Do probiotic preparations for humans really have efficacy?

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Microbial ecology studies have provided convincing evidence for the crosstalk between members of the autochthonous microbiota and the host immune system. The tight interrelationship between microbiota and host mucosal cells are mediated by microbial metabolites that are responsible for bacterial cell-to-cell communication by quorum-sensing mechanisms and also through the activation of eukaryotic cells following secretion of host defensins and modulation of cytokine expression profiles. All these host functions can be positively influenced by probiotic bacteria of human origin. However, few requirements for evaluating these strains for use in humans have been set according to their composition and metabolic activity. In this article, we have reported the scientific data published to date on the advantages of either mono- or multispecies probiotic products based on the outcome of the most significant clinical trials. According to published clinical trials, the efficacy of probiotic intervention for infectious or antimicrobial treatment-induced diarrhea, caused by different opportunistic bacterial or viral pathogens, was 48%. The probiotic preparations’ efficacy for silencing the clinical symptoms of IBS was 75% and for attenuating the inflammatory response during IBD was 83% whereas multistrain probiotics appear to have better efficacy.

Keywords: mono-strain probiotics; multistrain probiotics; multispecies probiotics; intestinal microbial ecology; infectious diarrhea; antibiotic treatment; Helicobacter pylori; Clostridium difficile infections; inflammatory bowel disease; irritable bowel syndrome

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At the turn of the last century, a probiotic preparation was defined as ‘a live non-pathogenic microbial feed or food supplement that beneficially affects the host by improving the intestinal microbial balance’ (1, 2). Later, according to the 2002 report of the Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food (ftp://ftp.fao.org/docrep/fao/009/a0512e/a0512e00.pdf), a new definition of a probiotic was adopted that stated ‘a probiotic is a viable microbial food component which has a demonstrated benefit on human health when given in specific amounts’ (3).

A mono-strain probiotic is defined as a product containing one strain of a well-defined microbial species, whereas multistrain probiotics contain more than one strain of the same species or genus. However, the term multispecies probiotic is often used for products containing microbial strains belonging to one or more genera as well (4).

Evaluation of probiotic efficacy

Currently, a probiotic product is a strain-specific preparation targeting different human metabolic functions to improve health by either supporting host physiologic activity or by reducing the risk of disease. It has been
generally accepted that the probiotic potential of different strains of the same species may have different probiotic effects (5–7). The project ‘Process for the Assessment of Scientific Support for Claims on Foods,’ (8, 9) several years ago proposed scientific tools for substantiating health claims made for probiotic products. Specifically, human clinical trial data based on objective measurements and defined probiotic culture characteristics needed to be evaluated to support a specific claim.

Today, there are emerging technologies for the discovery and measurement of the efficacy of a probiotic preparation based on changes in specific biomarkers. In February 2009, the International Life Sciences Institute Europe workshop, ‘Emerging Technologies for Efficacy Demonstration Task Force’ reviewed the advancements of these new technologies and discussed their role in food product development and in substantiating claims by relying on measurements of functional biomarkers of the host (http://www.ilsi.org/Europe/Pages).

In contrast, the European Food Safety Authority (EFSA) requirements for authorization of a specific health claim (Regulation (EC) No. 1924/2006) have considered appropriate human clinical trials and experimental interventions (randomized control trials (RCT); non-controlled RTs), quasi-experimental interventions (non-randomized either controlled or not), observational (cohort, case control, cross-sectional) and other studies (case reports). Additional studies using experimental animal models dealing with mechanisms by which the probiotic might be responsible for the claimed effect in a causal relationship were also proposed. Furthermore, ex vivo or in vitro studies based on either human or animal samples (case description) were also suggested as useful for these evaluations.

The confirmation of many of these effects on host physiologic function has been documented in a number of clinical trials in healthy volunteers or patients with specific diseases, despite the fact that the underlying causal relationships have yet to be established (10–12).

Selection of probiotics on the basis of the present knowledge on intestinal microbial ecology

The intestinal microbiota is a complex and dynamic mixture of microbes consisting of bacteria, archaea, protozoa, fungi, bacteriophages and other viruses. Based on molecular methods, current estimates indicate that intestinal microbiota in aggregate consists of $10^{14}$ viable microbes belonging to over 1,000 species, among which anaerobic bacteria predominate (13–18). There are also a wide variety of host, dietary and environmental factors that affect bacterial colonization of the gastrointestinal (GI) tract.

It has been demonstrated over several decades using culture-based methods that the major groups of fecal bacteria belong to the genera Bacteroides, Eubacterium, Clostridium, Ruminococcus, Fusobacterium, Bifidobacterium and Peptostreptococcus (19, 20). Recent molecular studies have revealed that the gut microbiota is largely dominated in adults by one member of the archaea, Methanobrevibacter smithii (21) and by members of two other bacterial phyla, the Bacteroidetes and the Firmicutes, that include the genera listed above. More specifically, three bacterial groups predominate within these phyla: the Bacteroides-Prevotella group, the Clostridium coccoides group, and the Clostridium leptum group (14).

Although there may be some confusion over the taxonomic designations of specific species, the recent molecular analyses largely confirm the results of previous culture-based research.

Beneficial properties of microbiota

Different biologic functions such as digestion of essential nutrients, maturation of intestinal epithelial cells, and impact on baseline physiologic parameters, including systemic effects on blood lipids, inhibition of harmful bacteria, and stimulation of the immune system have been attributed to the microbiota through careful scientific evaluations over many decades (22, 23). There is substantial evidence that modulation of pro- and anti-inflammatory responses, as revealed by cytokine profiles, is an important mechanism by which probiotics provide health benefits. It has been shown that cell surface molecules of Lactobacillus strains elicit strong tumor necrosis factor alpha-inducing activities in macrophages through Toll-like receptor 2 (24). In addition, the antimicrobial defensins, cathelicidins, eosinophil-derived neurotoxin, and AI-2 signaling compounds of Gram-positive and Gram-negative bacteria play important roles in both intra- and interspecies communication (25, 26).

Most beneficial microbes isolated from human microbiota and proposed as probiotics belong to the group of lactic acid-producing bacteria such as Lactobacillus spp., Bifidobacterium spp., Enterococcus spp. (3, 6) as well as strains of Escherichia coli, Bacillus spp., and Streptococcus spp. Research suggests that the close interrelation between host mucosal epithelial cells and microbiota (6, 27–29) is of utmost importance for good health.

Putative impact on health

In wealthy societies, the stress of living in a highly competitive environment, the increasing number of elderly people, and the concomitant reduction in physical activity and a diet consisting of high levels of fat, carbohydrate, and salt are considered risk factors for the increase in certain chronic diseases such as atherosclerosis, hypertension, type 2 diabetes, peptic ulcer disease, and neurodegenerative disorders. Furthermore, the large amount of disinfectants and antibiotics routinely used by Western societies represent significant risk factors associated with an increased number of humans...
suffering from GI tract-associated health problems. More specifically, during medical interventions that interrupt the balance of human resident microbiota, the sporadic numeric dominance of opportunistic pathogens may lead to translocation of these organisms into lymph nodes and systemic circulation through mucosal membranes of GI, respiratory, and urogenital tracts (30, 31). One example of this phenomenon is the parenteral administration of ceftriaxone to treat upper respiratory tract infections of children, which has been shown to induce a dysbiosis, that is, an imbalance in intestinal microbial flora characterized by a shift in the viable cell density of different bacterial species and a concomitant change in host physiology (32). This shift has a negative impact on host colonization resistance, which can result in the overgrowth of antibiotic-resistant strains of opportunistic pathogens (such as multidrug-resistant E. coli or vancomycin-resistant Enterococcus sp.) (33–35).

To correct the imbalance of microbiota composition caused by the use of antibiotics particularly in critically ill patients, the administration of probiotics has been suggested as a therapeutic intervention. In fact, several probiotic strains are intrinsically resistant to antimicrobials and can be used jointly with specific antibacterial treatments. Lactobacillus rhamnosus GG, a commercially available probiotic, is intrinsically resistant to metronidazole and vancomycin (both MIC >256) often used in the treatment of antibiotic-associated diarrhea and pseudomembranous colitis (36). Lactobacillus fermentum ME-3 (DSM14241) is suggested as a suitable probiotic additive in conjunction with ofloxacin (MIC 8 μg/ml) for the treatment of Salmonella enterica serovar Typhimurium infections (37).

Despite these examples, there is a need for evidence-based documentation supporting probiotic treatment using properly designed clinical trials. An example of the usefulness of this approach was published by Alberda and coworkers (38) who showed that patients receiving a combination of viable probiotic organisms during multiple organ dysfunction syndrome demonstrated greater enhancement in immune function than patients receiving either placebo or a non-viable probiotic formulation. In well-balanced microbial ecosystems, the proportions of anaerobic, microaerobic, and facultatively anaerobic bacteria are tightly linked. Therefore, a putative danger to health may result from a decreased abundance of anaerobes, such as Bacteroides, that are inversely associated with increased fasting glucose and obesity (39). It seems prudent that the consumption of high doses of lactic acid bacteria should be carefully monitored, to avoid the suppression of Bacteroidetes, particularly when given over extended periods of time.

The selection of probiotic Lactobacillus species for different age groups and the age-related shifts of some health indices, such as blood glucose content or the white blood cell (WBC) count, have recently been studied (40, 41). An example of this can be found in elderly subjects, where colonization of the gut with indigenous Lactobacillus reuteri has been shown to be associated with an increased peripheral WBC count. It is possible that in individuals with systemic infections, where the WBC is already elevated as a normal part of the inflammatory process, the introduction of the probiotic species L. reuteri may further provoke the inflammatory process, an additional increase in the WBC, and concomitant increase in the risk of complications for critically ill patients. The peripheral leukocyte count has also been used as a predictor of cardiovascular events and mortality suggesting that this may be a useful biomarker to evaluate as part of probiotic clinical trials (42). The diversity of intestinal Lactobacillus spp. in adults and elderly people is closely associated with shifts in specific metabolic markers. An example of this is the observation that Lactobacillus acidophilus is associated with increased blood glucose level in adults, whereas L. paracasei can decrease blood glucose levels in elderly (43).

**Composition of probiotic products**

The EFSA has set strict regulations regarding the hygienic, nutritional, and taste/smell indices of food/products carrying probiotics used for specific health claims (Regulation (EC) No. 1924/2006). However, this and other regulations usually do not address the compatibility of particular metabolic properties of probiotic strains with their functional efficacy in the host. For most probiotic strains, studies have mainly dealt with their safety and colonization ability but not with the actual efficacy of the probiotic product. Clearly, the effects of different metabolic properties of the probiotic product are not universally expressed in humans after consumption and may depend on different variables that are strain and host specific.

The confirmation of efficacy relies in large part on the reduction of clinical symptoms and maintenance of remission of acute disease as measured by non-objective indices, such as the perceived clinical outcome, for which the placebo effect can be a significant confounding variable. Thus, specific diagnostic biomarkers need to be developed to objectively document the efficacy of probiotic treatments.

**Mono-strain probiotics**

Mono-strain probiotics are defined as probiotics containing one strain of a probiotic species (4, 44). Different species of lactic acid bacteria produce many metabolites with documented antimicrobial effects such as lactic, acetic, and succinic acids. In addition to species differences, major strain-specific differences also exist. Some strains may additionally produce butyric acid, hydrogen peroxide, and bacteriocins that act as functional tools
that could be applied in humans (5). Recently (40, 45), Mikelsaar and co-workers listed some potentially new biomarkers produced by *L. fermentum* ME-3, including glutathione peroxidase and reductase. Among the activities of the soluble molecules produced by probiotic strains is the interference with pathogenic bacterial cell density by a category of soluble molecules, called quorum-sensing inhibitors (QSI). The inhibition process is dependent on the cellular density of the probiotic strain and the accumulation of soluble molecules at a specific concentration. QSI can modulate the expression of different virulence factors, such as antibiotic resistance and biofilm formation, making some pathogenic strains less virulent in a human host. This can be accomplished by inducing changes in the expression of cell surface molecules and consequently, by the shift of the adherence pattern from an aggregative to a diffuse one and the stimulation of bacterial cell endocytosis by epithelial cells, respectively (45).

Different testing systems have been proposed for the selection of monocultures of probiotic products belonging to *Bifidobacterium breve*, *Enterococcus faecium*, *L. casei*, *L. paracasei*, *L. rhamnosus*, *L. plantarum*, *L. fermentum*, *L. reuteri*, *Lactococcus lactis* of human origin with antimicrobial and immunomodulatory activity against pathogenic bacterial strains based on strain characteristics and intended use (46, 47).

For probiotic candidates, it is important to assess *in vitro* the presence of well-identified active compounds such as SCFAs, antimicrobial substances such as rhamnose, or other newly identified compounds (antioxidants, glutathione peroxidase and reductase, NO, polyamines, diacetyl, etc.) (40). Second, these compounds may also be produced as the result of the industrial fermentation or metabolized end products (*ex vitro*) also included as part of the probiotic products such as during the long ripening of cheese manufactured with probiotic bacteria (48).

**Multistrain probiotics**

Multistrain probiotics contain more than one strain of the same species or closely related species. Timmermann and co-authors (44) differentiated multispecies probiotics that contain strains of different probiotic species that belong to one or more genera. We keep the multistrain definition open for both variants as basically both describe a product with a composition that includes several strains of bacteria.

On the one hand, there may be antagonistic relationships between combinations of strains, if some strains of the probiotic preparation include *Lactobacillus* spp. that include subclass IIB plantaricin genes that suppress the growth of other species of lactobacilli (49). On the other hand, preliminary results demonstrate that some combinations of different bacterial species, due to increased concentrations of QS molecules, exhibit an increased probiotic potential, resulting in interference with pathogen growth and expression of virulence and antibiotic resistance markers in a synergistic manner (50, 51). Cocultivation studies for the development of multistrain probiotics should evaluate:

1) the optimal associations showing the absence of cross-antagonism between species;
2) the probiotic effect of selected probiotic combinations on specific pathogens *in vitro* and on different human cell lines;
3) the influence of combinations of probiotic strains on biofilm development;
4) immuno-modulatory activity, including the cytokine profile induced in cultured epithelial and immune cells by different strains separately and in combination;
5) the cytotoxicity of combined probiotic culture fractions on different human cell lines; and
6) the influence of combined microbial culture fractions on the expression of different soluble virulence factors and resistance markers of pathogens.

**Assessment of efficacy of mono- or multispecies products**

Under ideal conditions, different mono or multispecies probiotics should be characterized using strain or combination-specific metabolic properties. In the prevention of disease or during supportive treatment of various disorders and improvement of metabolic stress, the rationale for the choice of a particular mono-strain probiotic or multistrain probiotic combination should be described in peer-reviewed clinical trial studies. Unfortunately, there are no regulatory requirements defining the optimal number of viable organisms in a probiotic product required for use or the daily dose (single species or multispecies in different combinations) that is necessary for the achievement of documented evidence-based health effects for specific diseases (51).

During the early 1990s, a group of probiotic experts concluded that the optimal prophylactic probiotic culture is a mixed one; different strains can be targeted toward different symptoms and blended into a single preparation (52). Their hypothesis that multiple probiotic strains may provide a more effective therapy than a single strain was supported by both *in vitro* and *in vivo* studies.

Two mono-strain *Bifidobacterium longum* (BB536) and *Lactobacillus johnsonii* (La1) probiotics in a mixture were perioperatively administered to colorectal cancer patients. The evaluated strains differed in their functional properties: La1 and not BB536 adhered to colonic mucosa and affected the intestinal pathogens (53). These results suggested that a more strict evaluation of the role of single components of multispecies probiotic needed to be...
performed depending on the application for which it is intended.

To date, there are several probiotic products composed of multiple species of lactobacilli with diverse functional properties that are documented by in vitro and animal experiments that when used during clinical trials may meet the standards for health claims acceptable for EFSA (Regulation No. 1924/2004). There are also several examples of concordance between the metabolic properties of a single probiotic strain and the specific effects on human health.

**Design of clinical trials**

FAO has set specific criteria for conducting safety studies for drugs that include the use of the healthy volunteers to establish product safety, followed by a second phase study to document efficacy through clinical trials with patients using a randomized double-blinded placebo-controlled (DBPC) approach. The evaluation of the impact of probiotics needs to meet the same criteria also using objective measurements to document clinical safety and efficacy according to the claimed metabolic and functional properties of the specific probiotic strain.

Perhaps the most difficult challenge for research on probiotic products is providing proof of efficacy in overtly healthy individuals (Fig. 1). In theory, this can be accomplished by relying on data from biomarkers (morphological and/or physiological measurements of organ function) of the individual in response to probiotic stimuli. Regardless of the subjective criteria according to which a subject feels healthy, which are skewed by the placebo effect, objective measurements are needed that are able to reveal possible changes in cellular or biochemical parameters from pretreatment levels. It is reasonable to assume that different clinical, morphologic, and biochemical indices as measured in blood, urine, and feces may be helpful for testing the impact of probiotics on the health of the individual, especially in different age groups such as newborns, infants, adults, and elderly subjects.

An attempt to follow this approach is shown by a research that suggests that a GI microbiome altered by probiotics influences host lipid and energy metabolism in atherosclerotic or obese patients, as documented by the decrease in the total serum lipidomic profile after treatment with the mono-strain probiotic LGG (54).

In clinical trials, the effect of probiotic intervention on the prevention or treatment of specific diseases has mostly relied on well-documented effects, where the degree of shift in the metabolic and inflammatory processes of gut is associated with objectively measured symptoms. However, in irritable bowel syndrome (IBS) and Crohn’s disease, where the pathogenesis is not completely understood, the efficacy of probiotic treatment is often evaluated according to the perceived improvement of clinical symptoms without measurement of specific inflammatory markers.

One promising approach for assessing clinical efficacy is to evaluate the effect of probiotic interventions using meta-analyses according to the reported evidence of well-designed randomized clinical trials (RCT). This method, when applied in an appropriate manner, may aid in determining whether single or multiple probiotic strains are more effective as therapeutic interventions. Unfortunately, many papers conclude with a statement announcing that more data are required and the study will be continued. Moreover, most of the papers are case reports, uncontrolled studies in humans, or studies containing insufficient documentation on beneficial effects of the tested probiotics (55).

**Comparison of effects of mono-strain versus multistrain probiotics**

Probiotics have been documented to have activity in treating a variety of clinical conditions – ranging from infantile diarrhea, necrotizing enteroocolitis, antibiotic-associated diarrhea, relapsing Clostridium difficile colitis, Helicobacter pylori infections, inflammatory bowel disease, female urogenital infections, and surgical infections (55). We have differentiated the efficacy of single and multiple strains of probiotic intervention for patients with

![Fig. 1. Different stages of metabolic and inflammatory stress (modified according Fourth Interactive workshop Nutrition and Health Claims Europe: Challenges Ahead, 2009, Brussels).](image-url)
various GI diseases mainly relying on the alleviation of clinical symptoms as assessed during well-designed randomized double blinded placebo controlled (DBPC) clinical trials. Most of the data are derived from comprehensive meta-analyses (56–58) or recent references included in databases. Only studies reported between the years 2000 and 2010 were included, where clinical efficacy was documented through DBPC studies, randomized or not. The efficacy in prevention or treatment of acute watery diarrhea, antibiotic-induced intestinal microbiota imbalance, *H. pylori* infection, *C. difficile* infection, *H. pylori* infection, IBS, inflammatory bowel disease (IBD), and pouchitis was analyzed (Table 1).

### Infectious diarrhea
Diarrhea is defined by the World Health Organization as having three or more loose or liquid stools per day. Viral, bacterial, and parasitic agents are recognized as the causative agents of infectious diarrheal illnesses. A meta-analysis of randomized placebo-controlled trials published in the year 2006 (59) suggested that probiotics significantly reduced antibiotic-associated diarrhea by 52%, the risk of travelers’ diarrhea by 8%, and that of acute diarrhea due to diverse causes by 34%. Probiotics reduced the associated risk of acute diarrhea among children by 57% and by 26% among adults.

The most commonly used single bacterial strains for probiotic treatment are *L. rhamnosus* GG ATCC 53103(LGG), *Bifidobacterium animalis* ssp lactis ATCC 35624, and *L. reuteri* ATCC 55730 (Table 2). It is thought that the effect of these single bacteria rely on their immunomodulating efficacy for GI infections. There is convincing evidence that the immunomodulatory effect of the probiotic is strain and dose dependent, whereas the target infectious agent is also an important factor as recently reported by Guandalini (60). The protective effect varied significantly on the basis of the studied probiotic strains. When administered alone (61), *Lactobacillus reuteri* was providing better results as compared to *B. lactis* BB12. In hospitalized patients, the duration of acute rotavirus diarrhea was reduced by LGG due to the stabilization of indigenous microbiota, reduction of gut permeability caused by rotavirus infection, together with a significant increase of cells secreting IgA antibody directed at rotavirus (62–64). Beside the aforementioned properties, LGG stimulates local release of interferon and facilitates antigen transport to underlying lymphoid cells, thus increasing antigen uptake and localization via Peyer’s patches. Two potent inhibitory peptides, active against both Gram-positive and Gram-negative bacteria released from LGG in culture media, have been recently characterized by liquid chromatography and mass spectrometry (65). However, in four different studies, no prophylactic or treatment effect with LGG was found (66–69). In contrast, evidence of a significant protective effect consisting of a 14% reduction in the incidence of diarrhea in nearly 4,000 children aged 1–5 years has been reported after their treatment with the mono-strain probiotic *L. casei* Shirota (70).

Dubey and coworkers (71) reported that VSL-#3, a multistrain probiotic, administration in patients suffering from acute rotavirus diarrhea resulted in earlier recovery and reduced need for oral rehydration solutions as compared to untreated controls, indicating that the decreased stool volume losses during diarrhea were beneficial in patient recovery. Recently, the effect of the mono-strain probiotic *Saccharomyces boulardii* was compared with a mixed preparation containing *S. boulardii*, *L. acidophilus*, *L. rhamnosus*, and *B. longum* strains in the treatment of rotavirus diarrhea in children less than 2 years of age; a significant reduction in diarrhea and fever duration was found only when the mono-strain probiotic was used (72).

The differences in host response to probiotic intervention need to be carefully evaluated. The commensal human microbiota has developed sophisticated mechanisms to counteract the inflammatory pathways and to protect host from pathogens that may provide predictions about the effects of probiotics, that are difficult to assume in the absence of actual clinical trial information and data on improved biomarkers.

### Table 1. Comparative summary of DBPC trials on mono- versus multistrain probiotics

| Disease                        | Number of mono-strains effective/non-effective probiotic trials (references No) | Number of multi-strain effective/non-effective probiotic trials (references No) |
|-------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| Acute watery diarrhea         | 5 (61, 63, 64, 70, 72) / 6 (61, 66–69, 145)                                       | 3 (71, 146, 147) / 1 (72)                                                       |
| Antibiotic associated diarrhea, *C. difficile* infection, *H. pylori* infection, IBS, inflammatory bowel disease (IBD), and pouchitis |                                                                                  |                                                                                  |
| IBS                           | 6 (91, 93, 94, 96, 97, 99) / 3 (91, 92, 95)                                       | 6 (100–102, 105–107) / 1 (104)                                                  |
| IBD, pouchitis                | 2 (115, 120) / 3 (141–143)                                                     | 13 (121–130, 137, 148, 149) / 0                                               |
Table 2. Mono or multispecies probiotic products and functional targets in the host

| Disease/reference | Probiotic | DBPC Clinical trial (randomized) | Efficacy |
|------------------|-----------|----------------------------------|----------|
|                  | Monostrain | Multistrain | Participants | Duration/Dose | Significant improvement | Failure |
| Acute watery diarrhea | B. lactis Bb12 or L. reuteri | | 201 (4–10 months) | 12 weeks min. 10⁷ CFU | Smaller number of diarrheal episodes by L. reuteri | B. lactis had no effect |
| Weizman et al. (61) | | | 81 children aged 1–36 | 6 x 10⁹ CFU months 33.3% | Reduction of the risk of nosocomial diarrhea and rotavirus gastroenteritis | |
| Szajewska et al. (63) | L. rhamnosus GG (ATCC 53103) | Breast-fed infants | R, DBCT | | Positive effect | |
| Szajewska et al. (64) | L. rhamnosus GG (ATCC 53103) | | | | | |
| Basu et al. (66) | L. rhamnosus GG (ATCC 53103) | | | | | |
| Hojsak et al. (67) | L. rhamnosus GG (ATCC 53103) | | | | | |
| Mastretta et al. (68) | L. rhamnosus GG (ATCC 53103) | Infants 220 (1–18 months) | | 10¹⁰ CFU | No effect in rotavirus diarrhea | |
| Hatakka et al. (69) | L. rhamnosus GG (ATCC 53103) | | | 7 months 10⁵ CFU | No effect in acute diarrhea | |
| Sur. et al. (70) | L. casei strain Shirota | | | | | |
| Dubey et al. (71) | VSL#3/sharp/³ | | | | | |
| Grandy et al. (72) | S. boulardii | S. boulardii, L. acidophilus, L. rhamnosus and B. longum | | | | |
| Chouraqui et al. (145) | Acidified milk formula supplemented with B. lactis Bb12 | | | | | |

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| Disease/reference | Probiotic | DBPC Clinical trial (randomized) | Efficacy |
|------------------|-----------|--------------------------------|----------|
| Narayanappa (146) | Bifilac content: *S. faecalis* S JPC *C. butyricum* B. *mesentericus* JPC Lactic acid bacillus (*L. sporogenes*) | 80 (3 months-3 y) 30 million + 2 million + 1 million + 50 million CFU | Less dehydration and duration of rotavirus shedding |
| Lin et al. (147) | *L. casei* rhamnosus, *L. rhamnosus* T cell-1 capsules | 1062 preschool children 10^8 CFU and 10^10 CFU 3 and 6 months | Significant reduction in gastrointestinal infection |
| Helicobacter pylori infection | *L. paracasei* 8700:2, *B. longum* 46 and *L. fermentum* ME-3 with Raftilose p65 | 53 Adults with no complaints | Positive effect in suppression of systemic oxidative stress The enteric coated mix did not eradicate the pathogen |
| Cremonini et al. (81) | *L. rhamnosus* GG (ATCC 53103 or *S. bouardi*), with triple therapy administered | 85 asymptomatic subjects treated for one week and continued afterwards for an additional week | No effect on the clearing of *H. pylori* in all three variations |
| Wang et al. (82) | Bb12 and La5, | 59 *H. pylori*-positive individuals > 10^7 CFU/mL of each strain in yoghurt twice daily for 6 weeks, 11 subjects were given a placebo. | Significantly lower *H. pylori* values in those receiving the AB yogurt |
| Imbalance (IMB) modulation after antibiotic treatment | *L. rhamnosus* GG, | Infants and young children at least 10 billion | Monostrain: Efficacy in these settings is only modest: strain and dose dependent |
| Guandalini (60) | Comparison: 1. *L. rhamnosus* GG; 2. *B. lactis*; 3. *L. reuteri* | R, DBCT | After 2 weeks 5% diarrhea in GG group, 16% in placebo group |
| Arvola et al. (73) | *L. rhamnosus* GG, | 119 children with respiratory infections and single antibiotic therapy R, DBPC Capsule 2 ×10^10 2 weeks | }
| Disease/reference Probiotic DBPC Clinical trial (randomized) | Efficacy |
|-------------------------------------------------------------|---------|
| **Monostrain**                                              | **Multistrain** |
| **Participants**                                            | Duration/Dose |
| **Significant improvement**                                 | **Failure** |
| Zoppi et al. (74) I. *L. rhamnosus GG*, II. *E. faecium SF68* III. *S. boulardii* | I.-B. *bifidum* – L. *Acidophilus* II. *Yovis*<sup>x</sup> | Children | Multistrain: I and II counteracted-stool frequency caused by CFX – reduced fecal pH – improved IMB composition |
| Thomas et al. (75) L. *rhamnosus* GG (ATCC 53103)            | 267 Adults | 10 × 10⁹ CFU | Monostrains: I, II, III-No effect on stool frequency& consistency, only I induced some favorable shifts in IMB No reduction of occurrence of diarrhea (LGG-29.3% vs. 29.9% placebo) |
| Wullt et al. (76) L. *plantarum* 299v with metronidazole and metronidazole combination with placebo | 20 pt | Improvement of clinical symptoms response and normalization of ratio of anti/proinflammatory cytokines |
| **Irritable bowel syndrome IBS**                            |          |
| O’Mahony et al. (91) 1. *B. infantis* 35624                 | 80 (18-73) | 1 × 10¹⁰ CFU in a milk drink, 8w | Improvement of IBS symptoms |
| Ligaarden et al. (92) 2. *L. salivarius* UCC4331            | 16 adults | Daily dose 10¹⁰ | No relief of symptoms No relief of IBS symptoms |
| Whorwell et al. (93) 3. *B. infantis* 35624 3 different doses | 362 women (19-69) | 4w | Improvement of IBS symptoms |
| Gawronska et al. (94) L. *rhamnosus* GG (ATCC 53103)        | 37 (6-16) | 4w | Moderate treatment of IBS |
| Bausserman & Michail (95) L. *rhamnosus* GG (ATCC 53103)   | 50 children 6 weeks | R DBPC | LGG was not superior to placebo in the treatment of abdominal pain |
| Niedzielin et al. (96) L. *plantarum* strain 299v           | 40 (27-63) | 4w | Reduction in pain, bloating and constipation of 95% patients |
| Nobaek et al. (97) L. *plantarum* strain 299v+ rose hip syrup + oat flour | 60 (21-78) | 4w | Decrease of pain and flatulence |
| Disease/reference | Probiotic | DBPC Clinical trial (randomized) | Efficacy |
|-------------------|-----------|-----------------------------------|----------|
|                   | Monostrain | Multistrain | Participants | Duration/Dose | Significant improvement | Failure |
| Agrawal et al. (99) | B. lactis DN-173 010 | 34 female pt | 1.25 x 10^10 colony forming units (cfu) fermented milk | Improvements in abdominal girth and gastrointestinal transit, as well as reduced symptomatology |
| Saggioro (100)     | L. plantarum LP01, B. breve BR03, L. plantarum LP01, L. acidophilus LA02 | 70 adult pt | 4w 5 x 10^9 CFU/g | Reduction of pain and global severity of symptoms |
| Guyonnet et al. (101) | B. animalis DN-1730101 Str. thermophilus, Lb. bulgaricus | 267 adults | 6w (high dose 3 x 10^11 CFU/g) | Improvement of IBS symptoms: bloating and constipation |
| Kajander et al. (102) | L. rhamnosus GG, L. rhamnosus Lc705, Propionibacterium freudenreichii ssp. shermanii JS and B. breve Bb99. | 86 pt | 5 months | Alleviation of distension and abdominal pain in IBS Stabilization of the microbiota. |
| Drouault-Holowacz et al. (104) | Four Lactobacillus strains daily for 4 weeks | 100 children 10^{10} CFU | R DBPC | 42.6% probiotic versus 42.3% placebo in relieving symptoms of IBS |
| Kim et al. (105)   | VSL#3 mixture | 25 (19-70) | 8w Daily 450 x 10^9 | Reduction of abdominal bloating |
| Kim et al. (106)   | VSL#3 mixture | 31 pt 17 pt | 4w 8w | Reduction of flatulence scores |

**Inflammatory bowel disease**

| Disease/reference | Probiotic | DBPC Clinical trial (randomized) | Efficacy |
|-------------------|-----------|-----------------------------------|----------|
| Kruis et al. (115) | E. coli Nissle 1917 (E. coli K5) | 327 adult patients | Nissle strain 200 mg 10^9 Or Mesalazine 500 mg 3 x 12 months | Equal to mesalamine in maintaining remission of UC |
| Guslandi et al. (120) | S. boulardii + mesalamine | 32 patients with a mild to moderate Crohn’s disease | 1 g both mesalamine and probiotic or mesalamine for 6 months | Maintenance in the treatment of Crohn’s disease versus relapses 6.25% versus 37.5% placebo |
| Disease/reference | Probiotic | DBPC Clinical trial (randomized) | Efficacy |
|-------------------|-----------|---------------------------------|----------|
| Monostrain        | B. bifidum strain | Kato et al. (121)  | 20 pt | 12 weeks | Induction of remission and reduction of UC activity index |
| Multistain        | L. acidophilus fermented milk | Huynh HQ et al. (122) | Children | 1 sachet | Remission rate of 56% and combined remission/response rate of 61% |
|                  | VSL#3 mixture | Pascarella et al. (123) | Children | 29 | Induction and maintenance of remission in UC in 92.8% with VSL#3 and concomitant IBD therapy 36.4% with placebo |
|                  | VSL#3/Balsalazide | Tursi et al. (124, 148) | Adults | 90 | Combination of VSL#3 low dose balsalazide was most effective in maintenance of remission in 87% of cases |
|                  | VSL#3 mixture | Bibiloni et al. (125) | Children | 34 | Remission in active ulcerative colitis (UCDAI ≤ 2) 53% |
|                  | VSL#3 | Sood et al. (126) | Adult patients | 147 | Induces remission in patients with mild-to-moderate active ulcerative colitis (UCDAI ≤ 2) |
|                  | LGG + inulin | Isshikawa et al. (127) | Adults | 11 | Reduced exacerbation of UC symptoms, maintenance without relapses |
|                  | Lactobacillus GG | Schultz (142) | 11 patients with Crohn's disease | R DBPC 2 × 10^9 CFU/day | 6 months | No efficacy in relapses between probiotic and placebo groups |
|                  | B. breve, B. bifidum and L. acidophilus YIT 0168 | Ishikawa et al. (127) | Subjects with probiotic versus 11 control subjects | 100 ml/day | No efficacy in relapses between probiotic and placebo groups |
|                  | LGG + inulin | Bousvaros (141) | Adults | 10 | Combination of probiotic and placebo was effective in maintenance of remission in 87% of cases |
|                  | Lactobacillus GG | Schultz (142) | Patients with Crohn's disease | R DBPC 2 × 10^9 CFU/day | 6 months | No efficacy in relapses between probiotic and placebo groups |

Do probiotic preparations for humans really have efficacy?
| Disease/reference | Probiotic | DBPC Clinical trial (randomized) | Efficacy |
|-------------------|-----------|---------------------------------|----------|
|                   | Monostrain | Multistrain | Participants | Duration/Dose | Significant improvement | Failure |
| Marteau et al. (143) | L. johnsonii LA1 | | 98 patients with Crohn’s disease | R DBPC 4 x 10⁶ cfu/day | 6 months | No sufficient effect, to prevent endoscopic recurrence of Crohn’s disease. |
| Pouchitis | Gionchetti et al. (117, 149) | VSL#3 mixture | 40 patients (18 to 65 years) | 900 x 10⁹ CFU t(nightly) | 12 months | Prevention of onset of pouchitis 12 months = VSL#3 90% versus Placebo 60% (P < 0.05) Median IBDQ score, median stool frequency showed progressive improvement |
| Gionchetti et al. (128) | VSL#3 mixture | | 40 patients (18 to 65 years) | 900 x 10⁹ CFU twice daily | 9 months | Maintenance of remission at 9 months: VSL#3 85% remission versus Placebo 0% remission |
| Gionchetti et al. (129) | VSL#3 mixture | | 23 adults | 3600 billion 4 weeks | High doses of VSL-3 are effective in treatment of mild pouchitis |
| Mimura et al. (130) | VSL#3 mixture | | 36 patients at remission stage | 1,800 x 10⁹ CFU taken once nightly for 12 months or until relapse | Maintenance of remission by high dose of VSL-3 at 12 months: VSL#3 85% remission versus Placebo 6% |
| Kühlbacher et al. (137) | VSL#3 mixture | | 2 months | | Maintenance of remission |
Antibiotic therapy and Clostridium difficile infection (CDI)

One of the main targets of probiotic intervention studies is the restoration of GI microbiota composition and the correction of dysbiosis-associated markers of GI function following antibiotic therapy, when the active agent is secreted via the hepatobiliary circulation. Arvola et al. (73) have demonstrated that a probiotic strain, Lactobacillus GG, has been effective in the prevention of diarrhea in children receiving antimicrobial treatment for respiratory infections with a single dose. It has also been reported that third generation cephalosporins, particularly ceftriaxone, induce a decrease in Escherichia coli and Lactobacillus counts and an increase in Gram-positive cocci and Clostridium counts (74). The same authors found that microbial shifts were associated with a reduction in the activities of enzymes such as β-galactosidase and β-glucosidase, and with an increase in the activity of β-glucuronidase, an enzyme involved in the formation of toxic and carcinogenic compounds. It was concluded that parenterally administered ceftriaxone caused a significant dysbiosis that may be corrected by appropriate probiotic intervention.

In Table 2, different trials performed using six commercial probiotics tested for their ability to counteract the side effects associated with ceftriaxone therapy are reported and the results suggest the following:

1) Three mono-strain products containing Saccharomyces boulardii, Enterococcus faecium SF 68, or L. rhamnosus GG respectively; having no effect on stool frequency;
2) Two multistrain preparations containing Bifidobacterium bifidum and L. acidophilus, or a multispecies preparation containing a combination of B. breve, B. infantis, B. longum, L. acidophilus, L. casei, L. delbrueckii subsp. bulgaricus, L. plantarum, S. faecium, S. salicarius subsp. thermophilus decreased stool frequency.

The conclusion of this comparison was that probiotics containing multiple species of lactobacilli and bifidobacteria at high concentration are more effective in preventing dysbiosis induced by ceftriaxone treatment than mono-strain probiotic preparations (74). The reduction of stool frequency associated with ceftriaxone treatment, accompanied with a decreased fecal pH, clearly supports the efficacy of multi-strain probiotics in antibiotic-associated diarrhea and the concomitant dysbiosis associated with antibiotic use. However, due to some strain variability in antibiotic sensitivity of Lactobacillus sp., the reduction of antibiotic-associated diarrhea with mono-strain probiotics cannot be excluded because only a limited number of strains have been evaluated in clinical trials.

Antibiotic therapy is often associated with Clostridium difficile infection (CDI). Probiotics that have been proposed for prevention and treatment of antibiotic-associated diarrhea and CDI include different bacterial species (Bifidobacterium, LGG, L. rhamnosus, L. casei, L. plantarum 299v, E. faecium [SF68]), and yeasts (S. boulardii, S. cerevisiae). These commercially available probiotic formulations are commonly available as lyophilized capsules or in the form of a fermented drink. A well-designed study by Guandalini (60) compared the efficacy of probiotic intervention by mono-strain probiotics such as L. rhamnosus GG, B. lactis, and L. reuteri, reporting only a modest clinical benefit after antibiotic treatment. Thomas and co-authors (75) performed a prospective, randomized double-blind, placebo-controlled trial to assess the clinical efficacy of LGG in prevention of C. difficile diarrhea in patients taking antibiotics. No differences in the rate of occurrence of diarrhea were found between patients treated with the probiotic formulation and those receiving a placebo.

In 2003, Wullt and colleagues (76) performed a double-blind, placebo-controlled trial to analyze the ability of Lactobacillus plantarum 299v to prevent recurrent episodes of Clostridium difficile-associated diarrhea. The lactobacilli treatment had no side effects, but the small sample size did not allow any conclusions to be drawn concerning the efficacy of L. plantarum in patients with recurrences.

Overall, none of the published papers on this topic (77–79) unequivocally prove that the use of probiotics for the prevention and/or therapy of CDI is able to reconstitute the gut microflora and to prevent recurrences. At the present time, proof of probiotic efficacy for CDI is inconclusive and a significant number of prospective randomized trials are urgently needed.

Helicobacter pylori infection

It has been reported that Lactobacillus sp., Bifidobacterium sp., Saccharomyces sp., Clostridium sp. strains, etc. have inhibitory effects on H. pylori infection in vitro and in vivo studies (5, 47, 80). Cremonini et al. (81) analyzed three randomized, double-blind, placebo-controlled studies documenting a decrease in the side effects of triple antibiotic therapy for H. pylori colonization both with single and double probiotics, but no effect on the clearing of H. pylori was registered in all three variations. Wang et al. (82) observed that out of the two probiotic strains present in AB yogurt, Bifidobacterium Bb12, and Lactobacillus La5, only Bb12 exerted an inhibitory effect in vitro against H. pylori. If they were administered as yogurt (containing >10^7 CFU/mL of each strain), the authors reported a steeper decrease in H. pylori colonization in those individuals who were more intensely colonized with the pathogen. The study group consisted of 59 H. pylori-positive individuals receiving yogurt twice...
daily for 6 weeks, whereas 11 subjects were given a placebo. Besides LAB, the effect of the mono-strain probiotic Clostridium butyricum MIYAIRI588 (generally used in Japan for treatment of patients with antibiotic-associated diarrhea and C. difficile infection) has been also demonstrated using germ-free mice. The number of *H. pylori* in gastric mucosa was significantly reduced by coinfection with vegetative cells of *C. butyricum* (83).

Mikelsaar and coworkers studied healthy individuals without GI complaints who were persistently colonized with *H. pylori*. The enteric coated mix of *L. paracasei* 8700:2, *B. longum* 46, and *L. fermentum* ME-3 with the probiotic Rafilose p65, although having in vitro antagonistic properties did not exert any antimicrobial effect on *H. pylori* infection in a randomized, double-blind, placebo-controlled trial (40, 80). Seemingly, the target pathogen was not reached in the stomach.

Lee et al. (84) reported the use of probiotics as a non-antibacterial strategy during *H. pylori* infection where they serve as suppressors for proinflammatory cytokine signaling, exerting a beneficial antiinflammatory effect. Mikelsaar and coworkers found that the systematic oxidative stress (ox-S) caused by *H. pylori* infection in overtly healthy persons (Fig. 1) was suppressed by the antioxidative probiotic *L. fermentum* ME-3 consumed over a 3-week course of therapy. Specifically, blood antioxidative indices, such as lipid peroxidation, content of reduced glutathione, and total antioxidative activity, were reduced (80, 85). These same biomarkers are also often used as predictive factors for the development of atherosclerosis (86). The ability of *L. fermentum* ME-3 strain to induce IL-10 (87) seems to be an important factor in suppression of systemic inflammation during any chronic infection. The evaluation of small numbers of probiotic strains may limit the conclusions on the increased IL-10 response provoked by bifidobacteria and *E. coli* Nissle as compared to the lack of activity for the four strains of lactobacilli that have also been evaluated (88).

*In vitro* studies and clinical trials have shown that mono-strain probiotic-supplemented regimens increased eradication rates and reduced side effects during antibiotic therapy for *H. pylori*. Because probiotics are effective against antibiotic-resistant *H. pylori* strains, their use needs to be considered as a supportive intervention during antibiotic eradication of this pathogen (83).

In summary, the results of clinical trials suggest that probiotic supplementation during anti-*H. pylori* therapy decreases adverse side effects, resulting in better compliance with antibiotic therapy and less systemic effects.

**Irritable bowel syndrome**

Irritable bowel syndrome is a functional bowel disorder that manifests as chronic, recurrent abdominal pain, or discomfort associated with disturbed bowel habit in the absence of structural abnormalities likely to account for these symptoms. The epigenetic model of IBS incorporates proinflammatory markers, neuroendocrine alterations, and links with both psychosocial and infectious stresses (89). In addition to the role of GI infections, it is suggested that IBS patients have an abnormal composition and a temporary instability of their intestinal microbiota. The altered microbiota raises the possibilities of therapeutic interventions using selective antibiotic therapy or probiotic administration.

In contrast, ulcerative colitis and Crohn’s disease, the two distinct idiopathic pathologies of inflammatory bowel diseases, are characterized by documented gut epithelial lesions and alteration of intestinal microbiota, particularly *Bacteroides* spp. (90). Both diseases are spontaneously relapsing and generally accepted as immunologically mediated disorders of the GI tract. The etiology and risk factors of these complex inflammatory diseases of the bowel remain elusive. However, it should be noted that IBS and IBD patients account for 30–50% of office visits for gastroenterology services.

As stated previously, IBS is a debilitating disorder. The evidence for efficacy of most drug therapies in the treatment of IBS is weak. Recent meta-analyses confirm a role for probiotics in IBS, but also make it clear that the effects of probiotics in IBS, as elsewhere, are highly strain specific. Variability and the formulation of specific strains used as probiotic products vary dramatically depending on where they are produced. Lack of quality control for probiotics hampers the ability to make recommendations for their use.

A wide variety of probiotic trials have been conducted with mono- or multistrain probiotic interventions. However, a literature search has revealed a few comparative studies on mono strains of different probiotic genera or species under the same clinical trial conditions. A well conducted study by O’Mahony et al. (91) showed that if two strains, *Bifidobacterium infantis* ATCC 35624 or *Lactobacillus salivarius* UCC4331, were administered to separate groups of patients with IBS, only *B. infantis* 35624 alleviated the symptoms of disease (Table 2). The symptomatic response was associated with normalization of the ratio of the antiinflammatory to proinflammatory cytokines, suggesting an immune-modulating role for this strain of *B. infantis*. A possible confounding variable for this study was the use of a milk product for delivery of the probiotic and not a capsule. At the same time, there are several failures of clinical efficacy for IBS treatment employing other single-strain probiotics. Clinical trials of *L. plantarum* MF1298 (92) did not document relief of the IBS symptoms.

Hoveyda et al. (57) have published a systematic review and meta-analysis of randomized trials performed up to 2007 to evaluate efficacy of probiotics for alleviating...
symptoms in IBS patients. They identified 14 randomized placebo controlled trials that report modest improvement in overall symptoms after several weeks of treatment, with mono-strain probiotics generally demonstrating better results. In five trials, a combination probiotic containing different numbers and strains of bacteria were used as intervention while nine clinical trials evaluated single strains alone or combined with a prebiotic substrate. The mono-strains included a variety of species, including *B. infantis* (93) and *L. rhamnosus* GG (94, 95) used as single agents. Only *L. plantarum* 299v was evaluated in two trials (96, 97) with the results showing a reduction of pain, bloating, and flatulence. Brenner and coauthors (98) have evaluated several clinical studies of IBS on the basis of the following approved criteria: (1) Random Controlled Trials (RCTs), (2) adults with IBS as defined by Manning or Rome II criteria, (3) single or combination probiotics versus placebo, and (4) improvement in IBS symptoms and/or decrease in frequency of adverse events reported. Only *B. infantis* 35624 (91, 93) could be shown to provide a significant improvement in the composite score for abdominal pain/discomfort, bloating/distention, and/or bowel movement difficulty compared with placebo in two appropriately designed studies. No other probiotic in their studies demonstrated significant ability to improve IBS symptoms. In 2009, Agrawal and coworkers (99) reported that the probiotic *B. lactis* is able to improve abdominal distension and GI transit in IBS patient with constipation. These results support the acceleration of transit as a useful strategy for treating distension.

Several multispecies mixtures have been reported to have efficacy for the treatment of IBS. These studies (100) have combined *L. plantarum* LP 01 with *B. breve* BR 03 or *L. plantarum* LP 01 with *L. acidophilus* LA 02, *B. lactis* DN-173 010 mixed with yoghurt as a delivery system (101). Kajander et al. (102) evaluated *L. rhamnosus* GG, *L. rhamnosus* Lc705, *Propionibacterium freudenreichii* spp. shermanii JS, and *B. breve* Bb99 and documented a decrease in bowel symptoms, abdominal pain, and bloating. Later, the same group (103) found that *Ruminococcus torques* phylotype was decreased in the probiotic group during the intervention at 6 months. In addition, the clostridial phylotype *Clostridium thermosuccinogenes*, was stably elevated. The bacterial alterations detected were in accordance with previously observed alleviation of symptoms in IBS. No alleviation of IBS symptoms was found when evaluating a *Lactobacillus* multispecies probiotic (104) in French children. However, the probiotic combination VSL#3, composed of eight different strains, has been observed to decrease flatulence scores (105) and alleviate abdominal bloating (106) but did not bring relief for stool frequency-related symptoms, abdominal pain scores, and colonic transit time. Guandalini et al. (107) in a more recent randomized, double-blind, placebo-controlled crossover study reported that VLS#3 was effective in ameliorating symptoms including abdominal pain/discomfort in patients with IBS.

The World Gastroenterology Organization Global Guideline (108) has suggested both *B. lactis* DN-173 strain and VSL#3 for the treatment of IBS.

**Inflammatory bowel disease**

Over 1 million people suffer from IBD in the United States, whereas in the UK one-quarter million people are afflicted with IBD (109, 110). Current understanding of IBD pathogenesis includes the adherence of bacteria to the intestinal mucosa and bacterial invasion into mucus epithelial cells with a concomitant inflammatory response. This chronic bowel inflammation cannot subside as long as the mucus barrier remains defective. The inflammatory response interferes with epithelial cell tolerance to intestinal bacteria and leads to characteristic changes in the composition of the fecal microbiota (109). In biopsies and stool samples of 58 adolescents with inflammatory bowel disease, an increase in the total populations of aerobic bacteria but not of anaerobes was demonstrated (111). This corresponds with a significant decrease in the concentrations of propionic and butyric acids in the feces of patients with IBD. The authors postulated that different components of *Enterobacteriaceae*, especially their lipopolysaccharides, may also contribute to perpetuation of chronic colon inflammation. However, most of the microbiota adhering to the colonic mucosa surrounding the mucus layer comprises *Clostridium cocoides* and *Bifidobacterium* spp. These findings suggest that IBD is not caused by a specific intestinal bacterial cluster or species and that a disordered intestinal microflora may be involved in the pathogenesis of IBD. A role for hydrogen peroxide-producing colonic bacteria as causative agents of the inflammatory process in young adults suffering from IBD has been hypothesized (112).

Selected mono-strain probiotics have been proven to be clinically effective in maintaining remission in patients with ulcerative colitis. *E. coli* strain Nissle 1917 has been used as a probiotic for the treatment of inflammatory bowel disease, chronic constipation, and acute protracted diarrhea (113). The strain Nissle 1917 expresses a K5 capsule that mediates interactions with intestinal epithelial cells. Additionally, this strain exhibits a particular lipopolysaccharide with immunomodulating properties without showing immunotoxic effects associated with endotoxin. The induction of chemokines by the Nissle 1917 strain was observed *in vitro* following the interaction with the basolateral surface of Caco-2 cells, suggesting that this strain is effective in repairing the epithelial barrier (114, 115). The main result of the Nissle 1917 strain has been the maintenance of remission in patients
with ulcerative colitis equal to the effect of mesalazine (115). Campieri and Gionchetti (116–118) and Guslandi (119) have provided convincing evidence supporting the role of intestinal bacteria as a cause of ulcerative colitis. Guslandi et al. (120) have reported the efficacy of mono-strain probiotics (S. boulardii) together with mesalazine in the prevention of recurrences in Crohn’s disease and in the prolongation of remission that were superior to mesalazine alone. In addition, it has been shown that Bifidobacterium spp. and Lactobacillus spp. fermented milk reduced the exacerbation of ulcerative colitis symptoms (121).

In seven different trials using VSL#3, it has been demonstrated that a remarkable induction of remission for mild or moderate ulcerative colitis occurs, along with maintenance of remission in children (122, 123) and in adults (124–126). Ishikawa and coworkers (127), using a mixture of B. breve, B. bifidum, and L. acidophilus YIT 0168 in fermented milk, reported reduced exacerbation of ulcerative colitis symptoms in 21 adult patients with maintenance of remission. The prevention of flare-ups in chronic pouchitis has been also demonstrated with VSL#3 treatment (117, 128–130).

Based on these trials, it appears that the VSL#3 multispecies probiotic mixture is effective in the maintenance of remission in IBS, IBD, and pouchitis. However, Haller and Autenrieth (51) have raised the question as to which of the eight single bacterial strains from the VSL#3 mixture play a major role in these effects and whether the whole mixture is necessary to obtain the reported probiotic effects. In most studies with VSL#3, a significant increase in bifidobacteria has been observed in patient’s feces during treatment. High concentrations of some Bifidobacterium strains of the combination, such as B. infantis Y1 and B. breve Y8, were detected in feces by Brigidi and coworkers (131, 132) suggesting their putative role in beneficial shifts of specific biochemical markers such as β-galactosidase and urease. The induction of the anti-inflammatory cytokine IL-10 by Bifidobacterium genomic DNA in peripheral blood mononuclear cells (133) and by the probiotic cocktail of VSL#3 in dendritic cells (134) has also been assessed. VSL#3 DNA (133) attenuated the release of systemic TNF-α and colonic IFN-γ in experimental models (135) providing in vitro evidence for the limitation of the epithelial proinflammatory responses. In addition, it is possible that maintaining tight junction protein expression may prevent epithelial cell permeability (136) and serve as a clue to probiotic intervention in the successful treatment of IBD using VSL#3.

Additional studies indicate that probiotic therapy with VSL#3 increased (137) the richness and total number of microbiota, especially obligate anaerobes. The number of mucosal regulatory cells was expanded in patients with ileal pouch-anal anastomosis for ulcerative colitis (138), resulting in an antiinflammatory cytokine response. Appropriately, powered studies with different (combinations) of probiotics show positive results for reduction of symptoms, although a considerable placebo effect is also found. The attenuation of the proinflammatory immune response using probiotic bacteria through TLR signaling, IL-10 upregulation, and expression of COX-2 appear to provide a theoretical explanation for a pathophysiological mechanism for probiotic impact in inflammatory bowel diseases (139, 140).

None of the probiotics such as LGG (141, 142) or L. johnsonii La1 (143) tested to date have been shown to be effective in induction or in maintenance of remission in patients with Crohn’s disease.

A summary of comparisons between the efficacy of probiotic preparations in different diseases and the preferences for mono- or multistrain probiotics is provided in Table 1 where 25 trials with probiotic prevention or treatment concerning infectious or antimicrobial treatment-induced diarrhea, caused by different opportunistic bacterial or viral pathogens with 48% of efficacy are depicted. The monostrain probiotic preparations (17 trials) were effective in 41% of cases, whereas the multistrain ones (8 trials) expressed somewhat higher (63%) efficacy. In IBS (16 trials), the total efficacy was 75% while by applying monostrain preparations, the efficacy was 67% and multistrain probiotic preparations 86%. In 18 trials with patients of IBD, the high preference for multistrain probiotic efficacy (40% vs. 100%) was registered. The reasons for better efficacy of multistrain probiotic preparations seemingly derive from the large individual interrelations between microbiota and health markers of humans apparent also in case of GI diseases.

Health risks and beneficial effects associated with probiotics

Probiotics are live microorganisms, so it is possible that their administration may result in host infection under certain circumstances such as profound immunosuppression of the host. The risk of sepsis due to probiotic bacteria should be weighed against the potential for sepsis due to more pathogenic bacteria and the morbidity for diseases in which probiotic bacteria are used as therapeutic agents (36, 55). No experimental or clinical data concerning increased risk of infection by either mono- or multistrain probiotics have been published to date, although anecdotal cases of infection have been reported.

Well-designed placebo controlled studies of the infection rates during use of probiotic products for a specific condition in a particular target population are needed to address this issue (144). Results based on objective criteria are required for ascertaining the real health benefits and risks for the use of probiotics. In this regard, a careful selection of the probiotic agent, a dose
standardization, and a thorough knowledge of the beneficial functional effects are the most important issues for clearly defining appropriate use of probiotics as interventions in a variety of clinical syndromes.

Conclusions
1. Probiotic strains of specific species, either in mono- or multiculture, should have specific and well-defined metabolic and functional properties measurable by objective criteria. The probiotic effect should target a particular host function that has been altered through environmental stress, antibiotic utilization, or during specific clinical diseases that result in the alteration of the normal microbiota.

2. According to published clinical trials, the efficacy of probiotic intervention for infectious or antimicrobial treatment-induced diarrhea, caused by different opportunistic bacterial or viral pathogens, was 48%, whereas the mono-strain probiotics have expressed somewhat lower efficacy than the combinations of different species.

3. The probiotic preparations efficacy for silencing the clinical symptoms of IBS was 75% and for attenuating the inflammatory response during IBD was 83%, whereas multi-strain probiotics appear to have better efficacy.

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