for thiopurines and TNFα blocking agents.[56,57] Therapeutic interventions addressing possible causative factors, such as microbial dysbiosis, in the initiation or progression of IBD could therefore be of additive value.

Although the exact aetiology is not yet clear, the generally accepted hypothesis suggests that IBD arises from loss of oral tolerance to the commensal microbiota, resulting in chronic intestinal inflammation in genetically predisposed hosts. Although a detailed characterization of the intestinal microbiota in IBD is not yet available, many studies, as discussed in section 2, have dem-

onstrated differences in its composition in UC and CD compared with controls, as well as within both patient groups in relation to disease activity, location and genotype. Whether the observed changes are a cause or a consequence of the inflammation still has to be further elucidated. Nevertheless, manipulation of the indigenous intestinal microbiota composition and activity, the immune system and host barrier function, is the main rationale for intervention studies with probiotics in IBD.

Probiotics are defined as "live microorganisms, which when administered in adequate amounts confer a health benefit to the host."[58] They should be of human origin, genetically stable and able to survive passage through the GIT (i.e. low pH, bile and digestive enzymes). Furthermore, they must be safe, and postulated health effects must be proven in human intervention studies. Apart from single bacterial strains, such as *Lactobacillus* spp. or *Bifidobacterium* spp., multispecies products are increasingly applied. As different bacterial strains can have different effects, they may act complementarily or even synergistically.[59]

Several possible mechanisms of probiotics may contribute to a health effect in IBD. Probiotic bacteria may affect the composition of the microbial ecosystem by competition for nutrients and adhesion sites, by the production of antimicrobial substances and/or via cell-cell communication.[60,61] Furthermore, they may affect the host immune system by interaction of bacterial products, cell wall components or DNA with epithelial and gut-associated immune cells.[62] Subsequently, changes in cytokine production, modulation of dendritic cell function, increased natural killer cell activity and the induction of regulatory T cells and defensins have been demonstrated.[60,62,63] Finally, probiotics can contribute to the production of SCFAs (e.g. butyrate) or affect the intestinal barrier function by induction of mucin secretion, enhancement of tight-junction expression and functioning and reduction of epithelial cell apoptosis.[64-67] These mechanisms of action have mainly been studied *in vitro*, *ex vivo* or in animal studies, and it has to be acknowledged that effects are found to differ between probiotic strains.

First lessons on probiotics reducing chronic intestinal inflammation came from animal studies.[68] In IBD patients, most studies have focused on maintaining or inducing remission. The next sections provide a critical review of the available literature on probiotic intervention studies for the management of IBD, addressing clinical outcome parameters in adult patients.

4. Methods of Literature Review

A systematic search was conducted in PubMed up to 1 October 2011, using the following (truncated) keywords 'IBD, inflammatory bowel disease*, ulcerative, colitis, Crohn* or pouch*' in any combination with 'probiotic*, lactobacill* or bifidobact*'. The search was limited to full-text English written papers, resulting in 1421 hits. By screening the titles and abstracts, studies performed *in vitro,* in animals, among minors (<18 years of age), reviews and studies on prebiotics or synbiotics were subsequently excluded. Full text of the remaining 58 papers on probiotics in adult IBD patients was checked. Only those investigating clinical outcome, either as a primary or secondary outcome parameter, in adult IBD patients were considered eligible for evaluation. Clinical outcome was defined by (validated) clinical activity indices and endoscopic and/or histological scores. One additional paper was found by checking references of pertinent articles. Studies analysing subgroups of previously published trials were not included for the evaluation of clinical outcome to avoid over-representation, but will be discussed in case of mechanistic support. Finally, 43 studies were included in this systematic review. As aetiological factors and clinical presentation differs between IBD entities and phenotypes, studies investigating active (n = 13) or inactive UC (n = 8), CD patients (n = 8) or UC patients with an ileo-anal pouch anastomosis (IPAA) [n = 12] were discussed separately. The studies by Karimi et al.[69] and Lorea Baroja et al.[70] investigated IBD patients as one group (including both UC and CD), and were therefore not included in the further analyses (figure 1).

The methodological quality for each of the included studies was assessed using the following

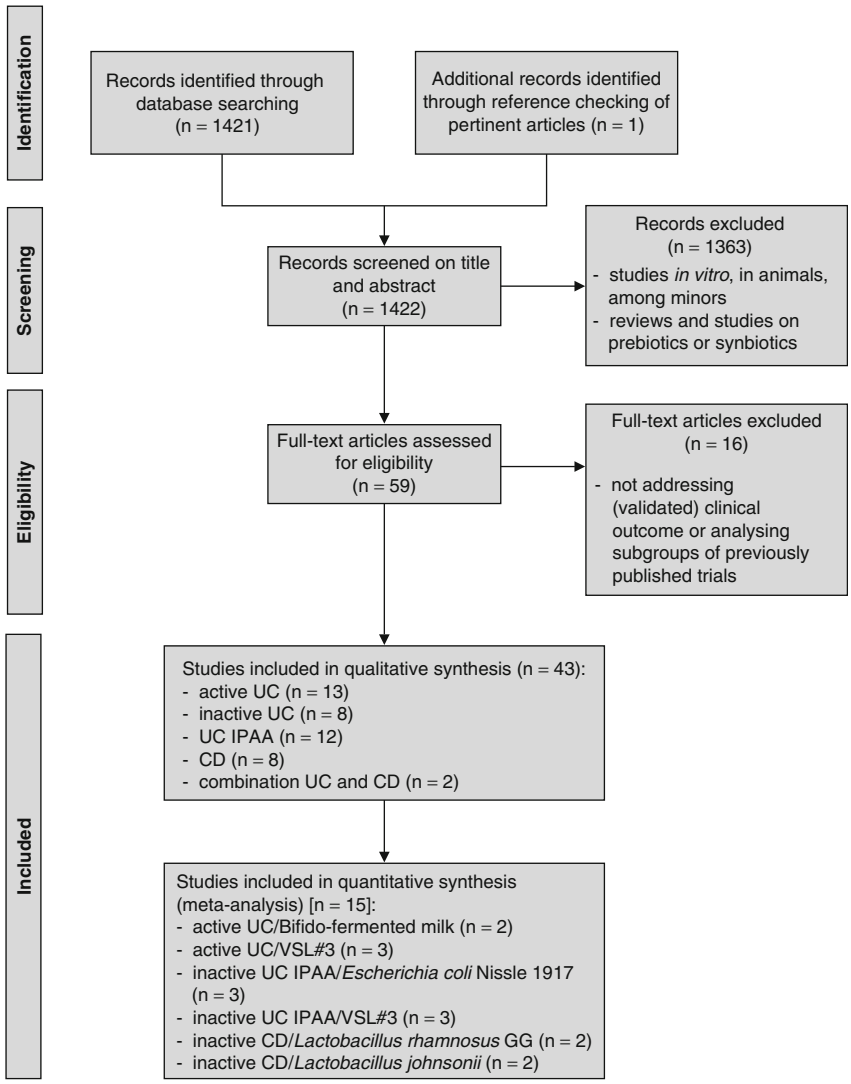

Fig. 1. Study selection process. **CD** = Crohn's disease; **IPAA** = ileo-anal pouch anastomosis; **UC** = ulcerative colitis.

components: presence or absence of a randomized controlled design; double blinding; intention-to-treat (ITT) analysis; and compliance check. Quality indicators not reported were considered to be absent. The information on methodological quality is available as Supplemental Digital Content, available at URL: http://links.adisonline.com/DGZ/A9. Information on probiotic strains used, dose and duration of treatment is included in the subgroup-specific tables for active UC, inactive UC, UC with an ileo-anal pouch and CD patients, respectively (tables I–IV).

4.1 Meta-Analyses

Subgroup-specific meta-analyses were only performed per probiotic, when more than one RCT addressing remission or relapse rate were available for a specific patient group. For each of the included studies, RRs and their 95% con-

Table I. Probiotic intervention studies in adult ulcerative colitis patients with active disease

Study, y of publication	Number of pts, disease activity	Design, duration	Intervention (daily dose[a])	Clinical outcome
Guslandi et al.,[71] 2003	25, mild to moderately active	Uncontrolled, 4 wk	*Saccharomyces boulardii* (750 mg) + mesalazine (3 g)	68% in remission (ITT) Decreased clinical activity ($p < 0.05$)
Ishikawa et al.,[72] 2003	21, mild to moderately active	RCT vs no additive tx, 12 mo	Bifido-fermented milk [*Bifidobacterium breve*, *Bifidobacterium bifidum* and *Lactobacillus acidophilus*] (10×10^9) vs no additive tx	Relapse rate: 27% vs 90% (ITT $p = 0.0075$) No differences in colonoscopic findings
Kato et al.,[73] 2004	20, moderately active	RCT vs PL, 12 wk	Bifido-fermented milk [*B. breve*, *B. bifidum* and *L. acidophilus*] (10×10^9) vs PL	40% vs 33% in remission (PP) Decreased clinical activity probiotic vs PL ($p < 0.05$) Decreased endoscopic/histological scores within probiotics group ($p < 0.01$)
Tursi et al.,[74] 2004	90, moderately active	RCT vs standard tx, 8 wk	VSL#3 (9×10^{11}) + balsalazide (2.25 g) vs balsalazide (4.5 g) vs mesalazine (2.4 g)	80% vs 77% vs 53.3% in remission (ITT $p < 0.02$) Faster remission induction: 4 vs 7.5 vs 13 d ($p < 0.01$)
Bibiloni et al.,[75] 2005	34, moderately active	Uncontrolled, 6 wk	VSL#3 (3.6×10^{12})	53% entered remission (ITT) 77% decreased ≥3 points in clinical activity index (ITT)
Tsuda et al.,[76] 2007	20, mild to moderately refractory active	Uncontrolled, 4 wk	BIO-THREE (*Streptococcus faecalis* 18 mg, *Clostridium butyricum* 90 mg, *Bacillus mesentericus* 90 mg) [n = 10: also 100 g dietary fibre daily]	45% in remission (ITT)
Soo et al.,[77] 2008	15, UC pts (active + inactive)	Uncontrolled, 5 wk	VSL#3 (1.8×10^{12})	Decrease in clinical disease activity (ITT $p = 0.02$)
Takeda et al.,[78] 2009	14, active	Uncontrolled, 24 wk	*Bifidobacterium longum* Bb536 ($2–3 \times 10^{11}$)	67% reached remission (ITT)
Sood et al.,[79] 2009	147, mild to moderately active	RCT vs PL, 12 wk	VSL#3 (3.6×10^{12}) vs PL	≥50% improved disease activity at wk 6 in 32.5% vs 10% (ITT $p = 0.001$) Remission at wk 12: 42.9% vs 15.7% (ITT $p < 0.001$)
Matthes et al.,[80] 2010	90, mild to moderately active	RCT vs PL, 8 wk	*Escherichia coli* Nissle 1917 enema (4×10^9) vs (2×10^9) vs (10^9) vs PL	Remission rates: 43.5% vs 47.8% vs 36.4% vs 35.0% (ITT $p = 0.4430$) 52.9% vs 44.4% vs 27.3% vs 18.2% (PP $p = 0.0446$)
Tursi et al.,[81] 2010	144, mild to moderately active	RCT vs PL, 8 wk	VSL#3 (3.6×10^{12}) vs PL	More subjects with ≥50% improvement in disease activity (PP $p = 0.010$, ITT $p = 0.031$) Remission rates 47.7% vs 32.4% (PP $p = 0.069$; ITT $p = 0.132$)
Nagasaki et al.,[82] 2010	1, active (unresponsive + severe infections)	Case report	*Bifidobacterium* spp. (6 mg), 1 wk	Improved physical condition/colonoscopic score

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Table I. Contd

Study, y of publication	Number of pts, disease activity	Design, duration	Intervention (daily dose[a])	Clinical outcome
D'Inca et al.,[83] 2011	26, mild active	RCT vs standard tx, 8 wk	5-ASA (2.4 g) vs 5-ASA + *Lactobacillus casei* (1.6×10^9) orally vs 5-ASA + *L. casei* (1.6×10^9) rectally	Improved clinical activity in 5-ASA group (ITT $p = 0.043$) Improved histology in both *L. casei* groups (ITT $p < 0.05$)

a Daily dose provided in CFU unless stated otherwise.

5-ASA = 5-aminosalicylic acid; **CFU** = colony-forming units; **ITT** = intention-to-treat analysis; **PL** = placebo; **PP** = per protocol analysis; **pts** = patients; **RCT** = randomized controlled trial; **tx** = treatment; **UC** = ulcerative colitis.

fidence intervals were calculated based upon the reported rates of relapses or maintenance of remission. Unless stated otherwise, rates of remission or relapses were based on ITT analyses.

To derive a pooled RR (RR_{pooled}) from individual studies, we used a random-effects meta-analysis model. Heterogeneity was quantified with the I-square index, which describes the proportion of total variation in study estimates due to heterogeneity.[112]

Statistical analyses were conducted using the 'metan' command within Stata version 11 (STATA Corp, College Station, TX, USA). The results were displayed as Forest Plots.

5. Results

5.1 Ulcerative Colitis (UC) Patients with Active Disease

Bennet et al.[113] were the first to treat a patient with active UC with antibiotics and a single rectal enema of the faecal microbiota of a healthy donor, and successfully induced remission for at least 6 months. A similar approach was published by Borody et al.[114] Subsequently, several probiotic intervention studies have been performed in active UC, with large variations in probiotic strains used, dose, duration and study designs (table I). Parts of these studies have also been reviewed by others.[115]

A case report[82] and single uncontrolled trials[71,76,78] were not considered sufficient to substantiate a beneficial effect of the probiotics studied.

Two randomized placebo-controlled trials were performed with Bifido-fermented-milk containing *Bifidobacterium breve* strain Yakult, *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in mild to moderately active UC patients.[72,73] Ishikawa et al.[72] found significantly fewer relapses in the probiotic versus the control group without additive treatment after 12 months' treatment (n = 21), as well as a significant decrease of the *Bacteroides vulgatus* – total *Bacteroides* ratio and decrease of butyrate within the probiotic group. No further changes in culture-based microbial analyses and faecal SCFAs were found. Kato et al.[73] found a significant decrease of clinical activity in the probiotic versus the placebo group after 12 weeks' treatment (n = 20), but did not find differences in relapse rate.[73] The RR_{pooled} was 2.70 (95% CI 0.47, 15.33), indicating that, overall, no significant benefit was found for Bifido-fermented milk in inducing remission in active UC patients (figure 2a).

A randomized, placebo-controlled, phase II dose-finding study was performed with *E. coli* Nissle 1917 in 90 patients with mild to moderately active UC.[80] They were the first to administer the probiotics rectally to increase the local action. Clinical remission rates showed a significant dose-dependent higher responder rate than placebo in the per-protocol (PP) but not in the ITT analysis. The latter was probably due to the high number of drop-outs because of protocol violations and discontinuation because of lack of efficacy. The number of side effects did not differ between groups. D'Inca et al.[83] compared orally or rectally administered *Lactobacillus casei* DG in combination with 5-ASA versus 5-ASA alone for 8 weeks in 26 patients with mildly active UC. The histological activity scores improved significantly within both *L. casei*-treated groups. Find-

ings were supported by changes in the microbiota and TLR-expression, but the subgroups were very small, and the clinical activity scored only improved significantly within the 5-ASA group.

The effect of VSL#3 consisting of four strains of *Lactobacillus* (*L. casei*, *L. plantarum*, *L. acidophilus* and *L. delbrueckii* subsp. *bulgaricus*), three strains of *Bifidobacterium* (*B. longum*, *B. breve* and *B. infantis*) and one strain of *Streptococcus* (*S. salivarius* subsp. *thermophilus*), has been investigated in two uncontrolled and three randomized controlled studies. The uncontrolled studies reported clinical remission in 53% of 34 patients with moderately active UC[75] and a significant decrease in clinical activity index[77] in a mixed group of patients with active and inactive UC (n = 15) after treatment with VSL#3. In a randomized, controlled, three-arm study including 90 patients with moderately active UC, Tursi et al.[74] compared the combination of VSL#3 with balsalazide versus balsalazide or mesalazine alone for 8 weeks and found significantly more patients entering clinical remission based on ITT analysis, as well as a faster induction of remission. Two other large, well designed, randomized, placebo-controlled trials found significantly more patients with mild to moderately active UC to reach at least 50% improvement in clinical activity indices after VSL#3 compared with placebo treatment for 12 (n = 147)[79] and 8 weeks (n = 144)[81] based on ITT analyses. In both studies, concomitant medication was continued in a stable dose. In contrast to the findings by Sood et al.,[79] the study by Tursi et al.,[81] which had a relatively high placebo response, could not find significant differences in remission rates. Major side effects did not occur in any of the studies using VSL#3. Further supportive mechanistic data mainly result from animal colitis models, showing an increase of luminal microbial diversity,[116] inhibition of pro-

Table II. Probiotic intervention studies in adult patients with ulcerative colitis in remission

Study, y of publication	Number of pts, disease activity	Design, duration	Intervention (daily dose[a])	Clinical outcome
Kruis et al.,[84] 1997	103, inactive	RCT vs standard tx, 12 wk	*Escherichia coli* Nissle 1917 (50×10^9) vs mesalazine (3×500 mg)	Similar relapse rate: 16.0% vs 11.3% (ITT, NS) 18.8 vs 13.25 (PP, NS)
Rembacken et al.,[85] 1999	83, inactive (after remission induction)	RCT vs standard tx, 12 mo	*E. coli* Nissle 1917 (50×10^9) vs mesalazine (3×400 mg)	Similar relapse rate: 67% vs 73% (ITT, NS)
Venturi et al.,[86] 1999	20, inactive (intolerant/allergic to 5-ASA)	Uncontrolled, 12 mo	VSL#3 (3×10^{12})	75% maintained remission (ITT)
Cui et al.,[87] 2004	30, inactive after inducing remission	RCT vs PL, 8 wk	Bifid triple viable capsule (1.26 g) vs PL	20% vs 93% relapses (ITT $p<0.01$)
Kruis et al.,[88] 2004	327, inactive	RCT vs standard tx, 12 mo	*E. coli* Nissle 1917 ($2–50\times10^9$) vs mesalazine (3×500 mg)	Similar relapse rate 45.1 vs 37.0% (ITT $p=0.013$) 36.4% vs 33.9% (PP $p=0.003$)
Zocco et al.,[89] 2006	187, inactive	RCT vs standard tx, 12 mo	*Lactobacillus rhamnosus* GG (18×10^9) vs mesalazine (2400 mg) vs *L.* GG (18×10^9) + mesalazine (2400 mg)	Similar relapse rate: 15% vs 20% vs 16% (ITT $p=0.77$) No difference in clinical, endoscopic and histological scores
Guslandi,[90] 2010	6, inactive (mesalamine intolerant)	Uncontrolled, 3 mo	*Saccharomyces boulardii* (500 mg) + rifaximin (400 mg)	Maintained remission based on clinical activity
Wildt et al.,[91] 2011	32, inactive	RCT vs PL, 52 wk	*Lactobacillus acidophilus* (La-5) + *Bifidobacterium animalis lactis* [Bb-12] (1.5×10^{11}) vs PL	Maintenance remission 25% vs 8% (ITT $p=0.37$)

a Daily dose provided in CFU unless stated otherwise.

5-ASA = 5-aminosalicylic acid; **CFU** = colony-forming units; **ITT** = intention-to-treat analysis; **NS** = not significant; **PL** = placebo; **PP** = per protocol analysis; **pts** = patients; **RCT** = randomized controlled trial; **tx** = treatment.

Table III. Probiotic intervention studies in adult ulcerative colitis patients with an ileo-anal pouch anastomosis

Study, y of publication	Number of pts, disease activity	Design, duration	Intervention (daily dose[a])	Clinical outcome
Active disease				
Kuzela et al.,[92] 2001	2, active pouchitis	Uncontrolled, 315/56 d	*Escherichia coli* Nissle 1917 ($2.5–5\times10^{10}$)	Both in remission from day 50 and 5, respectively
Gionchetti et al.,[93] 2007	23, mild to active pouchitis	Uncontrolled, 4 wk	VSL#3 (36×10^{11})	69% in remission (ITT) Decreased PDAI (ITT, $p<0.001$)
Inactive disease				
Gionchetti et al.,[94] 2000	40, after induction remission by antibiotics	RCT vs PL, 9 mo	VSL#3 (18×10^{11}) vs PL	Relapse rate 15% vs 100% (ITT $p<0.001$)
Kuisma et al.,[95] 2003	20, with history of pouchitis (subgroup had pouchitis)	RCT vs PL, 3 mo	*Lactobacillus rhamnosus* GG ($2–4\times10^{10}$) vs PL	No change in PDAI scores between groups (ITT)
Gionchetiti et al.,[96] 2003	40, 1 wk after ileostomy closure	RCT vs PL, 12 mo	VSL#3 (9×10^{11}) vs PL	10% vs 40% relapses ($p<0.05$) Increased PDAI in PL ($p<0.001$) but not VSL#3 group
Laake et al.,[97] 2003	10, in remission	Uncontrolled, 4 wk	*Lactobacillus acidophilus* (La-5) + *Bifidobacterium lactis* [Bb-12] (5×10^{10})	Significant change in endoscopic but not histological scores
Laake et al.,[98] 2004	41, in remission	Uncontrolled, 4 wk	*L. acidophilus* (La-5) + *B. lactis* [Bb-12] (both 5×10^{10})	22 completers Increased PDAI but not histological scores (PP $p=0.01$)
Mimura et al.,[99] 2004	36, in remission after 4 wk antibiotics	RCT vs PL, 12 mo	VSL#3 (18×10^{11}) vs PL	85% vs 6% maintained remission (ITT $p<0.0001$)
Gosselink et al.,[100] 2004	117, who underwent IPAA	CT vs historic group without tx, 3 y	*L. rhamnosus* GG (1.4×10^{10}) vs no tx	First pouchitis episode 7% vs 29% ($p=0.011$)
Laake et al.,[101] 2005	51, in remission (subgroup had pouchitis)	Uncontrolled, 4 wk	*L. acidophilus* (La-5) + *B. lactis* [Bb-12] (both 5×10^{10}) [n = 11 used dietary fibre preparation]	Improved endoscopic PDAI ($p=0.0001$) No improvement in symptoms (NS)
Shen et al.,[102] 2005	31, after induction remission by antibiotics (clinical setting)	Uncontrolled, 8 mo	VSL#3 (18×10^{11})	25 stopped, 6 completers Improved clinical PDAI (PP $p<0.001$) but not in endoscopic PDAI
Pronio et al.,[103] 2008	31, in remission	RCT vs no tx, 12 mo	VSL#3 (9×10^{11}) or no tx	28 completed the study Decreased PDAI within VSL#3 group (PP $p<0.01$)

a Daily dose provided in CFU unless stated otherwise.

CFU = colony-forming units; **IPAA** = ileo-anal pouch anastomosis; **ITT** = intention-to-treat analysis; **NS** = not significant; **PDAI** = Pouch Disease Activity Index; **PL** = placebo; **PP** = per protocol analysis; **pts** = patients; **(R)CT** = (randomized) controlled trial; **tx** = treatment.

inflammatory cytokines, induction of IL-10 and regulatory CD4+ T cells,[117] as well as an increased expression of tight junction proteins and a decrease of apoptosis[118] after treatment with VSL#3.

A meta-analysis was performed including the two studies comparing VSL#3 versus placebo[79,81] and the one by Tursi et al.[74] comparing the combination of VSL#3 with balsalazide versus mesalazine. The subsequent RR_{pooled} was 1.69 (95% CI 1.17, 2.43), indicating a significant benefit of VSL#3 over control in inducing remission in patients with active UC (figure 2b). However, I^2 indicates a moderate heterogeneity between the studies. The study by Tursi et al.[74] compared the combination of VSL#3 with balsalazide versus standard maintenance therapy, while the others compared VSL#3 versus placebo. After exclusion of the study by Tursi et al.,[74] a similar trend was found, but the RR_{pooled} was not found to be significant (1.88, 95% CI 0.96, 3.67).

5.2 UC Patients with Inactive Disease

Eight studies were identified that investigated probiotics in patients with inactive UC (table II). Two of them comprised single uncontrolled studies with *Saccharomyces boulardii*[90] and VSL#3,[86] respectively, and no conclusions can be drawn from them.

Three large, randomized, controlled, double-dummy trials compared *E. coli* Nissle 1917 with mesalazine (1200–1500 mg). They found similar relapse rates in both groups after 12 weeks[84] or 12 months[85,88] of intervention, based on ITT analyses. Furthermore, no differences were observed in side effects between the groups. The authors concluded that the *E. coli* Nissle was equally effective as mesalazine for maintaining remission. The findings were supported by animal studies, in which *E. coli* Nissle did result in a significant improvement of acute dextran-sulfate sodium-induced colitis. This was paralleled by a decrease of pro-inflammatory cytokines, in

Table IV. Probiotic intervention studies in adult patients with Crohn's disease

Study, y of publication	Numbers of pts, disease activity, disease location	Design, duration	Intervention (daily dose[a])	Outcome
Active disease				
Malchow,[104] 1997	23, active, all colon	RCT vs PL, 12 mo	Prednisolon + *Escherichia coli* Nissle 1917 (5×10^{10}) vs prednisolon + PL	75% vs 91.7% entered remission (ITT NS) 33.3% vs 63.6% relapse rate (ITT NS)
Matilla-Sandholm et al.,[105] 1999	20, active	Uncontrolled, 10 d	*Lactobacillus salivarius* UCC35624	No remission
Doman et al.,[106] 2008	3, active, disease locations unknown	Uncontrolled (case reports), 7–12 mo	*Lactobacillus acidophilus, Bifidobacterium, Lactobacillus casei* + *Streptococcus salivarius* subsp. *thermophilus* (8×10^{9})	Maintained remission but 1 wk abdominal pain (after 7 and 8 mo) in 2 of 3 pts
Inactive disease				
Guslandi et al.,[107] 2000	32, inactive, 9 ileum, 1 colon, 23 ileum + colon	RCT vs standard therapy, 6 mo	*Saccharomyces boulardii* (1 g) + mesalazine (2 g) vs mesalazine (3 g)	Relapse rate 6.25% vs 37.5% (ITT p = 0.04)
Prantera et al.,[108] 2002	45 inactive (10 d after curative resection), 35 ileum, 3 colon, 7 ileum + colon	RCT vs PL, 12 mo	*Lactobacillus rhamnosus* GG (12×10^{9}) vs PL	Clinical relapse in 16.6% vs 10.5% (PP p = 0.948) 60% vs 35.3% of those in remission had endoscopic recurrence (PP p = 0.297)
Marteau et al.,[109] 2006	98, after curative resection, 54 ileum, 4 colon, 40 ileum + colon	RCT vs PL, 6 mo	*Lactobacillus johnsonii* LA1 (4×10^{9}) vs PL	90 pts evaluated Similar recurrence rate: 49% vs 64% (ITT p = 0.15) No differences in endoscopic scores
Schultz et al.,[110] 2004	9, entering remission after steroids + antibiotics, disease location unknown	RCT vs PL, 6 mo	*L. rhamnosus* GG (2×10^{9}) vs PL	2 of 4 vs 3 of 5 relapsed (ITT)
Van Gossum et al.,[111] 2007	70, after curative ileo-caecal resection	RCT vs PL, 12 wk	*L. johnsonii* (10^{10}) vs PL	Endoscopic recurrence rate is 71 vs 63% (PP) No differences in endoscopic score or recurrence rate (ITT and PP)

a Daily dose provided in CFU unless stated otherwise.

CFU = colony-forming units; **ITT** = intention-to-treat analysis; **NR** = not reported; **NS** = not significant; **PP** = per protocol analysis; **RCT** = randomized controlled trial.

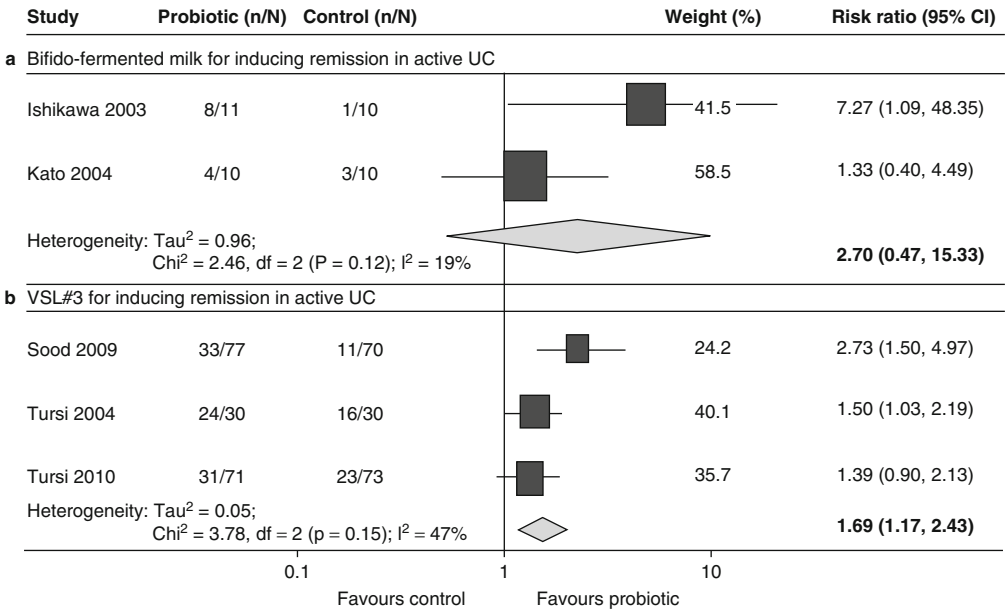

Fig. 2. Probiotics for inducing remission in active ulcerative colitis. Forest plot for comparison of (**a**) Bifido-fermented milk compared with placebo or no treatment and (**b**) VSL#3 compared with placebo in patients with (mild to moderately) active ulcerative colitis. Risk ratio > 1 indicates an increased risk of inducing remission based on intention-to-treat analyses. Ishikawa et al.,[72] Kato et al.,[73] Sood et al.,[79] Tursi et al.[74,81] **UC** = ulcerative colitis.

part mediated by TLRs[119,120] and by a regulation of the tight junction protein ZO-1 with a reduction of the intestinal permeability.[67] The RR_{pooled} was 1.08 (95% CI 0.86, 1.37), indicating that *E. coli* Nissle was not statistically significantly inferior to mesalazine in preventing relapses (i.e. or maintaining remission) [figure 3a].

In a large RCT including 187 patients,[89] *Lactobacillus rhamnosus* strain GG with or without mesalazine resulted in relapse rates and clinical, endoscopic and histological scores similar to those of mesalazine only. They did not have dropouts, nor did severe side effects occur. Possible mechanisms of actions were not assessed. In contrast, in a murine DSS model *L. rhamnosus* GG was found to aggregate colitis.[121]

Two placebo-controlled studies were published.[87,91] Cui et al.[87] studied 30 patients with inactive UC who were randomized to receive the multispecies probiotic 'bifido triple viable capsule' or placebo for 8 weeks. Significantly fewer relapses were found in the probiotic versus the placebo group, together with changes in the composition of the faecal microbiota, inhibition of mucosal nuclear factor (NF)-κB activation, decreases of TNFα and IL-1β and an increase of IL-10 tissue mRNA levels.[87] The beneficial effect in this single small study should be confirmed in large RCTs and is hampered by missing information on bacterial strains and numbers administered.

Wildt et al.[91] performed a randomized placebo-controlled trial comparing UC patients in remission treated with the multispecies Probio-Tec Ab 25 (containing *L. acidophilus* La-5 plus *Bifidobacterium animalis* subsp. *lactis* Bb-12) [n = 20] versus placebo (n = 12) for 52 weeks, but found no differences in maintenance of remission or in median time to relapses between both groups.

5.3 UC Patients with an Ileo-Anal Pouch Anastomosis

Twelve studies were identified that investigated the application of probiotics in UC patients with an IPAA (table III). IPAA is the restorative procedure of choice in UC patients who require

a surgical resection for therapy-refractory or corticosteroid-dependent disease. Pouchitis is the most frequent complication following pouch surgery. It is characterized by symptoms such as increased stool frequency and liquidity, abdominal cramping, urgency, tenesmus and occasionally rectal bleeding or fever and is associated with inflammation of the pouch mucosa. From a large study performed at the Mayo Clinic, the cumulative risk for the first episode of pouchitis at 1, 5 and 10 years following IPAA was found to be 15%, 36% and 46%, respectively.[122] Pouchitis is generally scored by the validated Pouchitis Disease Activity Index (PDAI), containing clinical, endoscopic and histological criteria.[123] The exact aetiology of pouchitis is not clear, but faecal stasis, mucosal ischaemia, bacterial dysbiosis and a disturbed immune response are considered to be involved.[124-126]

Although some studies included mixed patient groups, only two studies focused on inducing remission in patients with active pouchitis. However, both suffered from major methodological limitations, being a case report[92] and an uncontrolled trial.[93]

The most convincing evidence has been found for the multispecies probiotic VSL#3 in maintaining remission in IPAA patients. Three randomized placebo-controlled studies were performed, two by Giochetti and colleagues[94,96] and one by Mimura et al.[99] ITT analyses revealed significantly lower relapse rates after 9 or 12 months' intervention in UC patients with a pouch, either after inducing remission by antibiotics (n = 40 and n = 36)[94,99] or starting 1 week after ileostomy closure (n = 40).[96] No side effects were reported.

In all above-mentioned VSL#3 studies, the authors demonstrated increased faecal numbers of *S. salivarius*, lactobacilli and bifidobacteria, pointing to survival of the probiotic strains. Kühbacher et al.[127] performed molecular microbial analyses in a subgroup of the patients studied by Mimura et al.[99] They found a signif-

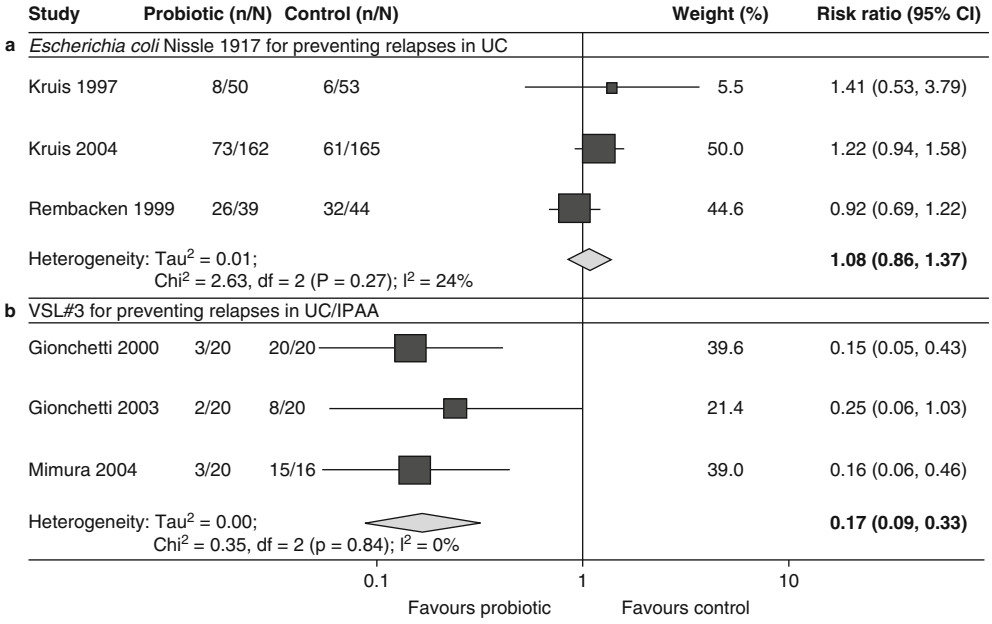

Fig. 3. Probiotics for preventing relapses in inactive ulcerative colitis/ileo-anal pouch anastomosis. Forest plot for comparison of (**a**) *Escherichia coli* Nissle 1917 compared with mesalazine in patients with inactive ulcerative colitis and for (**b**) VSL#3 compared with placebo in patients with inactive ulcerative colitis with an ileo-anal pouch anastomosis. Risk ratio > 1 indicates an increased risk of having a relapse based on intention-to-treat analyses. Kruis et al.,[84,88] Rembacken et al.,[85] Gionchetti et al.,[94,96] Mimura et al.[99] **IPAA** = ileo-anal pouch anastomosis; **UC** = ulcerative colitis.

icant increase of microbial diversity and total bacterial numbers, and a decrease of fungal diversity in biopsy samples of nine pouch patients in remission treated with VSL#3 for 12 months. Further mechanistic support was published by Ulisse et al.[128] and Lammers et al.,[129] who analysed a subgroup of patients from the study by Gionchetti et al.[96] A decrease of TNFα, IL-1α, interferon (IFN)-γ cytokine levels, inducible nitric oxide synthase and matrix metalloproteinase activity and an increase of IL-10 was found in biopsy samples of seven inactive pouch patients treated with VSL#3 for 9 months.[128] A significant decrease was also demonstrated for mRNA levels of IL-1β, IL-8 and IFNγ, as well as of the numbers of polymorphnuclear cells in tissue samples of eight VSL#3- compared with eight placebo-treated patients.[129] Finally, Pronio et al.[103] randomized another 31 pouch patients in remission to receive VSL#3 or no treatment for 12 months. They found a significant decrease in PDAI scores within the probiotic but not in the control group, paralleled by a significant decrease of tissue IL-1β mRNA, increase of tissue FOXP3 mRNA and an increased percentage of mucosal regulatory T cells in the probiotic versus the placebo group.

The three randomized placebo-controlled studies[94,96,99] were included in the meta-analysis, revealing a RR_{pooled} of 0.17 (95% CI 0.09, 0.33) [figure 3b]. Thereby, VSL#3 was found to be significantly better than placebo at preventing relapses in UC patients with an IPAA.

The promising results could not be confirmed in an uncontrolled clinical practice setting. Shen et al.[102] treated 31 antibacterial-dependent pouchitis patients with a 2-week antibacterial course to induce remission, and subsequently gave them VSL#3 for 8 months. They could not find a beneficial effect, and 25 of 31 patients stopped the intervention because of relapses or adverse symptoms.

Another multispecies probiotic, containing *L. acidophilus* (La-5) and *B. animalis* subsp. *lactis* (Bb-12), was studied by Laake and colleagues[97,98,101] and did result in a significant improvement in endoscopic and clinical scores in pouch patients in remission. However, all studies were uncontrolled and were further hampered by use of medication for bowel symptoms, use of dietary fibre and/or incomplete data in subgroups of patients.

Finally, two studies were published on *L. rhamnosus* GG with inconsistent results. A total of 20 inactive pouch patients with a history of recurrent pouchitis were randomized to receive *L. rhamnosus* GG or placebo for 3 months, but no significant effect was found on the PDAI.[95] Gosselink et al.[100] included 117 pouch patients of whom 39 received *L. rhamnosus* GG daily starting after IPAA surgery, and compared them with a historic 'untreated' control group of 78 patients. They found the first episode of pouchitis to be less frequent in the *L. rhamnosus* GG-treated versus the historic control group after a 3-year follow-up period. However, it has to be taken into account that a parallel placebo group was lacking and that surgical and medical interventions might have improved over time.

5.4 Crohn's Disease (CD) Patients

Only three small studies have been performed in patients with active CD (table IV), including an uncontrolled trial with *L. salivarius*[130] and a case report on three CD patients receiving a multispecies probiotic while on maintenance therapy.[106] The only placebo-controlled trial, conducted by Malchow,[104] reported a lower relapse rate after 12 months of follow-up when *E. coli* Nissle 1917 was added to prednisolon therapy.

Five studies were performed in inactive CD patients (table IV). Guslandi et al.[107] studied the effect of *S. boulardii* in combination with mesalazine versus mesalazine only in 32 patients with inactive CD and found a significantly lower relapse rate for the combination. They suggested that *S. boulardii* may contribute to this beneficial effect by its trophic effect on the intestinal mucosa and increased release of secretory IgA. Furthermore, *S. boulardii* has been found to improve the intestinal permeability in CD patients.[131,132] Although no side effects were reported, the beneficial effect of this single study should be confirmed in large controlled clinical trials before advice on routine use can be given.

L. rhamnosus GG was studied in two placebo-controlled trials that included 45 and 11 patients

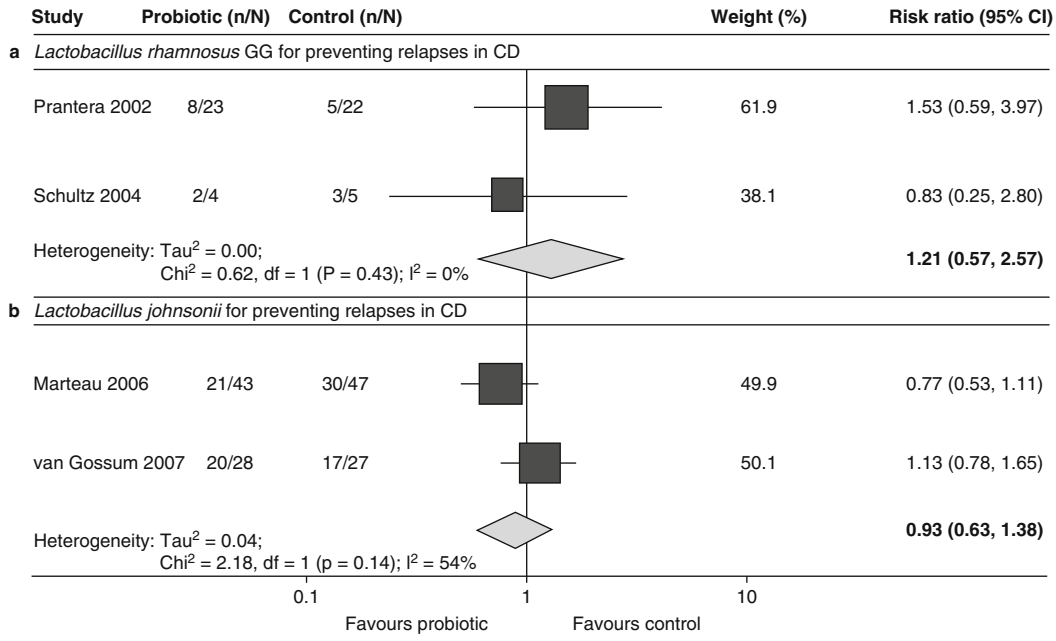

Fig. 4. Probiotics for preventing relapses in inactive Crohn's disease. Forest plot for comparison of (**a**) *L. rhamnosus* GG versus placebo and of (**b**) *L. johnsonii* compared with placebo in patients with inactive Crohn's disease. Risk ratio > 1 indicates an increased risk of having a relapse based on the endoscopic recurrence (per protocol analyses). Prantera et al.,[108] Schultz et al.,[110] Marteau et al.,[109] Van Gossum et al.[111] **CD** = Crohn's disease.

with inactive CD, either directly after curative resection or after induction of remission by steroids and antibiotics, respectively. Both suffered from rather high drop-out rates and did not find a significant benefit of the probiotic over placebo.[108,110] The effect of *Lactobacillus johnsonii* was assessed in two placebo-controlled trials that included 98 and 70 CD patients after curative resection. Neither study found differences in recurrence rates or endoscopic scores.[109,111] Furthermore, in the study by Van Gossum et al.,[111] the drop-out rate was rather high (21 out of 70). The RR_{pooled} for *L. rhamnosus* GG (1.21, 95% CI 0.57, 2.57) and *L. johnsonii* (0.93, 95% CI 0.63, 1.38) did confirm the lack of benefit of probiotic over placebo treatment in preventing relapses in patients with inactive CD (figure 4).

The majority of probiotic intervention studies in inactive CD did include patients with both ileal and/or colonic predominant disease and none of them performed analyses per disease location. The overall lack of positive results in CD may (in part) be caused by inter-individual differences between disease locations (e.g. ileum vs colon) as well as by differences at the genetic level (e.g. polymorphisms in *NOD2*/*CARD15*).

6. Summary and Conclusion

Mounting evidence suggests an important role for the intestinal microbiota in the chronic mucosal inflammation in IBD, and novel molecular approaches have further identified a microbial dysbiosis in IBD patients. Several mechanisms of action of probiotic products have been postulated, which may interfere with aetiological factors in IBD. Therefore, the interest of physicians and researchers in the application of probiotics in IBD is increasing. Since several probiotic products are commercially available and marketed as safe and beneficial for a wide variety of gastrointestinal diseases, the usefulness of probiotics is also a frequently asked question in the consultation room of IBD clinics.

Based on the current systematic review, level 1 evidence based on well designed randomized clinical trials and supporting the application of probiotics in the management of IBD is still limited. Studies are often not placebo controlled, are based on small numbers and suffer from high numbers of drop-outs. The latter stresses the importance of ITT analyses. Furthermore, different probiotic strains, combination of strains and dosages have been tested. As different strains are known to evoke different responses, it is generally acknowledged that results cannot be generalized. Therefore, meta-analyses were performed per probiotic applied to a specific patient group and could only be based on two or three studies per application.

E. coli Nissle 1917 was found to be comparable to standard treatment in preventing relapses in inactive UC (RR_{pooled} 1.08, 95% CI 0.86, 1.37). However, daily dosages of mesalazine were rather low (1200–1500 mg) and results should therefore be interpreted with care. Most convincing evidence was found for VSL#3 in inducing remission in patients with mild to moderately active UC (RR_{pooled} 1.69, 95% CI 1.17, 2.43) and for preventing relapses in inactive UC patients with an ileo-anal pouch (RR_{pooled} 0.17, 95% CI 0.09, 0.33). The findings were supported by mechanistic data from animal colitis models and pouch patients. However, moderate heterogeneity was found for the studies in active UC and the RR_{pooled} was not significant (1.88, 95% CI 0.96, 3.67) when one study with an active comparator instead of placebo was left out. The majority of studies with VSL#3 were performed by the same research group. Surprisingly, the efficacy of VSL#3 in pouch patients could not be confirmed in a clinical setting. This indicates that additional confirmation of the findings by independent groups, as well as the investigation of factors interfering with implementation in clinical practice, is warranted.

In CD, there is not sufficient evidence to support the use in daily clinical practice of any of the probiotics tested. As differences are reported in microbiota composition, defective immune responses (e.g. human β-defensin expression) and genetic susceptibility in CD patients with ileum versus colon involvement, these disease phenotypes should be studied separately and require a tailored probiotic selection.

Until now, most definitions of probiotics are based on 'live micro-organisms'.[133,134] However, evidence is growing that non-viable strains, secreted metabolites or even DNA-moieties of probiotic bacteria can also exert a beneficial effect.[62] Probably, the mechanism of action of a particular strain in a specific host situation will determine whether a strain has to be viable. Although clear information on minimal efficacious (viable) dosages are not available, at least 10^{8-10} colony-forming units per day are generally recommended.[58] Differences in dosages used further hamper a comparison between studies, and dose-effect studies are limited. Furthermore, promising *in vitro* data will not necessarily lead to positive results in clinical trials. For an optimal effect, the probiotic stains have to be able to adapt to the host environment as well as express the desired health-promoting effects. The number of bacteria consumed, as well as matrix of the product and the bacterial growth phase, will also affect their efficacy. For an adequate selection of appropriate probiotic strains or mixtures of strains, further mechanistic insight on the probiotic-host interaction is warranted for each application.

Probiotics are considered to be safe because of their long history of use in food fermentation and a low overall risk of infections due to lactic acid bacteria.[135,136] In the studies applying probiotics in IBD, no major side effects occurred. Most of the studies allowed concomitant medication in a stable dose, which is inevitable due to ethical reasons. However, one has to be careful extrapolating safety findings of probiotics as adjunctive therapy, especially when combined with immunosuppressive agents or biologicals. A few case reports have been published on *Lactobacillus* spp. causing hepatic abscess and sepsis in IBD patients.[137,138] Taking into account the increased mortality in a recent pancreatitis trial after intraduodenal administration of a multispecies probiotic,[139] and the limited safety data in immune-compromised individuals, care must be taken when using probiotics in severely ill sub-

jects, especially in those with an impaired intestinal barrier function such as patients with moderate to severe IBD.

In conclusion, further well designed studies based on ITT analyses by several independent research groups are still warranted to support the promising results for *E. coli* Nissle in inactive UC and for the multispecies product VSL#3 in active UC and inactive pouch patients. So far, no evidence is available yet to justify the use of probiotics in CD. Future studies should be designed carefully and should focus on specific disease subtypes and disease location. Further insight into the aetiology of IBD and the biological effects of probiotic strains will aid in tailored probiotic selection.

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Correspondence: Dr *D. Jonkers*, PhD, Division of Gastroenterology-Hepatology, Maastricht University Medical Centre, PO Box 5800, 6202 AZ Maastricht, the Netherlands. E-mail: d.jonkers@maastrichtuniversity.nl