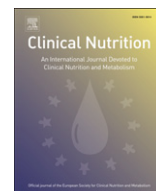




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Review

Therapeutical use of probiotic formulations in clinical practice

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SUMMARY

Background & Aims: The spreading of gastrointestinal diseases is growing all over the world. Although for some of them an effective therapeutic approach has been found, palliation rather than cure is very frequent due to a partial knowledge of their aetiology and pathogenesis. This review, analyzing the main clinical studies, aims at being a state of the art update of the use of probiotic formulations in daily practice.

Methods: In this review we include all the most significant clinical trials involving the use of probiotic formulations for the treatment of several pathologies.

Results: Dysbiosis has been observed in irritable bowel syndrome patients. Probiotics may exert a beneficial effect on Crohn's disease affected patients who have shown gut microbiota antigens and altered wall permeability. Moreover some probiotic formulations seem to enhance the therapy for *Helicobacter Pylori* reducing its pathogenic potential. Intestinal ecology imbalance has been also linked to cancer induction, allergy, skin and urogenital diseases. In addition probiotics administration seems to be particularly useful to ease post-operative complications.

Conclusion: Further future clinical trials, involving large numbers of patients, will be mandatory to achieve definite evidence of the preventive and curative role of probiotics in medical practice.

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1. Introduction

The gastrointestinal (GI) tract starts from the mouth, involves the oesophagus, the stomach, the small and large intestine and ends at the anus (Fig. 1). The GI tract has four main functions: ingestion, digestion, absorption, and defecation. In a normal human adult male, the GI tract is approximately 6.5 m long and can be divided into the upper tract which is made up of mouth, pharynx, oesophagus and stomach and the lower tract which is made up of the small intestine (duodenum, jejunum and ileum), the large intestine (cecum, colon and rectum) and anus.

1.1. Normal bacterial flora of the GI tract

Aerobic and anaerobic bacteria, yeast and fungi live into the GI tract which has more than 400 m² of surface area. All these organisms in a healthy intestinal tract live in a natural balance called symbiosis. There are more than 2000 species of commensal bacterial organisms within our bodies, the vast majority in the gut.¹

In fact the mammalian gut is considered one of the most densely populated ecosystems on Earth with a bacterial load in the region of 10¹² organisms/g of fecal material in the large intestine.² The several species of microorganisms in the adult human gut are known as the microbiota which may contain nearly 100 times the number of genes contained within the human genome. The genome of these collective organisms is called the microbiome. The longitudinal distribution of intestinal microorganisms increases in density progressing from the small bowel to colon (Fig. 2). Anaerobic bacteria benefit the host by performing metabolic functions including fermentation, providing short-chain fatty acids (SCFAs), producing vitamins, adding to the trophic action of the epithelium and aiding in the development of the immune system.³

The stomach harbours only very few bacteria (mainly acid-tolerant lactobacilli) because of the acidity due to the gastric juice. In the colon there are about a million times more bacteria than in the stomach due to a higher exposure to nutrients, slow transit, and low-redox potential.

About 50% of feces are made up of bacteria. While new bacteria are produced, the old ones are flushed out of the intestine and they will be finally present in the feces. The different strains of bacteria living in the GI tract are summarized in Table 1.

The normal flora which colonizes the GI tract exert several functions: 1) synthesizing and excreting vitamins in excess of their

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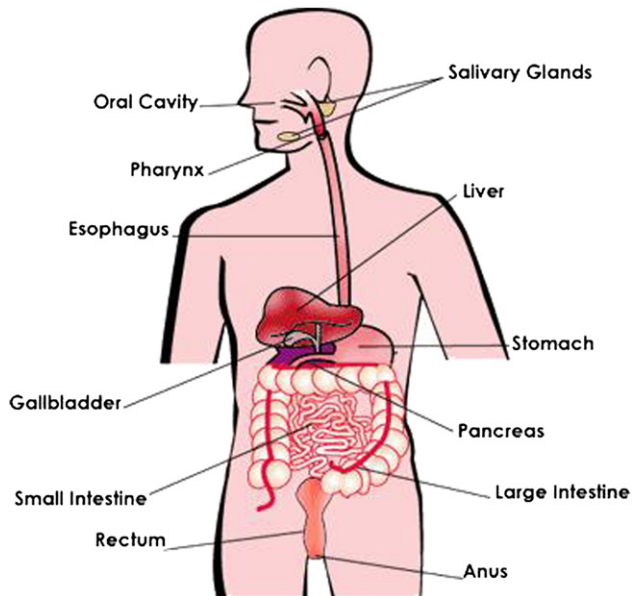


Fig. 1. Anatomy of the gastrointestinal tract.

Table 1
GI flora components.⁹

Oral cavity (saliva)	Streptococcus, Veillonella, Lactobacillus, Bifidobacterium, Fusobacterium, Staphylococcus, Bacteroides, Corynebacterium, Neisseria, Yeasts
Stomach	Streptococcus, Lactobacillus, Bifidobacterium, Bacteroides, Enterobacteriaceae, Yeasts
Duodenum	Streptococcus, Lactobacillus, Veillonella, Bacteroides, Bifidobacterium, Enterobacteriaceae, Yeasts
Ileum	Streptococcus, Lactobacillus, Bifidobacterium, Bacteroides, Clostridium, Enterobacteriaceae, Yeasts
Colon	Bacteroides, Eubacterium, Ruminococcus, Coprococcus, Peptostreptococcus, Bifidobacterium, Streptococcus, Enterobacteriaceae, Lactobacillus, Clostridium
Feces	Bacteroides, Eubacterium, Ruminococcus, Coprococcus, Peptostreptococcus, Bifidobacterium, Streptococcus, Enterobacteriaceae, Clostridium, Lactobacillus, Veillonella, Yeasts

colonize it during its passage through the birth canal. The host genotype is important in determining the populations of intestinal organisms which, after birth, are influenced by the baby exposure to numerous bacteria from the environment (e.g., skin, mouth, mother's milk). This initial microbiota is relatively unstable and changes during the initial period of life.

Reinhardt et al.⁸ report that infants are initially colonized by facultative anaerobes such as enterobacteria and gram-positive cocci, which are thought to create a reduced environment favorable for the establishment of obligate anaerobes, including *Bacteroides*, *Bifidobacterium*, and *Clostridium*.

Tennison et al.³ report that the type of birth delivery has a significant impact on the development of the gut microbiota in fact vaginal delivery allows infant exposure to maternal bacteria, i.e. the longer the birth process is the greater exposure you have, while infants born by cesarean delivery acquire bacteria by exposure to the mother as well as isolates transferred by nursing staff, other infants, air and equipment. Following birth, oral and cutaneous bacteria from the mother will be mechanically transferred to the infant by suckling, kissing and caressing. Moreover the Authors observed that breast feeding exposes the infant to bacteria, especially bifidobacteria, from milk ducts, nipple, and surrounding skin. Breast milk contains antimicrobial components and growth factors that stimulate the development and maturation of the intestinal mucosa. The

own needs, which can be absorbed as nutrients by their hosts (enteric bacteria secrete Vitamin K and Vitamin B12, and lactic acid bacteria produce certain B-vitamins); 2) preventing the pathogens colonization by competing for attachment sites or essential nutrients; 3) being likely to produce substances which inhibit or kill non indigenous species; 4) stimulating the development of certain tissues, i.e., the caecum and certain lymphatic tissues (Peyer's patches) in the GI tract; 5) stimulating the production of natural antibodies; 6) producing a variety of substances ranging from relatively non-specific fatty acids and peroxides to highly specific bacteriocins which inhibit or kill other bacteria.^{5,6} Gut microbiota are also known to influence energy balance and in turn, emerging evidence demonstrates the importance of gut microbiota in the pathophysiology of obesity.⁷

The initial acquisition of intestinal microbiota plays a key role in the development of immune processes and protection against pathogens. The human fetus is sterile in utero and microbes

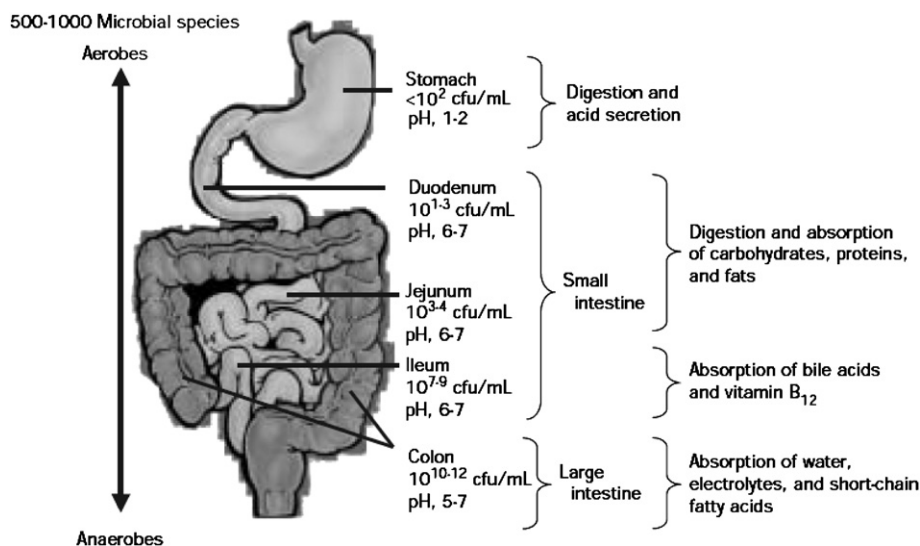


Fig. 2. Picture reported from "Dibaise et al.⁴ Gut Microbiota and Its Possible Relationship With Obesity". Key physiologic and microbiological features of the gut, relative concentrations of bacteria and the pH at various locations within the adult gut are shown (cfu = colony-forming unit).

Authors conclude that the development of infantile intestinal microbiota involves the initial vaginal exposure, transmission during neonatal care, subsequent breast feeding and finally, after the introduction of solid food, the bacterial profiles of breast and formula fed infants become similar to that of adults by the 1–2 years of life (conversion to adult microbiota).

1.2. Dysbiosis

The GI tract bacteria are all essential for its normal physiology but they are also potentially pathogenic. Dysbiosis occurs when the GI bacterial microflora ecology equilibrium is altered leading to an imbalance that can involve metabolic or immunologic feedback of the host. Round et al.¹⁰ describe a healthy microbiota as a balanced composition of many classes of bacteria such as symbionts (organisms with known health promoting functions), commensals (permanent residents which provide no benefit or detriment to the host) and pathobionts (permanent residents of the microbiota with the potential to induce pathology). The Authors underline that, in conditions of dysbiosis, there is an unnatural shift in the composition of the microbiota, which results in either a reduction in the numbers of symbionts and/or an increase in the numbers of pathobionts (Fig. 3). This condition can lead to mild subjective discomfort and even disease condition (e.g., dysbiosis can lead to non-specific inflammation which may predispose certain genetically susceptible people to inflammatory disease). The good balance of GI tract microflora is responsible for a lot of functions within the body such as vitamin production, hormonal activities, immunity and detoxification processes. In case of bacterial imbalance a release of toxic metabolic products is induced, followed by flatulence, bloating, intestinal pain and inflammation, cramping and constipation and/or diarrhea. It is important that a correct dysbiosis diagnosis is made because the only treatment of the symptoms will not lead to the solving of the real problem and will bring the patient to develop other diseases. In fact, intestinal dysbiosis should be considered as a possible cause, or a contributing factor in patients who have asthma, bronchitis, allergies, autoimmune disorders, breast and colon cancer, unexplained fatigue or neuropsychiatric symptoms. Systemic effects such as halitosis, adrenal stress, diarrhea, candida, leaky gut syndrome, colon cancer and breast cancer can be

considered a consequence of dysbiosis. Possible factors which can contribute to dysbiosis are: host genetics, lifestyle, exposure to microorganisms and medical practices, diet and stress. Birth in the sterile environment of hospitals can protect from exposure to dangerous pathogens, but can also prevent early exposure to health promoting bacteria. Overuse of vaccination and antibiotics, which do not distinguish between pathogenic or symbiotic microorganisms, could adversely alter the microbiota.¹⁰ Many chronic and degenerative diseases such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), rheumatoid arthritis, and ankylosing spondylitis are considered today to be linked to abnormalities in the bacteria microflora ecology (Fig. 4).

1.3. Probiotics, prebiotics and synbiotics

The term probiotic derives from the Greek/Latin word “pro” and the Greek word “bios”, meaning for life. The concept of probiotic was probably firstly introduced by the Russian Nobel laureate Elie Metchnikoff in 1907 (“The Prolongation of Life: Optimistic Studies”) where he proposed the idea that ingesting microbes could have beneficial effects for human beings, especially to treat digestive diseases.¹¹ The term “probiotic” was firstly used in 1965, by Lilly and Stillwell, to describe substances secreted by one organism which stimulates the growth of another. The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations have defined probiotics as “live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host.” Probiotics consist of bacteria or yeasts and can be considered functional foods that can re-colonize and restore the microflora symbiosis of the intestinal tract. Most probiotics are bacteria similar to those naturally found in people’s guts, especially in those of breastfed infants because they are known to have natural protection against many diseases. Most often, the bacteria come from two groups, *Lactobacillus* or *Bifidobacterium*. Each group involves different species (*Lactobacillus acidophilus*, *Bifidobacterium bifidus* etc.) which include different strains. Some common probiotics, such as *Saccharomyces boulardii*, are yeasts. Some examples of bacteria used in probiotics formulations are *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Lactobacillus salivarius*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus sporogenes*, *Bifidobacterium bifidum*, *Bifidobacteria longum*, *Bifidobacteria infantis*, *Streptococcus thermophilus* and *Homeostatic Soil Organisms* (HSO’s). A good probiotic must possess the following requirements: 1) being able to adhere to cells; 2) excluding or reducing pathogenic adherence; 3) being able to persist, multiply and produce acids, hydrogen peroxide and bacteriocins antagonistic to pathogen growth; 4) being able to be safe, noninvasive, noncarcinogenic and non-pathogenic; 5) being able to coaggregate as to form a normal balanced flora.¹² For an adequate amount of health benefits, a dose of 5 billion colony-forming unit (CFU) has been recommended for at least 5 days (5×10^9 CFU/day).¹³ Probiotics are available in food and dietary supplements (for example, capsules, tablets and powders) and in some other forms as well. In probiotic foods and supplements, the bacteria may have been present originally or added during preparation. Probiotics must survive gastric and bile acids in order to reach the intestinal tract, colonize the host epithelium, and exhibit a beneficial effect. Most conventional forms of lactobacilli-type probiotics are nonspore forming and, therefore, are inactivated by bile and low gastric pH. In addition, probiotics selected for commercial use must survive industrial manufacturing and storage to ensure long-term viability and activity. Most cells of conventional lactobacilli die at 70 °C, while spore-bearing lactic acid-forming bacteria do not show a decrease in viable cells even after heating in saline at 85 °C for 30 min. Therefore the survival of

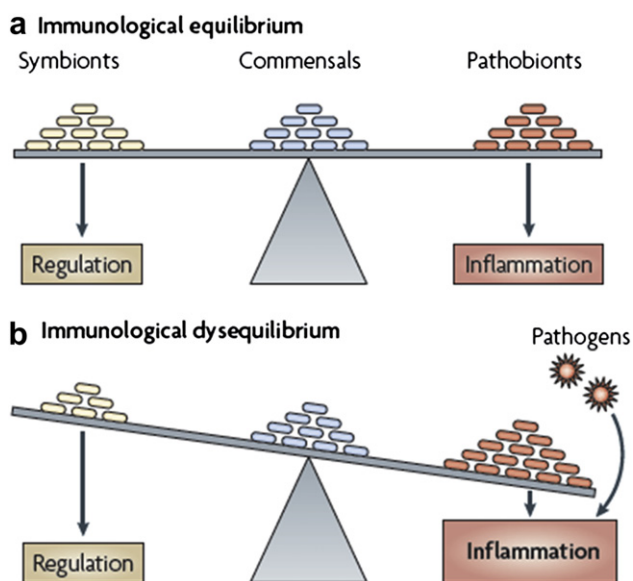


Fig. 3. Figure reported from Round et al.¹⁰ The gut microbiota shapes intestinal immune responses during health and disease.

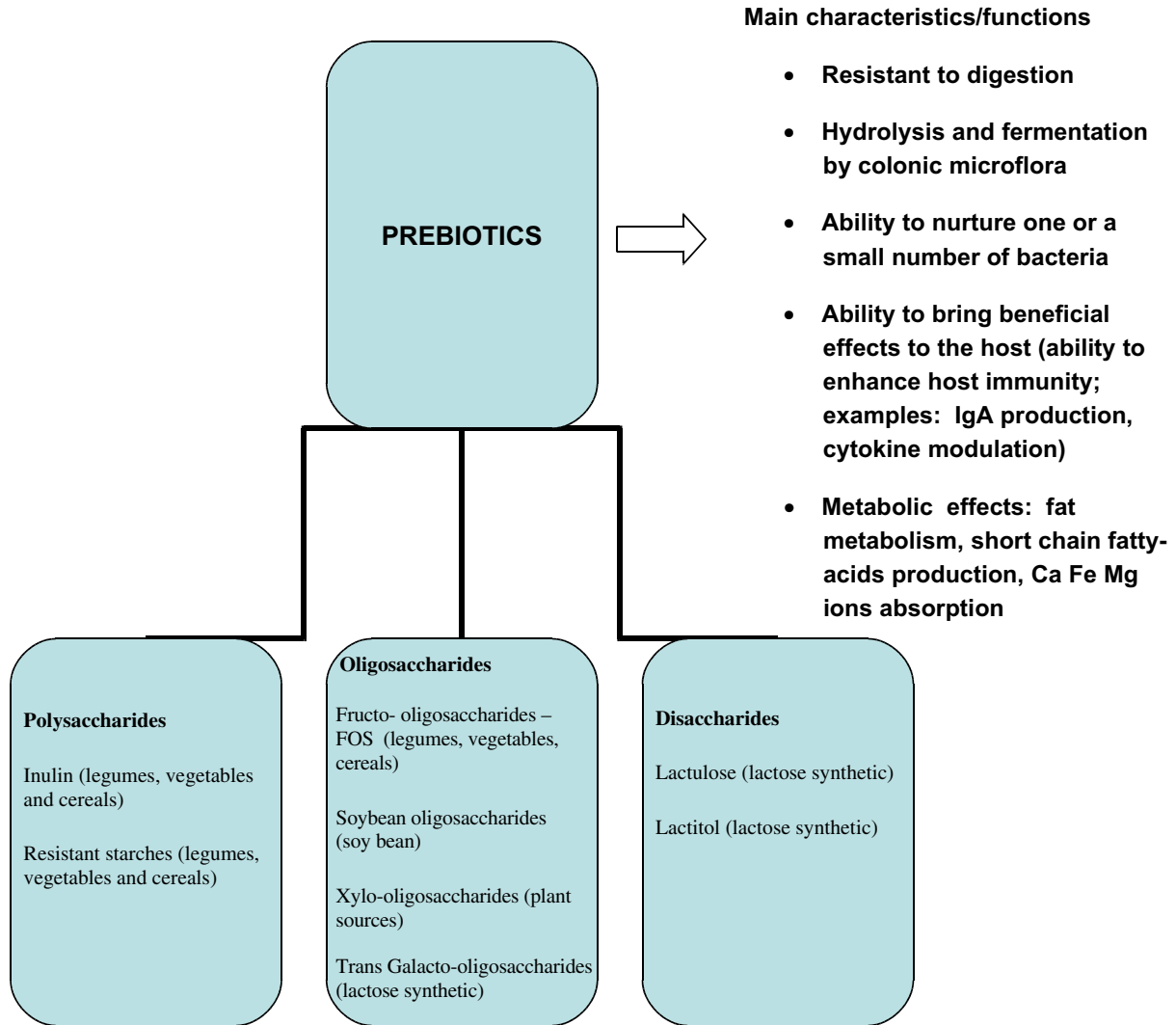


Fig. 4. Common prebiotics supplements and their main functions.

probiotics through the GI tract is strongly affected by stomach acidity, the length of exposure to acid, the concentration of and length of exposure to bile salts and the level of bile salt hydrolase activity. Probiotics must not be confused with prebiotics, non-digestible food ingredients that selectively stimulate the growth and/or activity of beneficial microorganisms already present in people's colons. The term prebiotic was first introduced by Gibson and Roberfroid in 1995 and is defined as "a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health".¹¹ When probiotics and prebiotics are mixed together, they form a synbiotic. A synbiotic is a supplement that contains both a prebiotic and a probiotic that work together to keep healthy the bacterial microflora of the human intestine. Fermented milks (yogurt and kefir) are considered to be true synbiotic products, that is, functional foods, since they contribute to restore the normal bacterial microflora also supplying the food it needs to survive. However not all these products promote symbiosis. The best synbiotic combinations currently available include bifidobacteria and fructooligosaccharides (FOS), *Lactobacillus GG* and inulins, and bifidobacteria and lactobacilli with FOS or inulins.

2. Short chain fructooligosaccharides(sc-FOS)

FOS are extracted from fruits and vegetables like bananas, onions, chicory root, garlic, asparagus, barley, wheat, jicama, tomatoes, onion rye, garlic and leeks. The Jerusalem artichoke and its relative, yacón have been found to have the highest concentrations of FOS. FOS are a group of linear fructose oligomers (oligosaccharides), able to escape digestion in the human upper intestine and reach the colon where they are totally fermented mostly to lactate, SCFAs (acetate, propionate and butyrate), and gas, like dietary fibres. As a consequence of their fermentation, their caloric value is approximately 2 Kcal/g. A fecal bulking effect of FOS has been observed in humans. An important property of sc-FOS is the stimulation of bifidobacterial growth (FOS chemical structure is made up of β -2-1-fructose-fructose which is recognized by the enzyme β -fructosidase which is typical of bifidobacteria that can easily metabolize it; the most of pathogens and putrefactive bacteria are not able to break FOS chemical bond making very selective the utilization of these oligosaccharides) specifically while suppressing the growth of potentially harmful species such as *Clostridium perfringens* in the colon. The prebiotic effect of sc-FOS is dose dependent and chain length related and is associated with

a decrease in fecal pH, an increase in fecal or colonic organic acids, a decrease in the production of nitrogenous end products in urine and stools, a decrease in fecal bacterial enzymatic activities and a modification in fecal neutral sterols. The sc-FOS enhance magnesium absorption in humans and have been shown, in animal models, to reduce colon tumour development by enhancing both colon butyrate concentrations and local immune system effectors.¹⁴ Buddington et al.¹⁵ studied the effects of dietary FOS in 12 healthy adult human subjects fed on a controlled diet for 42 days (4 g neosugar/d between days 7 and 32). FOS caused β -glucuronidase and glycocholic acid hydrolase activities to decrease 75% and 90% respectively. The Authors found that 4 g neosugar/d alters the fecal flora in a beneficial way decreasing the activities of some reductive enzymes. Williams et al. determined changes in the abundance of selected bacteria in anaerobic fecal samples from 10 adult human volunteers who consumed 4 g neosugar (mixture of sc-FOS by Nutrilite Products Inc.) daily for two weeks. The presence of FOS in the diet acts on the increasing of the growth of the host's existing microbiota encouraging the proliferation of beneficial bacteria groups such as lactobacilli and bifidobacteria.¹⁶ Bornet et al.¹⁷ report that in vitro and in vivo studies (in animals and humans) demonstrated that FOS are good substrates for the *Bifidobacteria spp* possessing β -fructoisidases and for *Bacteroides spp* and that they are bad substrates for *E. Coli* and *C. Perfringens*. FOS feeding is also related to an increase of bifidobacteria and lactobacilli and to a decrease of *C. Perfringens*. Bifidobacteria are responsible for acetic acid and lactic acid production which reduces the intestinal pH. This pH reduction restricts or prohibits the growth of many potential pathogens and putrefactive bacteria with a reduction of enzyme activities in stools (nitroreductase, decarboxylase etc.) with a deep impact on the metabolism of carcinogenic substances such as N-nitroso compounds, phenolic products of tyrosine and tryptophan and metabolites of biliary steroids. In conclusion FOS are able to act on bifidobacteria and could be a protective factor against colon cancer. Hidaka et al.¹⁸ performed several clinical studies based on the administration of FOS and conclude that these compounds are responsible for cholesterol reduction (reduction in LDL cholesterol contents), suppression of putrefaction, normalization of colon microbial disorders and alleviation of constipation. The Authors underline that their usefulness seems to be strictly related to the proliferation of bifidobacteria, to other saccharolytic intestinal bacteria and SCFAs produced by these organisms as well. Hidaka et al.¹⁹ performed some clinical studies using Neosugar G (prepared from sucrose through the action of the enzyme β -fructofuranosidase produced by *Aspergillus Niger*), Neosugar (a mixture of FOS purified from Neosugar G by removing the mono-saccharides) and disaccharides through a resin treatment. Applied foods containing Neosugar such as jellies and drinks were also prepared for the clinical studies. The clinical studies showed that FOS administration improved blood lipids in hyperlipidemia, suppressed the production of intestinal putrefactive substances and improved the intestinal flora with relief from constipation. Aumenta et al.²⁰ evaluated the effects of treatment with symbiotic of "zir fos" (*Bifidobacterium longum W11* + FOS Actilight) on chronic constipation in patients undergoing a weight loss diet (hypocaloric diet (1200/1400 cal.) based on 1 bag of symbiotic zir fos per day for the entire duration of the study and physical activity program). Patients' follow-up was available for up to 60 days. Two hundred and ninety-seven patients (79.4% women and 18.2% men, mean age 32.2) were included in the study. The Authors' data demonstrate the utility of symbiotics in improving constipation during hypocaloric diet in the treatment of obesity. Zunft et al.²¹ evaluated the benefits derived from the use of a symbiotic containing bifidobacteria and FOS as inulin in treating constipation in elderly. The double blinded randomized crossover study involved 49 elders

aged 66–74. The Authors observed a significant increase in the number of stool discharge in elders improving their bowel function and quality of life. Malaguarnera et al.²² designed a study to assess the clinical efficacy of *Bifidobacterium longum* plus FOS in the treatment of Minimal Hepatic Encephalopathy (MHE; this pathology describes patients with chronic liver disease or cirrhosis who have no clinical symptoms of brain dysfunction but perform worse on psychometric tests compared with healthy subjects. In this condition ammonia has been found to induce cerebral dysfunction. Increased intestinal ammonia production is due to bacterial urease activity and the production of other toxin metabolites, such as mercaptans, thioles). A total of 60 cirrhotic patients were randomly and equally divided into two groups receiving *Bifidobacterium* + FOS (17 males, 13 females; mean age, 46 \pm 11 years) or placebo (16 males, 14 females; mean age, 45 \pm 12 years), respectively. All patients underwent clinical and laboratory assessment psychometric tests and automated EEG analysis: neurophysiological assessment, liver function assessment, and neuropsychological assessment. The Authors observed an improvement in biochemical and neuropsychological tests of the group treated with *Bifidobacterium longum* and FOS. Scholz-Ahrens et al.²³ report that nondigestible oligosaccharides (NDOs) have been found to stimulate absorption of several minerals and improve mineralization of bone. Most of the scientific evidence for the functional effects of NDOs is based on animal experiments in which NDOs increase the availability of calcium, magnesium, zinc, and iron. This stimulatory effect of some NDOs is assumed to be mainly due to their prebiotic character. Prebiotics such as inulin, oligofructose, glucooligosaccharide, and galacto-oligosaccharide have been found to stimulate absorption and retention of several minerals, particularly magnesium, calcium, and iron. Most of these findings were obtained in studies performed on rats. Up to now the number of studies on the effect of NDOs on mineral metabolism in humans is limited; positive effects on calcium absorption seem to occur under conditions of increased calcium requirements (adolescence and postmenopause) Figs. 5–7.

2.1. The safety of probiotics: goods and flaws

The word probiotics refers to microorganisms that are able to confer health benefits on humans and that have been industrially prepared for nutritional and pharmaceutical use. The consumption of these products is growing very fast all over the world and probiotics are generally considered generally regarded as safe (GRAS).¹³ Indu et al.¹² suggest that probiotics are responsible for competitive exclusion of enteric pathogens from the gut lumen. The putative inhibitory mechanism should be the production of lactic acid and bacteriocins. Moreover they restore the normal intestinal flora during an antibiotic therapy, trigger cytokines synthesis from enterocytes attaching to their surfaces, produce toxic metabolites like hydrogen peroxide and synthesize butyric acid which increases the turnover of enterocytes and neutralizes dietary carcinogens. Probiotics exert several immunomodulatory functions acting on cellular and humoral responses. Furthermore they can stimulate Type 1 Helper T Cells (*L. Casei*), mononuclear cells leading to increased clearance of circulating pathogenic bacteria (*L. Acidophilus*), macrophages to produce TNF-alpha, IL-6 and NO production, increase clearance of circulating pathogenic bacteria by Kupffer Cells and they can produce antirotaviral IgA and anti-influenza IgG antibodies (*B. Breve*). Snyderman²⁸ reports three theoretical concerns regarding the safety of probiotics: firstly the occurrence of disease, due to bacteremia, sepsis or endocarditis, secondly the toxic or metabolic effects on the GI tract and thirdly the transfer of antibiotic resistance in the GI flora. The Author underlines that, although there are rare cases of bacteremia or

Lactobacillus species

- L. acidophilus*
- L. casei*
- L. fermentum*
- L. gasseri*
- L. johnsonii*
- L. lactis*
- L. paracasei*
- L. plantarum*
- L. reuteri*
- L. rhamnosus*
- L. salivarius*

Bifidobacterium species

- B. bifidum*
- B. breve*
- B. lactis*
- B. longum*
- B. animalis*
- B. infantis*

Streptococcus species

- S. thermophilus*

Yeasts

- Saccharomyces cerevisiae (boulardii)*

Mixtures

Lactobacillus acidophilus + *Lactobacillus casei*, *Lactobacillus rhamnosus* + *Lactobacillus reuteri*, VSL#3 (mixture of 1 strain of *Streptococcus thermophilus*, four *Lactobacillus* spp., & three *Bifidobacterium* spp. strains), *Lactobacillus acidophilus* + *Bifidobacterium bifidum*, *Lactobacillus helveticus* + *Lactobacillus rhamnosus*, *Bacillus clausii* strains

Fig. 5. Commercially used probiotic strains.

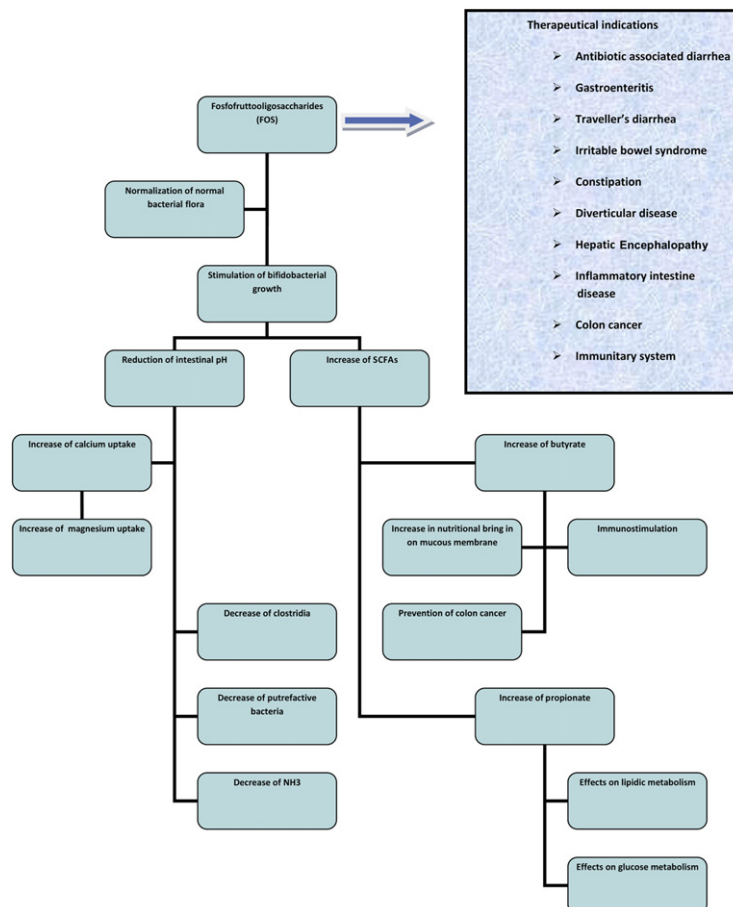


Fig. 6. FOS activities and therapeutical uses.²⁴

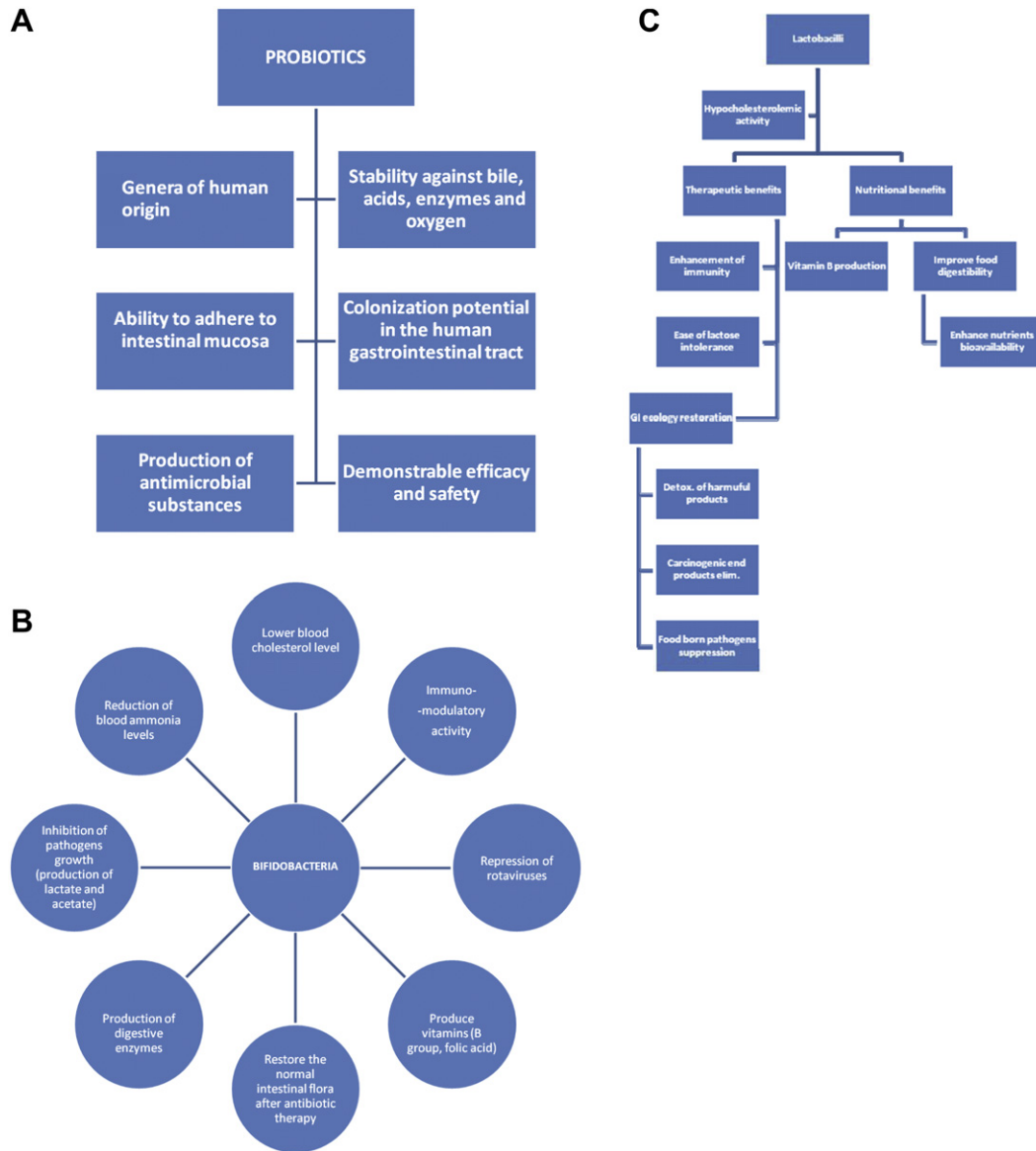


Fig. 7. A. Criteria for classifying a bacterial strain as a probiotic. B. Bifidobacteria benefits on human health. Main therapeutic and nutritional effects of Lactobacilli.^{25–27}

fungemia related to the use of saprophytic probiotics, epidemiologic evidence through clinical trials and epidemiologic survey suggests that they can be safely used. Wassenaar et al.²⁹ highlight that bacteria used for probiotics formulation should be completely safe although there could be some flaws: 1) sometimes virulence factors have been detected in probiotic bacterial strains; 2) horizontal gene transfer can result in acquisition of virulence genes or antimicrobial resistance in probiotic bacteria; 3) antimicrobial resistance in these bacteria can possibly aid the spread of unwanted resistance in endogenous bacterial populations. Liong et al.³⁰ observe that some bacteria strains, commonly used in probiotic formulations, such as *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Enterococcus*, and *Bifidobacterium* have been isolated from infection sites, leading to the problem that these probiotics can translocate. The Authors underlined that probiotics translocation hardly occurs in healthy humans (where it happens, detrimental effects are rare) but health-damaging effects of probiotics translocation can occur in immunocompromised patients. In these people probiotics might theoretically cause infections that need to be treated with

antibiotics but, unfortunately, the antibiotic resistance of some strains has increased the complexity of their eradication. Surely further investigations are required to analyze the mechanisms of probiotic translocation and infection. Safety assessments should be focused on these mechanisms so that the negative effects of probiotics do not outweigh the benefits.

2.2. Probiotics and immunity

Lammers et al.³¹ studied mucosal gene expression of the pleiotropic proinflammatory cytokines (interleukin(IL)-1beta, IL-6), TH1 cytokines (interferon-gamma (IFN-gamma), TNF-alpha, IL-12), regulatory cytokines (IL-10, transforming growth factor-beta), and the chemokine IL-8. In addition to assess the cytokines gene expression, the presence of polymorphonuclear cells in the mucosal tissue was evaluated. Patients who were treated with probiotics had significant lower mucosal mRNA expression levels of IL-1beta, IL-8, and IFN-gamma compared with placebo-treated patients. A lower number of polymorphonuclear cells was present

in the tissue of patients within the probiotic group compared with the number of polymorphonuclear cells in the tissue of patients receiving placebo and patients having an episode of pouchitis. This study highlights that probiotic treatment is able to regulate the mucosal immune response reducing mucosal levels of neutrophil-chemoattractant IL-8 and tissue influx of polymorphonuclear cells, and may further act by inhibit T cells activation, reinforce the barrier function and keep a tight control of the potent proinflammatory cytokine IL-1beta. A randomized double-blind study was designed by Marchan et al.³² Probiotic bacteria or placebo were administered for 1 month before delivery to mothers and for 6 months to infants with a family history of allergy. Plasma samples were analyzed for C-reactive protein (CRP), total IgA and IgE, food-specific IgA, IgG, and IgE, IL-2, IL-4, IL-6, IL-10, TNF-alpha, and IFN-gamma. The association of CRP with a decreased risk of eczema at 2 years of age in allergy-prone children supports the idea that chronic, low-grade inflammation protects from eczema. Low-grade inflammation induced by probiotics was characterized by elevation of IgE, IgA, and IL-10, a change typically observed in helminth infection associated induction of regulatory mechanisms. This study emphasizes the role of chronic microbial exposure as an immune modulator protecting from allergy. Stadbauer et al.³³ in an open-label study involving 12 patients with alcoholic cirrhosis (*Lactobacillus casei* Shirota (LcS) (6.5×10^9) 3 times daily for 4 weeks), 13 healthy controls and 8 cirrhotic patients who did not receive probiotics, report that probiotics restore neutrophil phagocytic capacity in cirrhosis, possibly by changing IL-10 secretion and TLR4 expression, warranting larger randomized controlled and mechanistic studies. Kekkonen et al.³⁴ evaluated the effect of stimulation provided by several probiotic bacterial strains (*Streptococcus*, *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Leuconostoc* and *Propionibacterium*) on cytokines production in human peripheral blood mononuclear cells (PBMC). The probiotic *S. thermophilus* and *Leuconostoc* strains were shown to be more potent inducers of Th1 type cytokines IL-12 and IFN-gamma than the probiotic *Lactobacillus* strains and the use of bacterial combinations did not result in enhanced cytokine production. The first study to investigate the effects of probiotics intervention on global lipidomic profiles in humans was designed by Kekkonen et al.³⁵ The study investigated the effect of three weeks' intervention with *Lactobacillus rhamnosus* on global serum lipidomic profiles and evaluated whether the changes in inflammatory variables (CRP, TNF-alpha and IL-6) are reflected in the global lipidomic profiles of healthy adults. The Authors observed that probiotic *Lactobacillus rhamnosus* intervention may lead to changes in serum global lipid profiles, as reflected in decreased glycerophosphatidylcholines (GPCCho), lysophosphatidylcholines (LysoGPCCho) and sphingomyelins (SM) and in the increasing of triacylglycerols (TAGs). Among the inflammatory variables, IL-6 was moderately associated with changes in global lipidomic profiles, with the top-ranked lipid associated with IL-6 which is the proinflammatory LysoGPCCho (20:4). There was a weak association between the lipidomic profiles and the two other inflammatory markers, TNF-alpha and CRP25. Takeda et al.³⁶ in a placebo-controlled crossover trial, observed the effect of LcS on natural killer (NK) cell activity in humans. NK cell activity exhibited a declining trend during the period of placebo ingestion, but NK cell activity increased after intaking fermented milk containing 4×10^{10} live LcS for 3 weeks. When human PBMC were cultured in the presence of heat-killed LcS, NK cell activity was enhanced. The ability of LcS to enhance NK cell activity and induce IL-12 production was correlated, and the addition of anti-IL-12 monoclonal antibody reduced the enhancement of NK cell activity triggered by LcS. In addition, the separation of NK cells from LcS-stimulated monocytes with membrane filter reduced NK cell activity to the intermediate level and almost deprived monocytes

of the ability to produce IL-12. The Authors demonstrated that LcS can enhance NK cell activity in vivo and in vitro in humans, and IL-12 may be responsible for enhancement of NK cell activity triggered by LcS.

Changes in the composition of the gut microbiota have been implicated in the pathogenesis of allergic disorders suggesting beneficial interactions between the intestinal immune system and specific bacterial strains. Ivory et al.³⁷ designed a double-blinded, placebo-controlled study with 10 seasonal allergic rhinitis (SAR) sufferers in each group. The Authors observed that volunteers, treated with LcS, showed a significant reduction in levels of antigen-induced IL-5, IL-6 and IFN-gamma production compared with volunteers supplemented with placebo. Meanwhile, levels of specific IgG increased and the IgE ones decreased in the probiotic group. So changes in antigen-induced production of cytokines were observed in patients treated with probiotics showing that probiotic supplementation modulates immune responses in SAR and may have the potential to alleviate the severity of symptoms.

Twetman et al.³⁸ randomly assigned forty-two healthy adults with moderate levels of gingival inflammation to one of three parallel arms: Group A/P was given one active and one placebo gum daily, Group A/A received two active chewing gums, and Group P/P two placebo gums. The chewing gums contained two strains of *Lactobacillus reuteri*: ATCC 55730 and ATCC PTA 5289 (1×10^8 CFU/gum, respectively). The Authors observed a reduction of proinflammatory cytokines (the levels of TNF-alpha and IL-8 decreased significantly ($p < 0.05$) in Group A/A compared with baseline after 1 and 2 weeks, respectively) in gingival crevicular fluid that points out that the probiotic approach can fight inflammation in the oral cavity.

2.3. Probiotics, skin disease and allergy

The skin is able to act as a physical barrier exerting several functions such as fluid homeostasis, thermoregulation, immunologic functions, neurosensory functions, metabolic functions and primary protection against infection. In case of thermal injury the burn can be colonized by several types of pathogens, i.e. primarily gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), gram-negative bacteria such as *Acinetobacter baumannii*-calcoacetis complex, *Pseudomonas aeruginosa*, *Klebsiella* species and fungal pathogens. The prevention of infection include early burn-eschar excision, topical and prophylactic antibiotics, and aggressive infection-control measures. The antimicrobial resistance of bacteria isolated from patients with burns has increased and bacterial colonization and infection remain the major causes of delayed healing and graft rejection following burns. Topical treatment is necessary to reduce the incidence of burn wound infection. The ability of the probiotic organism *Lactobacillus plantarum* to inhibit the pathogenic activity of *Pseudomonas aeruginosa*, both in vitro and in vivo, was tested by Valdéz et al.³⁹ In vitro (*Lactobacillus plantarum* whole cultures, culture filtrates acid filtrate and neutralised acid filtrate) and isolated, washed cells were tested for their effects on the production of the *P. aeruginosa* quorum-sensing signal molecules, acyl-homoserine-lactones (AHLs), and two virulence factors controlled by signal molecules such as elastase and biofilm. All were inhibited by *L. plantarum* cultures and filtrates, but not by isolated, washed cells. The acid *L. plantarum* growth medium itself had some inhibitory activity but the greatest activity was exerted by the whole culture. In vivo (a burned-mouse model was used) burns infected with *P. aeruginosa* were treated with *L. plantarum* at 3, 4, 5, 7 and 9 days post-infection. Samples from skin, liver and spleen taken after 5, 10 and 15 days demonstrated that *L. plantarum* had inhibited *P. aeruginosa* colonization. There was also an improvement in tissue repair, enhanced phagocytosis of *P.*

aeruginosa produced by tissue phagocytes and a decrease in apoptosis at 10 days. The Authors conclude that *L. plantarum* and/or its by-products are potential therapeutic agents for the local treatment of *P. aeruginosa* burn infections.

Peral et al.⁴⁰ cultured *L. plantarum* in De Man, Rogosa and Sharpe medium to provide an alternative method for burn treatment using Silver sulphadiazine (SD-Ag, a microbicidal agent) as a control. Eighty burned patients from the Plastic Surgery and Burns Unit were grouped into infected (delayed) second- and third-degree and non infected (early) third-degree burns and treated with *L. plantarum* or SD-Ag. The proportion of patients with delayed second-degree burns was 0.71 for *L. plantarum* and 0.73 for SD-Ag (relative rate: -2.72%) in comparison with the decrease in bacterial load ($<10^5$ bacteria/g of tissue), promotion of granulating tissue wound bed and healing. In early third-degree burns, the values were 0.75 for *L. plantarum* and 0.84 for SD-Ag (relative rate: -1.07%) in preventing wound infection and promotion of granulation tissue, 0.90 in graft taking for both treatments (relative rate: 0%) and 0.75 for *L. plantarum* and 0.77 for SD-Ag (relative rate: -2.60%) in healing. In delayed third-degree burns, values were 0.83 for *L. plantarum* and 0.71 for SD-Ag (relative rate: $+16.90\%$) compared to the decrease in the bacterial load ($<10^5$ bacteria/g of tissue) and providing a granulating tissue wound bed, 0.90 in graft taking for both treatments (relative rate: 0%) and 0.75 for *L. plantarum* and 0.64 for SD-Ag (relative rate: $+17.19\%$) in healing. This study suggests that the *L. plantarum* treatment could be a valid therapy for the topical treatment of burns.

Probiotic formulations have been widely studied for the treatment of atopic dermatitis (AD; a type of eczema), a pathology defined as an inflammatory, chronically relapsing, non-contagious and pruritic skin disease which is associated with elevated IgE levels and Th2 responses. AD in animal models and human studies has been investigated using different probiotic different strains such as *Bifidobacterium*, *Lactobacillus*, and *Lactococcus*. In several animal studies involving the use of probiotics it was observed suppression of specific or non-specific IgE production, reduction of infiltrated eosinophils and degranulated mast cells, potentiation of regulatory T cell cytokines such as IL-10 and TGF-beta relative to IL-4 and IL-5 and potentiation of Th1/Th2 activity along with reduced symptoms of AD. Several well-designed double-blind placebo-controlled human studies showed that some probiotic strains administered during perinatal period prevented the occurrence of AD but they cannot consistently show a reduction in specific or non-specific IgE or a change in specific immunomodulatory cytokines. The administration of selected strains of probiotics during the perinatal period may be helpful in the prevention of AD.⁴¹

Gueniche et al.⁴² designed a prospective, double-blind, placebo-controlled clinical study with a cream containing a 5% lysate of the non-pathogenic bacteria *Vitreoscilla filiformis*. Seventy-five volunteers with AD (6–70 years of age) were randomized to receive either *V. filiformis* cream 5% or vehicle cream daily for 30 days. The therapy efficacy was evaluated by the following parameters: SCOR of Atopic Dermatitis (SCORAD), transepidermal water loss (TEWL), assessment of microflora and the patient's assessment of itch and loss of sleep. *V. filiformis* lysate significantly decreased SCORAD levels ($P = 0.0044$) and pruritus ($P = 0.0171$). Active cream significantly decreased loss of sleep from day 0 to day 29 ($P = 0.0074$). *V. filiformis* lysate reduced *Staphylococcus aureus* colonization of the skin. The skin barrier as determined by TEWL also improved significantly with the cream alone. *V. filiformis* lysate significantly improved AD. This may be in part due to a reduction of *S. aureus*, but it seems to concern in most parts a direct immunomodulatory effect on skin-associated immune responses.

Zuccotti et al.⁴³ analyzed the possible causes related to the increase of diagnosis of allergy related diseases in the last few years. They have noticed firstly a reduced microbial stimulation

during infancy and early childhood which results in slower post-natal maturation of the immune system and development of an optimal balance between Th1 and Th2-like immunity (the hygiene hypothesis) and secondly an altered microflora that promotes the persistence of those Th2 cytokines (IL-4, IL-5, IL-3) which are predominant at birth and prevent the shift toward a Th1 response with IL-12 and IFN-gamma production.

Now we are going to report some of the several studies involving the use of probiotic formulations to evaluate their efficacy for the treatment of allergic diseases basing on their ability to change either the composition and/or the metabolic activities of the microbiota or modulate immune system reactivity in a way that benefits health.

Wickens et al.⁴⁴ designed a double-blind, randomized placebo-controlled study in which 512 pregnant women were randomized to take *Lactobacillus rhamnosus* HN001 (*L. rhamnosus*), *Bifidobacterium animalis* subsp. *lactis* strain HN019 or placebo daily from 35 weeks gestation until 6 months if breast-feeding, and their infants were randomized to receive the same treatment from birth to 2 years ($n = 474$). The infants' cumulative prevalence of eczema and point prevalence of atopy, using skin prick tests to common allergens, was assessed at 2 years. Infants receiving *L. rhamnosus* had a significantly ($P = 0.01$) reduced risk of eczema (hazard ratio (HR), 0.51; 95% CI, 0.30–0.85) compared with placebo, but this was not the case for *B. animalis* subsp. *lactis* (HR, 0.90; 95% CI, 0.58–1.41). There was no significant effect of *L. rhamnosus* (HR, 0.74; 95% CI, 0.46–1.18) or *B. animalis* subsp. *lactis* (HR, 0.82; 95% CI, 0.52–1.28) on atopy. *L. rhamnosus* (71.5%) was more likely than *B. animalis* subsp. *lactis* (22.6%) to be present in the feces at 3 months, although detection rates were similar by 24 months. The Authors conclude that supplementation with *L. rhamnosus*, but not *B. animalis* subsp. *lactis*, substantially reduced the cumulative prevalence of eczema, but not atopy, by 2 years.

Abrahamsson⁴⁵ performed a double-blind, randomized, placebo-controlled trial involving 232 families with allergic disease. The mothers received *L. reuteri* ATCC 55730 (1×10^8 CFU) daily from gestational week 36 until delivery. Their babies then continued with the same product from birth until 12 months of age and were followed up for another year to assess if the oral administration of probiotic *L. reuteri* may alleviate and even prevent eczema in infants with a family history of allergic disease. The cumulative incidence of eczema was similar, 36% in the treated versus 34% in the placebo group. However the *L. reuteri* group had less IgE-associated eczema during the second year, 8% versus 20% ($P = 0.02$). Skin prick test reactivity was also less common in the treated than in the placebo group, significantly so for infants with mothers with allergies, 14% versus 31% ($P = 0.02$). Wheeze and other potentially allergic diseases were not affected. The Authors concluded that the treated infants had less IgE-associated eczema at 2 years of age and therefore possibly ran a reduced risk to develop later respiratory allergic disease. Therefore probiotics may reduce the incidence of IgE-associated eczema in infancy.

Kukkonen et al.⁴⁶ studied the effect of a mixture of 4 probiotic bacterial strains (*Lactobacillus rhamnosus* GG (ATCC 53103), *L. rhamnosus* LC705 (DSM 7061), *Bifidobacterium breve* Bb99 (DSM 13692) and *Propionibacterium freudenreichii* ssp. *shermanii* JS (DSM 7076)) along with prebiotic galacto-oligosaccharides in preventing allergic diseases. One thousand two hundred and twenty three pregnant women carrying high-risk children were randomized to use a probiotic preparation or a placebo for 2–4 weeks before delivery. Their infants received the same probiotics plus galacto-oligosaccharides ($n = 461$) or a placebo ($n = 464$) for 6 months. Probiotic treatment compared with placebo showed no effect on the cumulative incidence of allergic diseases but tended to reduce

IgE-associated (atopic) diseases (odds ratio (OR), 0.71; 95% CI, 0.50–1.00; $P = 0.052$). Probiotic treatment reduced eczema (OR, 0.74; 95% CI, 0.55–0.98; $P = 0.035$) and atopic eczema (OR, 0.66; 95% CI, 0.46–0.95; $P = 0.025$). Lactobacilli and bifidobacteria more frequently ($P < 0.001$) colonized the guts of supplemented infants. Probiotic treatment showed no effect on the incidence of all allergic diseases for infants aged 2 but significantly prevented eczema and especially atopic eczema. The Authors observed an inverse association between atopic diseases and colonization of the gut through probiotics and concluded that the prevention of atopic eczema in high-risk infants is possible modulating the infants' gut microbiota with probiotics and prebiotics.

2.4. Probiotics in surgical practice

Postoperative complications in GI surgery could involve bacteremia and infectious complications. The main causes could be the translocation of the GI bacteria or its toxins as a consequence of bacterial overgrowth, the loss of intestinal epithelial integrity and the immunologic compromising of the host. Probiotics could be good candidate to fight against these factors through the competition with potential pathogens for nutrients or enterocyte adhesion sites, degradation of toxins, production of antimicrobial factors, and local and systemic immunomodulation. The aim of this short paragraph is to report some important clinical trials which could help to evaluate the usefulness of probiotics action to ease the complication rate in patients undergoing surgery on the GI tract. Some of the main important procedures which have been related to the use of probiotics are: first the ileal pouch-anal anastomosis (IPAA) which involves the removal of the entire colon and rectum, with preservation of the anus and sphincter muscles and is performed to avoid a permanent stoma (opening for collecting waste) in cases where the entire colon and rectum need to be removed usually in patients with chronic ulcerative colitis or pathologies associated with colon cancers; second the pancreaticoduodenectomy to treat tumors of the pancreatic head, neck or uncinat process; third the surgical resection for patients with localized tumors affecting the pancreas. Falk et al.⁴⁷ analyzed the possible role of probiotics for the treatment of pouchitis which is the inflammation of the ileal reservoir and the major complication of IPAA following proctocolectomy for ulcerative colitis (UC). This review reported that patients with pouchitis have an increased number of anaerobes and aerobes, less bifidobacteria and anaerobic lactobacilli and more clostridia than patients not affected by that condition. The Authors reported a double-blind study (40 patients with chronic pouchitis treated with VSL#3, a probiotic bacterial mix containing 4 lactobacilli strains, three bifidobacteria strains and one streptococcus strain) showing that probiotics can prevent relapse of pouchitis. Another study combining *Lactobacillus rhamnosus* with FOS confirmed the possible use of probiotic formulations to induce remission in this condition.

Pronio et al.⁴⁸ reported that VSL#3 administration in patients with IPAA modulates the Pouchitis Disease Activity Score and expands the number of mucosal regulatory T cells (open-label study in which 31 patients, without signs and symptoms of pouchitis, were randomized in different periods from surgery to 2 sachets of VSL#3 once daily or no treatment for 12 months). In particular the Authors observed a significant reduction in PDAI score and a significant increase in the percentage of mucosal CD4+CD25(high) and CD4+LAP-positive cells compared with baseline values. Tissue samples at different points showed a significant reduction in IL-1beta mRNA expression, and a significant increase in Foxp3 mRNA expression.

Rayes et al.⁴⁹ designed a prospective randomized monocentric double-blind trial involving 80 patients following pylorus-

preserving pancreatoduodenectomy (PPPD). Group A received a composition of 4 lactobacilli and 4 fibers; group B received placebo, only fibers, starting the day before surgery and continuing for 8 days. The study proved that early enteral nutrition, supplemented with a mixture of lactobacilli and fibers, reduces bacterial infection rates and antibiotic therapy following PPPD.

Nomura et al.⁵⁰ randomly allocated seventy patients with pancreaticobiliary diseases to two groups where the first group received probiotics and the second served as control before pancreaticoduodenectomy. The probiotics used in the study contained *Enterococcus faecalis T-110*, *Clostridium butyricum TO-A*, and *Bacillus mesentericus TO-A* and were first administered immediately after admission, 3–15 days before the operation, and then reintroduced on the second post-operative day. They were administered until hospital discharge. This study shows that the use of perioperative probiotics reduces post-operative infectious complications after pancreaticoduodenectomy, making it a promising potential adjunct therapy for patients undergoing high-risk hepato, biliary, and pancreatic surgery.

Reddy et al.⁵¹ designed a study where ninety-two patients were randomly assigned to one of four groups. Group 1 had mechanical bowel preparation (MBP) only, group 2 had neomycin + MBP, group 3 had synbiotics + neomycin + MBP, and group 4 had synbiotics + neomycin but no MBP. Changes in gut microflora were assessed by culturing nasogastric aspirates and polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis of fecal samples. Intestinal barrier function was determined by microbiological confirmation of bacterial translocation and measurement of intestinal permeability. The inflammatory response was monitored by measurement of serum CRP and IL-6, and septic morbidity was recorded prospectively. They investigated whether it was possible to modulate gut microflora and preserve intestinal barrier function during elective colorectal surgery by using combinations of oral antibiotics, synbiotics and MBP. The Authors did not observe a reduction in inflammatory response or septic morbidity. This study shows that a combination of MBP, neomycin and synbiotics reduces the prevalence of fecal Enterobacteriaceae and bacterial translocation.

Van Gossum et al.⁵² designed a multicenter clinical trial evaluating the efficacy of an oral administration of the probiotic *Lactobacillus johnsonii LA1* on early post-operative endoscopic recurrence of Crohn's disease (CD). The study involved seventy patients with CD enrolled prior to elective ileo-caecal resection and randomly assigned after surgery to daily treatment with either (LA1, Nestle, 10^{10} CFU) (group A, $n = 34$) or placebo (group B, $n = 36$) for 12 weeks. The Authors demonstrated that oral administration of the probiotic LA1 in patients with CD failed to prevent early endoscopic recurrence at 12 weeks after ileo-caecal resection.

Sugawara et al.⁵³ investigated the effect of perioperative oral administration of synbiotics upon intestinal barrier function, immune responses, systemic inflammatory responses, microflora, and surgical outcome in patients undergoing high-risk hepatobiliary resection. Patients with biliary cancer involving the hepatic hilus ($n = 101$) were randomized, before hepatectomy, into a group receiving post-operative enteral feeding with synbiotics (group A) or another receiving preoperative plus post-operative synbiotics (group B). Lactulose-mannitol (L/M) ratio, serum diamine oxidase (DAO) activity, NK cell activity, IL-6, fecal microflora, and fecal organic acid concentrations were determined before and after hepatectomy. The Authors concluded that preoperative oral administration of synbiotics can enhance immune responses, attenuate systemic post-operative inflammatory responses, and improve intestinal microbial environment. These beneficial effects likely reduce post-operative infectious complications after hepatobiliary resection for biliary tract cancer.

Woodgard et al.⁵⁴ focused on the issue of Roux-en-Y gastric bypass (RNYGB) surgery, a treatment for morbid obesity, which is responsible for altering GI flora leading to bacterial overgrowth and dysmotility. The Authors randomized forty-four patients undergoing RNYGB to either a probiotic or control group; 2.4 billion colonies of *Lactobacillus* were administered daily postoperatively to the probiotic group. Probiotic administration was shown to improve bacterial overgrowth, vitamin B12 availability, and weight loss after RNYGB supporting the hypothesis that altering the GI microbiota can influence weight loss.

2.5. Urogenital infections

Urogenital infections not caused by sexual transmission in women are still one of the most important medical issue. Recurrent urinary tract infection (UTI) is, in most cases caused by the uropathogens *E. Coli*; recurrent bacterial vaginosis (BV) is usually caused by *Gardnerella vaginalis*; recurrent yeast vaginosis is usually caused by *Candida albicans*. The predominant bacteria in the urinary tract of healthy women are lactobacilli. Infections are usually treated using antibiotics with the effect of decreasing the number of lactobacilli which cause GI symptoms, raise drug resistance and do not restore the urinary tract natural barrier to infections. Zuccotti et al.⁴³ reported some studies highlighting that probiotics could be a good alternative to antibiotic therapy due to their quality to adhere to uroepithelial cells and produce inhibitors of pathogenic growth and biosurfactant secretion. The same Authors reported that oral lactobacilli-based therapy under viable conditions showed that these bacteria are able to colonize the urinary tract after enteral colonization. Reid et al.⁵⁵ reported that a daily intake of probiotic strains *L. rhamnosus GR-1* and *Lactobacillus fermentum RC-14*, resulted in some asymptomatic BV patients reverting to a normal lactobacilli dominated vaginal microflora. The same Authors reported other two studies regarding BV: firstly a twice daily use of hydrogen peroxide producing 108 *L. acidophilus* in a product called Vivag for six days led to a 43% improvement compared to none at all in the placebo group; secondly yogurt containing *L. acidophilus*, delivered in a tampon to pregnant women, showed to treat BV and prevented infection at 2 months follow-up. Reid et al.⁵⁵ also reported a study which indicates that the recurrence of UTI can be reduced using one or two capsules (*L. Rhamnosus GR-1* and *L. Fermentum B-54*, replaced more recently by *RC-14*, freeze dried and placed in gelatine capsules, with dosage at 10⁹ per capsule) vaginally per week for one year, with no side effects or yeast infections. The Authors also explored the use of probiotics for the treatment of yeast vaginitis reporting that *L. Rhamnosus GR-1* and *L. Fermentum RC-14* are able to kill and inhibit the adhesion of yeast to vaginal cells. Moreover they reported a crossover study involving 33 patients with recurrent vaginitis treated with eight ounces of *L. Acidophilus* supplemented yogurt daily for six months and then switched to a yogurt free diet. The therapy resulted in 0.4 breakthrough infections compared with 2.5 per study term.

Czaja et al.⁵⁶ performed a phase I trial to assess the safety and tolerance of a *Lactobacillus vaginal* suppository for prevention of recurrent UTI involving premenopausal women with a history of recurrent UTI. They were randomized to use *Lactobacillus crispatus CTV-05* or placebo vaginal suppositories daily for five days. No severe adverse events occurred. Mild to moderate vaginal discharge and genital irritation were reported by women in both study arms. Seven women randomized to *L. crispatus CTV-05* developed pyuria without associated symptoms. Most women had high concentrations of vaginal H202-producing lactobacilli before randomization. *L. crispatus*, *Lactobacillus jensenii*, and *Lactobacillus gasseri* were the most common *Lactobacillus* species identified, with stable

prevalence over time. According to this study *L. crispatus* CTV-05 can be given as a vaginal suppository with minimal side effects to healthy women with a history of recurrent UTI although mild inflammation of the urinary tract was noted in some women.

Anukam et al.⁵⁷ enrolled 40 women diagnosed with BV (it is particularly common condition in black women and in Nigeria it is often caused by *Mycoplasma*, as well as *Atopobium*, *Prevotella* and *Gardnerella* sp.) by discharge, fishy odor, sialidase positive test and Nugent Gram stain scoring. They were randomized to receive either two dried capsules containing *L. rhamnosus GR-1* and *L. reuteri RC-14* each night for 5 days, or 0.75% metronidazole gel, applied vaginally twice a day, in the morning and evening. The follow-up at day 6, 15 and 30 showed a significant cure for BV in probiotic lactobacilli treated subjects compared to metronidazole treatment. This is the first report of an effective (90%) cure for BV using probiotic lactobacilli. The Authors conclude that, given the correlation between BV and HIV, and the high risk of the latter in Nigeria, intravaginal use of lactobacilli can provide women with a self-use therapy, similar to over-the-counter anti-yeast medication, for treatment of urogenital infections.

Martinez et al.⁵⁸ designed a study involving sixty-four Brazilian women diagnosed with BV. They were randomly assigned to receive a single dose of tinidazole (2 g) supplemented with either 2 placebo capsules or 2 capsules containing *L. rhamnosus GR-1* and *L. reuteri RC-14* every morning for 4 weeks. At the end of treatment (day 28), the probiotic group had a significantly higher cure rate for BV (87.5%) than the placebo group (50.0%) ($p = 0.001$). In addition, according to the Gram-stain Nugent score, more women were assessed at “normal” vaginal microbiota in the probiotic group (75.0% vs. 34.4% in the placebo group; $p = 0.011$). This study shows that probiotic lactobacilli can provide benefits to women being treated with antibiotics for an infectious condition.

Mastromarino et al.⁵⁹ enrolled thirty-nine women with BV in a double-blind, placebo-controlled clinical trial to receive either one *Lactobacillus*-containing tablet or placebo daily for 7 days. After the completion of therapy, all of the patients in the *Lactobacillus*-treated group ($n = 18$) were free of BV, showing a normal (83%) or intermediate (17%) vaginal flora, as compared with only two patients free of BV with intermediate flora (12%) from among the 16 placebo-treated women ($p < 0.001$). Two weeks after the completion of therapy, treatment was successful (score < 7) in 61% of *Lactobacillus*-treated patients as compared with 19% of those ones in the placebo group ($p < 0.05$). In the treatment group, the total number of symptomatic patients and the intensity of their symptoms, in particular vaginal malodour, were significantly reduced at both follow-up visits. The previously reported results brought the Authors to conclude that intravaginal administration of exogenous selected strains of lactobacilli can restore a normal vaginal microbiota and could be a useful choice for the treatment of BV.

Larsson et al.⁶⁰ investigated firstly if supplementary lactobacilli treatment could improve the initial cure rate after vaginal clindamycin therapy, and secondly, if lactobacilli as repeated adjunct treatment during 3 menstrual cycles could lengthen the time to relapse after initial cure. A hundred BV diagnosed women were offered vaginal clindamycin therapy followed by vaginal gelatine capsules containing either 109 freeze-dried lactobacilli or identical placebo capsules for 10 days during 3 menstrual cycles in a double-blind, randomized, placebo-controlled trial. The Authors concluded that supplementary treatment, combining two different strains of probiotic lactobacilli, does not improve the efficacy of BV therapy during the first month of treatment, but for women initially cured, adjunct treatment of lactobacilli during 3 menstrual cycles lengthens the time to relapse significantly (more women remained BV free at the end of the 6-month follow up).

2.6. Probiotics and renal diseases

Renal failure can be faced removing waste products and excess fluid using a mechanical method called dialysis. The two forms of dialysis used in medical practice are hemodialysis (HD) and peritoneal dialysis (PD). HD cycles blood through a machine that filters the blood and returns it to the body cleaned of waste. PD cycles fluid into and out of the abdomen using the individual's own peritoneum as a filter. Plasma uremic toxins levels such as phenol, p-cresol, and indican are produced in the intestine as bacterial putrefactive metabolites and accumulate to a great degree in the feces of HD patients and cannot be efficiently reduced by HD. Intestinal microflora is deranged in HD patients as we can see an increase in aerobic bacteria such as *Escherichia coli* and a decrease in anaerobic bacteria such as *Bifidobacterium*.

Yangco et al.⁶¹ reported the successful utilization of nitazoxanide and probiotics to treat multirecurrent *Clostridium difficile* infection (CDI) in a PD patient. A 58-year-old woman was admitted with hypotension, nausea and vomiting attributed to metronidazole therapy for CDI, her third CDI treatment regimen in 3 months. During her admission, the patient developed CDI and was started on a 6-week regimen of nitazoxanide and probiotics to assist in re-establishing the colonic flora. The regimen was well tolerated and the patient remained disease free at follow up, four months later.

Hida et al.⁶² demonstrated that Lebenin, a preparation consisting of antibiotic-resistant lactic acid bacteria, administered orally is able to reduce the levels of fecal putrefactive metabolites to levels comparable with those of healthy subjects. Moreover the plasma level of indican also significantly decreased in these Lebenin-treated patients. An analysis of the fecal microflora revealed that a disturbed composition of the microflora characterized by an overgrowth of aerobic bacteria is restored to normal by oral administration of Lebenin in HD patients. The Authors demonstrated that oral administration of lactic acid bacteria in uremic patients is effective in reducing the levels of uremic toxins, especially that of indican, in the blood by inhibiting bacterial production by means of correcting the intestinal microflora.

Taki et al.⁶³ demonstrated that the oral administration of *B. longum* in a gastroresistant seamless capsule to HD patients is effective in decreasing the pre-HD serum levels of homocysteine, indoxyl sulfate, and TAGs. The reduction in the serum level of homocysteine is mainly attributable to the supply of folate produced by *B. longum* in the human intestines.

3. Probiotics and GI diseases

3.1. Diverticular disease

Diverticular disease (DD) is an acquired weakening of the colon wall, and more rarely of the ileum and duodenum causing protrusions of the mucosa and submucosa through the muscular wall. These “pouches” occur in weak areas of the wall where blood vessels penetrate due to the high pressure inside the colon. Often they involve primarily the sigmoid region of the colon. The term “diverticulosis” refers to the asymptomatic condition and the term “diverticular disease” refers to the condition associated with symptoms. The term diverticulitis is used to indicate inflammation of the bowel wall.⁶⁴ Here we report two interesting studies designed by Tursi et al. evidencing the possible use of probiotics in the management of this condition.

Tursi et al.⁶⁵ designed a study to investigate whether balsalazide (a novel orally administered prodrug of 5-ASA in which an inert carrier molecule, 4-aminobenzoil- β -alanine, has been bonded to a molecule of 5-ASA. After administration, colonic bacteria split balsalazide into 5-ASA and 4-aminobenzoil- β -alanine, releasing

the active 5-ASA into the colon with minimal systemic absorption) and/or VSL#3 a probiotic mixture containing several billions of different bacterial strains, mainly *Lactobacillus* and *Bifidobacteria*, is effective in preventing diverticulitis recurrence. Thirty consecutive patients (19 males, 11 females, mean age 60.1 years, range 47–75 years) affected by uncomplicated diverticulitis of the colon were monitored. After obtaining remission, the patients were randomly assigned to one of the following groups as follows: group A, balsalazide 2.25 g daily for 10 days every month plus VSL#3 450 billions/day for 15 days every month and group B, VSL#3 alone 450 billions/day for 15 days every month. Primary endpoint was considered the maintaining of remission throughout a 12-month follow-up. Secondary end-points considered were: firstly the assessment of the overall scores at the end of the follow-up and secondly the effects of the two different treatments with regards to every symptom assessed. The Authors found that one group A patient (6.66%) showed relapse of symptoms at the 10th month of follow-up; at the end of follow-up, 11 patients were completely symptom-free (73.33%) whilst 2 patients complained of only mild, recurrent symptoms (13%); two group B patients (13.33%) showed relapse of the disease at the 5th and 8th month of follow-up, respectively; at the end of follow-up, 8 patients were completely symptom-free (60%), 2 patients complained of mild, recurrent symptoms (13.33%), 1 patient (6.66%) complained of mild but continuous symptoms; no side effects were recorded throughout the follow-up in both groups. This study showed that the combination probiotic/anti-inflammatory drug was found better than probiotic treatment in preventing relapse of uncomplicated diverticulitis of the colon, even if without statistical significance.

Tursi et al.,⁶⁶ after the 2007 pilot study, designed another study to assess if four different therapeutic schedules with mesalazine and/or probiotics are able to prevent the recurrence of symptomatic DD of the colon. This prospective, dose-finding study was conducted on 75 patients, enrolled in an open fashion: mesalazine 800 mg/daily (group M1) or mesalazine 1.6 gr 10 day/month (group M2); mesalazine 800 mg/daily + *L. casei* DG 16 billion/day for 10 day/month (group LM1) or mesalazine 1.6 gr + *L. casei* DG 16 billion/day for 10 day/month (group LM2); *L. casei* DG 16 billion/day for 10 day/month (group L). Seventy one patients completed the study (94.66%). Sixty six patients (88%) were symptom-free after the 24th month of treatment: 11 of group M1, 8 of group M2, 15 of group LM1, 12 of group LM2 and 20 in group L. Four patients (5.33%) suspended the treatment during the follow-up: all experienced recurrence of symptoms (100%), and two of them developed diverticulitis (50%). This study showed that Mesalazine and/or *L. casei* seem to be effective in maintaining remission of DD for long-time. The Authors found recurrence of the disease and complications in all the patients who suspended treatment.

3.2. Irritable bowel syndrome

In Europe and North America IBS is estimated to have an incidence of 10–15%. In Sweden, the most commonly cited figure is 13.5%. The prevalence of IBS is increasing in countries in the Asia–Pacific region, particularly in countries with developing economies. IBS mainly occurs between the ages of 15 and 65. The estimated prevalence of IBS in children is similar to that in adults.⁶⁷ IBS is a common disorder of the intestines associated with cramping, stomach pain, gas, bloating, and changes in bowel habits. IBS can be characterized by constipation, diarrhea or both. Diarrhea-predominant IBS (D-IBS) is characterized by an increased intestinal permeability accompanied by persistent low-grade immune activation in the gut presented as increased numbers of T lymphocytes, mast cells and enterochromaffin cells. In its normal condition, gut epithelial lining forms a relatively impermeable

barrier between luminal contents and submucosa required for intestinal homeostasis. This barrier is determined by complexes of proteins composing the junctional complexes. Tight junctions (TJs) are the most apical organelle of the epithelial junctional complexes and are crucial for the formation and function of epithelial barriers. TJs comprise numerous proteins, with the best characterized being zonula occludens (ZO)-1 and occludin.⁶⁸ The diarrhea form is the predominant form and brings the patient to experience abdominal pain, visible abdominal distension (bloating), bowel frequency particularly in association with pain, pain relieved by bowel action, rectal passage of mucous and sensation of incomplete evacuation. Spastic colon syndrome is an IBS variant which is mainly characterized by constipation. The constipation IBS variant let the patient experience the same symptoms except for the bowel frequency which is replaced by the constipation which is responsible for the pain. IBS seems to be due to a dysfunction of the muscles of the organs of the GI tract or the nerves controlling the organs. Nerves run for the entire length of the GI tract from the esophagus to the anus in the muscular walls of the organs reaching also the spinal cord and the brain. IBS is thought to result from an interplay of abnormal GI tract movements, increased awareness of normal bodily functions, and a change in of the nervous system communication between the brain and the GI tract. Although the causes of IBS are not yet known some factors are usually associated to this condition: genetic influences, food intake, endocrine imbalances, malabsorption, post-operative changes, altered GI motility, heightened sensory function of the intestine, or malfermentation of food residues and psychosomatic factors such as a psychological morbidity, stress and an abnormal illness behaviour.⁶⁹ IBS has a great negative impact on the patient's emotional health and quality of life especially for those who are facing this disease for their entire life. Symptoms can be alleviated by the patient's education, reassurance, and dietary modification but they are not a definitive resolution.⁶⁸ Before considering the most recent clinical trials for the treatment of IBS it is worth to remember some important studies faced before. In 1998 Vanderhoof et al.⁷⁰ reported a study developed in Poland using *L. Plantarum* 299 (LP299V). This bacterium is able to synthesize nitric oxide, an important mediator of gut motility, it may displace the attachment of other organisms from the intestinal mucosa and it seems to be able to reduce any inflammatory component associated with IBS. The study results showed a reduction of symptoms of abdominal pain and constipation when compared with placebo. The same Author⁷¹ reported another study to support the beneficial effect of LP299V. This small double-blinded placebo-controlled crossover study in

children with recalcitrant chronic, recurrent abdominal pain of childhood showed a significant reduction in the severity of abdominal pain in LP299V-treated patients compared with results in control subjects. Tsuchiya et al. realized a study to evaluate the efficacy of a symbiotic containing bifidobacteria ($4.95 \times 10^9/100$ ml) for the treatment of IBS. The Authors found that, after a 12 weeks treatment on 68 patients, the symbiotic was able to significantly reduce the intensity of the symptoms of IBS. O'Mahony et al.⁷² realized a study to compare the response of symptoms and cytokine ratios in IBS with ingestion of probiotic preparations containing a lactobacillus or bifidobacterium strain. Seventy-seven subjects with IBS were randomized to receive either *Lactobacillus salivarius* UCC4331 or *Bifidobacterium infantis* 35624, each in a dose of 1×10^{10} live bacterial cells in a malted milk drink or the malted milk drink alone as placebo for 8 weeks. *B. infantis* 35624 alleviates symptoms in IBS; this symptomatic response was associated with normalization of the ratio of an anti-inflammatory to a pro-inflammatory cytokine, suggesting an immune-modulating role for this organism in this disorder. Furrie et al.⁷³ designed a study based on the use of a symbiotic containing bifidobacteria to treat IBS. The symbiotic was successful in treating the pathology without evident adverse events and modulate the immunological parameters of the same. The treatment based on bifidobacteria and FOS improves the intestinal habits and the quality of patients' life.

Table 2, adapted from Guslandi et al.⁷⁴, summarizes some interesting studies involving the use of probiotics in the treatment of IBS.

Hun et al.⁷⁵ designed a controlled study to evaluate the effects of the probiotic *Bacillus coagulans* GBI-30, 6086 on IBS symptoms. *Bacillus coagulans* GBI-30, 6086 (Ganeden Biotech, Inc., Mayfield Heights, OH) is a patented strain of lactic acid producing bacteria that can sustain the low pH of stomach acid and become active in the intestine. Strains of *B. coagulans* produce coagulin, which is a heat-stable, protease-sensitive, bacteriocin-like inhibitory substance with activity against gram-positive bacteria. Spores of *Bacillus* are resistant to heat and hostile GI conditions and, therefore, are able to reach the intestine where they can germinate and proliferate within the host. *B. coagulans* GBI-30, 6086 maintains spore viability after 5 years of storage without the need for refrigeration (unpublished communication, Ganeden Biotech, Inc., Mayfield Heights, OH), making it particularly suitable for commercial use. This randomized, double-blind, parallel-group, placebo-controlled clinical trial involved 44 subjects who received either placebo or *B. coagulans* GBI-30, 6086 once a day for 8 weeks.

Table 2

Table reported from Guslandi et al. showing some studies involving the use of probiotics in the treatment of IBS.

Probiotic	Patient (n)	Symptoms	Probiotic effect VS Placebo
L. plantarum	60	Flatulence, Abdominal pain	Improved Same
	12 (crossover trial)	Flatulence Abdominal Pain	Same Improved
L. GG	40	Overall	Same
	24	Overall	Same
	50 (children) 37 (children)	Pain frequency Pain severity	Improved Same
L. reuteri	54	Overall	Same
S. boulardii	34	Overall	Improved
	62	Diarrhea	Improved
		Overall Diarrhea Pain	Improved Improved
VSL #3® is a Cocktail containing <i>Bifidobacteria</i> – <i>B. longum</i> , <i>B. infantis</i> and <i>B. breve</i> ; <i>Lactobacilli</i> – <i>L. acidophilus</i> , <i>L. casei</i> , <i>L. Bulgaricus</i> and <i>L. Plantarum</i> ; and <i>Streptococcus thermophilus</i> .	48	Bloating	Same

Self-assessments of the severity of IBS symptoms (abdominal pain and bloating) were recorded every day for 8 weeks. Because baseline values were significantly different between the 2 study groups, within-group analysis was conducted. All subjects met the Rome II Criteria for IBS with diarrhea, i.e. they must have had, during the 12 months prior to evaluation, and for a total of at least 12 weeks (not necessarily consecutively), abdominal discomfort or pain that had 2 of 3 features: relief with defecation, onset associated with a change in frequency of stool and onset associated with a change in appearance of stool. Individuals with any organic GI conditions or diseases, previous intestinal surgery, immunodeficiency, or lactose intolerance, or who were pregnant or lactating, were excluded from the study. Patients who had taken commercially available probiotic medications within 30 days of the study were also excluded. The Authors obtained as results the improvements from baseline abdominal pain and bloating scores in the *B coagulans* GBI-30, 6086 group which were statistically significant for all 7 weekly comparisons ($P < 0.01$); in the placebo group, only changes in abdominal pain scores at 6 and 8 weeks achieved statistical significance ($P < 0.05$); no treatment-related adverse events or serious adverse events were reported during the 8-week study period. The Authors, basing on the previous described results, suggest that the patented *B coagulans* GBI-30, 6086 probiotic might be a safe and effective option for the relief of abdominal pain and bloating for patients with IBS. These results justify the design of larger scale, controlled clinical trials to verify these findings.

Henck et al.⁷⁶ performed a clinical trial treating two hundred and ninety-eight patients with lower abdominal symptoms diagnosed as IBS for 8 weeks by the compound Symbioflor-2 (Symbiopharm GmbH, Herborn, Germany), an *E. coli* product ($N = 148$), or placebo ($n = 150$) in a double-blinded, randomized fashion. Patients were seen weekly by the physician, who assessed the presence of core IBS symptoms. Both an abdominal pain score (APS) as well as a general symptom score (GSS) were used as primary endpoints. Responders had to have complete absence of IBS core symptoms at $>$ or $= 1$ visit during treatment. The results showed that: the responder rate in GSS to the drug was 27/148 (18.2%) in comparison to placebo with 7/150 (4.67%) ($p = 0.000397$); the improvement in APS was 28/148 (18.9%) and 10/150 (6.67%) for placebo ($p = 0.001649$); the response was reached from the third visit onwards with both medication and placebo; post-hoc analysis revealed no significant differences in efficacy of the drug between the gender and different age groups. The Authors conclude that the probiotic Symbioflor-2 is effective and superior to placebo in reducing the typical symptoms of IBS.

Barrett et al.⁷⁷ aimed at determining whether *L. casei* strain *Shirota* (Yakult) can alter small intestinal bacterial overgrowth (SIBO), as tested by the lactulose breath test, and whether it is associated with changes in symptoms in IBS. Prior to the breath hydrogen test, dietary restriction (minimising intake of fibre and poorly absorbed short-chain carbohydrates) was advised for 24 h and the subjects fasted overnight. The patients consumed 15 g lactulose, made up to a 100 mL solution with water. Breath hydrogen samples were collected at baseline and every 15 min for at least 2 h. The Authors recruited 18 patients with IBS (Rome II criteria), who showed an early rise in breath hydrogen with lactulose (ERBHAL: defined as a rise of breath hydrogen of 10 ppm or greater above the baseline breath hydrogen on two consecutive 15-min samples, before 90 min following the ingestion of lactulose. The time of the first rise in breath hydrogen of 10 ppm or more was recorded). They consumed 65 mL of Yakult daily for 6 wks. Lactulose breath test was repeated at the end of the treatment period. Symptoms were recorded daily using a 10 cm visual analogue scale. Fourteen patients completed the study, 9 (64%) had reversal of ERBHAL, with the median time of first rise in breath hydrogen

increasing from 45 to 75 min ($P = 0.03$). There was no significant improvement in the symptoms score with probiotic therapy, except for wind ($P = 0.04$). Patients commencing with at least moderate symptoms and who no longer had ERBHAL at the end of treatment, showed improvement in the overall symptoms scores [median final score 5.3 (IQR 3.9–5.9), 55% reduction; $n = 6$] to a greater extent than those who had had persisting ERBHAL [final score 6.9 (5.0–7.0), 12% reduction; $n = 5$; $P = 0.18$]. This study shows that Yakult is effective in altering fermentation patterns in the small bowel, consistent in reducing SIBO. The loss of ERBHAL was associated with reduced symptoms. This study findings need to be confirmed by a randomized controlled trial.

Adriulli et al.⁷⁸ tested the efficacy of symbiotics in patients with D-IBS. Patients were randomized to a prebiotic ($n = 135$), or a symbiotic formulation containing *Lactobacillus paracasei* B21060 (Flortec, $n = 132$). Primary efficacy was the responder rate for pain and global relief of symptoms in the overall population and in patients with predominant diarrhea ($n = 47$). Post hoc time-trend analyses for changes within each treatment were carried out. The following results were obtained: patients with absent/mild pain amounted to 54.7% in the symbiotic group and to 57.4% in the prebiotic group at treatment week 4, and to 53.9% and 53.4% at the end of treatment; patients with amelioration of well-being were, respectively, 60.7% versus 61.7% at treatment week 4, and 63.3% versus 60.9% at the end of treatment; within each treatment group, patients with absent/mild pain increased in the Flortec and the prebiotic group, but time trend analyses were significant only for Flortec ($P = 0.019$); in D-IBS, Flortec significantly reduced bowel movements, pain, and IBS scores. The Authors concluded that: Flortec is encouraging in patients with D-IBS and is able to improve the pain and the patient's well-being even if in the future Flortec has to be compared with an inert placebo to establish its efficacy for the majority of IBS patients.

Zeng et al.⁶⁸ performed a study to determine the possible effect of lactic acid bacteria on the increased intestinal permeability in D-IBS. This treatment lasted 4 weeks in a randomized single blind placebo-controlled study with 30 D-IBS patients. Patients were given either a probiotic fermented milk (*Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium Longum*) or milk beverage containing no bacteria. The clinical symptoms were scored and intestinal permeability measured by a triple sugar test before and after treatment. Small bowel permeability was measured as the ratio of L/M recovery and colonic permeability was measured as the total mass of sucralose excretion (mg). After probiotics treatment, small bowel permeability decreased significantly from 0.038 at baseline to 0.023 ($P = 0.004$), the proportion of patients with increased small bowel permeability was lower than that one at baseline (28.6% vs. 64.3%, $P = 0.023$). However, colonic permeability improved neither in the probiotics group nor in the placebo group at week 4. Treatment with probiotics significantly decreased the mean global IBS scores compared with the baseline scores (9.62 ± 1.05 vs. 7.64 ± 1.24 , $P < 0.001$). Therefore short-term active lactic acid bacteria treatment for D-IBS improves mucosal barrier function.

Enck et al.⁷⁹ re-analyzed a study performed in 1988 and 1989 according to current IBS standards. Two hundred ninety-seven patients with lower abdominal symptoms diagnosed as IBS were treated for 8 weeks with the compound ProSymbioflor(R) (Symbiopharm GmbH, Herborn, Germany), an autolysate of cells and cell fragments of *Enterococcus faecalis* and *Escherichia coli*, or placebo in a double-blinded, randomized fashion. Patients were seen weekly by the physician, who assessed the presence of core IBS symptoms. Responders had at least a 50% GSS and in APS reports at $>/ = 1$ visit during treatment. The responder rate in GSS to the drug was 102/149 (68.5%) in comparison to placebo with 56/148 (37.8%)

($P < 0.001$), the improvement in APS was 108/149 (72.5%) and 66/148 (44.6%) respectively ($P = 0.001$). The number needed to treat was 3.27 for GSS and 3.59 for the APS report. Kaplan–Meier analysis revealed a mean response time of 4–5 weeks for active treatment and more than 8 weeks for placebo ($P < 0.0001$). This study leads the Authors to conclude that treatment of IBS with the bacterial lysate ProSymbioflor is effective and superior to placebo in reducing typical symptoms of IBS patients.

Drouault-Holowacz et al.⁸⁰ investigated the efficiency of a probiotic dietary supplement, containing four strains of lactic acid bacteria, on symptoms of IBS. One hundred and sixteen patients with IBS fulfilling the Rome II criteria were randomized in a parallel group, double-blind study to receive a placebo or a probiotic combination (1×10^{10} cfu) once daily for four weeks. The symptoms that were monitored weekly included discomfort, abdominal pain, and stool frequency and quality. Quality of life was assessed before and at the end of the treatment using the SF36 and FDD-quality-of-life questionnaires. The study showed the following results: one hundred subjects completed the study (48 in the probiotic combination group and 52 in the placebo group); the probiotic combination was not superior to the placebo in relieving symptoms of IBS (42.6% versus 42.3% improvement); the decrease of abdominal pain between the first and the fourth week of treatment was significantly higher in probiotic treated patients (–41.9 versus –24.2%, $P = 0.048$). Interesting findings were also observed from the IBS sub-groups such as a lower pain score at endpoint in patients with alternating bowel habits ($P = 0.023$) and an increase of stool frequency in the constipated sub-group from the first week of probiotic treatment ($P = 0.043$). The Authors conclude that the probiotic combination was not significantly superior to the placebo in relieving symptoms of IBS. Despite the apparent high placebo response, interesting findings were observed from IBS sub-groups in the field of abdominal pain and stool frequency.

Sinn et al.⁸¹ randomized 40 IBS patients into a placebo ($n = 20$) and probiotics group ($n = 20$). *Lactobacillus acidophilus*, SDC 2012 and 2013. These two lactobacilli were obtained from infants' feces and examined for their biological and biochemical characteristics. They showed the ability to survive in high acidity and medium containing bile acids as potent probiotics (unpublished data). These two selected strains were freeze dried by a microbial company (Culture systems, Inc. USA). The freeze-dried bacteria were mixed with an excipient and packed into capsules under good manufacturing processing (GMP) conditions (Natural F&P. Korea). In this study, the excipient was added to the blend of bacteria to achieve the desired dosage concentrations, 2×10^9 cfu/mL. Placebo capsules contained the excipient only. Four week treatment with *L. acidophilus*-SDC 2012, 2013 was associated with a reduced score for abdominal pain or discomfort compared to the baseline ($P = 0.011$). The percent reduction in abdominal pain or discomfort exceeded the placebo scores by more than 20% (23.8 and 0.2% for probiotics and placebo, respectively, $P = 0.003$). There was a significant difference in the proportion of responders between the probiotics and placebo groups ($P = 0.011$). There was no drop out or adverse events for either group during the study period. *Lactobacillus acidophilus*-SDC 2012, 2013 appeared to have a beneficial effect in patients with IBS. The observed benefits of *L. acidophilus*-SDC 2012, 2013 could come from anti-inflammatory effects or by repairing the dysfunctional relationship between the indigenous flora and the host. The Authors conclude that the exact mechanism of the beneficial effects of *L. acidophilus*-SDC 2012, 2013 requires further evaluation.

Bittner et al.⁸² designed a study to extend a previous 2-week assessment of a probiotic–prebiotic complex in patients with IBS. Prescript-Assist™ (P-A) is a probiotic–prebiotic complex reported to be associated with reduced signs and symptoms of several GI

disorders, particularly IBS. The probiotic component is a complex of 29 (*Anthrobacter agilis*, *A. citreus*, *A. globiformis*, *A. luteus*, and *A. simplex* *Acinetobacter calcoaceticus* *Azotobacter chroococcum* and *A. paspali* *Azospirillum brasiliense* and *A. lipoferum* *Bacillus brevis*, *B. marcerans*, *B. pumilus*, *B. polymyxa*, and *B. subtilis* *Bacteroides lipolyticum* and *B. succinogenes* *Brevibacterium lipolyticum* and *B. stationis* *Kurtha zopfii* *Myrothecium verrucaria* *Pseudomonas calcis*, *P. dentrificans*, *P. fluorescens*, and *P. glathei* *Phanerochaete chrysosporium* *Streptomyces fradiae*, *S. cellulosae*, and *S. griseoflavus*) soil-based microorganisms (SBOs), all class I etiologic agents (they present “no or minimal hazard under ordinary conditions”). In this open-label, partially controlled, 1-year extension study, data were collected from IBS patients who continued treatment following a 2-week study of the efficacy of the probiotic–prebiotic complex. Data were collected at 2 and approximately 60 weeks after the end of the original study. The Authors observed that a total of 25 patients entered the 2-week extension and 22 completed the approximately 60-week follow-up study. The results in the control group 2 weeks after crossover to treatment were similar to those from the original study, with reductions in IBS subsyndromes, i.e. general ill feelings/nausea ($P < 0.001$), indigestion/flatulence ($P < 0.001$), and marginally colitis ($P < 0.03$ [1-tailed]). Treatment was associated with a continued reduction in general ill feelings/nausea at 4 weeks ($P < 0.007$). At $>$ or = 52-week follow-up, the rate of remissions was 81.5%–100% ($P < 0.003$). Based on these results, treatment with this probiotic–prebiotic complex seems to be an option for short-term (2–4 weeks) and long-term (approximately 60 weeks) reductions in IBS symptoms.

Guyonette et al.⁸³ analyzed the effects of fermented milk containing *Bifidobacterium animalis* DN-173,010 and yogurt strains on the IBS in a multicenter, double-blind, controlled trial. The test product was a fermented milk (Activia, Danone), containing *B. animalis* DN-173, 010 (1.25×10^{10} CFU per pot) together with the two classical yogurt starters, *S. thermophilus* and *L. bulgaricus* ($1.2 \cdot 10^9$ cfu/pot). The control product was heat-treated yogurt containing non-living bacteria ($<10^4$ cfu/pot). Both the test and control products were without flavour, and had a similar appearance, colour, texture and taste. Each serving, corresponding to one pot, contained 125 g. Both products were specifically prepared for the study and provided by Danone Research (Palaiseau, France). A total of 274 primary care adults with constipation-predominant IBS (Rome II) were randomized to consume for 6 weeks either the test fermented milk or a heat-treated yogurt (control). HRQoL and digestive symptoms were assessed after 3 and 6 weeks on an intention-to-treat population of 267 subjects. The previously described results brought the Authors to the following results: the HRQoL discomfort score, the primary endpoint, was improved ($P < 0.001$) in both groups at weeks 3 and 6; the responder rate for the HRQoL discomfort score was higher (65.2 vs. 47.7%, $P < 0.005$), as it was higher the decrease in bloating score ($0.56 \pm$ (s.d.) 1.01 vs. 0.31 ± 0.87 , $P = 0.03$), at week 3 in the test vs. the control group. In those subjects with <3 stools/week, stool frequency increased ($P < 0.001$) over 6 weeks in the test vs. control group. This study suggests a beneficial effect of a probiotic food on discomfort HRQoL score and bloating in constipation-predominant IBS, and on stool frequency in subjects with <3 stools/week. In conclusion, the present large-scale study strongly suggests a beneficial effect of a probiotic food containing *B. animalis* DN-173010 on HRQoL discomfort score and bloating, and also on stool frequency in those subjects with <3 stools per week. Further studies aiming at confirming the results obtained and to elucidate mechanisms of such effects should be of special interest for providing additional scientific evidence to support the use of such probiotic food to alleviate IBS symptoms and improve HRQoL discomfort.

Gawrońska et al.⁸⁴ evaluated the efficacy of *L. rhamnosus GG* for treating functional abdominal pain disorders (FAPD) in children. A total of 104 children who fulfilled the Rome II criteria for functional dyspepsia (FD), or IBS, or functional abdominal pain (FAP) were enrolled in a double-blind, randomized controlled trial in which they received *L. rhamnosus GG* ($n = 52$), or placebo ($n = 52$) for 4 weeks. The patients in the *L. rhamnosus GG* group were more likely to have treatment success (no pain) than those in the placebo group (25% vs. 9.6%, relative benefit (RB) 2.6, 95% CI: 1.05–6.6, number needed to treat (NNT) 7, 95% CI: 4–123). For children with IBS ($n = 37$), those in the *L. rhamnosus GG* group were more likely to have treatment success than those in the placebo group (33% vs. 5%, RB 6.3, 95% CI: 1.2–38, NNT 4, 95% CI: 2–36) and reduced frequency of pain ($P = 0.02$), but not pain severity ($P = 0.10$). For the FD group ($n = 20$) and FAP group ($n = 47$), no differences were found. The Authors conclude that the *L. rhamnosus GG* appears to moderately increase treatment success, particularly among children with IBS.

Fanigliulo et al.⁸⁵ aimed at investigating the efficacy of rifaximin (a broad-spectrum, poorly absorbable antibiotic) on its own or in association with the probiotic strain of *B. longum W11* in reducing symptoms in patients with IBS. They performed a monocentric, prospective, randomized open trial including 70 patients randomized to two groups: Group A (41 patients) receiving rifaximin 200 (2 cp bid for ten days in a month) followed by a formulation of the probiotic strain of *B. longum W11* (one granulated suspension for 6 days on alternate weeks) and Group B (29 patients) receiving only rifaximin 200 (2 cp bid for ten days in a month). The clinical evaluation was performed at admission and after 2-months, taking into account the method of visual analogues. The Authors observed that at the 2-month follow-up, Group A patients reported a greater improvement of symptoms compared to patients in group B ($p = 0.010$) even if the physician's opinion at T1 did not confirm these results ($p = 0.07$). The previously reported results showed an increased colonization by means of *B. longum W11*, after the cyclic administration of rifaximin, which eradicates the bacterial overgrowth of the small intestine and may reduce symptoms, especially those related to bowel habit and stool frequency in patients with IBS. The abnormalities observed in the colonic flora of IBS suggest, in fact, that a probiotic approach will ultimately be justified.

Colecchia et al.⁶⁹ performed a study to evaluate the efficacy and safety of a symbiotic consisting of a probiotic, *B. longum W11*, and the short chain oligosaccharide prebiotic Fos Actilight, in patients with constipation-variant IBS. Six hundred and thirty six patients (250 men, 386 women) diagnosed with constipation-type IBS according to the Rome II criteria were enrolled in 43 centers and received the symbiotic at a dose of 3 g/die for at least 36 days. A validated questionnaire investigating symptoms and stool frequency was administered before and after treatment. The results were based on patients' responses to visual scale items; frequency increased significantly after treatment in the "no symptom" class from 3% to 26.7% for bloating and from 8.4% to 44.1% for abdominal pain ($P < 0.0001$); in the severest symptoms classes (moderate-severe), symptom frequency dropped significantly from 62.9% to 9.6% and from 38.8% to 4.1% for bloating and abdominal pain, respectively. Stool frequency significantly increased from 2.9 ± 1.6 times/week to 4.1 ± 1.6 times/week. The Authors conclude that the study product can increase stool frequency in patients with constipation-variant IBS and reduce abdominal pain and bloating in those ones with moderate-severe symptoms.

Whorwell et al.⁸⁶ assessed the efficacy of the probiotic bacteria *B. infantis 35624* on a large-scale, multicenter, clinical trial of women with IBS determining the optimal dosage of probiotic for administration in an encapsulated formulation. After a 2 weeks baseline,

362 primary care IBS patients, with any bowel habit subtype, were randomized to either placebo or freeze-dried, encapsulated *B. infantis* at a dose of 1×10^6 , 1×10^8 , or 1×10^{10} cfu/mL for 4 wks. IBS symptoms were monitored daily and scored on to a 6-point Likert scale with the primary outcome variable being abdominal pain or discomfort. A composite symptom score, the subject's global assessment of IBS symptom relief, and measures of quality of life (using the IBS-QOL instrument) were also recorded. The Authors report the following results: *B. infantis 35624* at a dose of 1×10^8 cfu was significantly superior to placebo and all other bifidobacterium doses for the primary efficacy variable of abdominal pain as well as the composite score and scores for bloating, bowel dysfunction, incomplete evacuation, straining, and the passage of gas at the end of the 4 week study; the improvement in global symptom assessment exceeded placebo for more than 20% ($p < 0.02$); two other doses of probiotic (1×10^6 and 1×10^{10}) were not significantly different from placebo; of these, the 1×10^{10} dose was associated with significant formulation problems. No significant adverse events were recorded. The Authors conclude that *B. infantis 35624* is a probiotic that specifically relieves many of the symptoms of IBS. At a dosage level of 1×10^8 cfu, it can be delivered by a capsule making it stable, convenient to administer, and amenable to widespread use. The lack of benefits observed with the other dosage levels of the probiotic highlights the need for clinical data in the final dosage form and dose of probiotic before these products are used in practice.

3.3. Inflammatory bowel disease clinical trials

IBD commonly refers to two chronic inflammatory diseases of the GI tract: UC and CD. The main difference between CD and UC is the location and nature of the inflammatory changes. CD can affect any part of the GI tract, from mouth to anus, although a majority of the cases start in the terminal ileum. UC affects the colon and the rectum. Microscopically, UC is restricted to the mucosa while CD affects the whole bowel wall. The World Gastroenterology Organization reports that: UC incidence has been increasing in Western countries since the Second World War and in low-incidence areas in eastern Europe, Asia and developing countries; CD incidence is <1 per 100,000 in Asia and South America, 1–3 per 100,000 in southern Europe, South Africa, 16 per 100,000 in New Zealand and Australia, 14 per 100,000 in Canada, 7 per 100,000 in the USA (based on data only from Olmsted County, Minnesota).⁸⁷ The pathogenesis of IBD is not completely understood. Genetic and environmental factors such as altered luminal bacteria and enhanced intestinal permeability play a role in the dysregulation of intestinal immunity, leading to GI injury. An abnormal activation of the mucosal immune system driven by the presence of the intestinal microbiota in a genetically predisposed patient seems to play a key role in this pathology. Especially in CD the intestinal microbiota is strongly suspected to play a role in initiating and triggering the immune system, leading to a characteristic inflammation. Furrie et al.⁸⁸ used a synbiotic combining a probiotic, *B. longum*, isolated from healthy rectal epithelium, and a prebiotic (Synergy 1), a preferential inulin-oligofructose growth substrate for the probiotic strain to treat UC patients. Treatment was employed in a double blinded randomized controlled trial using 18 patients with active UC for a period of one month. Clinical status was scored and rectal biopsies were collected before and after treatment, and transcription levels of epithelium related immune markers were measured. The Authors conclude that short-term synbiotic treatment of active UC resulted in improvement of the full clinical appearance of chronic inflammation in patients receiving this therapy.

Sokol et al.⁸⁹ determined the composition of the mucosa-associated microbiota of CD patients at the time of surgical resection and 6 months later using FISH analysis. They found that a reduction of a major member of Firmicutes, *Faecalibacterium prausnitzii*, is associated with a higher risk of post-operative recurrence of ileal CD. This study was conducted as part of a double-blind controlled trial that compared the efficiency of the probiotic *L. johnsonii* LA1 strain and a placebo to decrease endoscopic recurrence after curative surgery for CD. Although the whole human trial included 98 patients, the present study, included only a subset of these patients. *F. prausnitzii* A2–165 (DSM 17677), isolated from human fecal stool, was grown at 37 °C in LYHBHI medium (Brain–heart infusion medium supplemented with 0.5% yeast extract (Difco) and 5 mg/L hemin) supplemented with cellobiose (1 mg/ml; Sigma–Aldrich), maltose (1 mg/ml; Sigma), and cysteine (0.5 mg/ml; Sigma) in an anaerobic chamber. A lower proportion of *F. prausnitzii* on resected ileal Crohn mucosa was also associated with endoscopic recurrence at 6 months. The Authors observed the anti-inflammatory effects in both in vitro (cellular models) and in vivo [2,4,6-trinitrobenzenesulphonic acid (TNBS)-induced] colitis in mice to evaluate the immunomodulatory properties of *F. prausnitzii*. In Caco-2 cells transfected with a reporter gene for NF-κB activity. The study showed the following results: 1) *F. prausnitzii* had no effect on IL-1β-induced NF-κB activity, whereas the supernatant abolished it; 2) in vitro PBMC stimulation by *F. prausnitzii* led to significantly lower IL-12 and IFN-γ production levels and higher secretion of IL-10; 3) oral administration of either live *F. prausnitzii* or its supernatant markedly reduced the severity of TNBS colitis and tended to correct the dysbiosis associated with TNBS colitis, as demonstrated by real-time quantitative PCR (qPCR) analysis. In conclusion these results show that *F. prausnitzii* is an anti-inflammatory bacterial candidate. This bacterium exhibited anti-inflammatory effects, partly due to secreted metabolites blocking NF-κB activation and IL-8 secretion. In vivo effects were associated with a decrease in proinflammatory colonic cytokine synthesis and with the induction of anti-inflammatory cytokine secretion. Counter balancing dysbiosis, using the commensal bacterium *F. prausnitzii* as a candidate probiotic agent, appears to be a promising strategy in CD treatment. Further clinical studies are required to establish the diagnostic tools to define the best population of patients for this probiotic species, and especially whether the clinical benefit is more pronounced in patients with low levels of endogenous Firmicutes.

Vilela et al.⁹⁰ evaluated the influence of *Saccharomyces boulardii* on the intestinal permeability in CD. The Authors randomized thirty-four patients according to the Vienna classification for treatment with either placebo or *Saccharomyces boulardii*. The first group, consisting of 19 patients, received a placebo every 8 h as an oral capsule containing 200 mg cellulose, 6 mg sucrose and 2.4 mg magnesium stearate. The second group, consisting of 15 patients, received *S. Boulardii* every 8 h as an oral capsule formulation which contained 200 mg lyophilized *S. Boulardii* (about 4×10^8 cells), 6 mg sucrose and 2.4 mg magnesium stearate (Floratil®). A third group, consisting of 15 healthy volunteers, aged between 23 and 47 years, (mean age 36 years), who agreed to take part in the study, within the ethical norms of human research, were submitted to intestinal permeability tests in order to establish control values. They were not assigned to any kind of treatment. Baseline medications (mesalamine, azathioprine, prednisone, metronidazole and/or thalidomide) were maintained. Intestinal permeability (L/M ratio) was evaluated immediately before the beginning of treatment and at the end of the first and third treatment month. The Authors obtained the following results in volunteers, the L/M ratio was 0.005 ± 0.0037 , whereas this value was 0.021 ± 0.01 in patients with CD ($p = 0.001$). In the placebo group, there was an increase in

L/M ratio by 0.004 ± 0.010 ($p = 0.12$) at the end of the third month. In the *S. boulardii* group, there was an improvement in intestinal permeability, with a decrease in the L/M ratio by 0.008 ± 0.006 ($p = 0.0005$) in the same period.

The previously described results bring the Authors to the conclusion that patients with CD in remission present alterations in the integrity of the intestinal mucosal barrier according to L/M ratio. *S. Boulardii*, added to baseline therapy, improves intestinal permeability but complete normalization was not achieved.

Fujimori et al.⁹¹ realized a study to assess the clinical usefulness of combined probiotic and prebiotic therapy in the treatment of active CD. Ten active CD outpatients without history of operation for CD were enrolled (CD was diagnosed by established clinical, endoscopic, radiological and histological criteria.). Their mean (\pm) age was 27 ± 7 years and the main symptoms presented were diarrhea and abdominal pain. Patients' initial therapeutic regimen of aminosalicylates and prednisolone failed to achieve remission. Patients were thus initiated into a synbiotic therapy, consisting of both probiotics (75 billion CFU daily) and prebiotics (psyllium 9.9 g daily). Probiotics mainly comprised *Bifidobacterium* and *Lactobacillus*. Patients were free to adjust their intake of probiotics or prebiotics throughout the trial. Chron's Disease Activity Index (CDAI), International Organization for the Study of Inflammatory Bowel Disease (IOIBD) score and blood sample variables were evaluated and compared before and after the trial. The duration of the trial was 13.0 ± 4.5 months. By the end of therapy, each patient had taken a 45 ± 24 billion CFU daily probiotic dose, with six patients taking an additional 7.9 ± 3.6 g daily psyllium dose. Seven patients had improved clinical symptoms following combined probiotic and prebiotic therapy. Both CDAI and IOIBD scores were significantly reduced after therapy ($255-136$, $P = 0.009$; $3.5-2.1$, $P = 0.03$, respectively). Six patients had a complete response, one had a partial response, and three were non responders. Two patients were able to discontinue their prednisolone therapy, while four patients decreased their intake. There were no adverse events. High-dose probiotic and prebiotic cotherapy can be safely and effectively used for the treatment of active CD. In conclusion, our study shows that synbiotic therapy can safely reduce CD activity and achieve its remission. We found that synbiotic therapy is especially indicated for CD patients with frequent diarrhea. Further studies, examining such areas as fecal flora and SCFAs that compare CD patients with probiotics to CD patients with synbiotics, are expected. Larger scale studies and randomized controlled studies on the treatment of CD with probiotic therapy in combination with prebiotics are clearly necessary for a fuller appreciation of the therapeutic value of synbiotics.

Chermesh et al.⁹² designed a prospective multicenter, randomized study. Patients were randomized to active treatment or placebo in a 2:1 ratio. Active treatment consisted of Synbiotic 2000, which contains a mixture of prebiotics and probiotics, including 4 lactic acid bacteria and 4 fermentable fibers. The four lactic acid bacteria are 10^{10} *Pediococcus pentoseceus*, 10^{10} *L. raffinolactis*, 10^{10} *L. Paracasei susp paracasei* 19, and 10^{10} *L. plantarum* 2362; the 4 fermentable fibers are 2.5 g β-glucans, 2.5 g inulin, 2.5 g pectin, and 2.5 g resistant starch. The Authors checked if treatment with Synbiotic 2000 could prevent post-operative recurrence in patients with CD. Follow-up consisted of endoscopic, clinical, and laboratory parameters. Thirty patients were enrolled. No differences were found between the 2 treatment groups regarding gender, age at diagnosis, age at surgery, weight, smoking status, type of disease, length of the resected segment, or medical treatment prior to surgery. No difference in either endoscopic or clinical relapse rate was found between patients treated with once daily dose of Synbiotic 2000 or placebo. In our small study, Synbiotic 2000 had no effect on post-operative recurrence of patients with CD. Larger

studies in patients with the inflammatory type of CD undergoing surgery, using higher doses of probiotics cocktail, may prove effective. This study highlights that Synbiotic 2000 had no effect on post-operative recurrence of patients with CD.

Van Gossum et al.⁹³ designed a multicenter clinical trial evaluating the efficacy of an oral administration of the probiotic *L. johnsonii*, (LA1, Nestec) on early post-operative endoscopic recurrence of CD. Seventy patients with CD were enrolled prior to elective ileo-caecal resection and randomly assigned after surgery to daily treatment with either *Lactobacillus johnsonii*, (10^{10} CFU) (group A, $n = 34$) or placebo (group B, $n = 36$) for 12 weeks. The treatment consisted of the probiotic LA1 in freeze-dried form and blended with maltodextrin at 10^{10} CFU/day. The placebo was maltodextrin only. The LA1 powder was supplied in foil sachets (weight 2 g) containing 10^{10} CFU of probiotics. The placebo was a powder of the same appearance and weight, also in individual foil packets. Both probiotics and placebo were administered in combination with an enteral formula at 120 mL/day (ACD004, Nunspeet, Holland, Konolfingen, Switzerland). The identity of the treatment sachet was blind to patients, support staff, and investigators (numerical codes). Treatment codes were broken only by the statistician after completion of the trial. The primary objective was to assess the effect of LA1 on the endoscopic recurrence rate at 12 weeks. Stratification was performed according to smoking status at randomization. Seven and 14 patients were excluded in the LA1 and placebo groups, respectively. In intention-to-treat analysis, the mean endoscopic score was not significantly different between the two treatment groups at 3 months (LA1 versus placebo: 1.50 ± 1.32 versus 1.22 ± 1.37 , treatment effect: $P = 0.48$, smoke effect: $P = 0.72$). The percentage of patients with severe recurrence ($i3 + i4$) was 21% and 15% in the LA1 and placebo groups, respectively ($P = 0.33$). Using a per-protocol (PP) analysis, the mean endoscopic score was not significantly different between the two treatment groups (LA1 versus placebo groups: 1.44 ± 1.31 versus 1.05 ± 1.21 , $P = 0.32$). The percentage of patients with severe recurrence ($i3 + i4$) was 19% and 9% in the LA1 and placebo groups, respectively ($P = 0.054$). Clinical relapse rate Clinical relapse rate >150 , with an increase of clinical relapse rate >70 points or greater from baseline in the LA1 and placebo groups was 15% (4/27) and 13.5% (3/22), respectively (PP analysis: chi-square test, $P = 0.91$ and log-rank test: $P = 0.79$). Oral administration of the probiotic LA1 in patients with CD failed to prevent early endoscopic recurrence at 12 weeks after ileo-caecal resection.

Vasquez et al.⁹⁴ analyzed and compared the mucosa-associated bacteria (MAB) in non-inflamed and inflamed ileal mucosa of CD patients ($n = 22$). Tissue samples from the inflamed ileal mucosa and from the adjacent non-inflamed ileal mucosa were taken from surgical resection specimens. The MAB were investigated using fluorescence in situ hybridization with 7 group-specific probes and temporal temperature gradient gel electrophoresis (TTGE). Samples from both non-inflamed and inflamed mucosa were taken from 15 patients biopsies. The distribution of the bacterial populations was not different between non-inflamed and inflamed mucosa. The *Bacteroidetes phylum* was dominant and accounted for 29% of MAB (0–74%) in non-inflamed tissues and 32% (0–70%) in inflamed areas. The *Proteobacteria* represented 12% (0–70%) of MAB both in non-inflamed and inflamed areas. The *Clostridium coccoides* group (*Firmicutes phylum*) represented 15% of MAB in non-inflamed tissues versus 7% in inflamed areas. For most of the patients the similarity index between TTGE paired profiles was very high.

The Authors conclude that the dominant MAB do not differ between non-inflamed and inflamed ileal mucosa in CD. This fact argues against a localized dysbiosis to explain the patchy distribution of mucosal lesions.

3.4. *Helicobacter pylori* eradication

Helicobacter pylori (*H. pylori*) is a small curved to spiral rod-shaped bacterium found in the epithelial mucus surface of most patients with active gastritis. *H. pylori* is strongly associated with duodenal peptic ulceration and it is the main etiologic agent of chronic gastritis and gastric cancer and other gastric malignancies. Today the therapy to eradicate this bacterium is based on a combination of antibiotics and proton pump inhibitors (PPI). The (13)C-urea breath test is the commonly used test to diagnose the presence of *H. pylori* in the stomach. The test also finds application to understand if *H. pylori* has been eradicated through a treatment with antibiotics. The urea breath test is based on the ability of *H. pylori* to break down urea, a chemical made up of nitrogen and carbon, into carbon dioxide which then is absorbed from the stomach and eliminated in the breath. For the test, patients swallow a capsule containing urea made from an isotope of carbon. If *H. pylori* is present in the stomach, the urea is broken up and turned into carbon dioxide. The carbon dioxide is absorbed across the lining of the stomach and into the blood. It then travels in the blood to the lungs where it is excreted in the breath. Samples of exhaled breath are collected, and the isotopic carbon in the exhaled carbon dioxide is measured. If the isotope is detected in the breath, it means that *H. pylori* is present in the stomach. If the isotope is not found, *H. pylori* is not present. Probiotics seem to have a direct antimicrobial effect, as shown through in vitro studies, through competition with *H. pylori*, inhibition of adherence and production of metabolites and antimicrobial molecules. Implementation of probiotics with standard anti-*H. pylori* regimens can also improve patients' compliance with therapy, reducing the incidence of side effects associated with antibiotic treatment.

Kim et al.⁹⁵ performed a study to evaluate whether the addition of probiotics to PPI-based triple therapy increases the success in the eradication of *H. Pylori*. Three hundred and forty-seven *H. Pylori* infected patients were randomized into a triple-plus-yogurt group (yogurt group, $n = 168$) or a triple-only group (control group, $n = 179$). Triple therapy consisted of PPI b.i.d., clarithromycin 500 mg b.i.d., and amoxicillin 1 g b.i.d. for 7 days. The yogurt group received triple therapy for 1 week and one bottle of Will yogurt per day for 3 weeks, starting on the first day of the triple therapy. Will yogurt (a Korean brand) contains *L. acidophilus* HY2177, *L. casei* HY2743, *B. longum* HY8001, and *Streptococcus thermophilus*B-1. (13) C-urea breath test was performed for at least 4 weeks after completion of the triple therapy. Eradication rates, compliances, and adverse events were compared. With the intention to treat analysis the *H. Pylori* eradication rates in the yogurt group 79.2% (133 of 168) was similar to that in the control group 72.1% (129 of 179) ($p = 0.124$). However, by means of PP analysis, the eradication rate in the yogurt group, 87.5% (133 of 152) was higher than that in the control group, 78.7% (129 of 164) ($p = 0.037$). Common adverse events were metallic taste (11.8%) and diarrhea (8.6%). The frequency of adverse effects in the yogurt group 41.1% (69/168) were higher than in the control group, 26.3% (47 of 179) ($p = 0.003$). However, most adverse events were mild to moderate in intensity, and the severities of adverse effects were similar in both groups ($p = 0.401$). The Authors conclude that, the addition of a yogurt to triple therapy does not decrease the adverse effects of the triple therapy. However it increases *H. pylori* eradication rate in PP analysis, suggesting a possibility that the addition of Will yogurt, commercialized as a kind of food, into triple therapy, might be an option to increase the *H. pylori* eradication rate.

Imase et al.⁹⁶ examined intestinal microbiota changes during *H. pylori* eradication therapy and the preventive effect of CBM588 as a probiotic agent. Nineteen patients with gastro-duodenal ulcer were randomly divided into three groups: group A (without

probiotics), group B (with regular doses of *CBM588*) and group C (with double doses of *CBM588*). *CBM588* is a probiotic agent containing approximately 10^7 cfu per tablet. The incidence of diarrhea and soft stools during *H. pylori* eradication therapy was 43% in group A and 14% in group B, while none of the patients in group C reported diarrhea or soft stools. Both bacterial counts and detection rates of bifidobacteria and/or obligate anaerobe were decreased by eradication therapy. However, bacterial counts of obligate anaerobes in group C were significantly higher than in group A ($P < 0.05$). Additionally, during eradication therapy, *C. difficile* toxin A was detected in both group A and group B but not in group C. The Authors conclude that side effects associated with *H. Pylori* eradication therapy, including diarrhea and/or soft stools, are considered attributable to disturbances in the composition of intestinal microbiota caused by antibiotics, and to toxin A produced by *C. difficile*. The results of our study also suggest that these symptoms can be prevented by concomitant administration of a viable bacterial preparation containing *CBM588*.

Francavilla et al.⁹⁷ tested whether *Lactobacillus Reuteri ATCC 55730* reduces *H. Pylori* intragastric load in vivo, decreases dyspeptic *Reuteri* symptoms, and affects eradication rates after conventional treatment. In a double-blind placebo-controlled study, 40 *H. Pylori* positive subjects were given *L. Reuteri* or placebo once a day for 4 weeks. *L. reuteri* or placebo were both provided by Nóos (BioGaia AB, Sweden) as chewable tablets and included either *L. Reuteri* each tablet containing 10^8 CFU of *L. Reuteri ATCC 55730* or placebo which consisted of tablets identical in taste and appearance to the active study product except for the absence of freeze-dried *L. reuteri* (and cryoprotectants). Everyone underwent upper endoscopy, (13)C-urea breath test, and *H. Pylori* stool antigen determination at entry and (13)C-urea breath test and *H. Pylori* stool antigen (used as both qualitative and semiquantitative markers) after 4 weeks of treatment. Sequential treatment was administered subsequently to everyone. In vivo, *L. reuteri* reduces *H. pylori* load as semiquantitatively assessed by both (13)C-urea breath test delta-value and *H. Pylori* stool antigen quantification after 4 weeks of treatment ($p < 0.05$). No change was shown in patients receiving placebo. *L. Reuteri* administration was followed by a significant decrease in the Gastrointestinal Symptom Rating Scale as compared to pretreatment value ($p < 0.05$) that was not present in those ones receiving placebo ($p =$ not significant). No difference in eradication rates was observed. *L. Reuteri* effectively suppresses *H. pylori* infection in humans and decreases the occurrence of dyspeptic symptoms. Nevertheless, it does not seem to affect antibiotic therapy outcome. The Authors report that a 4-week supplementation with *L. reuteri* is effective in reducing *H. pylori* bacterial load in humans and theoretically may help to control gastric inflammation.

Cindoruk et al.⁹⁸ investigated the efficacy and safety of *S. Boulardii* in the prevention of side-effects related to *H. Pylori* eradication. The secondary aim of the study was to define the effect of *S. Boulardii* on the eradication success of anti-*H. pylori* therapy. One hundred and twenty-four patients with *H. Pylori* infection (male/female: 44/80, mean age: 48 ± 14.25 year) receiving 14 days of triple therapy (clarithromycin 500 mg b.i.d., amoxicillin 1000 mg b.i.d., and lansoprazole 30 mg b.i.d.) were randomly assigned to *S. Boulardii* or placebo. Dyspeptic symptoms were recorded using modified Glasgow Dyspepsia Questionnaire (GDQ). Side-effect profile and tolerability were assessed using a symptom-based questionnaire. *H. pylori* status was rechecked 6 weeks after completion of eradication therapy. *H. Pylori* eradication rate, although higher in the treatment group, was statistically similar in treatment and control groups: 71% (44/62) versus 59.7% (37/62), respectively ($p > 0.05$). Nine (14.5%) patients in the treatment group and 19 (30.6%) patients in the placebo group experienced

diarrhea ($p < 0.05$). Epigastric discomfort was more frequent in the control group [9 (14.5%) versus 27 (43.5%), respectively ($p < 0.01$)]. Diffuse abdominal pain, abdominal gas, taste disturbance, urticaria and nausea symptoms were similar in both groups. GDQ scores after treatment were significantly better for treatment group (mean \pm SD, range: 1.38 ± 1.25 (0–5) vs. 2.22 ± 1.44 (0–6), respectively; $p < 0.01$). The Authors conclude that *S. Boulardii* improves anti-*H. Pylori* antibiotic therapy-associated diarrhea, epigastric discomfort, and treatment tolerability. In addition, *Saccharomyces boulardii* supplement decreases post-treatment dyspepsia symptoms independent of *H. pylori* status. Addition of *S. boulardii* does not affect the rate of *H. pylori* eradication.

Scaccianoce et al.⁹⁹ enrolled 65 consecutive dyspeptic patients with *H. Pylori* infection. Patients received one of the following therapies: a) standard 7-day triple therapy; b) standard 7-day triple therapy plus *L. Reuteri* supplementation; c) the same 7-day triple therapy plus a probiotic mixture; d) a 14-day standard triple therapy plus a probiotic mixture. *H. pylori* eradication was checked using a (13)C-urea breath test performed 4–6 weeks after treatment. The Authors observed that the 14-day therapy plus probiotic mixture tended to achieve higher eradication rate (71%). The lowest incidence of side effects was observed following the 7-day therapy plus *L. Reuteri* (6%) and the highest one the 14-day therapy plus probiotic mixture (33%). The Authors underline that there is no significant statistically difference between the therapy regimens. This study shows that 7–14 days triple therapy with or without probiotic supplement fails to achieve acceptable *H. Pylori* eradication rates.

3.5. Cancerogenesis

Colon cancer is a multi-factorial and complex neoplasm involving both genetics and environmental factors. There seems to be a strong relationship between colon cancer, diet and intestinal microflora. The rupture of the intestinal microflora equilibrium due to a bad diet seems to be related to an increase in the risk of developing colon cancer. Liong³⁰ underlines the different properties attributed to probiotics and prebiotics in the past years such as anti-carcinogenic, antimutagenic properties, ability of modifying differentiation processes in tumor cells, production of SCFAs and alteration of tumor gene expressions but he also evidences that, despite all the positive findings, other researchers have also reported insignificant colon cancer protective effects. Certain bacterial species in the colon produce harmful substances that seem to be correlated to cancer. That is why it has been thought that probiotics may modulate several major intestinal functions potentially associated with the development of colon cancer preventing the growth of deleterious organisms, producing anti-carcinogenic substances and moving the balance of gut bacteria in favour of the ones beneficial for the organism. Colon cancer has been correlated to high fat diets and it is thought to be due to colon raised levels of bile acids which help digest fat. These salts are released into the small intestine, and also re-absorbed there, but some may pass into the colon. The break down products of bile may have a cytotoxic effect on the cells lining the colon, increasing cell proliferation and possibly cancer. Probiotic modulation of the intestinal microflora may affect the activity of one of the enzymes (7 α -dehydroxylase) forming these toxic products but probiotics may also reduce the toxicity of bile salts binding to them. While growing in the colon, probiotics seem to exert different functions such as controlling the growth of potentially harmful bacteria, binding to mutagens, preventing harmful enzyme activity in the gut (β -glucuronidase, nitroreductase), interacting with the cells of the colon, forming conjugated linoleic acid, a compound with anti-inflammatory properties that may inhibiting the development of cancer. It may also increase levels of butyric acid in the colon which

is an important energy source and growth regulator for colon cells, stimulating the activity of beneficial enzymes that inactivate carcinogens (glutathione S transferase) and the immune system, and produce products that have a beneficial effect on the cells of the colon (*Lactobacillus* and *Bifidobacterium* produce lactic acid and similar SCFAs increasing the gut lumen acidity). Moreover Takeda et al.¹⁰⁰ suggest that daily intake of *Lactobacillus casei* strain *Shirota* provides a positive effect on the activity of NK cells which seem to exert a key role in protecting the human organism against cancer.

Hatakka et al.¹⁰¹ examined fecal β -glucosidase, β -glucuronidase, and urease activities during administration of *L. rhamnosus* LC705 (LC705) together with *Propionibacterium freudenreichii* ssp *shermanii* JS (PJS). Thirty-eight healthy men participated in this randomized, double-blind, placebo-controlled, two-period crossover study with treatment periods of 4 weeks. Subjects consumed daily bacterial or placebo capsules. Bacterial capsules contained viable LC705 and PJS. LC705 (DSM7061) and PJS (DSM7067), which are commonly used in the manufacture of ripened, semi-hard cheeses. The freeze-dried bacteria were filled in gelatine capsules. The subjects consumed daily two capsules containing viable LC705 and PJS (2×10^{10} CFU of each strain daily) or two placebo capsules. The same amount of this particular bacterial combination had previously shown physiological effects in two human studies. The amount of bacteria in the capsules remained unchanged during the study. In addition to the bacteria, the capsules contained microcrystalline cellulose as a filler. The placebo capsules were of identical composition but without the bacteria. Subjects were instructed to take the capsules daily with the first meal of the day. The activities of β -glucosidase, β -glucuronidase and urease, recovery of LC705 and PJS, and counts of total lactobacilli and propionibacteria were determined from feces. The mean fecal counts of total lactobacilli and propionibacteria as well as LC705 and PJS strains were significantly increased during the administration of bacteria (3.5-, 13-, 80- and 11-fold, respectively). β -glucosidase activity decreased by 10% ($P = 0.18$) and urease activity by 13% ($P = 0.16$) during bacterial supplementation versus placebo. The change in β -glucosidase activity was negatively correlated with the change in propionibacteria counts ($R = -0.350$, $P = 0.039$), being -2.68 versus 0.94 nmol/min/mg protein in subjects with increased and unchanged/decreased propionibacteria, respectively ($P = 0.003$). In conclusion, the administration of LC705 and PJS was followed by an increase in the fecal counts of lactobacilli and propionibacteria and a decrease in the activity of β -glucosidase with increasing counts of propionibacteria. The Authors conclude that the administration of LC705 together with PJS was associated with a decrease in β -glucosidase activity related to the increasing counts of propionibacteria, although we found no significant influence on the activity of three bacterial enzymes, β -glucosidase, urease and β -glucuronidase, compared to placebo. To evaluate the effect of the carcinogenic potential of those enzymes and the safety of those strains further studies are warranted.

Roller et al.¹⁰² investigated whether daily intake of an SYN modulates immune functions. In a randomized double-blind, placebo-controlled trial, thirty-four colon cancer patients who had undergone 'curative resection' and forty polypectomized patients participated. Subjects of the SYN group daily received encapsulated bacteria (1×10^{10} CFU of *L. rhamnosus* GG and 1×10^{10} CFU of *Bifidobacterium lactis* Bb12 (Bb12)) and 10 g of inulin enriched with oligofructose. Controls received encapsulated maltodextrin and 10 g of maltodextrin. Prior to intervention (T1), and 6 (T2) and 12 weeks after the start of the intervention (T3), phagocytic and respiratory burst activity of neutrophils and monocytes, lytic activity of NK cells and production of IL-2, IL-10 and IL-12, as well as TNF- α and IFN- γ by activated PBMC were measured. In feces, the concentrations of transforming growth factor- β 1 and

prostaglandin E2 were measured. IL-2 secretion by activated PBMC from the polypectomized group increased significantly between T1 or T2 and T3 ($P < 0.05$). In the cancer group, SYN treatment resulted in an increased capacity of PBMC to produce IFN- γ at T3 ($P < 0.05$). Other immunity-related parameters were not affected by SYN treatment, neither in the cancer nor in the polypectomized group. In conclusion, supplementation with this SYN has minor stimulatory effects on the systemic immune system of the two study groups. The Authors conclude that no negative effects were observed in cancer and polypectomized patients with daily consumption of this SYN for 12 weeks, but more definitive testing is required to indicate conclusively that this product is safe. The SYN supplement had only minor effects on the immune system of the subjects in both groups. It is possible that SYN supplementation in humans preferentially affects the gut-associated lymphoid tissue rather than the systemic immune system.

Rafter et al.¹⁰³ verified whether the prebiotic concept (selective interaction with colonic flora of nondigested carbohydrates), as induced by a synbiotic preparation oligofructose-enriched containing inulin (BeneoSynergy1; ORAFI, Tienen, Belgium; SYN1), *L. rhamnosus* GG and Bb12, is able to reduce the risk of colon cancer in humans. The 12 week randomized, double-blind, placebo-controlled trial of a synbiotic food composed of the prebiotic SYN1 and probiotics *L. rhamnosus* GG and Bb12 was conducted in 37 colon cancer patients and 43 polypectomized patients. Treatment consisted of a synbiotic preparation that contained the prebiotic SYN1 and the probiotic strains Bb12 and *Lactobacillus delbreuckii* subspecies *rhamnosus* strain GG. SYN1 is an oligofructose-enriched inulin preparation. Fecal and blood samples were obtained before, during, and after the intervention, and colorectal biopsy samples were obtained before and after the intervention. The effect of synbiotic consumption on a battery of intermediate biomarkers for colon cancer was examined. Synbiotic intervention resulted in significant changes in fecal flora: *Bifidobacterium* and *Lactobacillus* increased and *Clostridium perfringens* decreased. The intervention significantly reduced colorectal proliferation and the capacity of fecal water to induce necrosis in colonic cells and improve epithelial barrier function in polypectomized patients. Genotoxicity assays of colonic biopsy samples indicated a decreased exposure to genotoxins in polypectomized patients at the end of the intervention period. Synbiotic consumption prevented an increased secretion of IL-2 by means of PBMC in the polypectomized patients and increased the production of interferon in the cancer patients. The Authors conclude that the synbiotic intervention results in significant alterations in the composition of the colonic bacterial ecosystem, which presumably has consequences for the metabolic activity of the colon. These results also provide indirect evidence that some of the consequences of the synbiotic intervention might be a decreased exposure of the epithelium to cytotoxic and genotoxic agents, a decreased colonic cell proliferation and an improved mucosa structure.

3.6. Diarrhea

Diarrhea is an increase in the water content, frequency, and volume of bowel movements. Probiotics could be important for the treatment of diarrhea illnesses (Viral Diarrhea, Antibiotic-Associated Diarrhea, *Clostridium difficile*- Related Diarrhea, Traveler's Diarrhea (TD)).

Viral gastroenteritis is characterized by watery diarrhea, vomiting, headache, fever, chills, and abdominal pain. The viruses that may be responsible for this condition are: rotaviruses, caliciviruses, adenoviruses, astroviruses, toroviruses, coronaviruses and pestiviruses. Citomegaloviruses are responsible for viral colitis. Rotavirus is the most common cause of viral diarrhea and can injure the

intestinal epithelium leading to malabsorption and can increase the secretion through the epithelium. The rotavirus has several ways of transmission: fecal–oral, through contaminated water supplies, poor hygiene, food and fomites. Saavedra et al.¹⁰⁴ reported that *Lactobacillus GG* has been observed to decrease the rate of rotavirus-associated diarrhea and rotaviral shedding. Moreover the Author reported an interesting study which highlights that 90% of children treated with *Lactobacillus GG* had an IgA specific Antibody-Secreting Cells (sASC) response to rotavirus versus 46% for placebo. Another study performed on 55 infants describes how infectious diarrhea was successfully treated adding a combination of *Bifidobacteria* and *Streptococcus thermophilus* to powdered formula. This study shows that the combination of *B. Bifidum* and *S. Thermophilus* also reduced the rate of rotavirus-associated diarrhea and decreased rotavirus shedding.

TD is a condition usually due to the ingestion of food and beverages contaminated by fecal matters. 80% of the cases are bacteria caused. The most common bacteria are enterotoxigenic *E. Coli* and enteroaggregative *E. Coli*, *Campylobacter jejuni*, *Salmonella* species and *Aeromonas* species. Some protozoa such as *Entamoeba histolytica* and *Giardia lamblia* or enteroviruses are generally minor causes of TD.¹⁰⁵

Episodes of diarrhea are frequent side effects of antibiotic therapy. Antibiotic therapy leads to an alteration of normal intestinal flora which may cause a loss of colonization resistance and an alteration of the metabolic functions of the normal flora. The first situation is followed by an abnormal growth of pathogenic organisms such as *C. Difficile*, *Candida albicans*, *K.caytoxa* and *Salmonella* spp which are responsible for toxigenic diarrhea. The second situation leads to a diminished digestion of non absorbable carbohydrates followed by osmotic water secretion and a diminished colic absorption of water and electrolytes caused by a diminished production of SCFAs resulting in diarrhea.

Dubey et al.¹⁰⁶ conducted a double-blind randomized placebo-controlled study to evaluate efficacy and tolerability of *VSL[sharp]3* (CD Pharma India) in the treatment of acute rotavirus diarrhea in children. The patients were randomly assigned to receive 4 days of oral treatment with *VSL[sharp]3* probiotic mixture or placebo in addition to usual care for diarrhea. Use of probiotic mixture *VSL [sharp]3* in acute rotavirus diarrhea resulted in earlier recovery and reduced frequency of oral rehydration salt (ORS) administration reflecting decreased stool volume losses during diarrhea without side effects.

Narayanappa et al.¹⁰⁷ evaluated the efficacy and safety of Bifilac in reducing the episodes (frequency) and duration of diarrhea induced by rotaviral infection and to evaluate the efficacy of Bifilac to ameliorate the associated symptoms like dehydration and duration of rotaviral shedding in feces. The Authors enrolled 80 children aged between 3 months and 3 years and divided them into 2 groups, one group received standard therapy + placebo, the other group received standard therapy + probiotic (Bifilac) randomly. Children were assessed for frequency and duration of diarrhea, a degree of dehydration, duration and volume of ORS therapy, duration and volume of Intra venous fluids and duration of rotaviral shedding. They conclude that synbiotic bifilac appears to be a safe and very effective adjuvant in the management of acute rotaviral diarrhea.

Henker et al.¹⁰⁸ determined whether the stool frequency of infants and toddlers suffering from acute diarrhea could be normalized more quickly by administering the probiotic *Escherichia coli Nissle 1917 (EcN)* solution rather than by administering a placebo. The Authors also assessed the safety of *EcN*. A total of 113 children (aged 2–47 months) with acute diarrhea (>3 watery or loose stools in 24 h) were randomized to either a group receiving the probiotic *EcN* suspension ($n = 55$) or a group receiving the placebo suspension ($n = 58$) in a confirmative, double-blind clinical

trial. Depending on the age of patients, 1–3 ml per day of verum suspension (10^8 viable *EcN* cells per millilitre) or placebo were administered orally. The causes of the diarrhea were viral rather than bacterial. *EcN* was found to be safe and well-tolerated, and it showed a significant superiority compared to the placebo in the treatment of acute diarrhea in infants and toddlers.

Basu et al.¹⁰⁹ evaluated the role of *L. rhamnosus GG* as probiotic in acute watery diarrhea (AWD). This randomized, controlled, blinded trial involved 684 patients. The intervention group ($n = 330$) received ORS with probiotic powder containing 60 million cells of *L. rhamnosus GG*, while the control group ($n = 332$) received ORS alone twice daily for a minimum period of 7 days or till diarrhea ceased. During the study period all patients received ORS and/or IV fluids for ongoing losses, and nutritional supplementation. None of them received any antibiotic or anti-diarrhoeal medication. Rotavirus was isolated in 75.85%. There was no significant difference between treatment groups in the daily frequency or duration of diarrhea or vomiting or in the length of hospital stay. No complication was observed from the use of *L. rhamnosus GG*. *L. rhamnosus GG* supplementation did not decrease the frequency and duration of diarrhea and vomiting in children with AWD, and does not reduce hospital stay in these patients.

The efficacy of a combination of *B. longum PLO3*, *L. rhamnosus KL53A* and *L. plantarum PLO2* for the prevention of antibiotic-associated diarrhea in children was evaluated by Szymanski et al.¹¹⁰. Seventy-eight children (age: 5 months to 16 years) with otitis media, and/or respiratory tract infections, and/or UTIs were enrolled in a double-blind randomized control trial in which they received standard antibiotic treatment plus a food supplement containing 10^8 CFU of *B. longum*, *L. rhamnosus* and *L. plantarum* ($n = 40$) or a placebo ($n = 38$) orally twice daily for the duration of antibiotic treatment. Patients receiving probiotics had a similar rate of diarrhea (>or = 3 loose or watery stools/day for > or = 48 h occurring during or up to 2 weeks after the antibiotic therapy) as those receiving placebo (relative risk 0.5, 95% CI 0.06–3.5). The mean number of stools per day was significantly lower in the experimental group (mean difference -0.3 stool/day, 95% CI -0.5 to -0.07). No adverse events were reported. Concluding the three probiotics do not significantly alter the rate of diarrhea, although they reduce the frequency of stools per day. The Authors underline the overall frequency of diarrhea was surprisingly low even though these results should be interpreted with caution.

Ruszczynski et al.¹¹¹ enrolled children with common infections (aged 3 months to 14 years) in a double-blind, randomized, placebo-controlled trial to assess the efficacy of administration of *L. rhamnosus* for the prevention of antibiotic-associated diarrhea in children. They received standard antibiotic treatment plus 2×10^{10} CFU of a probiotic (*L. rhamnosus* strains *E/N*, *Oxy* and *Pen*) ($n = 120$) or a placebo ($n = 120$), administered orally twice daily throughout antibiotic treatment. Any diarrhea (>or = 3 loose or watery stools/day for > or = 48 h occurring during or up to 2 weeks after the antibiotic therapy) occurred in nine (7.5%) patients in the probiotic group and in 20 (17%) patients in the placebo group (relative risk, RR 0.45, 95% CI 0.2–0.9). Three (2.5%) children in the probiotic group developed diarrhea caused by *Clostridium difficile* or otherwise unexplained diarrhea compared to nine (7.5%) in the placebo group (RR 0.33, 95% CI 0.1–1.06). No adverse events were observed. This study shows that administration of *L. rhamnosus* (strains *E/N*, *Oxy* and *Pen*) to children receiving antibiotics reduces the risk of any diarrhea.

Beausoleil et al.¹¹² designed a study to assess the efficacy and safety of a fermented milk combining *L. acidophilus* and *L. casei* that is widely available in Canada, in the prevention of antibiotic-associated diarrhea (AAD). This double-blind, randomized study involved 80 hospitalized patients which were randomly assigned to receive

either a lactobacilli-fermented milk or a placebo on a daily basis. AAD occurred in seven of 44 patients (15.9%) in the lactobacilli group and in 16 of 45 patients (35.6%) in the placebo group (OR 0.34, 95% CI 0.125 to 0.944; $P = 0.05$). The median hospitalization duration was eight days in the lactobacilli group, compared with 10 days in the placebo group ($P = 0.09$). The study shows that the daily administration of a lactobacilli-fermented milk was safe and effective in the prevention of antibiotic-associated diarrhea in hospitalized patients.

Hickson et al.¹¹³ designed a study involving 135 hospital patients (mean age 74) taking antibiotics. This randomized double-blind placebo-controlled study aimed at determining the efficacy of a probiotic drink containing *Lactobacillus* for the prevention of any diarrhea associated with antibiotic use and caused by *Clostridium difficile*. The patients received 100 g (97 ml) drink containing *L. casei*, *L. bulgaricus*, and *S. thermophilus* twice a day during a course of antibiotics and for one week after the course finished. The placebo group received a longlife sterile milkshake. 7/57 (12%) of the probiotic group developed diarrhea associated with antibiotic use compared with 19/56 (34%) in the placebo group ($P = 0.007$). Logistic regression to control for other factors gave an OR 0.25 (95% CI 0.07 to 0.85) for the use of the probiotic, with low albumin and sodium also increasing the risk of diarrhea. The absolute risk reduction was 21.6% (6.6%–36.6%), and the number needed to treat was 5 (3–15). No one in the probiotic group and 9/53 (17%) in the placebo group had diarrhea caused by *C. difficile* ($P = 0.001$). The absolute risk reduction was 17% (7%–27%), and the number needed to treat was 6 (4–14). They conclude that the consumption of a probiotic drink containing *L. casei*, *L. bulgaricus*, and *S. thermophilus* can reduce the incidence of AAD and *C. difficile* associated diarrhea.

Wenus et al.¹¹⁴ to study the preventive effect of a milk drink fermented with multistrain probiotics on AAD, designed a double-blind placebo-controlled study involving 87 patients randomized to ingestion of a fermented milk drink containing *L. rhamnosus* GG, *La-5* and *Bb-12* ($n = 46$) or placebo with heat-killed bacteria ($n = 41$), during a period of 14 days. Sixty-three patients completed the study according to the protocol; two patients (5.9%) in the treatment group and eight (27.6%) in the placebo group developed AAD ($P = 0.035$). The relative risk of developing AAD was 0.21 (95% CI: 0.05–0.93) when they were given probiotic milk drink. This study proves that a fermented multistrain probiotic milk drink may prevent four of five cases of AAD in adult hospitalized patients.

Koning et al.¹¹⁵ studied the effect of a multispecies probiotic on the composition and metabolic activity of the intestinal microbiota and bowel habits in forty-one healthy volunteers. They were given 500 mg amoxicillin twice daily for 7 days and were randomized to either 5 g of a multispecies probiotic, Ecologic AAD (10^9 cfu/g), or placebo, twice daily for 14 days. After collection, feces was analyzed as to the composition of the intestinal microbiota, and beta-glucosidase activity, endotoxin concentration, *C. difficile* toxin A, SCFAs, and pH were determined. Bowel movements were scored according to the Bristol stool form scale. Mean number of enterococci increased significantly from log 4.1 at day 0 to log 5.8 (day 7) and log 6.9 (day 14) cfu/g feces ($P < 0.05$) during probiotic intake. Although no other significant differences were observed between both intervention groups, within each group significant changes were found over time in both microbial composition and metabolic activity. Moreover, bowel movements with a frequency $> = 3$ per day for at least 2 days and/or a consistency $> = 5$ for at least 2 days were reported less frequently in the probiotic compared to the placebo group (48% vs 79%, $P < 0.05$). This study shows that, apart from an increase in enterococci, no significant differences in microbial composition and metabolic activity are observed in the probiotic compared with the placebo group. However, changes over time were present in both groups, which differed significantly

between the probiotic and the placebo arm, suggesting that the amoxicillin effect was modulated by probiotic intake. The intake of a multispecies probiotic significantly reduced diarrhea-like bowel movements in healthy volunteers receiving amoxicillin.

3.7. Mixed studies on Probiotics

Sabia et al.¹¹⁶ focused on plasmid transfer by mating, a process that can occur in the gut microbiota. This process is particularly important when antibiotic-resistant genes are involved. The Authors investigated the in vitro capability of two *L. plantarum* strains (one bacteriocin producer and one non producer) to interfere with the conjugation processes. Different matings were performed adding to the donor and recipient cells *L. plantarum* 35d bac and *L. plantarum* 396/1 bac as agents of interference. Conjugations added with a *Staphylococcus aureus* strain or without any agent of interference were used as controls. Both *lactobacillus* strains were able to decrease mating frequency. Statistically significant differences in the viable transconjugants were obtained in the presence and in the absence of the lactobacilli. The effect was almost the same with the two *L. plantarum* independent of bacteriocin production. In the trial performed with *Streptococcus aureus*, no decrease in mating frequency was observed, confirming that the capability to interfere with R-plasmid transfer ability can be a property of the tested *L. Plantarum* strains.

Hoesl et al.¹¹⁷ focused on some interesting studies involving the administration of *Oxalobacter Formigenes* (discovered in 1985), a bacterium responsible for the degradation of oxalate in the human body. Several studies showed a link between a lack of colonization by *O. Formigenes* and a recurrence of oxalate stone formation. Moreover, among the stone formers, a lack of *O. Formigenes* is associated with hyperoxaluria. A higher urinary oxalate excretion in female calcium oxalate formers with recurrent UTI may be associated with the application of antibiotics and a subsequent eradication of *O. Formigenes*. A permanent decolonization was also found in cystic fibrosis patients. The Authors analyzed some clinical trials involving humans who followed administration of 500 mg of *O. Formigenes* strain; HCL urinary oxalate excretion and oxalate/creatinine ratios were found to be reduced; *O. Formigenes* was used in a first time clinical trial involving 9 children, seven of whom still have functioning kidneys and two with end-stage kidney failure all affected by primary hyperoxaluria Type I, an inherited, life threatening disease characterized by recurrent oxalate stone formation, nephrocalcinosis and eventual liver and kidney failure. Administering daily 2 teaspoonfuls of a frozen cell paste of live *O. Formigenes* (IxOC-2) oxalate excretion in the urine of the children with functioning kidneys decreased between 20 percent and 50 percent followed by a gradual increase after the treatment was stopped. In the two children on renal dialysis a significant reduction in blood levels of oxalate was observed.

In 2006 Hoppe et al.¹¹⁸ designed a new study orally administering *Oxalobacter* for 4 weeks as frozen paste, IxOC-2 or as enteric-coated capsules (IxOC-3). Nine patients (five with normal renal function, one after liver-kidney transplantation, and three with renal failure) completed the IxOC-2 study. Seven patients (six with normal renal function and one after liver-kidney transplantation) completed the IxOC-3 study. The preliminary obtained data indicate that *Oxalobacter formigenes* is safe, leads to a significant reduction of either urinary or plasma oxalate, and is a potential new treatment option for primary hyperoxaluria.

Skovbjerg et al.¹¹⁹ designed a double-blind pilot/preliminary study involving 60 children with long-standing secretory otitis media (SOM = a pathology characterized by persistent fluid in the middle ear cavity) who were scheduled for insertion of tympanostomy tubes. They were randomized to nasal spray treatment with

Streptococcus sanguinis, *L. rhamnosus* or placebo for 10 days before surgery. Clinical evaluation was carried out after 10 days of treatment. Middle ear fluid (MEF) was collected during surgery for quantification of cytokines and detection of bacteria by culture and PCR. Nasopharyngeal swabs were obtained before treatment and at surgery. The Authors observed that complete or significant clinical recovery occurred in 7/19 patients treated with *S. sanguinis* compared to 1/17 patients in the placebo group ($p < 0.05$). In the *L. rhamnosus* treatment group, 3/18 patients were cured or much better ($p = 0.60$ compared with placebo). Spray treatment did not alter the composition of the nasopharyngeal flora or the cytokine pattern observed in the nasopharynx or MEF, except for a higher level of IL-8 found in the nasopharynx of *L. rhamnosus* treated children. Although the mechanisms for the effect remains to be investigated this study shows that spray treatment with *S. sanguinis* may be effective against SOM.

4. Conclusion

Probiotics seem to play an important role in the lumen of the gut elaborating antibacterial molecules such as bacteriocins. Moreover they seem to be able to enhance the mucosal barrier increasing the production of innate immune molecules, including goblet cell-derived mucins and trefoil factors and defensins produced by intestinal Paneth cells. Some strains promote adaptive immune responses (secretory immune globulin A, regulatory T cells, IL-10). Some probiotics have the capacity to activate receptors in the enteric nervous system, which could be used to promote pain relief in the setting of visceral hyperalgesia.¹²⁰ Moreover probiotics exert an important action improving the abnormalities of both the colonic flora and the intestinal microflora. They could be effective for treating various pathologies preventing the dysbiosis which characterizes or is associated with these conditions. Further future clinical trials, involving large numbers of patients, will be mandatory to achieve definite evidence of the preventive and curative role of probiotics in medical practice. Details about correct formulations in terms of amount of bacteria, viability and associated growth factors, will be required in order to standardize the administration schedule and achieve homogeneous, comparable results on selected cohorts of recruited patients.

Conflict of interest statement

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Statement of authorship

The authors hereby certify that all work contained in this review is original work of Tommaso Iannitti and Beniamino Palmieri. All the information taken from other articles, including tables and pictures, have been referenced in the "Bibliography" section. The authors claim full responsibility for the contents of the article.

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