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DESCRIPTION

[0001] To date, diseases which are correlated with a functional disorder of the gastrointestinal tract and/or which are correlated with undesirable gastrointestinal inflammatory activity are common diseases with widespread prevalence. Diseases of both groups are characterized by abdominal pain, discomfort, distension, bloating, diarrhoea, constipation, digestive disorder, urgency and/or reduced and/or increased number of bowel movements, feeling of incomplete evacuation and/or combinations thereof, thereby leading to a significantly reduced overall wellbeing of the persons affected. In particular, with respect to irritable bowel syndrome (IBS) an efficient treatment is still lacking (Brenner and Chey, 2009). Several medications for IBS have been withdrawn or restricted due to adverse health effects. Additionally, current therapies are mainly targeted at single symptoms and do not alleviate IBS and improve quality of life (QoL) in general.

[0002] It is widely believed that diseases which are correlated with a functional disorder of the gastrointestinal tract, such as IBS or inflammatory bowel diseases, are accompanied (i) with an imbalance of the intestinal microbiota (Kassinen *et al.*, 2007) and/or (ii) a dysfunctional intestinal barrier of the gastrointestinal tract (Marshall *et al.*, 2004). This is thought to result in a reduction of probiotic bacteria, such as *Bifidobacteria*, in the gastrointestinal tract. Additionally, it is believed that a dysfunctional intestinal barrier causes undesirable entrance of facultative pathogenic bacteria into the mucosa which can lead to gastrointestinal inflammatory activity, subsequently. Recent studies propose that this inflammation triggers typical IBS symptoms such as pain, motility and dysfunction of the intestine.

[0003] An imbalance of the intestinal microbiota is mainly characterised by a reduced number of non-pathogenic microorganisms of human origin, such as *Bifidobacteria*, whereas the number of facultative pathogenic microorganisms, such as *Enterobacteria*, is increased (Jian-Min *et al.*, 2004). This imbalance is believed to lead to an increased production of gaseous compounds and short-chain fatty acids, dysmotility, flatulence and pain of the persons affected. Thus, for bringing the intestinal microbiota back into balance or improving intestinal barrier, probiotic bacteria came into focus. "Probiotics" comprising probiotic bacteria of non-pathogenic human origin have been defined as living microbial food and drugs which upon ingestion in certain numbers exert health effects beyond inherent basic nutrition. Mixtures of various microorganisms, particularly species of *Lactobacillus* and *Streptococcus*, have traditionally been used in fermented dairy products or drugs to promote health.

[0004] Many studies have been performed on this issue, however, as an imbalance of the intestinal microbiota is only one side of the coin, it is not surprising that most therapies which simply focus on the delivery of probiotics to the gastrointestinal tract, have not been successful in alleviating all symptoms of patients suffering from a functional disorder of the gastrointestinal tract. For example, IBS is diagnosed by the Rome III criteria, which are basically: abdominal pain or discomfort on at least 3 days per month during the last three months with symptoms which started at least 6 months ago combined with at least two criteria of (a) ease through defecation, (b) starting associated with a change in stool frequency or (c) in stool consistency and is often accompanied by bloating or distension, and bowel movement difficulties, either constipation and/or diarrhoea. -Coming along with these symptoms, patients may suffer from impaired social and personal function and diminished quality of life. Nevertheless, even if the symptoms, such as pain/discomfort, distension/bloating, urgency are largely removed an improvement of the overall health related quality of life (QoL) of the patient is very desirable. To date, none of the treatments provided, including probiotics and others, have been able to improve the symptoms pain/discomfort, distension/bloating and digestive disorder simultaneously with the QoL.

[0005] In this respect, the second important aspect in the development of a functional disorder of the gastrointestinal tract has to be considered which concerns a dysfunctional intestinal barrier of the gastrointestinal tract characterised by an increased permeability thereof. One very popular theory in this respect is that perturbations in the gut allow pathogenic microorganisms - which might dominate in case of an imbalance of the intestinal microbiota - to pass the barrier and penetrate the mucosa, thereby causing inflammatory reactions. Based on this hypothesis, several studies have been performed which focus on the treatment and alleviation of the inflammatory reaction and its effects on the gastrointestinal tract. EP 1 141235 for example describes a *Bifidobacterium longum infantis* (*B. infantis*) strain UCC35624 for application in nutritional supplements and in drugs which showed a strong ability to stimulate an anti-inflammatory response in the host by decreasing IL-8 levels (Whorwell *et al.*, 2006) and normalizing the IL-10/IL-12 ratio (O'Mahony *et al.*, 2005). However, different sources question whether the effect of *B. infantis* can be attributed to direct anti-inflammatory properties. Further, although *Lactobacillus salivarius* has also shown strong anti-inflammatory effects *in vitro* and in mice model - comparable to *B. infantis* - it has not shown significant effects in alleviating IBS and its symptoms.

[0006] Most important, the efficacy of probiotics is strongly strain specific and only certain strains might be able to improve IBS and its symptoms. Up to date, several studies have examined the effects of probiotics on symptoms of diseases which are correlated with a functional disorder of the gastrointestinal tract, such as IBS (O'Mahony *et al.*, 2005, Kajander *et al.*, 2005;

Williams *et al.*, 2008; Guyonnet *et al.*, 2007). However, only a few could show a significant benefit. Additionally, no probiotic strain showed a significantly alleviating effect to symptoms of irritable bowel syndrome and a simultaneously improved quality of life. These findings could be attributed to the fact that the efficacy of probiotics is strongly strain specific. It is widely accepted that the properties of one probiotic strain cannot be transferred to another (Brenner and Chey, 2009). Therefore, it is important to acknowledge that the efficacy and effect of a single probiotic strain cannot be predicted which underlines the importance of testing and proving the efficacy of a single strain. For this purpose, methods are described in the experimental section.

[0007] Never before, a probiotic study has shown a reduction of the above mentioned symptoms (pain/distension/bloating/urgency/digestive disorder) and a simultaneous improvement of a patient's quality of life in a disease like IBS. Thus, there is a significant need for a therapy improving the symptoms pain/discomfort, distension/bloating, urgency and digestive disorder of a patient suffering from a functional disorder of the gastrointestinal tract, taking into account that the functionality of the intestinal wall is strengthened.

[0008] Therefore, the present invention provides a probiotic formulation comprising a strain of *Bifidobacterium bifidum* MIMBb75, deposited under deposit No. DSM 24514, for use in the treatment of irritable bowel syndrome, or a disease selected from the group consisting of irritable bowel movement, inflammatory bowel disease, Crohn's disease, ulcerative colitis, pouchitis, post infection colitis, diarrhoeal disease, *Clostridium difficile* associated diarrhoea, and combinations thereof. Said strain was isolated from a fecal sample of a healthy adult, general properties the strain were disclosed by Guglielmetti *et al.* ("Implication of an outer surface lipoprotein in adhesion of *Bifidobacterium bifidum* to Caco-2 cells", Applied and Environmental Microbiology, vol. 74, no. 15, 2008, pages 4695-4702 and "Study of the adhesion of *Bifidobacterium bifidum* MIMBb75 to human intestinal cell lines", Curr. Microbiology, vol. 59, 2009, pages 167-172).

[0009] In the present invention *Bifidobacterium bifidum* MIMBb75, deposited under deposit No. DSM 24514, significantly improved the symptoms abdominal pain/discomfort, distension/bloating, urgency and digestive disorder of a gastrointestinal disease, in particular of IBS. The alleviation of general IBS along with an improvement of the single symptoms pain/discomfort, distension/bloating, urgency and digestive disorder with a simultaneous improvement of QoL with the strain for use according to the invention was an unexpected result as it is known and accepted in the prior art that the effectiveness of a probiotic is highly strain specific and even close related members of one *Bifidobacterium* strain species regularly do not have comparable properties or are even inactive in gastrointestinal diseases (Brenner and Chey, 2009). Without intention of being bound to one theory, the inventors believe that the exceeding and unexpected efficiency of the *Bifidobacterium bifidum* MIMBb75 as defined in the claims is due to its ability to prevent the passage of pathogenic bacteria through the intestinal barrier of the gastrointestinal tract and their penetration of the mucosa. Assuming that the dysfunction of the intestinal barrier is due to "holes" or weakened parts in the intestinal wall through which the pathogenic microorganisms might enter the mucosa, the inventors believe that adhesion of *Bifidobacterium bifidum* MIMBb75 cells to the intestinal wall reduce the permeability of the barrier thereby improving the intestinal wall in a "hole plugging" mode of action. Further, it is very important that a reduction of the symptoms or the disease itself is also maintained during the washout-phase, i.e. the phase after end of therapy, wherein for example no further probiotic formulation for use according to the invention is administered to the subject or patient. In case of the strain contained in the probiotic formulation for use according to the invention, the positive effect of the strain is maintained in case of global IBS symptoms, abdominal pain/discomfort, distension/bloating and digestive disorder during the washout-phase as will be explained in detail below and is also presented in the Example and Figure section.

[0010] Thus, the present invention provides a probiotic formulation comprising a strain of *Bifidobacterium bifidum* MIMBb75, deposited under deposit No. DSM 24514, for use in the treatment of irritable bowel syndrome, or a disease selected from the group consisting of irritable bowel movement, inflammatory bowel disease, Crohn's disease, ulcerative colitis, pouchitis, post infection colitis, diarrhoeal disease, *Clostridium difficile* associated diarrhoea, and combinations thereof. Said strain is especially for use as probiotic, in foodstuff, medical device and/or medicament in alleviating, preventing and/or treating abdominal pain, bloating, obstipation, digestive disorder, reduced number of bowel movements, increased number of bowel movements, feeling of incomplete evacuation global IBS symptoms and/or combinations thereof. The usefulness of the strain in alleviating, preventing and/or treating a subject suffering from abdominal pain, discomfort, distension, digestive disorder, bloating, urgency and/or reduced and/or increased number of bowel movements, feeling of incomplete evacuation, global IBS symptoms and/or combinations thereof can be assessed using a 7-point Likert scale which is commonly used in clinical studies and is also disclosed herein. The quality of life (QoL) of a patient may be assessed using the standard SF-12 questionnaire which is also common to a person skilled in the art in clinical studies and an accepted standard to measure the QoL. Moreover, in case of global IBS symptoms, abdominal pain/discomfort, distension/bloating the positive effect of the strain disclosed herein is maintained during the washout-phase as will be explained in detail below and is also presented in the Example and Figure section.

[0011] According to the invention, the probiotic formulation as defined in the claims is used for treating irritable bowel syndrome,

or a disease selected from the group consisting of irritable bowel movement, inflammatory bowel disease, Crohn's disease or ulcerative colitis, pouchitis, post infection colitis, diarrhoeal disease, *Clostridium difficile* associated diarrhoea, and combinations thereof, preferably the disease is irritable bowel movement.

[0012] In alternative embodiments, the cells of the strain are viable or non-viable. With non-viable cells product preparation is simpler, cells may be incorporated easily into pharmaceuticals and storage requirements are much less limited than in case of viable cells. Cells may be killed thermally or by exposure to altered pH or subjection to pressure.

[0013] The invention thus provides a probiotic formulation comprising a strain of *Bifidobacterium bifidum* MIMBb75, deposited under deposit No. DSM 24514 for use in a treatment as defined in the claims.

[0014] In a preferred embodiment, the probiotic formulation further comprises at least one prebiotic. Preferably, the prebiotic is inulin or a fructooligosaccharide.

[0015] In any of the embodiments mentioned above, the probiotic formulation may further comprise at least one pharmaceutically acceptable compound, an ingestible carrier, an adjuvant, a bacterial component, a drug entity, a biological compound and/or a protein and/or peptide, in particular a protein and/or peptide that is rich in glutamine/glutamate, a lipid, a carbohydrate, a vitamin, mineral and/or trace element; optionally said at least one pharmaceutically acceptable compound is a member selected from the group consisting of one or more vitamins, such as vitamins of the B group, one or more minerals, such as calcium or magnesium, one or more carbohydrates, such as lactose, maltodextrin, inulin, dextrose, mannitol, maltose, dextrin, sorbitol, fructose, and a mixture thereof. Preferably, the ingestible carrier is a capsule, tablet, powder or a food product; optionally the food product is a dairy product, acidified milk, yoghurt, frozen yoghurt, yoghurt production, such as fermented yoghurt drink, drinking yoghurt, cheese, fermented cream, milk based desserts fermented milk or humanised milk, milk powder, milk concentrate, cheese spread, dressing, beverage and others.

[0016] In one embodiment, the probiotic formulation further comprises a protein and/or peptide, in particular a protein and/or peptide that is rich in glutamine/glutamate, a lipid, a carbohydrate, a vitamin, mineral and/or trace element.

[0017] In any of the above embodiments, the strain may be present in the probiotic formulation at more than 10^1 cfu per capsule or tablet or per unit of powder or food product, optionally not less than 10^2 , 10^3 , 10^4 10^5 or 10^6 colony forming units (cfu) per capsule or tablet or per unit of powder or food product, preferably the strain is present at more than 10^7 cfu per capsule or tablet or per unit of powder or food product, more preferably the strain is present at more than 10^8 cfu per capsule or tablet or per unit of powder or food product, even more preferably the strain is present at more than 10^9 cfu or more than 10^{10} cfu per capsule or tablet or per unit of powder or food product. A "unit of powder" denotes an amount of powder which comprises more than 10^1 cfu, optionally not less than 10^2 , 10^3 , 10^4 10^5 or 10^6 colony forming units (cfu), preferably more than 10^7 cfu, more preferably more than 10^8 cfu, even more preferably more than 10^9 cfu or more than 10^{10} cfu. Thus, a unit of powder represents the amount which comprises the standard daily dose of a patient, however, the daily dose may be increased or reduced due to several reasons and in several ways which are described below. A typical amount by weight of a unit of powder should be easily ingestible and digestible, ranging from mg to several gram, for example 5 g, 10 g, 15 g or more. A unit of powder may also be mixed with any food product mentioned herein and also with another food product which is regarded suitable by a person skilled in the art. Thus, the amount of cfu per amount by weight of powder may be much higher than the standard daily dose in an amount of powder which is regarded easily ingestible and digestible. Here, the powder will have to be diluted with other components allowed in the probiotic formulation as described above. A "unit of food product" or "food product" in general denotes a typical amount by weight or volume which is regarded as standard or typical quantity of the particular food product used as ingestible carrier, a typical amount proposed as consumption portion to be eaten or drunk, a packaging unit or comparable. A "unit of ingestible carrier" hence denotes a tablet, capsule, suppository or comparable or a unit of powder or unit of food product which denotes a typical amount by weight or volume which is regarded as standard or typical quantity of the particular food product used as ingestible carrier, a typical amount proposed as consumption portion to be eaten or drunk, a packaging unit or comparable.

[0018] In any of the above embodiments, the daily amount of the strain administered to a patient in the probiotic formulation, preferably, is not less than 10^1 cfu per capsule or tablet or per unit of powder or food product, optionally not less than 10^2 , 10^3 , 10^4 , 10^5 or 10^6 cfu, preferably not less than 10^7 cfu, more preferably not less than 10^8 cfu even more preferably not less than 10^9 cfu or not less than 10^{10} cfu.

[0019] The probiotic formulation according to any of the above embodiments is for use in the treatment of irritable bowel

syndrome, or a disease, e.g., selected from the group consisting of irritable bowel movement, inflammatory bowel disease, Crohn's disease, ulcerative colitis, , pouchitis, post infection colitis, diarrhoeal disease *Clostridium difficile* associated diarrhoea and combinations thereof. Preferably the disease is irritable bowel syndrome. Said strain is especially for use as probiotic, in foodstuff, medical device and/or medicament in alleviating, preventing and/or treating abdominal pain, bloating, obstipation, digestive disorder, reduced number of bowel movements, increased number of bowel movements, feeling of incomplete evacuation, global IBS symptoms and/or combinations thereof.

[0020] A method for producing the probiotic formulation for use according to the invention, comprises at least the following steps:

1. a) fermenting and/or growing the strain *Bifidobacterium bifidum* MIMBb75, deposited under deposit No. DSM 24514 in a protein-rich liquid growth medium,
2. b) harvesting the cells by centrifugation, stabilising, freeze-drying, milling and sieving of the cells,
3. c) optionally mixing/blending the cells with one or more members of the group consisting of a prebiotic, a pharmaceutically acceptable compound, an adjuvant, a bacterial component, a drug entity, a biological compound, a protein and/or peptide or else, and
4. d) introducing the cells of step b) or the mixture of step c) in an ingestible carrier.

[0021] The *Bifidobacterium bifidum* MIMBb75, deposited under deposit No. DSM 24514 belongs to the genus of *Bifidobacteria* which are gram-positive, non-motile, often branched anaerobic bacteria generally present in the gastrointestinal tract and vagina. They are commonly isolated from the faeces of healthy infants and adults. If it is referred to the strains, also cells of the strain are meant. *Bifidobacteria* have already been describes to aid in digestion, are associated with a lower incidence of allergies and may also play a role in preventing some forms of tumour growth. *Bifidobacteria* belong to the family of *Bifidobacteriaceae* of *Actinobacteria*. Deposit of *Bifidobacterium bifidum* MIMBb75 was made at the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) on 26 January 2011 and deposited under deposit No. DSM 24514.

A "disease" is any condition of a subject, also referred to as patient or human, that is altered in comparison to the condition of a subject which is regarded healthy. In general, a disease as defined in the claims is correlated with a functional disorder of the gastrointestinal tract, with undesirable gastrointestinal inflammatory activity, with an imbalance of the intestinal microbiota, a reduction of *bifidobacteria* in the gastrointestinal tract, a dysfunctional intestinal barrier of the gastrointestinal tract and/or combinations thereof. The diseases which are addressed by the strain in the probiotic formulation can be selected from the group consisting of irritable bowel movement, inflammatory bowel disease, Crohn's disease, ulcerative colitis, irritable bowel syndrome, pouchitis, post infection colitis, diarrhoeal disease *Clostridium difficile* associated diarrhoea and combinations thereof. A "symptom" or "individual symptom" is the way a disease of a subject is manifested, such as abdominal pain, discomfort, distension, bloating, digestive disorder, urgency and/or reduced and/or increased number of bowel movements, feeling of incomplete evacuation, global IBS symptoms and/or combinations thereof and an overall reduced quality of life. "Global IBS symptoms" comprise an overall assessment of all individual symptoms mentioned above regarding IBS. A "patient" (= person or human or animal) is a non-healthy subject which ingests the probiotic formulation for use according to the invention comprising a strain of *Bifidobacterium bifidum* MIMBb75, deposited under deposit No. DSM 24514 for treatment reasons as the patient, person, human or animal suffers from any disease as defined in the claims. Thus, a patient (= person) is in need of a new therapy such as of a therapy with the strain of *Bifidobacterium bifidum* MIMBb75, deposited under deposit No. DSM 24514.

[0022] The probiotic formulation for use in the present invention is for use as probiotic, in foodstuff and/or medicament and comprises a strain of *Bifidobacterium bifidum* MIMBb75, deposited under deposit No. DSM 24514. The probiotic formulation for use in the present invention may also be regarded as pharmaceutical formulation. "Foodstuff" denotes in principle any form of substance or material that is ingestible and digestible by human and preferably also by animal. In general, this comprises substances or material which can be eaten or drunk to provide nutritional support for the body and/or pleasure. In case of human, these substances may be of plant, microbial or animal origin. Optionally comprised in the probiotic formulation may be at least one pharmaceutically acceptable compound, an ingestible carrier, an adjuvant, a bacterial component, a drug entity, a biological compound and/or a protein and/or peptide, in particular a protein and/or peptide that is rich in glutamine/glutamate, a lipid, a carbohydrate, a vitamin, mineral and/or trace element; optionally said at least one pharmaceutically acceptable compound is a member selected from the group consisting of one or more vitamins, such as vitamins of the B group, one or more minerals, such as calcium or magnesium, one or more carbohydrates, such as lactose, maltodextrin, inulin, dextrose, mannitol, maltose, dextrin, sorbitol, fructose, and a mixture thereof. Medicaments and other therapeutic goods in general are subject to more strict regulations and approval processes. These approval processes are not necessary in case of a foodstuff. As already explained above, a "probiotic" in the sense of the invention comprises probiotic bacteria of non-pathogenic human origin and has been defined as living microbial food and medical supplements which upon ingestion in certain numbers exert health effects beyond inherent basic nutrition. Mixtures of various microorganisms, particularly species of *Lactobacillus* and *Streptococcus*, have

traditionally been used in fermented dairy products to promote health. The probiotic for use in the present invention comprises the *Bifidobacterium bifidum* MIMBb75 strain as defined in the claims. However, also other strains which have been regarded as being useful probiotics may additionally be present in the probiotic formulation for use in the present invention as bacterial component. These are for example probiotic strains of the species *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Streptococcus* or mixtures thereof. Thus, a "bacterial component" denotes a viable or non-viable strain or cell of a probiotic strain as mentioned above and may further be used for compounds, such as biological molecules, polysaccharides, lipids or else of bacterial origin or being produced by bacterial fermentation or expression. Even dead material of any bacterial species may be understood as bacterial compound.

[0023] The probiotic formulation for use in the invention may further comprise at least one prebiotic or a combination of two, three, four or even more different prebiotics. A "prebiotic" in the sense of the invention is meant to serve as nutrient to the strain for use in the invention and to keep them in a viable and healthy state after thawing from freeze-drying. Per gram of microbial cells, i.e. per gram of strain, in general, 2 - 5 g, preferably -2.5 g, more preferably at least 2.5 g, even more preferably at least 3 g, most preferably at least 4 g of prebiotic is necessary. In general, by humans non-digestible food ingredients, such as certain carbohydrates, are used as prebiotic. Examples for a prebiotic comprise carbohydrates, preferably oligosaccharides, inulin, fructooligosaccharides, galactooligosaccharides, psyllium, oligofructose, isomaltoligosaccharides, xylooligosaccharides, soyoligosaccharides, maltodextrin, glucooligosaccharides, mannanoligosaccharides, arabinogalactan, arabinxytan, lactosucrose, gluconan, lactulose, polydextrose, oligodextran, gentiooligosaccharide, pectic oligosaccharide, xanthan gum, gum arabic, hemicellulose, resistant starch and its derivatives, and mixtures and/or combinations thereof.

[0024] The probiotic formulation for use in the invention may further comprise at least one pharmaceutically acceptable compound, at least one ingestible carrier, at least one adjuvant, at least one bacterial component, at least one drug entity, at least one biological compound and/or at least one protein and/or peptide, in particular a protein and/or peptide that is rich in glutamine/glutamate, a lipid, a carbohydrate, a vitamin, mineral and/or trace element.

[0025] A "pharmaceutically acceptable compound" denotes a liquid, solid or gaseous chemical or biological compound which is acceptable in a pharmaceutical composition or formulation characterised by a well tolerability by the human and also animal physiology and body being pharmacologically inactive or having no harmful effect on the physiology of the recipient. At least one, two, three, four, five or even more different pharmaceutically acceptable compounds may be present in the probiotic formulation for use in the invention in different amounts. The amount may be adjusted by the manufacturer according to the specific needs of the *Bifidobacterium bifidum* strain which is used and according to a certain application, way of administration or dosage regimen. Examples of pharmaceutically acceptable compounds include prebiotics, carbohydrates, lipids, vitamins, minerals, trace elements, amino acids, nucleic acids, maltodextrin, inulin, lactose, glucose, sucrose, maltose, dextrin, dextrose, fructose, sorbitol, fructooligosaccharide, mannitol, corn starch, crystalline cellulose, gum arabic, calcium phosphate, alginates, calcium silicate, microcrystalline cellulose, cellulose, polyvinyl pyrrolidone, tragacanth gum, gelatin, preferably bovine gelatin, syrup, aminosalicilate, sulfasalazine, 5-aminosalicylic acid, 4-aminosalicylic acid, benzalazine, dihydrochloride salt, olsalazine, balsalazide, bismuth subsalicylate, methyl cellulose, carboxymethyl cellulose, methylhydroxybenzoic acid esters, propylhydroxybenzoic acid esters, talc, magnesium stearates, inert polymers, water and mineral oils, iodine, magnesium, magnesium aspartate-ascorbate complex, magnesium amino acid chelate, zinc, zinc amino acid chelate, selenium, selenium amino acid complex, copper, copper amino acid chelate, manganese, manganese amino acid chelate, chromium, chromium polynicotinate, molybdenum, molybdenum amino acid chelate, potassium, potassium aspartate-ascorbate complex, choline, choline bitartrate, inositol, vanadium, vanadyl sulfate, boron, boron aspartate-citrate, citrus bioflavonoids, modified cellulose gum, silica, vegetarian stearine, titanium dioxide, magnesium stearate, preferably said at least one, two, three, four, five or even more pharmaceutically acceptable compound is a member selected from the group consisting of one or more vitamins, such as B vitamins, one or more minerals, such as magnesium and calcium, one or more carbohydrates, gelatin, preferably bovine gelatin, mannitol, dextrin, fructose, sorbitol, a prebiotic, maltose, maltodextrin, inulin, dextrose, iron, lactose and a mixture thereof. Moreover, the probiotic formulation may comprise pharmaceutically acceptable fillers, binders, lubricants, wetting agents, disintegrants, emulsifying agents, suspending agents, preservatives, sweetening agents and flavoring agents which are known to a person skilled in the art.

[0026] The probiotic formulation for use in this invention may be formulated such that after administration to a patient, the strain or cells are released rapidly, continuously or slowly. Examples of vitamins which may be comprised in the probiotic formulation for use in the invention are water-soluble and water-insoluble vitamins, such as vitamin A (e.g. retinol, retinal and carotenoids including beta carotene), vitamin B₁ (thiamine), vitamin B₂ (riboflavin), vitamin B₃ (e.g. niacin, niacinamide, nicotinamide), vitamin B₅ (pantothenic acid), vitamin B₆ (e.g. pyridoxine, pyridoxamine, pyridoxal), vitamin B₇ (biotin), vitamin B₉ (e.g. folic acid, folinic acid), vitamin B₁₂ (e.g. cyanocobalamin, hydroxycobalamin, methylcobalamin), vitamin C (ascorbic acid), vitamin D (e.g. ergocalciferol, cholecalciferol), vitamin E (e.g. tocopherols, tocotrienols), vitamin K (e.g. phyloquinone, menaquinones),

preferably vitamins of the B group are comprised in the probiotic formulation for use in the invention, such as vitamin B₁ (thiamine), vitamin B₂ (riboflavin), vitamin B₃ (e.g. niacin, niacinamide, nicotinamide), vitamin B₅ (pantothenic acid), vitamin B₆ (e.g. pyridoxine, pyridoxamine, pyridoxal) vitamin B₇ (biotin), vitamin B₉ (e.g. folic acid, folinic acid), vitamin B₁₂ (e.g. cyanocobalamin, hydroxycobalamin, methylcobalamin) and mixtures thereof. Examples of minerals comprised in the probiotic formulation for use in the invention are magnesium, calcium, zinc, selenium, iron, copper, manganese, chromium, molybdenum, potassium, vanadium, boron, titanium, preferably magnesium and/or calcium are present. A "trace element" is a chemical element which is only needed in very low quantities for the growth, development and/or physiology of the organism, preferably of a human organism. "Carbohydrates" are organic compounds consisting only of carbon, hydrogen and oxygen and having the empirical formula C_m(H₂O)_n, wherein the hydrogen to oxygen atom ratio is 2:1. "Lipids" are at least partially water-insoluble biological compounds due to a long hydrophobic carbohydrate part. Lipids are very important party of cell membranes in biological systems.

[0027] An "ingestible carrier" in the sense of the invention is a carrier which is used for administration of the probiotic formulation for use in the invention to a patient helping to ingest the probiotic formulation and the strain. The term "dairy product" as used herein is meant to include a medium comprising milk of animal and/or vegetable origin. Milk of animal origin includes milk from cow, sheep, goat and buffalo. As milk of vegetable origin there can be mentioned any fermentable substance of vegetable origin which can be used according to the invention, in particular originating from soybeans, rice or cereals. A possible ingestible carrier is a member selected from the group comprising a capsule, tablet, powder, granule, troche, cachet wafer capsule, elixir, emulsion, solution, syrup, suspension, soft and hard gelatin capsule, suppository, aseptic packed powder or a food product; optionally the food product is a dairy product, acidified milk, yoghurt, frozen yoghurt, yoghurt production, such as fermented yoghurt drink, drinking yoghurt, cheese, fermented cream, milk based desserts fermented milk or humanised milk, milk powder, milk concentrate, cheese spread, dressing, beverage and others. An "adjuvant" in the sense of the invention is a pharmacological or immunological agent that modifies the effect of other agents, such as drugs or vaccines or of the probiotic formulation for use in the present invention while having few - if any - direct effects when given alone.

[0028] "Drug entity" relates to a chemical or biological compound that is pharmaceutically active while being pharmaceutically tolerable to the patient, such as bisacodyl, loperamide, aminosalicilate, sulfasalazine, 5-aminosalicylic acid, 4-aminosalicylic acid, benzalazine, dihydrochloride salt, olsalazine, balsalazide, bismuth subsalicylate or mixtures thereof. Such compound may be any compound that promotes the beneficial effects of the probiotic formulation for use according to the invention either directly or indirectly. Directly means that the strain of *Bifidobacterium bifidum* MIMBb75 itself is protected, its growth or adhesion to the intestinal barrier is supported. Indirectly means that additional substances or compounds, i.e. drug entities, which are known to be useful in prophylaxis and/or treatment of a disease correlated with a functional disorder of the gastrointestinal tract, with undesirable gastrointestinal inflammatory activity, with an imbalance of the intestinal microbiota, a reduction of *Bifidobacteria* in the gastrointestinal tract and/or a dysfunctional intestinal barrier of the gastrointestinal tract, or for use in alleviating, preventing and/or treating a subject suffering from abdominal pain, discomfort, distension, bloating, digestive disorder, urgency and/or reduced and/or increased number of bowel movements, feeling of incomplete evacuation and/or combinations thereof may be comprised alone or in combination with other drug entities in the probiotic formulation. In particular, a drug entity may be comprised in the probiotic formulation which is a known medicament for treating irritable bowel syndrome, or a disease selected from the group consisting of irritable bowel movement, inflammatory bowel disease, Crohn's disease, ulcerative colitis, pouchitis, post infection colitis, diarrhoeal disease, *Clostridium difficile* associated diarrhoea, and combinations thereof. Examples of such a drug entity known to a person skilled in the art and may be found in Rote Liste, 2010, Germany, or any other pharmaceutical register. A "biological compound" may be any biological compound such as a carbohydrate, amino acid, lipid, nucleic acid, protein, peptide, cell compartment, phospholipids, polyether, plant, animal, or microbial compound. "Protein", "peptide" or "polypeptide" is to be understood according to their general meaning being a linear composition of proteinogenic or non-proteinogenic amino acids, wherein proteins may also comprise one or more subunits and catalytically relevant substances, such as vitamins, minerals or substances.

[0029] "Colony forming unit" (cfu) is a measure of viable bacterial cells of the strain of *Bifidobacterium bifidum* for use in the invention. The probiotic formulation for use in the invention may comprise the strain at more than 10¹ cfu, optionally not less than 10², 10³, 10⁴, 10⁵ or 10⁶ colony forming units (cfu) per capsule or tablet or per unit of powder or food product, preferably the strain is present at more than 10⁷ cfu per capsule or tablet or per unit of powder or food product, more preferably, the strain is present at more than 10⁸ cfu per capsule or tablet or per unit of powder or food product, even more preferably, the strain is present at more than 10⁹ cfu per capsule tablet or per unit of powder or food product. The daily amount of the strain administered to a patient in the probiotic formulation is not less than 10¹ cfu, optionally not less than 10², 10³, 10⁴ 10⁵ or 10⁶ cfu, preferably not less than 10⁷ cfu, more preferably not less than 10⁸ cfu, even more preferably not less than 10⁹ cfu or less than 10¹⁰ cfu. The daily amount may be increased depending on the physiological condition of the patient, i.e. in case of severe symptoms.

Here, the amount may be increased in that the daily amount is doubled, tripled or multiplied. On the contrary, the daily amount may be reduced, halved or divided by two, three or four, for example in case of intolerance or additional medication or the daily amounts as mentioned before may not be administered daily but every second or third day or weekly. The probiotic formulation may be taken at any time which is regarded convenient by the patient, such as in the morning, midday, in the afternoon or in the evening, preferably, the time is in the morning, for example after wake up, or in the evening, for example before going to sleep. If the probiotic formulation comprises an ingestible carrier being a food product, the administration is performed according to the nature of the ingestible carrier, i.e. food product. If the probiotic formulation comprises an ingestible carrier such as a capsule of tablet or comparable ingestible carrier, the administration is performed orally accompanied by a beverage or water, preferably by water.

[0030] In general, the probiotic formulation for use in the invention is administered according to the specific form of the probiotic, foodstuff or medicament. Thus, normally, the probiotic formulation for use in the invention is administered orally, preferably parenterally, or through the rectum.

[0031] The method for producing the probiotic formulation for use in the invention comprises at least the following steps:

1. a) fermenting and/or growing of a strain of *Bifidobacterium bifidum* MIMBb75, deposited under deposit No. DSM 24514, in a protein-rich liquid growth medium,
2. b) harvesting the cells of the above mentioned strains by centrifugation, stabilising, freeze-drying, milling and sieving of the cells,
3. c) optionally mixing/blending the cells with one or more members of the group consisting of a prebiotic, a pharmaceutically acceptable compound, an adjuvant, a bacterial component, a drug entity, a biological compound, a protein and/or peptide, and
4. d) introducing the cells of step b) or the mixture of step c) in an ingestible carrier.

[0032] First it should be noted that fermentation and growing of the *Bifidobacterium bifidum* strain for use in the invention may be performed by standard means and methods which are known to a person skilled in the art, such as described in "Probiotics and Health Claims", Wolfgang Kneifel, Seppo Salminen, John Wiley & Sons; 1. Edition (7 January 2011).

[0033] In general, a medium for growing and/or fermenting the *Bifidobacterium bifidum* strain for use in the invention comprises at least water, dextrose, yeast extract and minerals. The standard medium used for growing and/or fermenting the strain is MRS broth (Difco, Detroit, MI, USA) supplemented with 0.05% L-cysteine hydrochloride (cMRS). The skilled person is well aware of the fact that also other media may be used for growing, fermenting and pre-culture of bacterial organisms, such as strains of *Bifidobacterium bifidum*. Optionally, prior to step a) the strain is grown in a pre-culture in a shake flask. For this purpose, a volume of at least 200 mL, preferably of at least 300 mL, more preferably of at least 400 mL, even more preferably of at least 500 mL and most preferably of at least 600 mL of standard medium as mentioned above is inoculated with at least a single cell of the strain. This pre-culture is generally grown over night anaerobically at 37°C at 220 rpm.

[0034] The pre-culture may completely or partially then be used for inoculation of a higher fermentation in a pre-fermenter in a volume of at least 500 L, preferably of at least 600 L, more preferably of at least 700 L, even more preferably of at least 800 L, most preferably of at least 1000 L. After fermentation of the strain in a pre-fermenter, the cell culture containing said strain is at least partially transferred to the main fermenter having at least a volume of at least 5000 L, preferably of at least 7500 L, more preferably of at least 10,000 L, even more preferably of at least 15,000 L and most preferably of at least 20,000 L. Also in the pre-fermenter and main fermenter, the above mentioned standard medium is used for fermentation and growing of the *Bifidobacterium bifidum* strain for use in the invention. Fermentation and growing is stopped and the cells are harvested after depletion of the carbohydrates comprised in the medium which serve as nutrient for the strain. In general, the cell density after depletion is at least 10^1 cfu, optionally not less than 10^2 , 10^3 , 10^4 10^5 or 10^6 cells/mL, preferably at least 10^7 cells/mL, more preferably at least 10^8 cells/mL, even more preferably at least 10^9 cells/mL or at least 10^{10} cells/mL. The skilled person knows methods for cell density or biomass determination, such as biomass determination as dry weight using standard drying procedures and gravimetric determination of preweighted glass tubes or by measurement of the optical density using a spectrophotometer (such as Hitachi U-1100, Japan) at an absorption of 578 nm.

[0035] The pre-fermenter and main fermenter may be derived from a standard bioreactor suitable for culturing, fermenting, growing and/or processing of the strain of *Bifidobacterium bifidum* as defined in the claims. Suppliers of standard bioreactors are, for example, Applikon Biotechnology B.V. (Schiedam, The Netherlands), Infors (Bottmingen, Switzerland), Bioengineering (Wald, Switzerland) and Sartorius Stedim Biotech GmbH (Göttingen, Germany). Both, pre-fermentation and main fermentation are

performed in batch mode which means generally that no medium or other components are replenished during fermentation.

[0036] After main fermentation, the cells are harvested by centrifugation, such as by using a disc centrifuge or separator having an operating capacity of around 2000 - 15,000 L/h as provided by GEA Westfalia Separator Group GmbH (Oelde, Germany) or else. The cells are stabilised, freeze-dried, milled and sieved using standard applications and means which are well known to a person skilled in the art, subsequently. Freeze-drying gives a concentrate having a volume of 500 L to 1,000 L, preferably of 600 L to 800 L and having a weight of approximately 100 kg to 200 kg, preferably of ~150 kg.

[0037] Afterwards, the cells are optionally mixed with a prebiotic and/or at least one or more members of the group consisting of a pharmaceutically acceptable compound, an ingestible carrier, an adjuvant, a bacterial component, a drug entity, a biological compound and/or a protein and/or peptide and/or mixtures thereof, in particular a protein and/or peptide that is rich in glutamine/glutamate, a lipid, a carbohydrate, a vitamin, mineral and/or trace element; optionally said at least one pharmaceutically acceptable compound is a member selected from the group consisting of one or more vitamins, such as vitamins of the B group, one or more minerals, such as calcium or magnesium, one or more carbohydrates, such as lactose, maltodextrin, inulin, dextrose, mannitol, maltose, dextrin, sorbitol, fructose, and a mixture thereof in different amounts.

[0038] For obtaining the probiotic formulations for use in the invention, in general, 15 - 40 g, preferably 18 - 35 g, more preferably 20 - 30 g, even more preferably 22 - 28 g, most preferably 25 g of the *Bifidobacterium bifidum* cells, or strain are mixed with 50 - 100 g, preferably with 60 - 90 g, more preferably with 65 - 85 g, even more preferably with 70 - 80 g, most preferably with 75 g of one or more members of the group consisting of a prebiotic, carbohydrate, a pharmaceutically acceptable compound or mixtures thereof as nutrient for the cells, or strain, and are optionally mixed with 30 - 70 g, preferably with 35 - 65 g, more preferably with 40 - 60 g, even more preferably with 45 - 55 g, most preferably with 50 g of one or more members of the group consisting of a pharmaceutically acceptable compound, an ingestible carrier, an adjuvant, a bacterial component, a drug entity, a biological compound and/or a protein and/or peptide, in particular a protein and/or peptide that is rich in glutamine/glutamate, a lipid, a carbohydrate, a vitamin, mineral, trace element and or mixtures thereof for improvement of the pourability of the probiotic formulation. Preferably, 15 - 40 g, preferably 18 - 35 g, more preferably 20 - 30 g, even more preferably 22 - 28 g, most preferably 25 g of *Bifidobacterium bifidum* cells, or strain for use in the invention are mixed with 50 - 100 g, preferably with 60 - 90 g, more preferably with 65 - 85 g, even more preferably with 70 - 80 g, most preferably with 75 g of a carbohydrate, carbohydrate mixture, prebiotic or mixture thereof, and optionally with 30 - 70 g, preferably with 35 - 65 g, more preferably with 40 - 60 g, even more preferably with 45 - 55 g, most preferably with 50 g of one or more members of the group consisting of a pharmaceutically acceptable compound, a carbohydrate, a vitamin, mineral, trace element and or mixtures thereof. More preferably, 15 - 40 g, preferably 18 - 35 g, more preferably 20 - 30 g, even more preferably 22 - 28 g, most preferably 25 g of *Bifidobacterium bifidum* cells, or strain for use in the invention are mixed with 50 - 100 g, preferably with 60 - 90 g, more preferably with 65 - 85 g, even more preferably with 70 - 80 g, most preferably with 75 g of a carbohydrate, inulin, a fructooligosaccharide or a mixture thereof, and optionally with 30 - 70 g, preferably with 35 - 65 g, more preferably with 40 - 60 g, even more preferably with 45 - 55 g, most preferably with 50 g of one or more members of the group consisting of a vitamin, such as a vitamin of the B group, one or more minerals, such as calcium or magnesium, one or more carbohydrates, such as lactose, maltodextrin, inulin, dextrose, mannitol, fructooligosaccharide, mannitol, maltose, dextrin, sorbitol, fructose, and a mixture thereof. Even more preferably, 25 g of *Bifidobacterium bifidum* cells, or strain for use in the invention are mixed with 75 g of a member selected from the group consisting of a carbohydrate, prebiotic, maltodextrin, inulin, dextrose, mannitol, maltose, dextrin, sorbitol, fructose, and a mixture thereof, and are mixed with 50 g of a member selected from the group consisting of cellulose, pharmaceutically acceptable compound, an ingestible carrier, an adjuvant, a bacterial component, a drug entity, a biological compound and/or a protein and/or peptide, in particular a protein and/or peptide that is rich in glutamine/glutamate, a lipid, a carbohydrate, a vitamin, mineral, trace element and a mixture thereof. Most preferably, 25 g of *Bifidobacterium bifidum* cells, or strain for use in the invention are mixed with 75 g maltodextrin, and are mixed with 50 g of cellulose. Mixing/blending the components with the cells, or strain for use in the invention after fermenting and/or growing the strain, harvesting, stabilising, freeze-drying, milling and sieving, may be performed by standard techniques, applications and means which are known to a person skilled in the art, preferably a column blender is used. Of course, also higher or lower amounts can be used in mixing/blending depending on the volume of the device used for mixing/blending as long as the ratio of the components corresponds to the ratios mentioned above. A typical ratio for example is 25% strain, 75% prebiotic or carbohydrate or maltodextrin and 50% pharmaceutically acceptable compound, such as cellulose.

[0039] For example, 50 mg - 300 mg, preferably 75 mg - 250 mg, more preferably 100 mg - 200 mg, even more preferably 120 mg - 175 mg, most preferably 150 mg of the *Bifidobacterium bifidum* cells, or strain for use in the invention or of the above mentioned probiotic formulations for use in the invention are introduced into one unit of ingestible carrier, for example into a capsule, tablet, troche, cachet wafer capsule, elixir, emulsion, solution, syrup, suspension, soft and hard gelatin capsule, suppository, aseptic packed powder or a food product, such as a dairy product, acidified milk, yoghurt, frozen yoghurt, yoghurt production, such as fermented yoghurt drink, drinking yoghurt, cheese, fermented cream, milk based desserts fermented milk or humanised milk, milk powder, milk concentrate, cheese spread, dressing, beverage and others, preferably 50 mg, preferably 75

mg, more preferably 100 mg, even more preferably 120 mg and most preferably 150 mg of the *Bifidobacterium bifidum* cells, or strain for use in the invention or of the above mentioned mixtures are introduced into a capsule, tablet troche, cachet wafer capsule, soft and hard gelatin capsule, suppository, more preferably into a capsule, soft and hard gelatin capsule. Thereby, after introduction into the unit of ingestible carrier, i.e. the capsule, tablet, powder, granule, troche, cachet wafer capsule, elixir, emulsion, solution, syrup, suspension, soft and hard gelatin capsule, suppository, aseptic packed powder or food product, such as a dairy product, acidified milk, yoghurt, frozen yoghurt, yoghurt production, such as fermented yoghurt drink, drinking yoghurt, cheese, fermented cream, milk based desserts fermented milk or humanised milk, milk powder, milk concentrate, cheese spread, dressing and/or beverage comprises more than 10^1 cfu, optionally not less than 10^2 , 10^3 , 10^4 10^5 or 10^6 cfu, preferably more than 10^7 cfu, more preferably more than 10^8 cfu, even more preferably more than 10^9 cfu or more than 10^{10} cfu. In any case the amount by weight may be adjusted according to the concentration of cfu, so that the amount of the *Bifidobacterium bifidum* cells, or strain for use in the invention in the final product, such as capsule, tablet, powder, granule, troche, cachet wafer capsule, elixir, emulsion, solution, syrup, suspension, soft and hard gelatin capsule, suppository, aseptic packed powder or food product corresponds to more than 10^1 cfu, optionally not less than 10^2 , 10^3 , 10^4 10^5 or 10^6 cfu, preferably more than 10^7 cfu, more preferably more than 10^8 cfu, even more preferably more than 10^9 cfu or more than 10^{10} cfu. In case that the amount of cfu is less than 10^1 cfu, optionally not less than 10^2 , 10^3 , 10^4 10^5 or 10^6 cfu, 10^7 cfu, 10^8 cfu, even 10^9 cfu or less than 10^{10} cfu, the patient may adapt to aforementioned amounts of cfu by administering more than one unit of ingestible carrier.

[0040] In an exemplary embodiment, 25 mg of *Bifidobacterium bifidum* MIMBb75 deposited under deposit No. DSM 24514 cells, i.e. at least 10^9 cfu *Bifidobacterium bifidum* MIMBb75 deposited under deposit No. DSM 24514, 75 mg maltodextrin and 50 mg cellulose are introduced into one gelatine capsule, preferably in a bovine gelatine capsule. Preferably, the aforementioned described capsule with 25 mg of *Bifidobacterium bifidum* MIMBb75 deposited under deposit No. DSM 24514 cells, comprises at least 10^9 cfu *Bifidobacterium bifidum* MIMBb75 deposited under deposit No. DSM 24514, 75 mg maltodextrin and 50 mg cellulose is administered once daily. Ingestible carriers in general should be of standard to high quality, should fulfil the hygiene requirements of food supplements and may be obtained from various manufacturers. Capsules, for example bovine gelatine capsules, may for example be obtained from Capsugel (Bornem, Belgium).

[0041] In another preferred embodiment, 25 mg of *Bifidobacterium bifidum* MIMBb75 deposited under deposit No. DSM 24514 cells, preferably comprising at least 10^9 cfu *Bifidobacterium bifidum* MIMBb75 deposited under deposit No. DSM 24514 and 2 g maltodextrin are mixed and are considered as unit of powder. Preferably, the aforementioned described unit of powder with 25 mg of *Bifidobacterium bifidum* MIMBb75 deposited under deposit No. DSM 24514 cells, i.e. at least 10^9 cfu *Bifidobacterium bifidum* MIMBb75 deposited under deposit No. DSM 24514 is administered once daily.

[0042] Independent of the particular ingestible carrier chosen by the patient, the daily amount of the *Bifidobacterium bifidum* cells, or strain administered to a patient in the probiotic formulation is not less than 10^1 cfu, optionally not less than 10^2 , 10^3 , 10^4 10^5 or 10^6 cfu, preferably not less than 10^7 cfu, more preferably not less than 10^8 cfu, even more preferably not less than 10^9 cfu or not less than 10^{10} cfu. Alternative doses are discussed above.

EXAMPLES

1. Double-Blind, randomized and placebo-controlled study to assess the efficiency of *Bifidobacterium bifidum* MIMBb75 in patients having irritable bowel syndrome

1.1 Overview

[0043] 122 patients were successfully randomised to receive either placebo (N=62) or *Bifidobacterium bifidum* MIMBb75 (N=60) (also regarded as "*Bifidobacteria* group"). Subjects consumed either 1×10^9 cfu/capsule or placebo once a day for four weeks. The severity of IBS and individuall IBS symptoms was recorded daily on a 7-point Likert scale.

1.2 Study Population

[0044] Patients were recruited from principal investigators and by advertisement. The nutritional study protocol has been presented to the Ethics Committee of the Bavarian Chamber of Physicians. For inclusion, clinically inconspicuous subjects aged between 18 and 68 years with mild to moderate IBS (Rome III criteria) have been considered. Individuals with inflammatory organic gastrointestinal disease, systemic diseases, cancer, autoimmune diseases, diabetes, known lactose intolerance or immunodeficiency, known further abdominal surgery except appendectomy, no disease free sigmoidoscopy or colonoscopy in the last five years when older 50 years, diagnosed hyperthyroidism, use of antipsychotics or systemic corticosteroids for at least 3 months prior to study start, major psychiatric disorder, celiac disease or pregnancy had been excluded.

1.3 Study Design

[0045] This study was performed as a prospective, multi-centre, randomised, double-blind, placebo-controlled, two-arm nutritional study. Throughout the study, patients recorded daily their global IBS symptoms as well as individual IBS symptoms using a patient diary. Additionally, patients have been questioned at physician site for global and individual IBS symptoms (visit 2-4) and quality of life (visit 3 and 4). Physician visits took place at screening, after two weeks (run-in phase), after 6 weeks (end of treatment) and after 8 weeks (end of wash-out phase) (Figure 1).

[0046] After patients have given their written informed consent, they qualified for the screening examination at day 1 (visit 1), which included a complete medical history and physical examination. A blood sample was taken for analysis in a central laboratory, including a pregnancy test. At the screening visit, patients were instructed to maintain their eating and life style habits throughout the study. A patient diary has been handed out.

[0047] At the second visit (day 15), diaries were reviewed. Patients who had at least 2 days with mild to moderate pain during the second week of run-in and who fulfilled all inclusion criteria and who did not violate any of the exclusion criteria were 1:1 randomized to receive either *B. bifidum* MIMBb75 or placebo. The treatment was allocated according to a computer-generated blocked randomisation list with a block size of 4. The block size was not disclosed to the investigators. During the intervention period, patients received either one capsule comprising probiotic formulation daily for 4 weeks or an identical appearing placebo. The allocation was blinded to both patients and site staff.

[0048] At the end of the treatment phase (visit 3, day 43), investigators collected unused study product and empty sachets in order to confirm compliance. Diaries were collected and reviewed.

[0049] After the nutritional supplement-free wash-out phase (visit 4, day 57), a complete physical examination was performed and a blood sample was taken. Bisacodyl and loperamide were allowed as rescue medication. Other probiotics and medications that could influence the efficacy of the study product were not allowed.

1.4 Probiotic Preparation

[0050] Nutritional supplement was prepared under good manufacturing process (GMP) conditions. *B. bifidum* MIMBb75 was grown in a protein-rich liquid growth medium, harvested through centrifugation, stabilized, freeze-dried, milled and sieved. The dry powder bacteria were mixed with a pharmaceutically acceptable compound and filled into capsules of 1×10^9 cfu. Placebo capsules appeared identical and contained maltodextrin.

1.5 Endpoint definitions

[0051] The prospectively defined primary efficacy variable was the subject's global assessment of IBS symptoms using a 7-point Likert scale. Patients were asked to answer the daily question "If you consider your IBS symptoms (e.g. abdominal pain/discomfort, distension/bloating, digestive disorder, urgency, bowel habit) in general, how have you been affected by these symptoms during the last 24 hours?" Possible answers ranged from 0 (not at all), 1 (very mild), 2 (mild), 3 (moderate), 4 (strong), 5 (very strong) to 6 (intolerable).

[0052] Secondary efficacy variables included the individual symptoms of IBS alone, such as "abdominal pain/discomfort", "distension/bloating", "digestive disorder" and "urgency", recorded on the same 7-point Likert scale. The individual symptom

scores were additionally combined into a composite symptom score as the arithmetic mean of three individual symptom scores. Furthermore, the reduced and/or increased number of bowel movements, feeling of incomplete bowel evacuation and intake of other medication were reported daily in the diary.

[0053] At the end of treatment and again at the end of the study, physicians questioned the patients regarding the global assessment of efficacy and tolerability. Efficacy was assessed by the following question: "Please consider how you felt during the 4 week treatment regarding your overall well-being, and symptoms of abdominal discomfort/pain and altered bowel habit. Compared to the way you usually felt before taking the study medication, how would you rate your relief of symptoms during the last 4 weeks?" Possible answers were: "completely relieved (1), considerably relieved (2), somewhat relieved (3), unchanged (4) or worse (5)". Both "completely relieved" and "considerably relieved" were defined as "adequate relief". Health related quality of life was assessed by the use of the SF-12 questionnaire prior to treatment and at the end of the treatment.

[0054] Adverse events were recorded throughout the study and the global assessment of tolerability has been questioned at physician visit 3 and 4. Laboratory values and vital signs were examined at the screening visit and at the end of study.

1.6 Statistical methods

3.6.1 Simple size estimation

[0055] A reduction of the subject's global assessment (SGA) of at least 20% on the 7-point Likert scale was considered a relevant treatment effect. Based on published data (Whorwell, 2006), a difference of 0.6 points in the SGA of IBS symptoms between *B. bifidum* MIMBb75 and placebo on the 7-point Likert scale was anticipated (e.g. 3.0 in the placebo group and 2.4 in the *Bifidobacteria* group). Standard deviation was estimated with 1.0 using the same data. With these assumptions, a Wilcoxon-Mann-Whitney test with a two-sided significance level of $\alpha = 0.05$ and a power of $1-\beta = 0.8$, a sample size of 47 patients per group was required. With an estimated drop-out rate of 15-20% after randomisation, 110 randomised patients were planned and 132 patients were recruited to account for possible withdrawals prior to study start.

1.6.2 Statistical analysis

[0056] The primary objective of this study was to prove a significant reduction of the SGA of general IBS symptoms at the end of treatment in the *Bifidobacteria* group vs. placebo. The SGA was calculated for each subject as arithmetic mean at baseline, during the treatment period and during the wash-out phase. To account for possible differences in the baseline values, the change from baseline calculated as mean score during 4 weeks of treatment minus mean score during the run-in phase (week 1-2) was defined as primary target criterion. The non-parametric Van Elteren test stratified by study centres was used for the comparison of treatment arms. $P < 0.05$ was considered statistically significant.

[0057] The primary analysis was based on the intent-to-treat population where all successfully randomised patients were included. Missing post-baseline values were imputed by the baseline value for the primary target criterion and these patients were evaluated as non-responders. An additional per protocol analysis was performed for supportive purposes.

[0058] Descriptive analyses of secondary target criteria were based on available data. Treatment differences were tested by use of the non-parametric Wilcoxon test for continuous variables or by Fishers exact test for binary variables. All p-values are two-sided.

[0059] Secondary efficacy variables included response based on a 50% rule of symptom relief during treatment (at least improvement in two out of four weeks within the treatment period and improvement defined as at least one point reduction from baseline). All statistical analyses were performed using SAS version 9.1.3 for windows, SAS Institute Inc., Cary, NC, USA.

1.7 Results

1.7.1 Subjects

[0060] A total of 132 patients were included into the study and 122 patients were successfully randomised to receive either placebo (N=62) or *B. bifidum* MIMBb75 (N=60). All randomized patients were analyzed for intent to treat (ITT) (N=122). One patient with no post randomization visit was excluded from the analysis of adverse events. A total of 103 patients (49 placebo, 54 verum) were analysed as per protocol (Figure 2).

1.7.2 Baseline Characteristics

[0061] In terms of baseline characteristics, there were no significant differences between the groups. 21.5% were classified as diarrhoea-predominant IBS, 19.8% as constipation-predominant IBS and 58.7% as alternators with no significant differences between the *Bifidobacteria* and the placebo group.

[0062] Demographics were well balanced between the treatment groups with about 67% female patients and mean weight of 71 kg corresponding to a BMI of 24. Patients were on average 41 years in the placebo group and 37 years in the *Bifidobacteria* group (Table 1).

Table 1: Demographic characteristics of the intent to treat (ITT)-population. SD - standard deviation, BMI - Body mass index.

	Placebo	Verum
	N(%) or mean \pm SD	N(%) or mean \pm SD
N=122 (62+60)		
Age	40.98 \pm 12.80	36.65 \pm 12.42
Female sex	41 (66.1)	41 (68.3)
Height (cm)	169.50 \pm 8.75	170.78 \pm 9.47
Weight (kg)	70.79 \pm 15.54	70.45 \pm 16.02
BMI	24.60 \pm 5.19	24.02 \pm 4.45
IBS Type (N=122 (61+60))		
Diarrhoea predominant	12 (19.4)	14 (23.3)
Constipation predominant	15 (24.2)	9 (15.0)
Alternating type	34 (54.8)	37 (61.7)

1.7.3 Subject's global assessment (SGA) of IBS symptoms

[0063] The primary endpoint was the reduction of the SGA of IBS symptoms on the subject's global assessment diary. *B. bifidum* MIMBb75 significantly improved global IBS symptoms by -0.88 points [95% confidence interval (CI): -1.07; -0.69] (from 2.95 in the run in phase to 2.07 in the treatment phase) compared to only -0.16 points [95% CI: -0.32; 0.00] (from 2.79 in the run in phase to 2.63 in the treatment phase) in the placebo group ($p < 0.0001$) using the 7-point Likert scale. The evaluation of the SGA on a weekly basis showed a significant benefit for patients within the *Bifidobacteria* group for every single week starting the second week of treatment till the end of the study (Figure 3).

1.7.4 Secondary endpoints

[0064] Secondary endpoints included changes in individual IBS symptoms, such as "pain/discomfort", "distension/ bloating", "digestive disorder", "urgency", "reduced and/or increased number of bowel movements" and "feeling of incomplete evacuation", on a 7-point Likert scale. *B. bifidum* MIMBb75 showed significant reduction of pain/discomfort by -0.82 points [95% CI: -1.01; -0.63] vs. -0.18 [95% CI: -0.35; -0.01] in the placebo group ($p < 0.0001$), and distension/bloating by -0.92 points [95% CI: -1.15; -0.69] vs. -0.21 [95% CI: -0.37; -0.05] in the placebo group ($p < 0.0001$) during treatment. The reduction persisted during the wash-out phase. Urgency was significantly reduced by -0.67 points [95% CI: -0.86; -0.48] vs. -0.21 [95% CI: -0.35; -0.07] in the placebo group ($p = 0.0001$) during treatment but not during wash-out. No effects could be detected for frequency of bowel movement and feeling of incomplete bowel evacuation (Figure 4).

[0065] The evaluation of the individual IBS symptoms pain/discomfort and distension/bloating on a weekly basis showed a significant benefit for patients within the *Bifidobacteria* group compared to placebo for every single week beginning the second week of treatment till the end of the study. A significant difference in urgency between *Bifidobacteria* and placebo group was shown between week four and six (Figures 5 and 6).

[0066] Digestive disorder was measured by the item "bowel movement satisfaction" in the questionnaire at physician site. Bowel movement satisfaction decreased from 3.89 to 2.44 in the *Bifidobacteria* group vs. 3.69 to 6.47 in the placebo group ($p=0.0002$) after treatment. The reduction persisted during washout phase (2.33 in the *Bifidobacteria* group vs. 3.47 in placebo group, $p<0.0001$).

1.7.5 Composite score

[0067] A composite score was calculated for the individual IBS symptoms (pain/discomfort, distension/bloating, urgency). During the run in phase, the score was comparable in both groups. The patients within the *Bifidobacteria* group significantly benefited from the consumption of *B. bifidum* MIMBb75 vs. placebo (-0.80 in the *Bifidobacteria* group; -0.20 in the placebo group; $p<0.0001$). This improvement was also preserved during the wash-out phase (-0.85 in the *Bifidobacteria* group; -0.31 in the placebo group; $p<0.0001$).

1.7.6 Treatment responders

[0068] Overall responders were defined as patients experiencing an improvement of the average weekly score of at least 1 point on the Likert scale for the primary parameter (SGA of IBS symptoms) in at least two out of the 4 weeks treatment period (50% rule). Abdominal pain responders were defined using the same 50% rule for at least one point average improvement for the assessment of "pain/discomfort". Overall responder rates were 56.7% in the *Bifidobacteria* group and only 21.0% in the placebo group ($p=0.0001$). The difference between the treatment arms was only a little bit less pronounced when considering only the symptom "pain/discomfort" where responder rates were calculated to be 48.3% in the *Bifidobacteria* and only 24.2% in the placebo group ($p=0.008$) (Figure 7).

1.7.7 Global efficacy at physician site

[0069] The overall assessment of efficacy was significantly better in the *Bifidobacteria* group compared to placebo. At the end of treatment 43.3% of the patients in the *Bifidobacteria* group achieved adequate relief compared to only 8.1% in the placebo group ($p<0.0001$). At end of the study adequate relief was reported for 46.7% in the *Bifidobacteria* and 11.3% of the patients in the placebo group ($p<0.0001$; Figure 8).

1.7.8 Health related quality of life

[0070] The evaluation of the SF-12 sum scores showed a significant gain in quality of life within the *Bifidobacteria* group. Physical health sum improved by 3.99 in the *Bifidobacteria* group and by only 1.08 in the placebo group compared to baseline ($p=0.0185$). Mental health sum improved by 5.78 in the *Bifidobacteria* group and by only 1.58 in the placebo group compared to baseline ($p=0.0083$).

1.7.9 Adverse events

[0071] Only 36 adverse events were reported with suspected relationship to the study product, 13 in the placebo and 23 in the treatment group, but no significant differences could be detected in the side effects profile of *B. bifidum* MIMBb75 vs. placebo.

1.7.10 Summary

[0072] *B. bifidum* MIMBb75 significantly reduced the subject's global assessment (SGA) of IBS symptoms by -0.88 points [95%

CI: -1.07; -0.69] compared to only -0.16 [95% CI: -0.32; 0.00] points in the placebo group ($p < 0.0001$). *B. bifidum* MIMBb75 also significantly improved the individual IBS symptoms pain/discomfort, distension/bloating, digestive disorder and urgency. The evaluation of the SF-12 sum scores showed a significant gain in quality of life within the *Bifidobacteria* group. Furthermore, adequate relief was reported by 46.7% of patients in the *Bifidobacteria* and only by 11.3% of patients in the placebo group ($p < 0.0001$). Overall responder rates were 56.7% in the *Bifidobacteria* group and only 21.0% in the placebo group ($p = 0.0001$). *B. bifidum* MIMBb75 was well tolerated and the adverse events were not different from placebo.

1.8 Conclusion

[0073] *B. bifidum* MIMBb75 effectively alleviates global IBS as well as improves individual IBS symptoms. Considering the high efficacy of *B. bifidum* MIMBb75 in IBS along with the good side effect profile, *B. bifidum* MIMBb75 is a promising candidate for IBS therapy.

[0074] This randomised, double blind, placebo-controlled study indicates that *B. bifidum* MIMBb75 has beneficial effects in the treatment of IBS. In this study, *B. bifidum* MIMBb75 significantly improved global IBS and its related symptoms such as pain/discomfort and bloating compared to placebo. Moreover, *B. bifidum* MIMBb75 also significantly improved quality of life. These benefits persisted within the consumption-free wash-out phase. Overall responder rates were predominantly high with 56.7% in the *Bifidobacteria* group compared to only 21.0% in the placebo group ($p = 0.0001$). At the end of the study adequate relief was reported for 46.7% in the *Bifidobacteria* and only 11.3% of the patients in the placebo group ($p < 0.0001$).

[0075] Up to date, several studies have examined the effects of *Bifidobacteria* on IBS and its symptoms. However, only a few could show a significant benefit. Additionally, to the inventors' knowledge, no probiotic strain could show to significantly alleviate irritable bowel syndrome and simultaneously improve quality of life. While some studies might have missed to show efficacy due to small sample size and randomisation errors, several different probiotic strains did repeatedly show no significant improvement in IBS. Recently, Brenner et al. (2009) published a systematic review of randomised controlled trials (RCTs) aimed at the evaluation of the efficacy, safety, and tolerability of probiotics in the treatment of IBS. A total of 16 RCTs were included in the analysis. Of those, exclusively one *Bifidobacteria* strain showed efficacy for improvement of IBS symptoms in two appropriately designed studies. These findings can be attributed to the fact, that the efficacy of probiotics is strongly strain specific and that only few strains might be able to show efficacy in IBS.

[0076] In conclusion, *B. bifidum* MIMBb75 improves global IBS as well as its IBS symptoms along with a good side effect profile.

BRIEF DESCRIPTION OF THE DRAWINGS

[0077]

Figure 1:

Study schematic.

Figure 2:

Diagram of study flow.

Figure 3:

Comparison of effects of placebo and *B. bifidum* MIMBb75 on global IBS symptoms (by SGA, recorded on a 0-6 scale) on a weekly basis. Significant improvement of global IBS symptoms in the *Bifidobacteria* group vs. placebo.

Figure 4:

Comparison of the reduction of IBS symptoms (*B. bifidum* MIMBb75 vs. placebo) on mean score changes from baseline to treatment phase.

Figure 5:

Comparison of effects of placebo and *B. bifidum* MIMBb75 on pain/discomfort (recorded on a 0-6 Likert scale) on a weekly basis. Significant improvement in the *Bifidobacteria* group vs. placebo group.

Figure 6:

Comparison of effects of placebo and *B. bifidum* MIMBb75 on distension/bloating (recorded on a 0-6 scale) on a weekly basis. Significant improvement in the *Bifidobacteria* group vs. placebo group.

Figure 7:

Overall responders during treatment (ITT).

Figure 8:

Adequate relief after treatment (ITT).

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- EP1141235A [0005]

Non-patent literature cited in the description

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- Study of the adhesion of Bifidobacterium bifidum MIMB75 to human intestinal cell lines *Curr. Microbiology*, 2009, vol. 59, 167-172 [0008]
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- **BRENNER et al.** systematic review of randomised controlled trials (RCTs), 2009, [0075]

BIFIDOBACTERIUM BIFIDUM STAMMER TIL BRUG I GASTROINTESTINALE
SYGDOMME

Patentkrav

- 5 **1.** Probiotisk formulering omfattende en stamme af *Bifidobacterium bifidum*
MIMBb75, deponeret under deponerings Nr. DSM 24514, til anvendelse i
behandlingen af irritable tarmsyndrom, eller en sygdom valgt fra gruppen
bestående af irritable tarmbevægelse, inflammatorisk tarmsygdom, Crohn's
sygdom, ulcerøs colitis, pouchitis, postinfektøs colitis, diarréfremkaldende
10 sygdom, *Clostridium difficile* forbundet diarré, og kombinationer deraf.
- 2.** Den probiotiske formulering til anvendelse ifølge krav 1, hvor behandlingen
omfatter lindring, forebyggelse og/eller behandling af abdominal smerte,
oppustethed, forstoppelse, fordøjelsesslidelse, reduceret antal af tarmbevægelser,
15 øget antal af tarmbevægelser, følelse af ufuldstændig tømning, eller
kombinationer deraf.
- 3.** Den probiotiske formulering til anvendelse ifølge et hvilket som helst af
kravene 1-2, yderligere omfattende mindst inulin og/eller et fruktooligosaccharid.
20
- 4.** Den probiotiske formulering til anvendelse ifølge et hvilket som helst af
kravene 1-3, yderligere omfattende mindst en farmaceutisk acceptabel
forbindelse; en spiselig bærer; en adjuvant; en bakteriel komponent; en
lægemiddelentitet; en biologisk forbindelse; og/eller et protein, og/eller et peptid,
25 fortrinsvis et protein og/eller peptid der er rigt på glutamin/glutamat; et lipid; et
kulhydrat; et vitamin; mineral og/eller sporelement.
- 5.** Den probiotiske formulering til anvendelse ifølge krav 4, hvor den mindst ene
farmaceutisk acceptable forbindelse er et medlem valgt fra gruppen bestående af
30 et eller flere vitaminer, fortrinsvis vitaminer af B gruppen; et eller flere mineraler,
fortrinsvis calcium, eller magnesium; et eller flere kulhydrater, fortrinsvis et
kulhydrat valgt fra gruppen bestående af laktose, maltodextrin, inulin, dextrose,
mannitol, maltose, dextrin, sorbitol, fruktose, og en blanding deraf.

- 6.** Den probiotiske formulering til anvendelse ifølge krav 4 eller 5, hvor den spiselige bærer er en kapsel, tablet, pulver eller en madvare.
- 7.** Den probiotiske formulering til anvendelse ifølge krav 6, hvor madvaren er et
5 mejeriprodukt, surmælk, yoghurt, frossen yoghurt, mælkepulver, mælkekoncentrat, smøreost, dressing eller drik.
- 8.** Den probiotiske formulering til anvendelse ifølge et hvilket som helst af kravene 6 til 7, hvor stammen er tilstede i mere end 10^1 cfu per kapsel eller tablet
10 eller per enhed af pulver eller madvare.
- 9.** Den probiotiske formulering til anvendelse ifølge et hvilket som helst af kravene 6 til 7, hvor stammen er tilstede i mere end 10^2 , 10^3 , 10^4 , 10^5 eller 10^6 cfu per kapsel eller tablet eller per enhed af pulver eller madvare.
15
- 10.** Den probiotiske formulering til anvendelse ifølge et hvilket som helst af kravene 6 til 9, hvor den daglige mængde af stammen der skal indgives til en patient ikke er mindre end 10^1 cfu per kapsel eller tablet eller per enhed af pulver eller madvare.
20
- 11.** Den probiotiske formulering til anvendelse ifølge et hvilket som helst af kravene 6 til 10, hvor den daglige mængde af stammen der skal indgives til en patient ikke er mindre end 10^2 , 10^3 , 10^4 , 10^5 eller 10^6 cfu per kapsel eller tablet eller per enhed af pulver eller madvare.

DRAWINGS

Figure 1

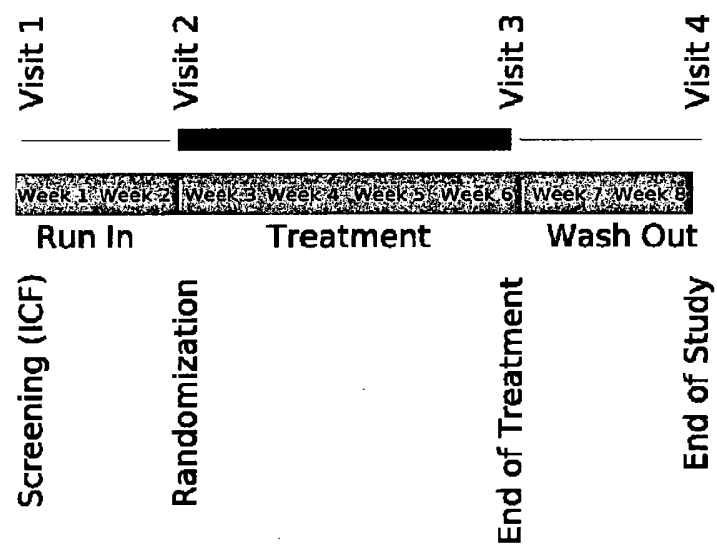


Figure 2

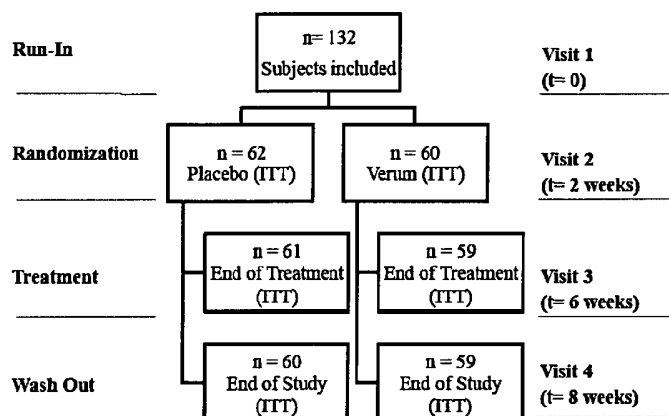


Figure 3

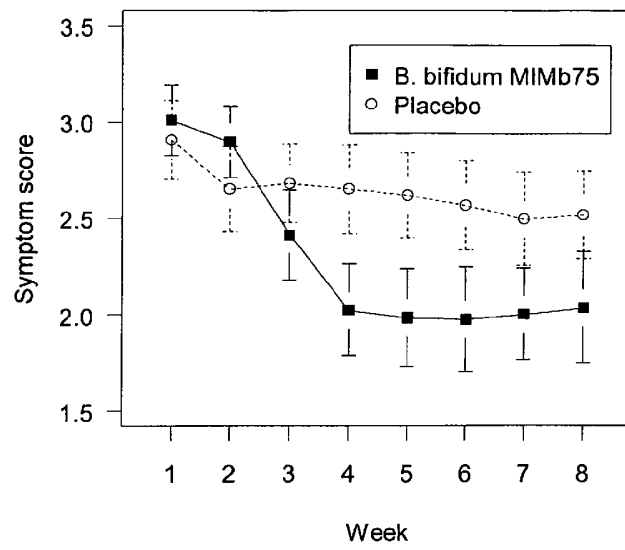


Figure 4

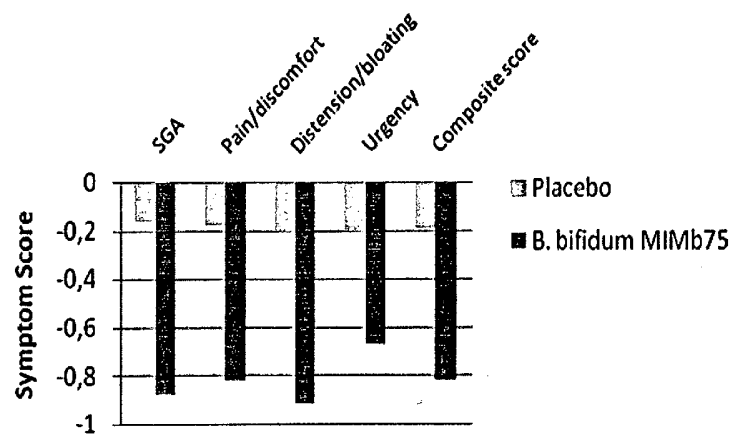


Figure 5

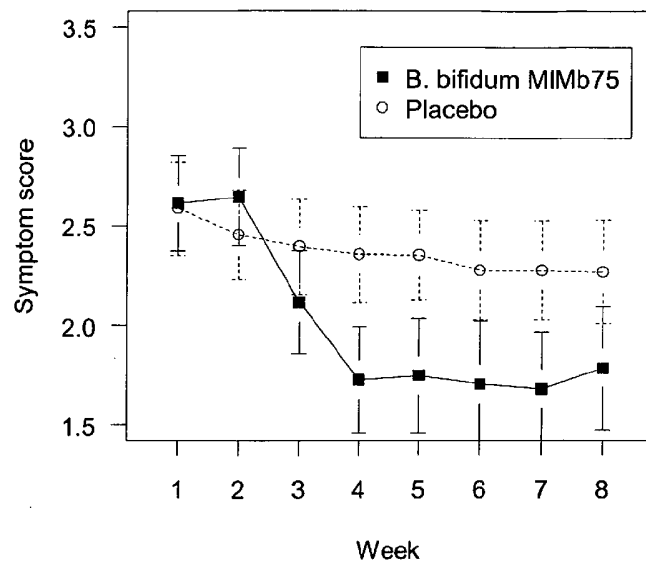


Figure 6

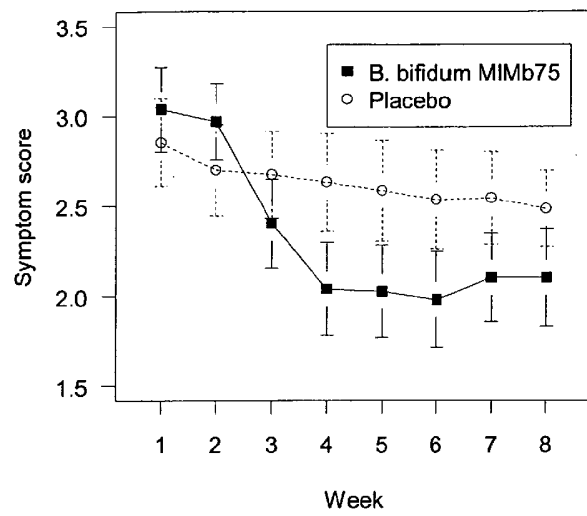


Figure 7

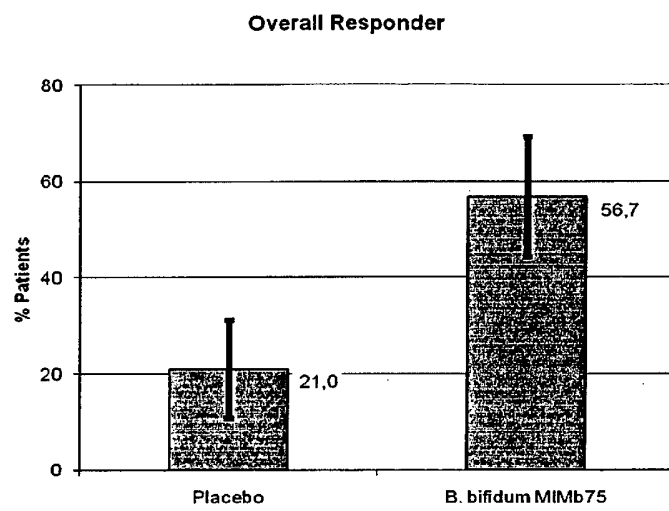


Figure 8

