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(54) Benævnelse: **SAMMENSÆTNINGER OMFATTENDE EN BAKTERIESTAMME AF BLAUTIA HYDROGENOTROPHICA TIL ANVENDELSE I BEHANDLINGEN ELLER FOREBYGGELSEN AF DIARRÉ ELLER FORSTOPPELSE**

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Fortsættes ...

DESCRIPTION

TECHNICAL FIELD

[0001] This invention is in the field of compositions comprising a bacterial strain of the genus *Blautia hydrogenotrophica* having a 16s rRNA sequence that is at least 95% identical to SEQ ID NO:5, for use in a method of treating or preventing diarrhea and/or constipation.

BACKGROUND TO THE INVENTION

[0002] The human intestine is thought to be sterile *in utero,* but it is exposed to a large variety of maternal and environmental microbes immediately after birth. Thereafter, a dynamic period of microbial colonization and succession occurs, which is influenced by factors such as delivery mode, environment, diet and host genotype, all of which impact upon the composition of the gut microbiota, particularly during early life. Subsequently, the microbiota stabilizes and becomes adult-like [1]. The human gut microbiota contains more than 1500 different phylotypes dominated in abundance levels by two major bacterial divisions (*phyla*), the Bacteroidetes and the Firmicutes [2-3]. The successful symbiotic relationships arising from bacterial colonization of the human gut have yielded a wide variety of metabolic, structural, protective and other beneficial functions. The enhanced metabolic activities of the colonized gut ensure that otherwise indigestible dietary components are degraded with release of by-products providing an important nutrient source for the host and additional health benefits. Similarly, the immunological importance of the gut microbiota is well-recognized and is exemplified in germfree animals which have an impaired immune system that is functionally reconstituted following the introduction of commensal bacteria [4-6].

[0003] Dramatic changes in microbiota composition have been documented in gastrointestinal disorders such as inflammatory bowel disease (IBD). For example, the levels of *Clostridium* cluster XIVa and *Clostridium* cluster XI *(F. prausnitzii)* bacteria are reduced in IBD patients whilst numbers of *E. coli* are increased, suggesting a shift in the balance of symbionts and pathobionts within the gut [7-11].

[0004] In recognition of the potential positive effect that certain bacterial strains may have on the animal gut, various strains have been proposed for use in the treatment of various diseases (see, for example, [12-15]). A number of strains, including mostly *Lactobacillus* and *Bifidobacterium* strains, have been proposed for use in treating various bowel disorders (see [16] for a review). Strains of the genus *Blautia* have also been proposed for use in modulating the microbial balance of the digestive ecosystem in IBS patients (WO 01/85187). However, the relationship between different bacterial strains and different diseases, and the precise effects of particular bacterial strains on the gut and at a systemic level and on any particular types of diseases, are poorly characterised.

[0005] There is a requirement for the potential effects of gut bacteria to be characterised so that new therapies using gut bacteria can be developed.

[0006] US 2010/0247489 describes the use of mineral nutrients to treat digestive disorders. US'789 proposes optionally also using numerous different genera of bacteria to prevent a wide variety of abdominal symptoms but does not link any bacteria to any diseases or symptoms.

[0007] WO 2016/086206 suggests that bacteria in the order Clostridiales can be used to treat or prevent various conditions but does not link any bacteria to any diseases or symptoms.

[0008] WO 2012/142605 proposes that it may be possible to treat a number of different diseases with a combination of microorganisms. WO'605 suggests a large number of possible bacterial species that could be employed, but there is no teaching in WO'605 as to how any of the proposed bacterial species could be used to treat any of the diseases proposed.

[0009] WO 02/07741 suggests using various bacteria from the class *Clostridia* to treat gastro-intestinal disorders but does not link any bacteria to any diseases or symptoms. WO 2016/086209 suggests using a large number of possible bacteria in the order Clostridiales to decrease the secretion of pro-inflammatory cytokines and/or increase the secretion of anti-inflammatory cytokines, but there is no teaching as to how any of the proposed bacterial species could be used to treat any of the diseases proposed.

SUMMARY OF THE INVENTION

[0010] The inventors have developed new therapies for treating and preventing diarrhea and/or constipation. In particular, the inventors have identified that bacterial strains from the genus *Blautia* can be effective for reducing diarrhea and/or constipation. As described in the examples, oral administration of compositions comprising *Blautia hydrogenotrophica* may reduce diarrhea and/or constipation in patients having irritable bowel syndrome (IBS). Therefore, in a first embodiment, the invention provides a composition comprising a bacterial strain of the genus *Blautia hydrogenotrophica* having a 16s rRNA sequence that is at least 95% identical to SEQ ID NO:5, for use in a method of treating or preventing diarrhea and/or constipation.

[0011] In preferred embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia hydrogenotrophica* having a 16s rRNA sequence that is at least 95% identical to SEQ ID NO:5, for use in a method of treating or preventing diarrhea and/or constipation in a subject diagnosed with Crohn's disease, ulcerative colitis, or, more preferably, IBS. In some embodiments, the subject diagnosed with IBS has IBS with both diarrhea and constipation. In some embodiments, the subject diagnosed with IBS has IBS with diarrhea but without constipation or with only a small amount of constipation. In some embodiments, the subject diagnosed with IBS has IBS with constipation but without diarrhea or with only a small

amount of diarrhea.

[0012] The bacterial strain in the composition is of *Blautia hydrogenotrophica,* as defined in the claims. Closely related strains may also be used, such as bacterial strains that have a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Blautia hydrogenotrophica.* Preferably, the bacterial strain has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:5. Most preferably, the bacterial strain in the composition is the *Blautia hydrogenotrophica* strain deposited under accession number DSM 10507/14294.

[0013] In certain embodiments, the composition of the invention is for oral administration. Oral administration of the strains of the invention can be effective for treating diarrhea and/or constipation. Also, oral administration is convenient for patients and practitioners and allows delivery to and / or partial or total colonisation of the intestine.

[0014] In certain embodiments, the composition of the invention comprises one or more pharmaceutically acceptable excipients or carriers.

[0015] In certain embodiments, the composition of the invention comprises a bacterial strain that has been lyophilised. Lyophilisation is an effective and convenient technique for preparing stable compositions that allow delivery of bacteria, and is shown to provide effective compositions in the examples.

[0016] In certain embodiments, the invention provides a food product comprising the composition as described above.

[0017] In preferred embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia hydrogenotrophica* having a 16s rRNA sequence that is at least 95% identical to SEQ ID NO:5, for use in a method of treating or preventing a disease associated with *Enterobacteriaceae* infection, such as *E. coli* infection. In certain embodiments, the compositions of the invention are for use in treating or preventing diarrhea, gastroenteritis, urinary tract infection or neonatal meningitis.

[0018] In further preferred embodiments, the compositions of the invention are for use in reducing the level of *Enterobacteriaceae* in the gastrointestinal tract, preferably the level of *E. coli,* in the treatment or prevention of IBS, Crohn's disease, ulcerative colitis, functional dyspepsia, diarrhea, gastroenteritis, urinary tract infection or neonatal meningitis.

[0019] In particularly preferred embodiments, the compositions of the invention are for use a method of treating or preventing diarrhea associated with *Enterobacteriaceae* infection, such as *E. coli* infection, or are for use in reducing the level of *Enterobacteriaceae* in the gastrointestinal tract, preferably the level of *E. coli,* in the treatment or prevention of diarrhea.

BRIEF DESCRIPTION OF DRAWINGS

[0020]

Figure 1: Changes in patient symptoms during dosing period (days 1-16) of Phase I clinical trial.

Figure 2: Changes in patient symptoms during washout period of Phase I clinical trial.

Figure 3: Hydrogen breath test Cmax results for day 1, day 2, day 15 and day 16 for IBS patients treated with Blautix (**Figure 3a**) and IBS patients treated with placebo (**Figure 3b**). **Figure 3c** is a graph comparing the percentage of Blautix treated patients with a reduction in hydrogen between the mean of days 15 and 16 and the mean of days 1 and 2 to the percentage of placebo treated patients with a reduction in hydrogen between these time points.

Figure 4a: Hydrogen uncorrected and hydrogen corrected breath test paired data for day 1 and day 15 for Blautix treated IBS patients; **Figure 4b:** graph comparing mean hydrogen uncorrected breath test results for day 1 and day 15 for the Blautix treatment group; **Figure 4c:** graph comparing mean hydrogen corrected breath test results for day 1 and day 15 for the Blautix treatment group.

Figure 5a: Hydrogen uncorrected and hydrogen corrected breath test paired data for day 1 and day 15 for placebo treated IBS patients; **Figure 5b:** graph comparing mean hydrogen uncorrected breath test results for day 1 and day 15 for the placebo group; **Figure 5c:** graph comparing mean hydrogen corrected breath test results for day 1 and day 15 for the placebo group.

Figure 6: Graphs comparing the mean hydrogen breath test results from day 1 and day 15 for the Bautix treatment group (Verum) and placebo group (**Figure 6a:** uncorrected hydrogen; **Figure 6b:** corrected hydrogen).

DISCLOSURE OF THE INVENTION

Bacterial strains

[0021] The compositions of the invention comprise a bacterial strain of the genus *Blautia hydrogenotrophica* having a 16s rRNA sequence that is at least 95% identical to SEQ ID NO:5. The examples demonstrate that bacteria of the *Blautia* genus are useful for treating or preventing diarrhea and/or constipation.

[0022] Examples of *Blautia* strains include *Blautia hydrogenotrophica, B. stercoris, B. faecis, B.*

coccoides, B. glucerasea, B. hansenii, B. luti, B. producta, B. schinkii and *B. wexlerae*. The *Blautia* species are Gram-reaction-positive, non-motile bacteria that may be either coccoid or oval and all are obligate anaerobes that produce acetic acid as the major end product of glucose fermentation [17]. Blautia may be isolated from the human gut, although *B. producta* was isolated from a septicaemia sample.

[0023] *Blautia hydrogenotrophica* (previously known as *Ruminococcus hydrogenotrophicus*) has been isolated from the guts of mammals, is strictly anaerobic, and metabolises H_2/CO_2 to acetate, which may be important for human nutrition and health. The type strain of *Blautia hydrogenotrophica* is S5a33 = DSM 10507 = JCM 14656. The GenBank accession number for the 16S rRNA gene sequence of *Blautia hydrogenotrophica* strain S5a36 is X95624.1 (disclosed herein as SEQ ID NO:5). This exemplary *Blautia hydrogenotrophica* strain is described in [17] and [18]. The S5a33 strain and the S5a36 strain correspond to two subclones of a strain isolated from a faecal sample of a healthy subject. They show identical morphology, physiology and metabolism and have identical 16S rRNA sequences.

[0024] Thus, in some embodiments, the *Blautia hydrogenotrophica* for use in the invention has the 16S rRNA sequence of SEQ ID NO:5.

[0025] The *Blautia hydrogenotrophica* bacterium deposited under accession number DSM 10507 and also under accession number DSM 14294 was tested in the Examples and is also referred to herein as strain BH. Strain BH was deposited with the Deutsche Sammlung von Mikroorganismen [German Microorganism Collection] (Mascheroder Weg 1b, 38124 Braunschweig, Germany) in 26th January 1996 as *"Ruminococcus hydrogenotrophicus"* under accession number DSM 10507 and also under accession number DSM 14294 as "S5a33" on 14th May 2001. The depositor was INRA Laboratoire de Microbiologie CR de Clermont-Ferrand/Theix 63122 Saint Genes Champanelle, France. Ownership of the deposits has passed to 4D Pharma Plc by way of assignment.

[0026] Bacterial strains closely related to the strain tested in the examples are also expected to be effective for treating or preventing diarrhea and/or constipation. In certain embodiments, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Blautia hydrogenotrophica*. Preferably, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:5.

[0027] Bacterial strains that are biotypes of the bacterium deposited under accession number DSM 10507/14294 are also expected to be effective for treating or preventing diarrhea and/or constipation. A biotype is a closely related strain that has the same or very similar physiological and biochemical characteristics.

[0028] Strains that are biotypes of a bacterium deposited under accession number DSM 10507/14294and that are suitable for use in the invention may be identified by sequencing

other nucleotide sequences for a bacterium deposited under accession number DSM 10507/14294. For example, substantially the whole genome may be sequenced and a biotype strain for use in the invention may have at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity across at least 80% of its whole genome (e.g. across at least 85%, 90%, 95% or 99%, or across its whole genome). For example, in some embodiments, a biotype strain has at least 98% sequence identity across at least 98% of its genome or at least 99% sequence identity across 99% of its genome. Other suitable sequences for use in identifying biotype strains may include hsp60 or repetitive sequences such as BOX, ERIC, $(GTG)_5$, or REP or [20]. Biotype strains may have sequences with at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of a bacterium deposited under accession number DSM 10507/14294, NCIMB 42381 or NCIMB 42486. In some embodiments, a biotype strain has a sequence with at least 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of the *Blautia hydrogenotrophica* strain deposited as DSM 10507/14294 and comprises a 16S rRNA sequence that is at least 99% identical (e.g. at least 99.5% or at least 99.9% identical) to SEQ ID NO:5. In some embodiments, a biotype strain has a sequence with at least 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of the *Blautia hydrogenotrophica* strain deposited as DSM 10507/14294 and has the 16S rRNA sequence of SEQ ID NO:5.

[0029] Alternatively, strains that are biotypes of a bacterium deposited under accession number DSM 10507/14294 and that are suitable for use in the invention may be identified by using the accession number DSM 10507/14294 deposit, and restriction fragment analysis and/or PCR analysis, for example by using fluorescent amplified fragment length polymorphism (FAFLP) and repetitive DNA element (rep)-PCR fingerprinting, or protein profiling, or partial 16S or 23s rDNA sequencing. In preferred embodiments, such techniques may be used to identify other *Blautia hydrogenotrophica* strains.

[0030] In certain embodiments, strains that are biotypes of a bacterium deposited under accession number DSM 10507/14294 and that are suitable for use in the invention are strains that provide the same pattern as a bacterium deposited under accession number DSM 10507/14294 when analysed by amplified ribosomal DNA restriction analysis (ARDRA), for example when using Sau3AI restriction enzyme (for exemplary methods and guidance see, for example,[21]). Alternatively, biotype strains are identified as strains that have the same carbohydrate fermentation patterns as a bacterium deposited under accession number DSM 10507/14294.

[0031] Other *Blautia* strains that are useful in the compositions and methods of the invention, as defined in the claims, such as biotypes of a bacterium deposited under accession number DSM 10507/14294, may be identified using any appropriate method or strategy, including the assays described in the examples. For instance, strains for use in the invention may be identified by culturing bacteria and administering to rats to test in the distension assay. In particular, bacterial strains that have similar growth patterns, metabolic type and/or surface antigens to a bacterium deposited under accession number DSM 10507/14294 may be useful in the invention. A useful strain will have comparable microbiota modulatory activity to the DSM

10507/14294 strain. In particular, a biotype strain will elicit comparable effects on diarrhea and/or constipation to the effects shown in the Examples, which may be identified by using the culturing and administration protocols described in the Examples.

[0032] A particularly preferred strain of the invention is the *Blautia hydrogenotrophica* strain deposited under accession number DSM 10507/14294. This is the exemplary BH strain tested in the examples and shown to be effective for treating disease. Therefore, the invention provides a cell, such as an isolated cell, of the *Blautia hydrogenotrophica* strain deposited under accession number DSM 10507/14294, or a derivative thereof, for use in therapy, in particular for the diseases described herein.

[0033] A derivative of the strain deposited under accession number DSM 10507/14294 may be a daughter strain (progeny) or a strain cultured (subcloned) from the original. A derivative of a strain of the invention may be modified, for example at the genetic level, without ablating the biological activity.

[0034] In particular, a derivative strain of the invention is therapeutically active. A derivative strain will have comparable microbiota modulatory activity to the original DSM 10507/14294 strain. In particular, a derivative strain will elicit comparable effects on diarrhea and/or constipation to the effects shown in the Examples, which may be identified by using the culturing and administration protocols described in the Examples. A derivative of the DSM 10507/14294 strain will generally be a biotype of the DSM 10507/14294 strain.

[0035] References to cells of the *Blautia hydrogenotrophica* strain deposited under accession number DSM 10507/14294 encompass any cells that have the same safety and therapeutic efficacy characteristics as the strains deposited under accession number DSM 10507/14294, and such cells are encompassed by the invention.

[0036] In preferred embodiments, the bacterial strains in the compositions of the invention are viable and capable of partially or totally colonising the intestine.

Therapeutic uses

[0037] In preferred embodiments, the compositions of the invention are for use in treating diarrhea and/or constipation. In some embodiments the composition is for use in treating a patient having both diarrhea and constipation. In some embodiments the composition is for use in treating a patient having diarrhea without constipation. In some embodiments the composition is for use in treating a patient having constipation without diarrhea. Although patients often exhibit either diarrhea or constipation, a single patient may exhibit both conditions at different times. Patients having IBS and/or inflammatory diseases of the intestine often exhibit diarrhea and/or constipation. Accordingly, the invention provides the composition of the invention for use in treating a patient diagnosed with or suspected of having IBS or an inflammatory disease of the intestine. For example, IBS patients may experience alternating

cycles of diarrhea and constipation or an IBS patient may be characterised as an IBS patient with diarrhea or an IBS patient with constipation. In some embodiments, a composition of the invention may be used to treat a patient having IBS, a patient having IBS with diarrhea or a patient having IBS with constipation. For example, in some embodiments, the subject diagnosed with IBS has IBS with both diarrhea and constipation. In some embodiments, the subject diagnosed with IBS has IBS with diarrhea but without constipation or with only a small amount of constipation. In some embodiments, the subject diagnosed with IBS has IBS with constipation but without diarrhea or with only a small amount of diarrhea. In some embodiments, the inflammatory disease is of the small intestine, colon or rectum. Diarrhea is a symptom of Crohn's disease and ulcerative colitis. Accordingly, a composition of the invention may be used to treat a patient having Crohn's disease or ulcerative colitis.

[0038] As demonstrated in the examples, bacterial compositions of the invention may be effective for reducing diarrhea and/or constipation.

[0039] In preferred embodiments, the compositions of the invention are for use in treating or preventing diarrhea and/or constipation associated with Crohn's disease, ulcerative colitis or, more preferably, IBS. In preferred embodiments, the compositions of the invention are for use in treating or preventing diarrhea and/or constipation in a subject diagnosed with Crohn's disease, ulcerative colitis or, more preferably, IBS. In preferred embodiments the compositions of the invention are for use in treating or preventing diarrhea and/or constipation in the treatment of Crohn's disease, ulcerative colitis or, more preferably, IBS. In some embodiments, the patient has abdominal pain and/or bloating in addition to diarrhea and/or constipation. In such embodiments, the patient may have any combination of two, three or all four of these conditions. For example, the patient may have diarrhea, constipation, abdominal pain and bloating; diarrhea, constipation and abdominal pain; diarrhea, constipation and bloating; diarrhea, abdominal pain and/or bloating without constipation; constipation, abdominal pain and/or bloating without diarrhea.

[0040] In certain embodiments, the composition may be in the form of a bacterial culture. In some embodiments, the composition may preferably be a lyophilisate.

[0041] In some embodiments, the composition of the invention is for use in treating diarrhea and/or constipation in a subject having an increased level of hydrogen in their breath relative to a healthy subject. In some embodiments, the composition of the invention is for use in reducing the hydrogen level in the breath of a subject having diarrhea and/or constipation. The subject is preferably a subject diagnosed as having IBS and/or an inflammatory disease of the intestine. In some embodiments, the inflammatory disease is of the small intestine, colon or rectum. In some embodiments, the subject has been diagnosed with Crohn's disease, ulcerative colitis or, more preferably, IBS, for example one of the types of IBS described herein. Without being bound by theory, it is speculated that the increased hydrogen level arises from the mechanisms underlying the inflammation in the intestine. The examples show that treatment with a composition of the invention reduces the level of hydrogen detected in hydrogen breath tests. Accordingly, the hydrogen levels are preferably assessed using a

hydrogen breath test. The hydrogen breath test is well known in the art and so the skilled person will know how to conduct such a test. In some embodiments, the patient is administered lactulose as the substrate for the test.

[0042] The hydrogen breath test is also a useful tool for monitoring the effectiveness or likely effectiveness of treatment or prevention using a composition of an invention. For example, a reduction in the level of hydrogen detected in a subject's breath following treatment or prevention by a composition of the invention may indicate that the treatment is having a therapeutic or preventative effect. Accordingly, in some embodiments the methods and uses of the invention further comprise monitoring the hydrogen level in a subject's breath during and/or following treatment or prevention with a composition of the invention and thereby assessing the effectiveness or likely effectiveness of treatment or prevention.

[0043] For example, hydrogen levels may be monitored at one or more (e.g. 1, 2, 3, 4 or more than 4) times, for example, including before treatment, at the start of treatment, during treatment, at the end of treatment and/or following treatment, as desired. In some embodiments, the level of hydrogen in the subject's breath at the end and/or following the dosing period (during which the composition is administered to the subject) is compared to the level at the start and/or before the dosing period and a reduction in the level indicates the effectiveness or likely effectiveness of the treatment or prevention. For example, in embodiments in which the dosing period is 16 days, it may be desirable to take measurements at day 1 and day 16, or for example at day 1, day 2, day 15 and day 16. In some embodiments, multiple measurements are taken and the mean of those measurements obtained (for example, the mean of day 1 and day 2 and the mean of day 15 and day 16). In some embodiments, a reduction in at least 40 ppm in the hydrogen level Cmax indicates that the treatment or prevention is effective or likely to be effective. In some embodiments, the hydrogen level in the subject's breath is measured only once, for example, at the end of or following treatment, and the finding that the level is at or close to a predetermined level is indicative that the treatment or prevention is likely to have been effective. The hydrogen breath test is a standard assay and so predetermined levels are known in the art.

[0044] In certain embodiments, the compositions of the invention are for use in preventing diarrhea and/or constipation in a subject that is receiving or has received or is about to receive (for example, later the same day, the next day, or within 2, 3, 4, 5, 6, 7, 10, 14 or 21 days) antibiotic treatment or that is suffering from or has suffered from bacterial gastroenteritis. Antibiotic treatment and bacterial gastroenteritis are associated with changes in the gut microbiota that may precede diarrhea and/or constipation and that may be prevented by the compositions of the invention. The compositions of the invention may be administered simultaneously, separately or sequentially with an antibiotic treatment. For example, the compositions of the invention may be administered concurrently with an antibiotic treatment.

[0045] In preferred embodiments, treatment with compositions of the invention results in a reduction in diarrhea and/or constipation.

[0046] Treatment or prevention of diarrhea and/or constipation may refer to, for example, an alleviation of the severity of symptoms or a reduction in the frequency of exacerbations or the range of triggers that are a problem for the patient.

[0047] In certain embodiments, the compositions of the invention are for use in treating or preventing a disease associated with *Enterobacteriaceae* infection, such as *E. coli* infection. In certain embodiments, the compositions of the invention are for use in treating or preventing diarrhea, gastroenteritis, urinary tract infection or neonatal meningitis. In such embodiments the *Enterobacteriaceae* may be a pathogenic strain.

[0048] In certain embodiments, the compositions of the invention are for use in treating or preventing a disease associated with increased levels of *Enterobacteriaceae,* such as *E. coli.* In certain embodiments, the compositions of the invention are for use in treating or preventing diarrhea, gastroenteritis, urinary tract infection or neonatal meningitis. In such embodiments, the *Enterobacteriaceae* may be a commensal or non-pathogenic strain.

[0049] In preferred embodiments, the compositions of the invention are for use in reducing the level of *Enterobacteriaceae* in the gastrointestinal tract, preferably the level of *E. coli,* in the treatment or prevention of a disease associated with increased levels of *Enterobacteriaceae,* such as IBS, Crohn's disease, ulcerative colitis, functional dyspepsia, diarrhea, gastroenteritis, urinary tract infection or neonatal meningitis. *Enterobacteriaceae* and in particular *E. coli* are known to be potential triggers for, or known to exacerbate, Crohn's disease and ulcerative colitis [22-24], so the effect shown in the examples for the compositions of the invention may be beneficial in the treatment of these conditions.

[0050] In certain embodiments, the compositions of the invention are for use in treating or preventing IBS, Crohn's disease, ulcerative colitis, functional dyspepsia, diarrhea, gastroenteritis, urinary tract infection or neonatal meningitis by reducing the level of *Enterobacteriaceae* in the gastrointestinal tract.

Modes of administration

[0051] Preferably, the compositions of the invention are to be administered to the gastrointestinal tract in order to enable delivery to and / or partial or total colonisation of the intestine with the bacterial strain of the invention. Generally, the compositions of the invention are administered orally, but they may be administered rectally, intranasally, or via buccal or sublingual routes.

[0052] In certain embodiments, the compositions of the invention may be administered as a foam, as a spray or a gel.

[0053] In certain embodiments, the compositions of the invention may be administered as a suppository, such as a rectal suppository, for example in the form of a theobroma oil (cocoa

butter), synthetic hard fat (e.g. suppocire, witepsol), glycero-gelatin, polyethylene glycol, or soap glycerin composition.

[0054] In certain embodiments, the composition of the invention is administered to the gastrointestinal tract via a tube, such as a nasogastric tube, orogastric tube, gastric tube, jejunostomy tube (J tube), percutaneous endoscopic gastrostomy (PEG), or a port, such as a chest wall port that provides access to the stomach, jejunum and other suitable access ports.

[0055] The compositions of the invention may be administered once, or they may be administered sequentially as part of a treatment regimen. In certain embodiments, the compositions of the invention are to be administered daily. The examples demonstrate that administration provides successful colonisation and clinical benefits in treatment of diarrhea and/or constipation.

[0056] In certain embodiments, the compositions of the invention are administered regularly, such as daily, every two days, or weekly, for an extended period of time, such as for at least one week, two weeks, one month, two months, six months, or one year. The examples demonstrate that BH administration may not result in permanent colonisation of the intestines, so regular administration for extended periods of time may provide greater therapeutic benefits.

[0057] In some embodiments the compositions of the invention are administered for 7 days, 14 days, 16 days, 21 days or 28 days or no more than 7 days, 14 days, 16 days, 21 days or 28 days. For example, in some embodiments the compositions of the invention are administered for 16 days.

[0058] In certain embodiments of the invention, treatment according to the invention is accompanied by assessment of the patient's gut microbiota. Treatment may be repeated if delivery of and / or partial or total colonisation with the strain of the invention is not achieved such that efficacy is not observed, or treatment may be ceased if delivery and / or partial or total colonisation is successful and efficacy is observed.

[0059] In certain embodiments, the composition of the invention may be administered to a pregnant animal, for example a mammal such as a human in order to prevent diarrhea and/or constipation developing in her child *in utero* and / or after it is born.

[0060] The compositions of the invention may be administered to a patient that has been diagnosed with diarrhea and/or constipation or a disease or condition associated with diarrhea and/or constipation, or that has been identified as being at risk of diarrhea and/or constipation. The compositions may also be administered as a prophylactic measure to prevent the development of diarrhea and/or constipation in a healthy patient.

[0061] The compositions of the invention may be administered to a patient that has been identified as having an abnormal gut microbiota. For example, the patient may have reduced

or absent colonisation by *Blautia,* and in particular *Blautia hydrogenotrophica, Blautia stercoris* or *Blautia wexlerae.*

[0062] The compositions of the invention may be administered as a food product, such as a nutritional supplement.

[0063] Generally, the compositions of the invention are for the treatment of humans, although they may be used to treat animals including monogastric mammals such as poultry, pigs, cats, dogs, horses or rabbits. The compositions of the invention may be useful for enhancing the growth and performance of animals. If administered to animals, oral gavage may be used.

[0064] In some embodiments, the subject to whom the composition is to be administered is an adult human. In some embodiments, the subject to whom the composition is to be administered is an infant human.

Compositions

[0065] Generally, the composition of the invention comprises bacteria as defined in the claims. In preferred embodiments of the invention, the composition is formulated in freeze-dried form. For example, the composition of the invention may comprise granules or gelatin capsules, for example hard gelatin capsules, comprising a bacterial strain of the invention as defined in the claims.

[0066] Preferably, the composition of the invention comprises lyophilised bacteria. Lyophilisation of bacteria is a well-established procedure and relevant guidance is available in, for example, references [25-27]. Lyophilisate compositions may be particularly effective. In preferred embodiments, the compositions of the invention comprises lyophilised bacteria and is for the treatment of diarrhea and/or constipation associated with IBS.

[0067] Alternatively, the composition of the invention may comprise a live, active bacterial culture. The examples demonstrate that cultures of the bacteria of the invention are therapeutically effective.

[0068] In some embodiments, the bacterial strain in the composition of the invention has not been inactivated, for example, has not been heat-inactivated. In some embodiments, the bacterial strain in the composition of the invention has not been killed, for example, has not been heat-killed. In some embodiments, the bacterial strain in the composition of the invention has not been attenuated, for example, has not been heat-attenuated. For example, in some embodiments, the bacterial strain in the composition of the invention has not been killed, inactivated and/or attenuated. For example, in some embodiments, the bacterial strain in the composition of the invention is live. For example, in some embodiments, the bacterial strain in the composition of the invention is viable. For example, in some embodiments, the bacterial strain in the composition of the invention is capable of partially or totally colonising the

intestine. For example, in some embodiments, the bacterial strain in the composition of the invention is viable and capable of partially or totally colonising the intestine.

[0069] In some embodiments, the composition comprises a mixture of live bacterial strains and bacterial strains that have been killed.

[0070] In preferred embodiments, the composition of the invention is encapsulated to enable delivery of the bacterial strain to the intestine. Encapsulation protects the composition from degradation until delivery at the target location through, for example, rupturing with chemical or physical stimuli such as pressure, enzymatic activity, or physical disintegration, which may be triggered by changes in pH. Any appropriate encapsulation method may be used. Exemplary encapsulation techniques include entrapment within a porous matrix, attachment or adsorption on solid carrier surfaces, self-aggregation by flocculation or with cross-linking agents, and mechanical containment behind a microporous membrane or a microcapsule. Guidance on encapsulation that may be useful for preparing compositions of the invention is available in, for example, references [28-29].

[0071] The composition may be administered orally and may be in the form of a tablet, capsule or powder. Encapsulated products are preferred because *Blautia* are anaerobes. Other ingredients (such as vitamin C, for example), may be included as oxygen scavengers and prebiotic substrates to improve the delivery and / or partial or total colonisation and survival *in vivo*. Alternatively, the probiotic composition of the invention may be administered orally as a food or nutritional product, such as milk or whey based fermented dairy product, or as a pharmaceutical product.

[0072] The composition may be formulated as a probiotic.

[0073] A composition of the invention includes a therapeutically effective amount of a bacterial strain of the invention. A therapeutically effective amount of a bacterial strain is sufficient to exert a beneficial effect upon a patient. A therapeutically effective amount of a bacterial strain may be sufficient to result in delivery to and / or partial or total colonisation of the patient's intestine.

[0074] A suitable daily dose of the bacteria, for example for an adult human, may be from about 1×10^3 to about 1×10^{11} colony forming units (CFU); for example, from about 1×10^7 to about 1×10^{10} CFU; in another example from about 1×10^6 to about 1×10^{10} CFU; in another example from about 1×10^7 to about 1×10^{11} CFU; in another example from about 1×10^8 to about 1×10^{10} CFU; in another example from about 1×10^8 to about 1×10^{11} CFU.

[0075] In certain embodiments, the dose of the bacteria is at least 10^9 cells per day, such as at least 10^{10}, at least 10^{11}, or at least 10^{12} cells per day.

[0076] In certain embodiments, the composition contains the bacterial strain in an amount of

from about 1 x 10^6 to about 1 x 10^{11} CFU/g, respect to the weight of the composition; for example, from about 1 x 10^8 to about 1 x 10^{10} CFU/g. The dose may be, for example, 1 g, 3g, 5g, and 10g.

[0077] Typically, a probiotic, such as the composition of the invention, is optionally combined with at least one suitable prebiotic compound. A prebiotic compound is usually a non-digestible carbohydrate such as an oligo- or polysaccharide, or a sugar alcohol, which is not degraded or absorbed in the upper digestive tract. Known prebiotics include commercial products such as inulin and transgalacto-oligosaccharides.

[0078] In certain embodiments, the probiotic composition of the present invention includes a prebiotic compound in an amount of from about 1 to about 30% by weight, respect to the total weight composition, (e.g. from 5 to 20% by weight). Carbohydrates may be selected from the group consisting of: fructo- oligosaccharides (or FOS), short-chain fructo-oligosaccharides, inulin, isomalt-oligosaccharides, pectins, xylo-oligosaccharides (or XOS), chitosan-oligosaccharides (or COS), beta-glucans, arable gum modified and resistant starches, polydextrose, D-tagatose, acacia fibers, carob, oats, and citrus fibers. In one aspect, the prebiotics are the short-chain fructo-oligosaccharides (for simplicity shown herein below as FOSs-c.c); said FOSs-c.c. are not digestible carbohydrates, generally obtained by the conversion of the beet sugar and including a saccharose molecule to which three glucose molecules are bonded.

[0079] The compositions of the invention may comprise pharmaceutically acceptable excipients or carriers. Examples of such suitable excipients may be found in the reference [30]. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art and are described, for example, in reference [31]. Examples of suitable carriers include lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol and the like. Examples of suitable diluents include ethanol, glycerol and water. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as, or in addition to, the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s). Examples of suitable binders include starch, gelatin, natural sugars such as glucose, anhydrous lactose, free-flow lactose, beta-lactose, corn sweeteners, natural and synthetic gums, such as acacia, tragacanth or sodium alginate, carboxymethyl cellulose and polyethylene glycol. Examples of suitable lubricants include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Preservatives, stabilizers, dyes and even flavouring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid, cysteine and esters of p-hydroxybenzoic acid, for example, in some embodiments the preservative is selected from sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used. A further example of a suitable carrier is saccharose. A further example of a preservative is cysteine.

[0080] The compositions of the invention may be formulated as a food product. For example, a food product may provide nutritional benefit in addition to the therapeutic effect of the invention, such as in a nutritional supplement. Similarly, a food product may be formulated to enhance the taste of the composition of the invention or to make the composition more attractive to consume by being more similar to a common food item, rather than to a pharmaceutical composition. In certain embodiments, the composition of the invention is formulated as a milk-based product. The term "milk-based product" means any liquid or semi-solid milk- or whey- based product having a varying fat content. The milk-based product can be, e.g., cow's milk, goat's milk, sheep's milk, skimmed milk, whole milk, milk recombined from powdered milk and whey without any processing, or a processed product, such as yoghurt, curdled milk, curd, sour milk, sour whole milk, butter milk and other sour milk products. Another important group includes milk beverages, such as whey beverages, fermented milks, condensed milks, infant or baby milks; flavoured milks, ice cream; milk-containing food such as sweets.

[0081] In some embodiments, the compositions of the invention, as defined in the claims, comprise one or more bacterial strains of the genus *Blautia* and do not contain bacteria from any other genus, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another genus.

[0082] In certain embodiments, the compositions of the invention contain a single bacterial strain or species and do not contain any other bacterial strains or species. Such compositions may comprise only *de minimis* or biologically irrelevant amounts of other bacterial strains or species. Such compositions may be a culture that is substantially free from other species of organism. In some embodiments, such compositions may be a lyophilisate that is substantially free from other species of organism.

[0083] In certain embodiments, the compositions of the invention, as defined in the claims, comprise one or more bacterial strains of the genus *Blautia,* for example, a *Blautia hydrogenotrophica,* and do not contain any other bacterial genus, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another genus. In certain embodiments, the compositions of the invention comprise *Blautia hydrogenotrophica,* as defined in the claims, and do not contain any other bacterial species, or comprise only *de minimis* or biologically irrelevant amounts of bacteria from another species. In certain embodiments, the compositions of the invention comprise a single strain of *Blautia hydrogenotrophica,* as defined in the claims, and do not contain any other bacterial strains or species, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another strain or species.

[0084] In some embodiments, the compositions of the invention comprise more than one bacterial strain or species. For example, in some embodiments, the compositions of the invention comprise more than one strain from within the same species (e.g. more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40 or 45 strains), and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise less

than 50 strains from within the same species (e.g. less than 45, 40, 35, 30, 25, 20, 15, 12, 10, 9, 8, 7, 6, 5, 4 or 3 strains), and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise 1-40, 1-30, 1-20, 1-19, 1-18, 1-15, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-50, 2-40, 2-30, 2-20, 2-15, 2-10, 2-5, 6-30, 6-15, 16-25, or 31-50 strains from within the same species and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise more than one species from within the same genus (e.g. more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, 23, 25, 30, 35 or 40 species), and, optionally, do not contain bacteria from any other genus. In some embodiments, the compositions of the invention comprise less than 50 species from within the same genus (e.g. less than 50, 45, 40, 35, 30, 25, 20, 15, 12, 10, 8, 7, 6, 5, 4 or 3 species), and, optionally, do not contain bacteria from any other genus. In some embodiments, the compositions of the invention comprise 1-50, 1-40, 1-30, 1-20, 1-15, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-50, 2-40, 2-30, 2-20, 2-15, 2-10, 2-5, 6-30, 6-15, 16-25, or 31-50 species from within the same genus and, optionally, do not contain bacteria from any other genus. The invention comprises any combination of the foregoing.

[0085] In some embodiments, the composition comprises a microbial consortium. For example, in some embodiments, the composition comprises the *Blautia* bacterial strain, as defined in the claims, as part of a microbial consortium. For example, in some embodiments, the *Blautia* bacterial strain is present in combination with one or more (e.g. at least 2, 3, 4, 5, 10, 15 or 20) other bacterial strains from other genera with which it can live symbiotically *in vivo* in the intestine. For example, in some embodiments, the composition comprises a bacterial strain of *Blautia hydrogenotrophica* in combination with a bacterial strain from a different genus. In some embodiments, the microbial consortium comprises two or more bacterial strains obtained from a faeces sample of a single organism, e.g. a human. In some embodiments, the microbial consortium is not found together in nature. For example, in some embodiments, the microbial consortium comprises bacterial strains obtained from faeces samples of at least two different organisms. In some embodiments, the two different organisms are from the same species, e.g. two different humans. In some embodiments, the two different organisms are an infant human and an adult human. In some embodiments, the two different organisms are a human and a non-human mammal.

[0086] In some embodiments, the composition of the invention additionally comprises a bacterial strain that has the same safety and therapeutic efficacy characteristics as the *Blautia hydrogenotrophica* strain deposited under accession number DSM 10507/14294, but which is not the *Blautia hydrogenotrophica* strain deposited under accession number DSM 10507/14294, or which is not a *Blautia hydrogenotrophica* or which is not a *Blautia.*

[0087] In some embodiments in which the composition of the invention comprises more than one bacterial strain, species or genus, the individual bacterial strains, species or genera may be for separate, simultaneous or sequential administration. For example, the composition may comprise all of the more than one bacterial strain, species or genera, or the bacterial strains, species or genera may be stored separately and be administered separately, simultaneously or sequentially. In some embodiments, the more than one bacterial strains, species or genera are

stored separately but are mixed together prior to use.

[0088] In some embodiments, the bacterial strain for use in the invention is obtained from human adult faeces. In some embodiments in which the composition of the invention comprises more than one bacterial strain, all of the bacterial strains are obtained from human adult faeces or if other bacterial strains are present they are present only in *de minimis* amounts. The bacteria may have been cultured subsequent to being obtained from the human adult faeces and being used in a composition of the invention.

[0089] In some embodiments, the one or more *Blautia* bacterial strains is/are the only therapeutically active agent(s) in a composition of the invention as defined in the claims. In some embodiments, the bacterial strain(s) in the composition is/are the only therapeutically active agent(s) in a composition of the invention.

[0090] The compositions for use in accordance with the invention may or may not require marketing approval.

[0091] In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is lyophilised. In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is spray dried. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is live. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is viable. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is capable of partially or totally colonising the intestine. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is viable and capable of partially or totally colonising the intestine.

[0092] In some cases, the lyophilised or spray dried bacterial strain is reconstituted prior to administration. In some cases, the reconstitution is by use of a diluent described herein.

[0093] The compositions of the invention can comprise pharmaceutically acceptable excipients, diluents or carriers.

[0094] In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain of the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat a disorder when administered to a subject in need thereof; and wherein the disorder is diarrhea and/or constipation, such as diarrhea and/or constipation associated with Crohn's disease, ulcerative colitis or, more preferably, IBS.

[0095] In certain embodiments, the invention provides the above pharmaceutical composition,

wherein the amount of the bacterial strain is from about 1×10^3 to about 1×10^{11} colony forming units per gram with respect to a weight of the composition.

[0096] In certain embodiments, the invention provides the above pharmaceutical composition, wherein the composition is administered at a dose of 1 g, 3 g, 5 g or 10 g.

[0097] In certain embodiments, the invention provides the above pharmaceutical composition, wherein the composition is administered by a method selected from the group consisting of oral, rectal, subcutaneous, nasal, buccal, and sublingual.

[0098] In certain embodiments, the invention provides the above pharmaceutical composition, comprising a carrier selected from the group consisting of lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol and sorbitol.

[0099] In certain embodiments, the invention provides the above pharmaceutical composition, comprising a diluent selected from the group consisting of ethanol, glycerol and water.

[0100] In certain embodiments, the invention provides the above pharmaceutical composition, comprising an excipient selected from the group consisting of starch, gelatin, glucose, anhydrous lactose, free-flow lactose, beta-lactose, corn sweetener, acacia, tragacanth, sodium alginate, carboxymethyl cellulose, polyethylene glycol, sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate and sodium chloride.

[0101] In certain embodiments, the invention provides the above pharmaceutical composition, further comprising at least one of a preservative, an antioxidant and a stabilizer.

[0102] In certain embodiments, the invention provides the above pharmaceutical composition, comprising a preservative selected from the group consisting of sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid.

[0103] In certain embodiments, there is provided the pharmaceutical composition of the invention, wherein the composition does not comprise any minerals, or more specifically, does not comprise any metals with an atomic number greater than 33, for example, wherein the composition does not comprise any minerals from the group consisting of selenium, molybdenum, tungsten, selenium compounds, molybdenum compounds and tungsten compounds.

[0104] In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is lyophilised.

[0105] In certain embodiments, the invention provides the above pharmaceutical composition, wherein when the composition is stored in a sealed container at about 4°C or about 25°C and the container is placed in an atmosphere having 50% relative humidity, at least 80% of the bacterial strain as measured in colony forming units, remains after a period of at least about: 1

month, 3 months, 6 months, 1 year, 1.5 years, 2 years, 2.5 years or 3 years.

[0106] In some embodiments, the composition of the invention is provided in a sealed container comprising a composition as described herein. In some embodiments, the sealed container is a sachet or bottle. In some embodiments, the composition of the invention is provided in a syringe comprising a composition as described herein.

[0107] The composition of the present invention may, in some embodiments, be provided as a pharmaceutical formulation. For example, the composition may be provided as a tablet or capsule. In some embodiments, the capsule is a gelatine capsule ("gel-cap").

[0108] In some embodiments, the compositions of the invention are administered orally. Oral administration may involve swallowing, so that the compound enters the gastrointestinal tract, and/or buccal, lingual, or sublingual administration by which the compound enters the blood stream directly from the mouth.

[0109] Pharmaceutical formulations suitable for oral administration include solid plugs, solid microparticulates, semi-solid and liquid (including multiple phases or dispersed systems) such as tablets; soft or hard capsules containing multi- or nano-particulates, liquids (e.g. aqueous solutions), emulsions or powders; lozenges (including liquid-filled); chews; gels; fast dispersing dosage forms; films; ovules; sprays; and buccal/mucoadhesive patches.

[0110] In some embodiments the pharmaceutical formulation is an enteric formulation, i.e. a gastro-resistant formulation (for example, resistant to gastric pH) that is suitable for delivery of the composition of the invention to the intestine by oral administration. Enteric formulations may be particularly useful when the bacteria or another component of the composition is acid-sensitive, e.g. prone to degradation under gastric conditions.

[0111] In some embodiments, the enteric formulation comprises an enteric coating. In some embodiments, the formulation is an enteric-coated dosage form. For example, the formulation may be an enteric-coated tablet or an enteric-coated capsule, or the like. The enteric coating may be a conventional enteric coating, for example, a conventional coating for a tablet, capsule, or the like for oral delivery. The formulation may comprise a film coating, for example, a thin film layer of an enteric polymer, e.g. an acid-insoluble polymer.

[0112] In some embodiments, the enteric formulation is intrinsically enteric, for example, gastro-resistant without the need for an enteric coating. Thus, in some embodiments, the formulation is an enteric formulation that does not comprise an enteric coating. In some embodiments, the formulation is a capsule made from a thermogelling material. In some embodiments, the thermogelling material is a cellulosic material, such as methylcellulose, hydroxymethylcellulose or hydroxypropylmethylcellulose (HPMC). In some embodiments, the capsule comprises a shell that does not contain any film forming polymer. In some embodiments, the capsule comprises a shell and the shell comprises hydroxypropylmethylcellulose and does not comprise any film forming polymer (e.g. see [32]).

In some embodiments, the formulation is an intrinsically enteric capsule (for example, Vcaps® from Capsugel).

[0113] In some embodiments, the formulation is a soft capsule. Soft capsules are capsules which may, owing to additions of softeners, such as, for example, glycerol, sorbitol, maltitol and polyethylene glycols, present in the capsule shell, have a certain elasticity and softness. Soft capsules can be produced, for example, on the basis of gelatine or starch. Gelatine-based soft capsules are commercially available from various suppliers. Depending on the method of administration, such as, for example, orally or rectally, soft capsules can have various shapes, they can be, for example, round, oval, oblong or torpedo-shaped. Soft capsules can be produced by conventional processes, such as, for example, by the Scherer process, the Accogel process or the droplet or blowing process.

Culturing methods

[0114] The bacterial strains for use in the present invention can be cultured using standard microbiology techniques as detailed in, for example, references [33-35].

[0115] The solid or liquid medium used for culture may for example be YCFA agar or YCFA medium. YCFA medium may include (per 100ml, approximate values): Casitone (1.0 g), yeast extract (0.25 g), $NaHCO_3$ (0.4 g), cysteine (0.1 g), K_2HPO_4 (0.045 g), KH_2PO_4 (0.045 g), NaCl (0.09 g), $(NH_4)_2SO_4$ (0.09 g), $MgSO_4 \cdot 7H_2O$ (0.009 g), $CaCl_2$ (0.009 g), resazurin (0.1 mg), hemin (1 mg), biotin (1 µg), cobalamin (1 µg), *p*-aminobenzoic acid (3 µg), folic acid (5 µg), and pyridoxamine (15 µg).

General

[0116] The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.,* references [36-43], *etc.*

[0117] The term "comprising" encompasses "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

[0118] The term "about" in relation to a numerical value *x* is optional and means, for example, *x*±10%.

[0119] The word "substantially" does not exclude "completely" *e.g.* a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word

"substantially" may be omitted from the definition of the invention.

[0120] References to a percentage sequence identity between two nucleotide sequences means that, when aligned, that percentage of nucleotides are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of ref. [44]. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in ref. [45].

[0121] Unless specifically stated, a process or method comprising numerous steps may comprise additional steps at the beginning or end of the method, or may comprise additional intervening steps. Also, steps may be combined, omitted or performed in an alternative order, if appropriate.

[0122] Various embodiments of the invention are described herein. It will be appreciated that the features specified in each embodiment may be combined with other specified features, to provide further embodiments. In particular, embodiments highlighted herein as being suitable, typical or preferred may be combined with each other (except when they are mutually exclusive).

MODES FOR CARRYING OUT THE INVENTION

Example 1 - Changes in patient symptoms during Phase I clinical trial

[0123] A Phase I clinical trial was conducted in which *Blautia hydrogenotrophica* ("Blautix", strain deposited under accession number DSM 10507 and also under accession number DSM 14294) was administered to human patients having irritable bowel syndrome (IBS). Patients were administered Blautix during a dosing period (days 1-16) with the washout period being day 19-23. Blautix was found to be both safe and well tolerated. Four symptoms were monitored, of which two were diarrhea and constipation. The study recorded whether patients experienced an improvement in, no change in or worsening of each of these symptoms. Results from patients administered Blautix were compared with those obtained using patients administered a placebo. Symptoms were monitored at three time points: day 1, day 15/16 and at the end of the study. The results are shown in Figures 1 and 2.

[0124] When the patients' reported symptoms at day 16 were compared to the baseline from day 1, 82% of 17 IBS patients receiving Blautix reported an improvement in symptoms (Figure 1). Improvement of symptoms, two of which are diarrhea and constipation, supports the use of Blautix for treating or preventing diarrhea and/or constipation.

[0125] 50% of patients receiving placebo reported an improvement in symptoms (Figure 1). High placebo response rates are an established phenomenon in IBS clinical studies. Xifaxan was recently approved to treat IBS based on much smaller improvements over placebo (see: http://www.accessdata.fda.gov/spl/data/5ab6fceb-4d22-4480-81fc-8bc28c16770d/5ab6fceb-4d22-4480-81fc-8bc28c16770d.xml).

[0126] A worsening of symptoms at the study completion (day 19-23) compared to symptoms present upon dosing completion (day 16) is expected based on the teaching presented here. This worsening of symptoms was seen in the Phase I clinical trial: 41% of IBS patients reported worsening of symptoms following cessation of Blautix dosing (Figure 2). The worsening of symptoms, two of which are diarrhea and constipation, following cessation of Blautix dosing therefore also supports the use of Blautix in treating or preventing diarrhea and/or constipation.

Example 2 - Hydrogen breath test results

[0127] Breath hydrogen levels are a biomarker of Blautix activity - MoA involves metabolism of endogenous H_2 to produce acetate. Human subjects were administered lactulose and hydrogen (H_2) levels (Cmax) were sampled at four time points: day 1, day 2, day 15 and day 16. Hydrogen uncorrected results were converted to hydrogen corrected results.

[0128] Some patients were excluded from the analysis. There were three reasons why a subject was not included in the hydrogen breath test analysis: 1) They produced a CMAX hydrogen breath test result of <20 on one of the four sampling days and were therefore deemed to have not responded to the test; 2) They were methane producers (taken as producing more methane than hydrogen in the breath test), this affects the hydrogen response; and/or 3) there was an aberrant value obtained in the hydrogen breath test (232 ppm). Subjects 3.12 (Blautix), 3.24 (Blautix), 4.07 (Blautix) were excluded as non-responders. Subjects 3.03 (Blautix) and 3.08 (Placebo) were excluded as methane producers (4.07, mentioned as excluded above, was also a methane producer). Subject 4.09 (Placebo) was excluded due to an aberrant value.

[0129] The results of the corrected hydrogen analysis from the end of the dosing period (day 15/16) were compared to those from the baseline (day 1/2). 10 out of 12 patients (83%) receiving Blautix had a reduction in hydrogen levels over this period (Figure 3a and 3c). In contrast, 3 out of 6 (50%) patients receiving placebo had reduced hydrogen levels (Figure 3b and 3c). These percentages are similar to the percentages of patients exhibiting an improvement in symptoms following Blautix treatment or administration of placebo.

[0130] Figure 4 shows the uncorrected and corrected hydrogen results for the Blautix (Verum) treatment group together with a statistical analysis of the results. The mean values for both uncorrected and corrected H_2 were found to differ between day 1 and day 15. After 13.5 days of treatment, a statistically significant ($p < 0.05$) decrease of H_2 in Cmax breath test was

detected after lactulose stimulation. In contrast, for the placebo group, the mean was found to be equivalent for day 1 and day 15 ($p > 0.05$) (Figure 5). Thus, the mean for the treatment group (also referred to as the VERUM group), decreases between day 1 and day 15 whereas the mean for the placebo group is equivalent between day 1 and day 15 for both the uncorrected hydrogen results and the corrected hydrogen results (Figure 6).

Example 3 - Stability testing

[0131] A composition described herein containing at least one bacterial strain described herein is stored in a sealed container at 25°C or 4°C and the container is placed in an atmosphere having 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90% or 95% relative humidity. After 1 month, 2 months, 3 months, 6 months, 1 year, 1.5 years, 2 years, 2.5 years or 3 years, at least 50%, 60%, 70%, 80% or 90% of the bacterial strain shall remain as measured in colony forming units determined by standard protocols.

Sequences

[0132]

SEQ ID NO:1 (*Blautia stercoris* strain GAM6-1 16S ribosomal RNA gene, partial sequence - HM626177)

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   1 tgcaagtcga gcgaagcgct tacgacagaa ccttcggggg aagatgtaag ggactgagcg
  61 gcggacgggt gagtaacgcg tgggtaacct gcctcataca ggggataac agttggaaac
 121 ggctgctaat accgcataag cgcacggtat cgcatgatac agtgtgaaaa actccggtgg
 181 tatgagatgg acccgcgtct gattagctag ttggaggggt aacggcccac caaggcgacg
 241 atcagtagcc ggcctgagag ggtgaacggc cacattggga ctgagacacg gcccagactc
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 361 gcgtgaagga agaagtatct cggtatgtaa acttctatca gcagggaaga aaatgacggt
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SEQ ID NO:3 (consensus 16S rRNA sequence for *Blautia stercoris* strain 830)

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caatgggcga aaccctgatg cagcgacgcc gcgtgaagga agaagtatct cggtatgtaa  420
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Patentkrav

1. Sammensætning omfattende en bakteriestamme af slægten *Blautia hydrogenotrophica* med en 16s rRNA-sekvens der er mindst 95% identisk med SEQ ID NO:5, til anvendelse i en fremgangsmåde til behandling eller forebyggelse af diarré og/eller forstoppelse.

2. Sammensætningen til anvendelse ifølge krav 1, hvor diarré og/eller forstoppelse er associeret med IBS og/eller en inflammatorisk sygdom af tarmen.

3. Sammensætningen til anvendelse ifølge krav 2, hvor den inflammatoriske sygdom er i tyndtarmen, kolon eller rektum.

4. Sammensætningen til anvendelse ifølge et hvilket som helst af de foregående krav, hvor diarré og/eller forstoppelse er associeret med IBS, Crohn's sygdom eller ulcerativ colitis.

5. Sammensætningen til anvendelse ifølge et hvilket som helst af kravene 1 til 3, hvor sammensætningen er til anvendelse

(a) i behandling eller forebyggelse af diarré og/eller forstoppelse hos et individ diagnosticeret med IBS, Crohn's sygdom eller ulcerativ colitis; og/eller

(b) i en fremgangsmåde til behandling af diarré, hvor individet har diarré uden forstoppelse; og/eller

(c) i en fremgangsmåde til behandling af forstoppelse, hvor individet har forstoppelse uden diarré.

6. Sammensætningen til anvendelse ifølge et hvilket som helst af de foregående krav, hvor individet yderligere har abdominalsmerte og/eller opsvulmethed

og/eller hvor individet har et øget hydrogenniveau i deres respiration i forhold til et sundt individ.

7. Sammensætningen til anvendelse ifølge et hvilket som helst af de foregående krav, hvor anvendelsen yderligere omfatter reduktion af hydrogenniveauet i individets ånde.

8. Sammensætningen til anvendelse ifølge et hvilket som helst af de foregående krav, hvor anvendelsen yderligere omfatter overvågning af hydrogenniveauet i individets ånde under og/eller efter behandlingen eller forebyggelsen og derved vurdere den sandsynlige effektivitet af behandling eller forebyggelse.

9. Sammensætning omfattende en bakteriestamme af slægten *Blautia hydrogenotrophica* med en 16s rRNA-sekvens der er mindst 95% identisk med SEQ ID NO:5, til anvendelse i en fremgangsmåde til reduktion af niveauet af *Enterobacteriaceae* i gastrointestinalkanalen i behandlingen eller forebyggelsen af diarré.

10. Sammensætningen til anvendelse ifølge krav 1, hvor diarré er associeret med *Enterobacteriaceae*-infektion.

11. Sammensætningen til anvendelse ifølge krav 12 eller krav 13, hvor *Enterobacteriaceae* er E. coli.

12. Sammensætningen til anvendelse ifølge et hvilket som helst af de foregående krav, hvor bakteriestammen har en 16s rRNA-sekvens der er mindst 97%, 98%, 99%, 99,5% eller 99,9% identisk med SEQ ID NO:5 eller som har 16s-rRNA-sekvensen af SEQ ID NO:5.

13. Sammensætningen til anvendelse ifølge krav 1, til anvendelse i en fremgangsmåde til behandling eller forebyggelse af diarré og/eller forstoppelse hos et individ diagnosticeret med IBS.

14. Sammensætningen til anvendelse ifølge et hvilket som helst af de foregående krav, hvor

(a) sammensætningen er til oral administration; og/eller

(b) sammensætningen omfatter én eller flere farmaceutisk acceptable excipienser eller bærere; og/eller

(c) bakteriestammen er lyofiliseret; og/eller

(d) bakteriestammen er levedygtig; og/eller

(e) sammensætningen omfatter en enkelt stamme af slægten *Blautia*; og/eller

(f) sammensætningen omfatter *Blautia*-bakteriestammen som del af et mikrobielt konsortium.

15. Fødevareprodukt omfattende sammensætningen ifølge et hvilket som helst af de foregående krav, til anvendelsen ifølge et hvilket som helst af de foregående krav.

DRAWINGS

FIG. 1

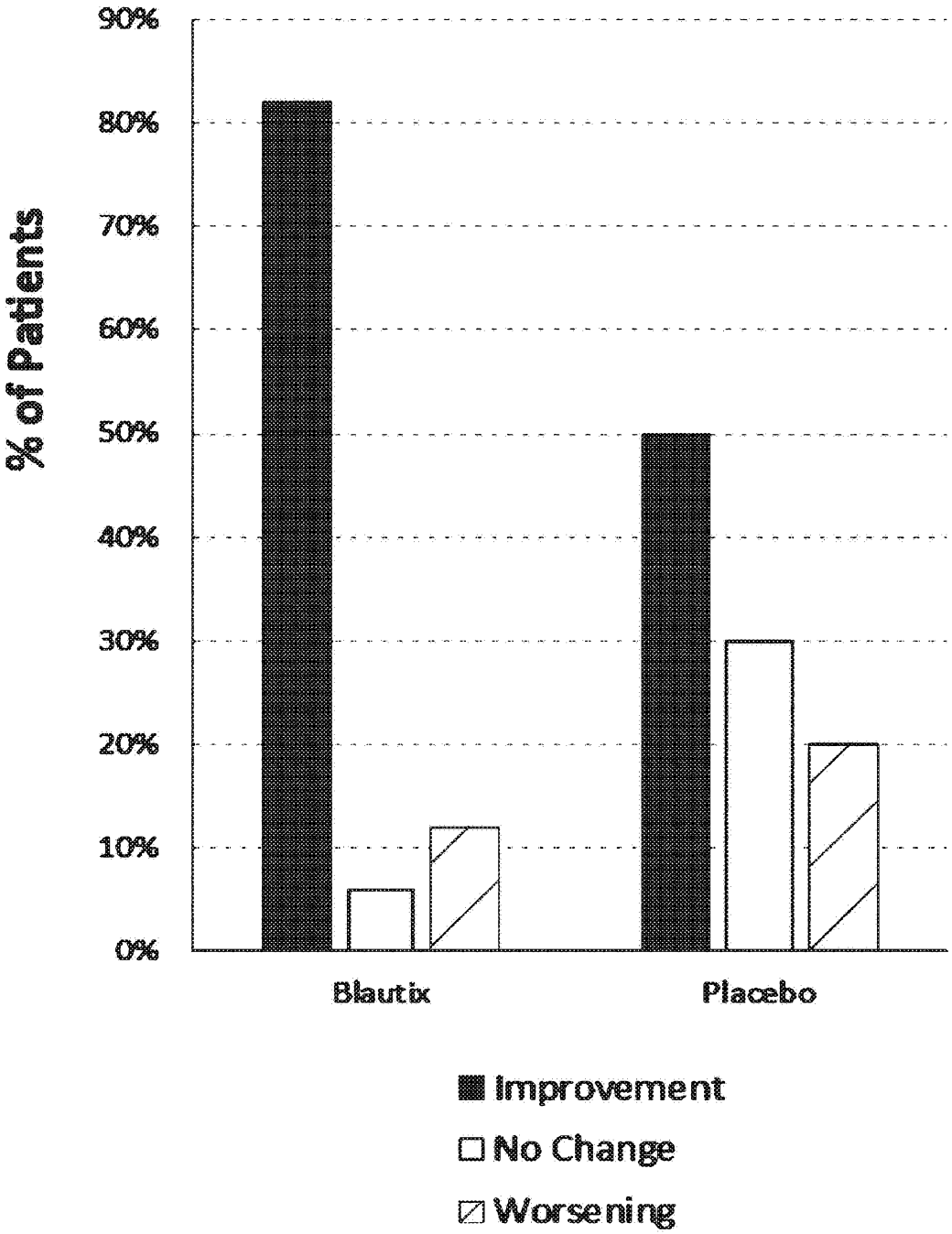

FIG. 2

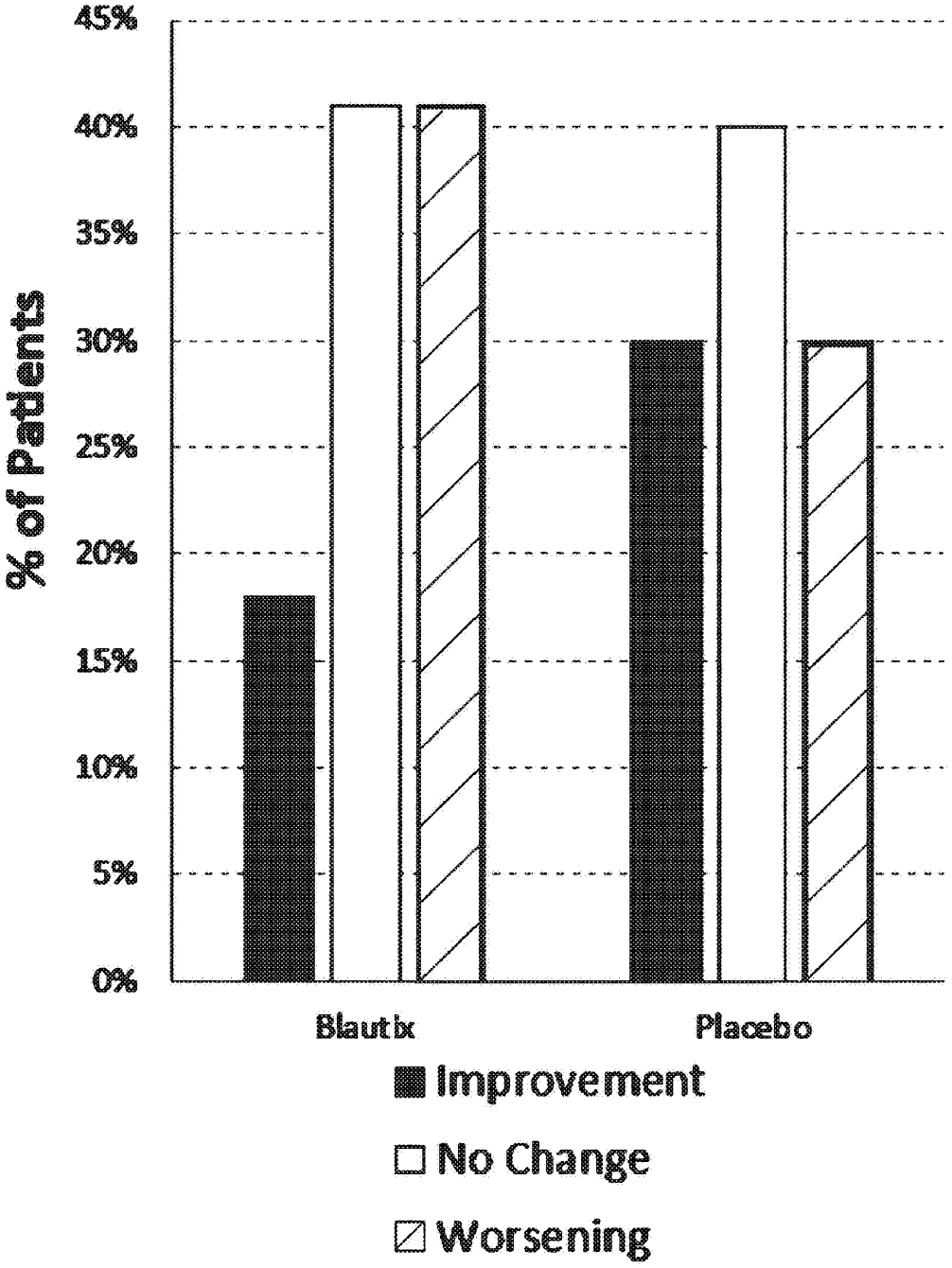
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
% of Patients
Blautix
Placebo
Improvement
No Change
Worsening

FIG. 3a Blautix 10/12 show reduction in hydrogen (83%)

	DAY 1	DAY 2	MEAN	DAY 15	DAY 16	MEAN		DIFF
Patient ID	CMAX	CMAX		CMAX	CMAX			CMAX
3.02	123.0	94.0	108.5	34.0	74.0	54.0		-54.5
3.04	70.0	32.0	51.0	25.0	46.0	35.5		-15.5
3.05	163.0	116.0	139.5	167.0	111.0	139.0		-0.5
3.07	71.0	69.0	70.0	46.0	80.0	63.0		-7.0
3.09	121.0	76.0	98.5	66.0	65.0	65.5		-33.0
3.11	75.0	98.0	86.5	128.0	76.0	102.0		15.5
3.13	118.0	41.0	79.5	85.0	59.0	72.0		-7.5
3.15	155.0	99.0	127.0	63.0	87.0	75.0		-52.0
3.17	134.0	210.0	172.0	139.0	107.0	123.0		-49.0
3.19	72.0	53.0	62.5	87.0	85.0	86.0		23.5
3.21	144.0	139.0	141.5	75.0	126.0	100.5		-41.0
3.22	59.0	71.0	65.0	55.0	34.0	44.5		-20.5
Mean	108.8	91.5	100.1	80.8	79.2	80.0		-20.1
SD	37.3	48.4		43.5	26.7			26.2
Median	119.5	85.0		70.5	78.0			-18.0
				Patients with reduction in H_2				10 (83%)

FIG. 3b Placebo 3/6 show reduction in hydrogen (50%)

	DAY 1	DAY 2	MEAN		DAY 15	DAY 16	MEAN		DIFF
Patient ID	CMAX	CMAX			CMAX	CMAX			CMAX
3.06	126.0	91.0	108.5		86.0	131.0	108.5		0.0
3.10	124.0	168.0	146.0		107.0	88.0	97.5		-48.5
3.14	55.0	79.0	67.0		53.0	68.0	60.5		-6.5
3.16	98.0	123.0	110.5		123.0	151.0	137.0		26.5
3.23	58.0	113.0	85.5		79.0	99.0	89.0		3.5
4.13	58.0	69.0	63.5		25.0	38.0	31.5		-32.0
MEAN	86.5	107.2	96.8		78.8	95.8	87.3		-9.5
SD	33.8	36.0	31.2		35.7	41.2	37.0		26.8
MEDIAN	78.0	102.0	97.0		82.5	93.5	93.3		-3.3
						Patients with reduction in H_2			3 (50%)

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FIG. 3c

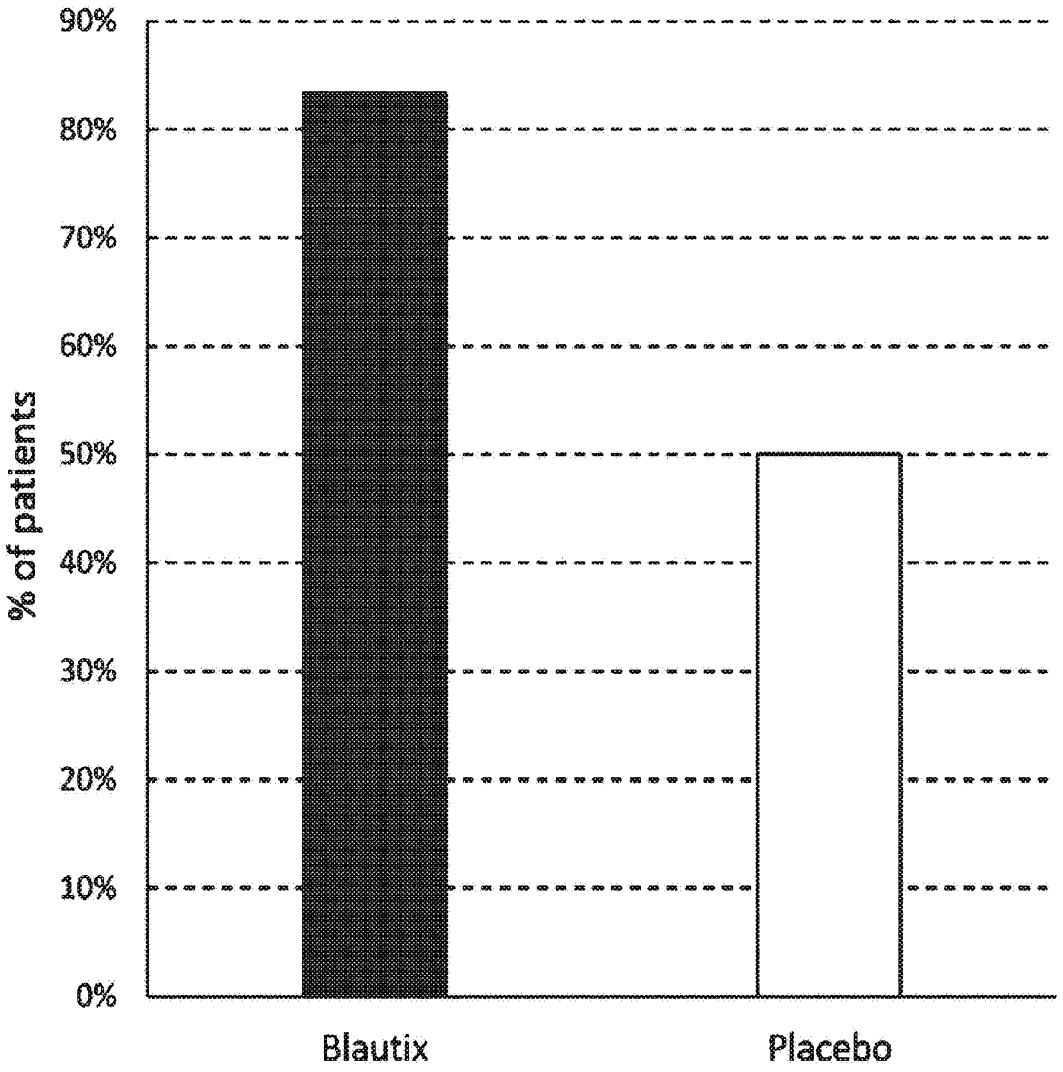

FIG. 4a

VERUM group

RANDOM NUMBER	DAY1 CMAX	DAY15 CMAX			H2 Corrected	RANDOM NUMBER	DAY1 CMAX	DAY15 CMAX	
3.02	89.8	23.0				3.02	123.0	34.0	
3.04	68.0	21.0				3.04	70.0	25.0	
3.05	130.4	112.8				3.05	163.0	167.0	
3.07	56.8	37.6				3.07	71.0	46.0	
3.09	96.8	52.8				3.09	121.0	66.0	
3.11	63.0	104.9				3.11	75.0	128.0	
3.12	12.3	38.7				3.12	16.0	53.0	
3.13	110.3	82.5				3.13	118.0	85.0	
3.15	115.7	53.8				3.15	155.0	63.0	
3.17	97.8	83.7				3.17	134.0	139.0	
3.19	51.1	66.1				3.19	72.0	87.0	
3.21	144.0	71.4				3.21	144.0	75.0	
3.22	52.7	43.9				3.22	59.0	55.0	
3.24	27.1	26.7				3.24	29.0	27.0	
		T Test unilateral	0.02032643					T Test unilateral	0.04798585
	Day 1	Day 15					Day 1	Day 15	
MEAN	79.7	58.5				MEAN	96.4	75.0	
SD	38.7	29.5				SD	46.5	43.0	
N	14	14				N	14	14	
MEDIAN	78.9	53.3				MEDIAN	96.5	64.5	
MIN	12.3	21.0				MIN	16.0	25.0	
MAX	144.0	112.8				MAX	163.0	167.0	

FIG. 4b

Hydrogen uncorrected

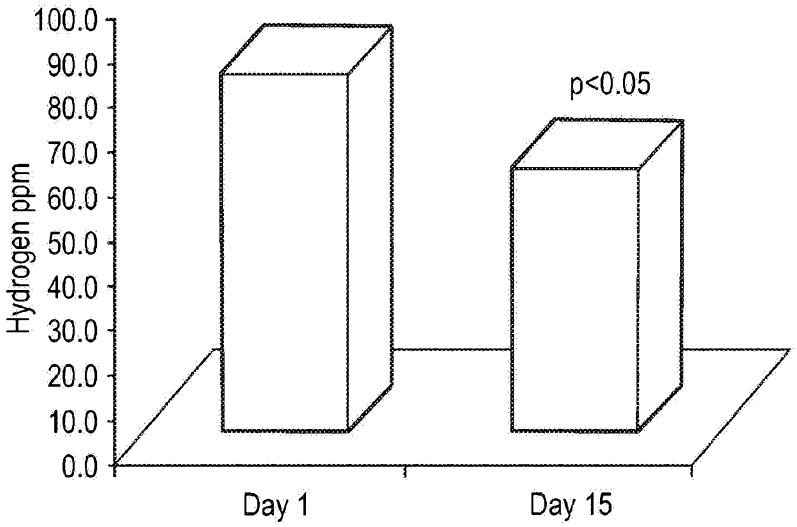

FIG. 4c

Hydrogen corrected

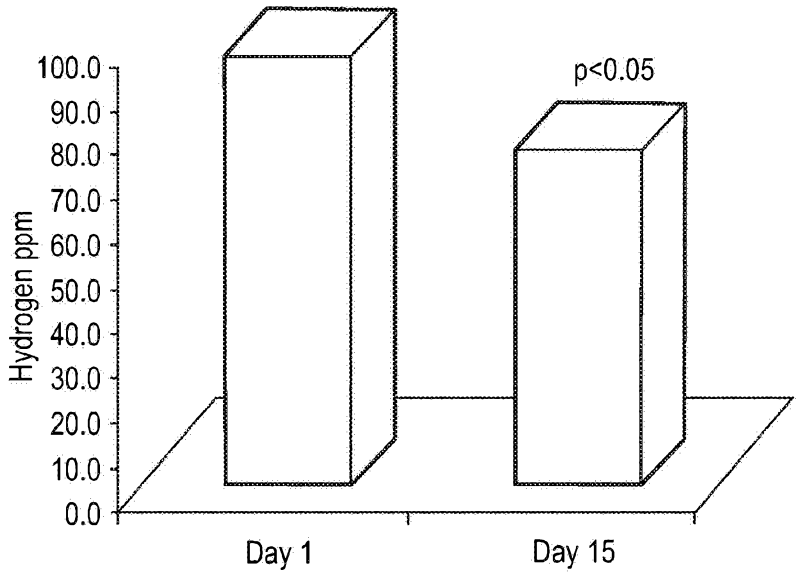

FIG. 5a

PLACEBO Group

RANDOM NUMBER	DAY1 CMAX	DAY15 CMAX			H2 corrected	RANDOM NUMBER	DAY1 CMAX	DAY15 CMAX	
3.06	103.3	81.9				3.06	126.0	86.0	
3.08	42.3	43.3				3.08	55.0	58.0	
3.10	97.6	84.3				3.10	124.0	107.0	
3.14	35.0	42.4				3.14	55.0	53.0	
3.16	59.0	80.9				3.16	98.0	123.0	
3.23	41.1	62.2				3.23	58.0	79.0	
4.13	53.2	25.0				4.13	58.0	25.0	
		T Test unilateral	0.41620609					T Test unilateral	0.27096549
	DAY1	DAY15					DAY1	DAY15	
MEAN	61.7	60.0				MEAN	82.0	75.9	
SD	27.7	23.5				SD	33.1	33.5	
N	7	7				N	7	7	
MEDIAN	53.2	62.2				MEDIAN	58.0	79.0	
MIN	35.0	25.0				MIN	55.0	25.0	
MAX	103.3	84.3				MAX	126.0	123.0	

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FIG. 5b

Hydrogen uncorrected placebo

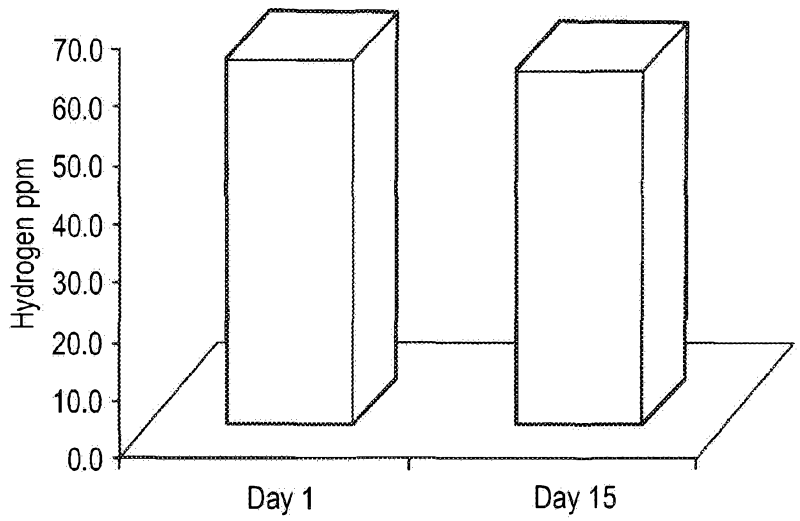

FIG. 5c

Hydrogen corrected placebo

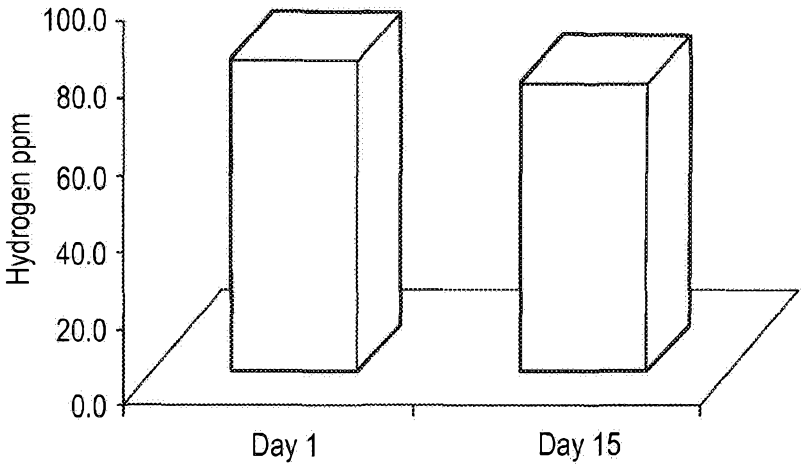

FIG. 6a

	DAY 1	DAY 15	N
VERUM Group	79.7	58.5	14
PLACEBO Group	61.7	60	7

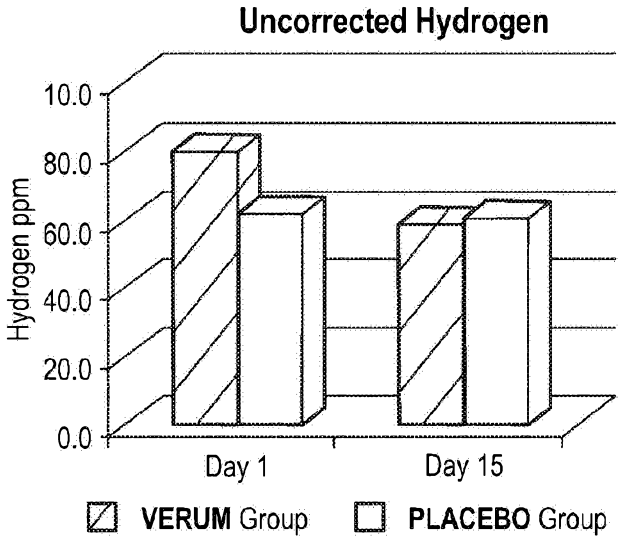

FIG. 6b

	DAY 1	DAY 15	N
VERUM Group	96.4	75	14
PLACEBO Group	82	75.9	7