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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

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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 antiinflammatory 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

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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 reuterine, 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

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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 enterocolitis, 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

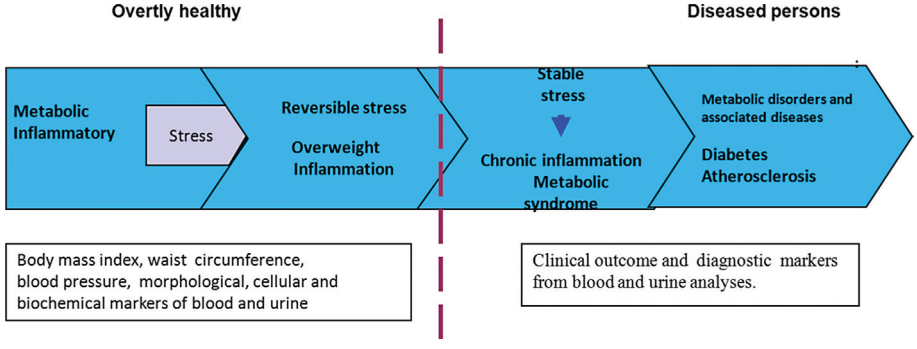

Fig. 1. Different stages of metabolic and inflammatory stress (modified according Fourth Interactive workshop Nutrition and Health Claims Europe: Challenges Ahead, 2009, Brussels).

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 (*referesnces 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	**2** (*60, 73*) / **4** (*74–76, 81*)	**2** (*74, 82*) / **2** (*80, 81*)
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**

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, 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.

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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						
Weizman et al. (61)	*B. lactis* Bb12 or *L. reuteri*		201 (4–10 months)	12 weeks min. 10^7 CFU	Smaller number of diarrheal episodes by L.r*euteri*	*B. lactis* had no effect
Szajewska et al. (63)	*L. rhamnosus* GG (ATCC 53103		81 children aged 1–36 months 33.3%;	6 $\times 10^9$ CFU	Reduction of the risk of nosocomial diarrhea and rotavirus gastroenteritis	
Szajewska et al. (64)	*L. rhamnosus* GG (ATCC 53103		Breast-fed infants	R, DBCT	Positive effect	
Basu et al. (66)	*L. rhamnosus* GG (ATCC 53103		634 Indian children	6×10^7 7 days + ORS		No decrease in frequency, duration of diarrhea and vomiting, no reduction of the hospital stay
Hojsak et al. (67)	*L. rhamnosus* GG (ATCC 53103)		281 children aged 1 to 7 years	10^9 CFU 3 months		No effect to vomiting and diarrheal episodes or number of days with gastrointestinal symptoms
Mastretta et al. (68)	*L. rhamnosus* GG (ATCC 53103		Infants 220 (1–18 months)	10^{10} CFU		No effect in rotavirus diarrhea
Hatakka et al. (69)	*L. rhamnosus* GG (ATCC 53103)		571 (1–6 years)	7 months 10^5 CFU		No effect in acute diarrhea
Sur. et al. (70)	*L. casei* strain Shirota		3758 Indian children (1–5y)	12 weeks probiotic drink + 12 w follow up	After 24 w 14% reduction of diarrhea ($p < 0.01$)	
Dubey et al. (71)		VSL#3/sharp/[3]	224 Indian Children	4 days	VSL#3 resulted in earlier recovery and decreased stool volume losses of rotavirus diarrhea	
Grandy et al. (72)	*S. boulardii*	*S, boulardii, L. acidophilus, L. rhamnosus* and *B. longum*	Less than 2 y of age		The significant decrease of duration of diarrhea and fever by monostrain	No result with multistrain
Chouraqui et al. (145)	Acidified milk formula supplemented with *B. lactis* Bb12		90 infants younger than 8 months	min. dose 10^8 CFU		No prevention of infant diarrhea in residential care settings

Table 2 (*Continued*)

Disease/reference	Probiotic		DBPC Clinical trial (randomized)		Efficacy	
	Monostrain	Multistrain	Participants	Duration/Dose	Significant improvement	Failure
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						
Mikelsaar et al. (80) Hütt et al. (85)		*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. boulardii*, with triple therapy administered	*B. lactis* and *L. acidophilus* combination with triple therapy	85 asymptomatic subjects treated for one week and continued afterwards for an additional week	R, DBCT		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.	R, DBCT	Significantly lower H. pylori values in those receiving the AB yogurt	
Imbalance (IMB) modulation after antibiotic treatment						
Guandalini (60)	*Comparison: 1.L. rhamnosus* GG; *2. B. lactis; 3. L. reuteri*.		Infants and young children	at least 10 billion	Monostrain: Efficacy in these settings is only modest: strain and dose dependent	
Arvola et al. (73)	*L. rhamnosus* GG,		119 children with respiratory infections and single antibiotic therapy	R,DBPC Capsule 2×10^{10} 2 weeks	After 2 weeks 5% diarrhea in GG groupo, 16% in placebo group	

Table 2 (*Continued*)

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*®	Children		Multistrain: I and II counteracted-stool frequency caused by CFX – reduced fecal pH – improved IMB composition	Monostrains: I, II, III-No effect on stool frequency& consistency, only I induced some favorable shifts in IMB
Thomas et al. 75)	*L. rhamnosus* GG (ATCC 53103)		267 Adults	10×10^9 CFU		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			Recurrence of clinical symptoms decreased to 36% under probiotic and to 30% under metronidazole and placebo (NS)
Irritable bowel syndrome IBS						
O′Mahony et al. (91)	1. *B. infantis* 35624		80 (18–73)	1×10^{10} CFU in a milk drink, 8w	Improvement of clinical symptoms response and normalization of ratio of anti/proinflammatory cytokines	
	2. *L. salivarius* UCC4331					No relief of symptoms
Ligaarden et al. (92)	*Lb. plantarum* MF1298		16 adults	Daily dose 10^{10}		No relief of IBS symptoms
Whorwell et al. (93)	*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	

Table 2 (*Continued*)

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	1.25×10^{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	70 adult pt	4w 5×10^9 CFU/g)	Reduction of pain and global severity of symptoms	
		L. plantarum LP01, *L. acidophilus* LA02		4w 5×10^9 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 [89]dose 3×10^{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	100 children 10^{10} CFU daily for 4 weeks	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 ×10e9	Reduction of abdominal bloating	No differences in the mean GI transit time
Kim et al. (106)		VSL#3 mixture	31 pt 17 pt	4 w 8 w	Reduction of flatulence scores	No differences in bloating, stool-related symptoms, abdominal scores, colonic transit.
Inflammatory bowel disease						
Kruis et al. (115)	*E. coli Nissle 1917* *(E. coli K5)*		327 adult patients	Nissle strain 200 mg 10^9 Or Mesalazine 500 mg 3×12 months	Equal to mesalamine in maintaining remission of UC	
Guslandi et al. (120)	*S. boulardii* + mesalamine		32 patients with a mild to moderateCrohn'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	

Table 2 (*Continued*)

Disease/reference	Probiotic		DBPC Clinical trial (randomized)		Efficacy	
	Monostrain	Multistrain	Participants	Duration/Dose	Significant improvement	Failure
Kato et al. (121)		*B. bifidum* strain *Yakult L. acidophilus* fermented milk 4/3 of $\geq$3 points)	20 pt	10^9 12 weeks	Induction of remission and reduction of UC activity index	
Huynh HQ et al. (122)		VSL#3 mixture	18 Children	1 sachet 450 B weight-based dose twice daily 8 weeks	Remission rate of 56% and combined remission/ response rate of 61%	
Pascarella et al. (123)		VSL#3 mixture + mesalazine 50 mg/Kg/die placebo +: mesalazine 50 mg/Kg/die +	Children 29	12 months	Induction and maintenance of remission in UC in 92,8% with VSL#3 and concomitant IBD therapy 36,4% with placebo	
Tursi et al. (124, 148)		VSL#3/Balazalide combination or Balazalide or mesalazine	90 adults	3600×10^9 CFU daily 8 weeks	Combination of VSL#3 + low dose balsalazide was most effective in maintenance of remission in 87% of cases Balsalazide = 80.7% Mesalamine = 72.7%	
Bibiloni et al. (125)		VSL#3 mixture	34 pt mean age of 35 years	$1{,}800 \times 10^9$ CFU taken twice daily 6 weeks	Remission in active ulcerative colitis (UCDAI $\leq$ 2) = 53%.	
Sood et al. (126)		VSL#3	147 adult patients		Induces remission in patients with mild-to-moderately active ulcerative colitis	
Ishikawa et al. (127)		*B. breve, B. bifidum and L. acidophilus* YIT 0168 i	11 subjects with probiotic versus 10 control	100 ml/day	Reduced exacerbation of UC symptoms, maintains remissions	
Bousvaros (141)	*LGG + inulin*		75 patients with Crohn's disease	R DBPC 2×10^{10} CFU/ day 24 months		No efficacy in relapses between probioticand placebo
Schultz (142)	Lactobacillus GG		11 patients with Crohn's disease	R DBPC 2×10^9 CFU/ day 6 months		No efficacy in relapses between probiotic and placebo groups

Table 2 (*Continued*)

Disease/reference	Probiotic		DBPC Clinical trial (randomized)		Efficacy	
	Monostrain	Multistrain	Participants	Duration/Dose	Significant improvement	Failure
Marteau et al. (143)	L. johnsoniii LA1		98 patients with Crohn's disease	R DBPC 4×10^9 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)	900×10^9 CFU tn 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)	900×10^9 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 4weeks	High doses of VSL-3 are effective in treatment of mild pouchitis	
Mimura et al. (130)		VSL#3 mixture	36 patients at remission stage	$1{,}800 \times 10^9$ CFU taken once nightly for 12 months or until relapse	Maintainance of remission by high dose of VSL-3 at 12 months: VSL#3 85% remission versus Placebo 6%	
Kühbacher 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 ß-galactosidase and ß-glucosidase, and with an increase in the activity of ß-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. salivarius 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 multistrain 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 Raftilose 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 possibile 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

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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 mucosal 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 coccoides* 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 lipopolysaccaride 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 monostrain 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* Y 8, 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 antiinflammatory 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

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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 measureable 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.

Conflict of interest and funding

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References

1. Fuller R. Probiotics in man and animals. J Appl Bacteriol 1989; 66: 365–78.
2. Elmer GW, Surawicz CM, McFarland LV. Biotherapeutic agents. A neglected modality for the treatment and prevention of selected intestinal and vaginal infections. JAMA 1996; 275: 870–6.
3. Reid G, Jass J, Sebulsky MT, McCormick JK. Potential uses of probiotics in clinical practice. Clin Microbiol Rev 2003; 16: 658–72.
4. Timmerman HM, Niers LE, Ridwan BU, Koning CJ, Mulder L, Akkermans LM, et al. Design of a multispecies probiotic mixture to prevent infectious complications in critically ill patients. Clin Nutr 2007; 26: 450–9.
5. Ouwehand AC, Salminen S, Isolauri E. Probiotics: an overview of beneficial effects. Antonie Van Leeuwenhoek 2002; 82: 279–89.
6. Ljungh A, Wadstrom T. Lactic acid bacteria as probiotics. Curr Issues Intest Microbiol 2006; 7: 73–89.
7. van Hemert S, Meijerink M, Molenaar D, Bron PA, de Vos P, Kleerebezem M, et al. Identification of *Lactobacillus plantarum* genes modulating the cytokine response of human peripheral blood mononuclear cells. BMC Microbiol 2010; 10: 293.
8. Contor L, Asp NG. Process for the assessment of scientific support for claims on foods (passclaim) phase two: moving forward. Eur J Nutr 2004; 43(Suppl 2): II3–6.
9. Aggett PJ, Antoine JM, Asp NG, Bellisle F, Contor L, Cummings JH, et al. Passclaim: consensus on criteria. Eur J Nutr 2005; 44(Suppl 1): i5–30.
10. Floch MH, Walker WA, Guandalini S, Hibberd P, Gorbach S, Surawicz C, et al. Recommendations for probiotic use–2008. J Clin Gastroenterol 2008; 42(Suppl 2): S104–8.
11. Mercenier A, Lenoir-Wijnkoop I, Sanders ME. Physiological and functional properties of probiotics. Bul Int Dairy Federation 2008; 429: 2–6.
12. Sanders ME, Marco ML. Food formats for effective delivery of probiotics. Ann Rev Food Sci Technol 2010; 1: 65–85.
13. Zoetendal EG, Cheng B, Koike S, Mackie RI. Molecular microbial ecology of the gastrointestinal tract: from phylogeny to function. Curr Issues Intest Microbiol 2004; 5: 31–47.
14. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science 2005; 308: 1635–8.
15. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science 2005; 307: 1915–20.
16. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 2006; 124: 837–48.
17. Dethlefsen L, Eckburg PB, Bik EM, Relman DA. Assembly of the human intestinal microbiota. Trends Ecol Evol 2006; 21: 517–23.
18. Ventura M, Turroni F, Canchaya C, Vaughan EE, O'Toole PW, van Sinderen D. Microbial diversity in the human intestine and novel insights from metagenomics. Front Biosci 2009; 14: 3214–21.
19. Moore WE, Holdeman LV. Special problems associated with the isolation and identification of intestinal bacteria in fecal flora studies. Am J Clin Nutr 1974; 27: 1450–5.
20. Simon GL, Gorbach SL. Intestinal flora in health and disease. Gastroenterology 1984; 86: 174–93.
21. Huys G, Vanhoutte T, Joossens M, Mahious AS, De Brandt E, Vermeire S, et al. Coamplification of eukaryotic DNA with 16s rrna gene-based pcr primers: possible consequences for population fingerprinting of complex microbial communities. Curr Microbiol 2008; 56: 553–7.
22. McFarland LV. Normal flora: diversity and functions. Microb Ecol Health Dis 2000; 12: 193–218.
23. Canny GO, McCormick BA. Bacteria in the intestine, helpful residents or enemies from within? Infect Immun 2008; 76: 3360–73.
24. Matsuguchi T, Takagi A, Matsuzaki T, Nagaoka M, Ishikawa K, Yokokura T, et al. Lipoteichoic acids from lactobacillus strains elicit strong Tumor Necrosis Factor alpha-inducing activities in macrophages through Toll-like receptor 2. Clin Diagn Lab Immunol 2003; 10: 259–66.
25. Yang D, Biragyn A, Hoover DM, Lubkowski J, Oppenheim JJ. Multiple roles of antimicrobial defensins, cathelicidins, and

eosinophil-derived neurotoxin in host defense. Annu Rev Immunol 2004; 22: 181–215.
26. Moslehi-Jenabian S, Gori K, Jespersen L. Ai-2 signalling is induced by acidic shock in probiotic strains of *Lactobacillus* spp. Int J Food Microbiol 2009; 135: 295–302.
27. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. Nature 2009; 457: 480–4.
28. Falk PG, Hooper LV, Midtvedt T, Gordon JI. Creating and maintaining the gastrointestinal ecosystem: what we know and need to know from gnotobiology. Microbiol Mol Biol Rev 1998; 62: 1157–70.
29. Neish AS. Microbes in gastrointestinal health and disease. Gastroenterology 2009; 136: 65–80.
30. Leibovici L, Arav-Boger R, Danon YL. A clinical model for diagnosis of urinary tract infection in young women. Arch Intern Med 1993; 153: 1011–5.
31. Otto GP, Sossdorf M, Claus RA, Rodel J, Menge K, Reinhart K, et al. The late phase of sepsis is characterized by an increased microbiological burden and death rate. Crit Care 2011; 15: R183.
32. Welling GW, Meijer-Severs GJ, Helmus G, van Santen E, Tonk RH, de Vries-Hospers HG, et al. The effect of ceftriaxone on the anaerobic bacterial flora and the bacterial enzymatic activity in the intestinal tract. Infection 1991; 19: 313–6.
33. Wlodarska M, Finlay BB. Host immune response to antibiotic perturbation of the microbiota. Mucosal Immunol 2010; 3: 100–3.
34. Sekirov I, Finlay BB. The role of the intestinal microbiota in enteric infection. J Physiol 2009; 587: 4159–67.
35. Croswell A, Amir E, Teggatz P, Barman M, Salzman NH. Prolonged impact of antibiotics on intestinal microbial ecology and susceptibility to enteric salmonella infection. Infect Immun 2009; 77: 2741–53.
36. Salminen MK, Rautelin H, Tynkkynen S, Poussa T, Saxelin M, Valtonen V, et al. Lactobacillus bacteremia, species identification, and antimicrobial susceptibility of 85 blood isolates. Clin Infect Dis 2006; 42: e35–44.
37. Truusalu K, Mikelsaar RH, Naaber P, Karki T, Kullisaar T, Zilmer M, et al. Eradication of *Salmonella typhimurium* infection in a murine model of typhoid fever with the combination of probiotic *Lactobacillus fermentum* ME-3 and ofloxacin. BMC Microbiol 2008; 8: 132.
38. Alberda C, Gramlich L, Meddings J, Field C, McCargar L, Kutsogiannis D, et al. Effects of probiotic therapy in critically ill patients: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr 2007; 85: 816–23.
39. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006; 444: 1027–31.
40. Mikelsaar M, Zilmer M. *Lactobacillus fermentum* ME-3-an antimicrobial and antioxidative probiotic. Micr Ecol Health Dis 2009; 1: 1–27.
41. Mikelsaar M, Stsepetova J, Hutt P, Kolk H, Sepp E, Loivukene K, et al. Intestinal *Lactobacillus* sp. is associated with some cellular and metabolic characteristics of blood in elderly people. Anaerobe 2010; 16: 240–6.
42. Margolis KL, Manson JE, Greenland P, Rodabough RJ, Bray PF, Safford M, et al. Leukocyte count as a predictor of cardiovascular events and mortality in postmenopausal women: the women's health initiative observational study. Arch Intern Med 2005; 165: 500–8.
43. Stsepetova J, Sepp E, Kolk H, Loivukene K, Songisepp E, Mikelsaar M. Diversity and metabolic impact of intestinal Lactobacillus species in healthy adults and the elderly. Br J Nutr 2011; 105: 1235–44.
44. Timmerman HM, Koning CJ, Mulder L, Rombouts FM, Beynen AC. Monostrain, multistrain and multispecies probiotics–a comparison of functionality and efficacy. Int J Food Microbiol 2004; 96: 219–33.
45. Lazar V, Balotescu C, Vassu T, Barbu V, Smarandache D, Sasarman E, et al. Experimental study on rats of the probiotic effect of some lactic acid bacteria previously selected for their in vitro capacity to interfere with salmonella enteritidis infection. Roumanian Biotech Lett 2005; 10: 2123–33.
46. Jacobsen CN, Rosenfeldt Nielsen V, Hayford AE, Moller PL, Michaelsen KF, Paerregaard A, et al. Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. By in vitro techniques and evaluation of the colonization ability of five selected strains in humans. Appl Environ Microbiol 1999; 65: 4949–56.
47. Hutt P, Shchepetova J, Loivukene K, Kullisaar T, Mikelsaar M. Antagonistic activity of probiotic lactobacilli and bifidobacteria against entero- and uropathogens. J Appl Microbiol 2006; 100: 1324–32.
48. Songisepp E, Kullisaar T, Hutt P, Elias P, Brilene T, Zilmer M, et al. A new probiotic cheese with antioxidative and antimicrobial activity. J Dairy Sci 2004; 87: 2017–23.
49. Maldonado A, Ruiz-Barba JL, Floriano B, Jimenez-Diaz R. The locus responsible for production of plantaricin s, a class IIb bacteriocin produced by *Lactobacillus plantarum* LPCO10, is widely distributed among wild-type *L. plantarum* strains isolated from olive fermentations. Int J Food Microbiol 2002; 77: 117–24.
50. Lazar V., Miyazaki Y., Hanawa T., Chifiriuc M., Ditu L., Marutescu L. et al. The influence of some probiotic cultures supernatants on the growth rate and virulence expression of several selected enteroaggregative *E. coli* (EAGGEC) clinical strains. Rom Arch Microbiol Immunol 2009; 68(4): 207–14.
51. Haller D, Autenrieth IB. Microbe-host interaction in chronic diseases. Int J Med Microbiol 2010; 300: 1–2.
52. Sanders ME. Summary of conclusions from a consensus panel of experts on health attributes of lactic cultures: significance to fluid milk products containing cultures. J Dairy Sci 1993; 76: 1819–28.
53. Gianotti L, Morelli L, Galbiati F, Rocchetti S, Coppola S, Beneduce A, et al. A randomized double-blind trial on perioperative administration of probiotics in colorectal cancer patients. World J Gastroenterol 2010; 16: 167–75.
54. Kekkonen RA, Sysi-Aho M, Seppanen-Laakso T, Julkunen I, Vapaatalo H, Oresic M, et al. Effect of probiotic *Lactobacillus rhamnosus* GG intervention on global serum lipidomic profiles in healthy adults. World J Gastroenterol 2008; 14: 3188–94.
55. Gupta V, Garg R. Probiotics. Indian J Med Microbiol 2009; 27: 202–9.
56. Ewaschuk JB, Dieleman LA. Probiotics and prebiotics in chronic inflammatory bowel diseases. World J Gastroenterol 2006; 12: 5941–50.
57. Hoveyda N, Heneghan C, Mahtani KR, Perera R, Roberts N, Glasziou P. A systematic review and meta-analysis: probiotics in the treatment of irritable bowel syndrome. BMC Gastroenterol 2009; 9: 15.
58. Haller D, Antoine JM, Bengmark S, Enck P, Rijkers GT, Lenoir-Wijnkoop I. Guidance for substantiating the evidence for beneficial effects of probiotics: probiotics in chronic inflammatory bowel disease and the functional disorder irritable bowel syndrome. J Nutr 2010; 140: 690S–7S.
59. Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S, Black RE. Efficacy of probiotics in prevention of acute diarrhoea: a meta-

Citation: Microbial Ecology in Health & Disease 2011, **22**: 10128 - DOI: 10.3402/mehd.v22i0.10128

analysis of masked, randomised, placebo-controlled trials. Lancet Infect Dis 2006; 6: 374–82.
60. Guandalini S. Probiotics for children with diarrhea: an update. J Clin Gastroenterol 2008; 42(Suppl 2): S53–7.
61. Weizman Z, Asli G, Alsheikh A. Effect of a probiotic infant formula on infections in child care centers: comparison of two probiotic agents. Pediatrics 2005; 115: 5–9.
62. Isolauri E, Rautava S, Kalliomaki M, Kirjavainen P, Salminen S. Probiotic research: learn from the evidence. Allergy 2002; 57: 1076–7; author reply 1078.
63. Szajewska H, Kotowska M, Mrukowicz JZ, Armanska M, Mikolajczyk W. Efficacy of *Lactobacillus rhamnosus* GG in prevention of nosocomial diarrhea in infants. J Pediatr 2001; 138: 361–5.
64. Szajewska H, Skorka A, Ruszczynski M, Gieruszczak-Bialek D. Meta-analysis: *Lactobacillus rhamnosus* GG for treating acute diarrhoea in children. Aliment Pharmacol Ther 2007; 25: 871–81.
65. Lu R, Fasano S, Madayiputhiya N, Morin NP, Nataro J, Fasano A. Isolation, identification, and characterization of small bioactive peptides from *Lactobacillus rhamnosus* GG conditional media that exert both anti-gram-negative and gram-positive bactericidal activity. J Pediatr Gastroenterol Nutr 2009; 49: 23–30.
66. Basu S, Chatterjee M, Ganguly S, Chandra PK. Efficacy of *Lactobacillus rhamnosus* GG in acute watery diarrhoea of indian children: a randomised controlled trial. J Paediatr Child Health 2007; 43: 837–42.
67. Hojsak I, Snovak N, Abdovic S, Szajewska H, Misak Z, Kolacek S. Lactobacillus GG in the prevention of gastrointestinal and respiratory tract infections in children who attend day care centers: a randomized, double-blind, placebo-controlled trial. Clin Nutr 2010; 29: 312–6.
68. Mastretta E, Longo P, Laccisaglia A, Balbo L, Russo R, Mazzaccara A, et al. Effect of Lactobacillus GG and breastfeeding in the prevention of rotavirus nosocomial infection. J Pediatr Gastroenterol Nutr 2002; 35: 527–31.
69. Hatakka K, Savilahti E, Ponka A, Meurman JH, Poussa T, Nase L, et al. Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. BMJ 2001; 322: 1327.
70. Sur D, Manna B, Niyogi SK, Ramamurthy T, Palit A, Nomoto K et al. Role of probiotic in preventing acute diarrhoea in children: a community-based, randomized, double-blind placebo-controlled field trial in an urban slum. Epidemiol Infect 2010: 1–8.
71. Dubey AP, Rajeshwari K, Chakravarty A, Famularo G. Use of VSL#3 in the treatment of rotavirus diarrhea in children: preliminary results. J Clin Gastroenterol 2008; 42(Suppl 3, Pt 1): S126–9.
72. Grandy G, Medina M, Soria R, Teran CG, Araya M. Probiotics in the treatment of acute rotavirus diarrhoea. A randomized, double-blind, controlled trial using two different probiotic preparations in bolivian children. BMC Infect Dis 2010; 10: 253.
73. Arvola T, Laiho K, Torkkeli S, Mykkanen H, Salminen S, Maunula L, et al. Prophylactic Lactobacillus GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. Pediatrics 1999; 104: e64.
74. Zoppi G, Cinquetti M, Benini A, Bonamini E, Minelli EB. Modulation of the intestinal ecosystem by probiotics and lactulose in children during treatment with ceftriaxone. Curr Ther Res 2001; 62: 418–35.
75. Thomas MR, Litin SC, Osmon DR, Corr AP, Weaver AL, Lohse CM. Lack of effect of Lactobacillus GG on antibiotic-associated diarrhea: a randomized, placebo-controlled trial. Mayo Clin Proc 2001; 76: 883–9.
76. Wullt M, Hagslatt ML, Odenholt I. *Lactobacillus plantarum* 299v for the treatment of recurrent Clostridium difficile-associated diarrhoea: a double-blind, placebo-controlled trial. Scand J Infect Dis 2003; 35: 365–7.
77. Miller M. The fascination with probiotics for *Clostridium difficile* infection: lack of evidence for prophylactic or therapeutic efficacy. Anaerobe 2009; 15: 281–4.
78. McFarland LV. Evidence-based review of probiotics for antibiotic-associated diarrhea and *Clostridium difficile* infections. Anaerobe 2009; 15: 274–80.
79. Bauer MP, van Dissel JT. Alternative strategies for *Clostridium difficile* infection. Int J Antimicrob Agents 2009; 33(Suppl 1): S51–6.
80. Mikelsaar M, Hütt P, Kullisaar T, Zilmer K, Zilmer M. Double benefit claims for antimicrobial and antioxidative probiotic. Micr Ecol Health Dis 2008; 20: 184–8.
81. Cremonini F, Di Caro S, Covino M, Armuzzi A, Gabrielli M, Santarelli L, et al. Effect of different probiotic preparations on anti-helicobacter *pylori* therapy-related side effects: a parallel group, triple blind, placebo-controlled study. Am J Gastroenterol 2002; 97: 2744–9.
82. Wang KY, Li SN, Liu CS, Perng DS, Su YC, Wu DC, et al. Effects of ingesting lactobacillus- and bifidobacterium-containing yogurt in subjects with colonized helicobacter pylori. Am J Clin Nutr 2004; 80: 737–41.
83. Kamiya S, Takahashi M, Manzoku T, Oka K, Osaki T, Hanawa T, et al. Probiotics and *Helicobacter pylori*. Micr Ecol Health Dis 2006; 18: 177–80.
84. Lee JS, Paek NS, Kwon OS, Hahm KB. Anti-inflammatory actions of probiotics through activating suppressor of cytokine signaling (SOCS) expression and signaling in Helicobacter pylori infection: a novel mechanism. J Gastroenterol Hepatol 2010; 25: 194–202.
85. Hutt P, Andreson H, Kullisaar T, Vihalemm T, Unt E, Kals J, et al. Effects of a synbiotic product on blood antioxidative activity in subjects colonized with Helicobacter pylori. Lett Appl Microbiol 2009; 48: 797–800.
86. Stocker R, Keaney JF, Jr. Role of oxidative modifications in atherosclerosis. Physiol Rev 2004; 84: 1381–478.
87. Truusalu K, Kullisaar T, Hutt P, Mahlapuu R, Aunapuu M, Arend A, et al. Immunological, antioxidative, and morphological response in combined treatment of ofloxacin and *Lactobacillus fermentum* ME-3 probiotic in Salmonella typhimurium murine model. Apmis 2010; 118: 864–72.
88. Helwig U, Lammers KM, Rizzello F, Brigidi P, Rohleder V, Caramelli E, et al. *Lactobacilli, Bifidobacteria* and *E. coli* Nissle induce pro- and anti-inflammatory cytokines in peripheral blood mononuclear cells. World J Gastroenterol 2006; 12: 5978–86.
89. Dinan TG, Cryan J, Shanahan F, Keeling PW, Quigley EM. Ibs: an epigenetic perspective. Nat Rev Gastroenterol Hepatol 2010; 7: 465–71.
90. Noor SO, Ridgway K, Scovell L, Kemsley EK, Lund EK, Crawford J, et al. Ulcerative colitis and irritable bowel patients exhibit distinct abnormalities of the gut microbiota. BMC Gastroenterol 2010; 10: 134.
91. O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, et al. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. Gastroenterology 2005; 128: 541–51.
92. Ligaarden SC, Axelsson L, Naterstad K, Lydersen S, Farup PG. A candidate probiotic with unfavourable effects in subjects with irritable bowel syndrome: a randomised controlled trial. BMC Gastroenterol 2010; 10: 16.

93. Whorwell PJ, Altringer L, Morel J, Bond Y, Charbonneau D, O'Mahony L, et al. Efficacy of an encapsulated probiotic *Bifidobacterium* infantis 35624 in women with irritable bowel syndrome. Am J Gastroenterol 2006; 101: 1581–90.
94. Gawronska A, Dziechciarz P, Horvath A, Szajewska H. A randomized double-blind placebo-controlled trial of *Lactobacillus* GG for abdominal pain disorders in children. Aliment Pharmacol Ther 2007; 25: 177–84.
95. Bausserman M, Michail S. The use of Lactobacillus GG in irritable bowel syndrome in children: a double-blind randomized control trial. J Pediatr 2005; 147: 197–201.
96. Niedzielin K, Kordecki H, Birkenfeld B. A controlled, double-blind, randomized study on the efficacy of *Lactobacillus plantarum* 299v in patients with irritable bowel syndrome. Eur J Gastroenterol Hepatol 2001; 13: 1143–7.
97. Nobaek S, Johansson ML, Molin G, Ahrne S, Jeppsson B. Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. Am J Gastroenterol 2000; 95: 1231–8.
98. Brenner DM, Chey WD. *Bifidobacterium infantis* 35624: a novel probiotic for the treatment of irritable bowel syndrome. Rev Gastroenterol Disord 2009; 9: 7–15.
99. Agrawal A, Houghton LA, Reilly B, Morris J, Whorwell PJ. Bloating and distension in irritable bowel syndrome: the role of gastrointestinal transit. Am J Gastroenterol 2009; 104: 1998–2004.
100. Saggioro A. Probiotics in the treatment of irritable bowel syndrome. J Clin Gastroenterol 2004; 38: S104–6.
101. Guyonnet D, Chassany O, Ducrotte P, Picard C, Mouret M, Mercier CH, et al. Effect of a fermented milk containing Bifidobacterium animalis DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults in primary care: a multicentre, randomized, double-blind, controlled trial. Aliment Pharmacol Ther 2007; 26: 475–86.
102. Kajander K, Myllyluoma E, Rajilic-Stojanovic M, Kyronpalo S, Rasmussen M, Jarvenpaa S, et al. Clinical trial: Multispecies probiotic supplementation alleviates the symptoms of irritable bowel syndrome and stabilizes intestinal microbiota. Aliment Pharmacol Ther 2008; 27: 48–57.
103. Lyra A, Krogius-Kurikka L, Nikkila J, Malinen E, Kajander K, Kurikka K, et al. Effect of a multispecies probiotic supplement on quantity of irritable bowel syndrome-related intestinal microbial phylotypes. BMC Gastroenterol 2010; 10: 110.
104. Drouault-Holowacz S, Bieuvelet S, Burckel A, Cazaubiel M, Dray X, Marteau P. A double blind randomized controlled trial of a probiotic combination in 100 patients with irritable bowel syndrome. Gastroenterol Clin Biol 2008; 32: 147–52.
105. Kim HJ, Camilleri M, McKinzie S, Lempke MB, Burton DD, Thomforde GM, et al. A randomized controlled trial of a probiotic, VSL#3, on gut transit and symptoms in diarrhoea-predominant irritable bowel syndrome. Aliment Pharmacol Ther 2003; 17: 895–904.
106. Kim HJ, Vazquez Roque MI, Camilleri M, Stephens D, Burton DD, Baxter K, et al. A randomized controlled trial of a probiotic combination VSL#3 and placebo in irritable bowel syndrome with bloating. Neurogastroenterol Motil 2005; 17: 687–96.
107. Guandalini S, Magazzu G, Chiaro A, La Balestra V, Di Nardo G, Gopalan S, et al. VSL#3 improves symptoms in children with irritable bowel syndrome: a multicenter, randomized, placebo-controlled, double-blind, crossover study. J Pediatr Gastroenterol Nutr 2010; 51: 24–30.
108. World Gastroenterology Organisation. (2009). WGO Practice Guideline. Irritable bowel syndrome: a global perspective. Milwaukee, USA: WGO; pp. 1–20.
109. McFarland LV, Dublin S. Meta-analysis of probiotics for the treatment of irritable bowel syndrome. World J Gastroenterol 2008; 14: 2650–61.
110. McFarland LV. State-of-the-art of irritable bowel syndrome and inflammatory bowel disease research in 2008. World J Gastroenterol 2008; 14: 2625–9.
111. Swidsinski A, Loening-Baucke V, Herber A. Mucosal flora in Crohn's disease and ulcerative colitis-an overview. J Physiol Pharmacol 2009; 60(Suppl 6): 61–71.
112. Strus M, Gosiewski T, Fyderek K, Wedrychowicz A, Kowalska-Duplaga K, Kochan P, et al. A role of hydrogen peroxide producing commensal bacteria present in colon of adolescents with inflammatory bowel disease in perpetuation of the inflammatory process. J Physiol Pharmacol 2009; 60(Suppl 6): 49–54.
113. Sonnenborn U, Schulze J. The non-pathogenic *Escherichia coli* strain Nissle 1917-features of a versatile probiotic. Microb Ecol Health Dis 2009; 21: 122–58.
114. Hafez M, Hayes K, Goldrick M, Warhurst G, Grencis R, Roberts IS. The K5 capsule of *Escherichia coli* strain Nissle 1917 is important in mediating interactions with intestinal epithelial cells and chemokine induction. Infect Immun 2009; 77: 2995–3003.
115. Kruis W, Fric P, Pokrotnieks J, Lukas M, Fixa B, Kascak M, et al. Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. Gut 2004; 53: 1617–23.
116. Campieri M, Gionchetti P. Bacteria as the cause of ulcerative colitis. Gut 2001; 48: 132–5.
117. Gionchetti P, Amadini C, Rizzello F, Venturi A, Poggioli G, Campieri M. Diagnosis and treatment of pouchitis. Best Pract Res Clin Gastroenterol 2003; 17: 75–87.
118. Gionchetti P, Rizzello F, Poggioli G, Pierangeli F, Laureti S, Morselli C, et al. Oral budesonide in the treatment of chronic refractory pouchitis. Aliment Pharmacol Ther 2007; 25: 1231–6.
119. Guslandi M. Of germs in inflammatory bowel disease and of how to fight them. J Gastroenterol Hepatol 2003; 18: 115–6.
120. Guslandi M, Mezzi G, Sorghi M, Testoni PA. *Saccharomyces boulardii* in maintenance treatment of crohn's disease. Dig Dis Sci 2000; 45: 1462–4.
121. Kato K, Mizuno S, Umesaki Y, Ishii Y, Sugitani M, Imaoka A, et al. Randomized placebo-controlled trial assessing the effect of bifidobacteria-fermented milk on active ulcerative colitis. Aliment Pharmacol Ther 2004; 20: 1133–41.
122. Huynh HQ, deBruyn J, Guan L, Diaz H, Li M, Girgis S, et al. Probiotic preparation VSL#3induces remission in children with mild to moderate acute ulcerative colitis: a pilot study. Inflamm Bowel Dis 2009; 15: 760–8.
123. Pascarella F, Giannetti E, Quaglietta L, Baldassano RN, Staiano A. Effect of a probiotic preparation (VSL#3) on induction and maintenance of remission in children with ulcerative colitis. Am J Gastroenterol 2009; 104: 437–43.
124. Tursi A, Brandimarte G, Giorgetti GM, Forti G, Modeo ME, Gigliobianco A. Low-dose balsalazide plus a high-potency probiotic preparation is more effective than balsalazide alone or mesalazine in the treatment of acute mild-to-moderate ulcerative colitis. Med Sci Monit 2004; 10: PI126–31.
125. Bibiloni R, Fedorak RN, Tannock GW, Madsen KL, Gionchetti P, Campieri M, et al. VSL#3probiotic-mixture induces remission in patients with active ulcerative colitis. Am J Gastroenterol 2005; 100: 1539–46.

Citation: Microbial Ecology in Health & Disease 2011, **22**: 10128 - DOI: 10.3402/mehd.v22i0.10128

126. Sood A, Midha V, Makharia GK, Ahuja V, Singal D, Goswami P, et al. The probiotic preparation, VSL#3 induces remission in patients with mild-to-moderately active ulcerative colitis. Clin Gastroenterol Hepatol 2009; 7: 1202–9–1209 e1.
127. Ishikawa H, Akedo I, Umesaki Y, Tanaka R, Imaoka A, Otani T. Randomized controlled trial of the effect of bifidobacteria-fermented milk on ulcerative colitis. J Am Coll Nutr 2003; 22: 56–63.
128. Gionchetti P, Rizzello F, Venturi A, Brigidi P, Matteuzzi D, Bazzocchi G, et al. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. Gastroenterology 2000; 119: 305–9.
129. Gionchetti P, Rizzello F, Morselli C, Poggioli G, Tambasco R, Calabrese C et al. High-dose probiotics for the treatment of active pouchitis. Dis Colon Rectum 2007; 50: 2075–82, discussion 2082–4.
130. Mimura T, Rizzello F, Helwig U, Poggioli G, Schreiber S, Talbot IC, et al. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. Gut 2004; 53: 108–14.
131. Brigidi P, Vitali B, Swennen E, Bazzocchi G, Matteuzzi D. Effects of probiotic administration upon the composition and enzymatic activity of human fecal microbiota in patients with irritable bowel syndrome or functional diarrhea. Res Microbiol 2001; 152: 735–41.
132. Brigidi P, Vitali B, Swennen E, Altomare L, Rossi M, Matteuzzi Dz. Specific detection of bifidobacterium strains in a pharmaceutical probiotic product and in human feces by polymerase chain reaction. Syst Appl Microbiol 2000; 23: 391–9.
133. Lammers KM, Brigidi P, Vitali B, Gionchetti P, Rizzello F, Caramelli E, et al. Immunomodulatory effects of probiotic bacteria DNA: Il-1 and il-10 response in human peripheral blood mononuclear cells. FEMS Immunol Med Microbiol 2003; 38: 165–72.
134. Hart AL, Lammers K, Brigidi P, Vitali B, Rizzello F, Gionchetti P, et al. Modulation of human dendritic cell phenotype and function by probiotic bacteria. Gut 2004; 53: 1602–9.
135. Bibiloni R, Simon MA, Albright C, Sartor B, Tannock GW. Analysis of the large bowel microbiota of colitic mice using pcr/dgge. Lett Appl Microbiol 2005; 41: 45–51.
136. Menningen R, Nolte K, Rijcken E. Probiotic mixture vsl#3 protects the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis in a murine model of colitis. Am J Physiol Gastrointest Liver Physiol 2009; 296: G1140–9.
137. Kuhbacher T, Ott SJ, Helwig U, Mimura T, Rizzello F, Kleessen B, et al. Bacterial and fungal microbiota in relation to probiotic therapy (VSL#3) in pouchitis. Gut 2006; 55: 833–41.
138. Pronio A, Montesani C, Butteroni C, Vecchione S, Mumolo G, Vestri A, et al. Probiotic administration in patients with ileal pouch-anal anastomosis for ulcerative colitis is associated with expansion of mucosal regulatory cells. Inflamm Bowel Dis 2008; 14: 662–8.
139. Bamias G, Nyce MR, De La Rue SA, Cominelli F. New concepts in the pathophysiology of inflammatory bowel disease. Ann Intern Med 2005; 143: 895–904.
140. Otte JM, Mahjurian-Namari R, Brand S, Werner I, Schmidt WE, Schmitz F. Probiotics regulate the expression of cox-2 in intestinal epithelial cells. Nutr Cancer 2009; 61: 103–13.
141. Bousvaros A, Guandalini S, Baldassano RN, Botelho C, Evans J, Ferry GD, et al. A randomized, double-blind trial of Lactobacillus GG versus placebo in addition to standard maintenance therapy for children with crohn's disease. Inflamm Bowel Dis 2005; 11: 833–9.
142. Schultz M, Timmer A, Herfarth HH, Sartor RB, Vanderhoof JA, Rath HC. Lactobacillus GG in inducing and maintaining remission of Crohn's disease. BMC Gastroenterol 2004; 4: 5.
143. Marteau P, Lemann M, Seksik P, Laharie D, Colombel JF, Bouhnik Y, et al. Ineffectiveness of *Lactobacillus johnsonii* La1 for prophylaxis of postoperative recurrence in crohn's disease: a randomised, double blind, placebo controlled getaid trial. Gut 2006; 55: 842–7.
144. Rijkers GT, Bengmark S, Enck P, Haller D, Herz U, Kalliomaki M, et al. Guidance for substantiating the evidence for beneficial effects of probiotics: current status and recommendations for future research. J Nutr 2010; 140: 671S–6S.
145. Chouraqui JP, Van Egroo LD, Fichot MC. Acidified milk formula supplemented with *Bifidobacterium lactis*: impact on infant diarrhea in residential care settings. J Pediatr Gastroenterol Nutr 2004; 38: 288–92.
146. Narayanappa D. Randomized double blinded controlled trial to evaluate the efficacy and safety of bifilac in patients with acute viral diarrhea. Indian J Pediatr 2008; 75: 709–13.
147. Lin JS, Chiu YH, Lin NT, Chu CH, Huang KC, Liao KW, et al. Different effects of probiotic species/strains on infections in preschool children: a double-blind, randomized, controlled study. Vaccine 2009; 27: 1073–9.
148. Tursi A. Balsalazide plus high-potency probiotic preparation (VSL#3) in the treatment of acute mild-to-moderate ulcerative colitis and uncomplicated diverticulitis of the colon. J Clin Gastroenterol 2008; 42(Suppl 3, Pt 1): S119–22.
149. Gionchetti P, Rizzello F, Helwig U, Venturi A, Lammers KM, Brigidi P, et al. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. Gastroenterology 2003; 124: 1202–9.

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