COMPOSITIONS COMPRISING BACTERIAL BLAUTIA STRAINS FOR TREATING VISCERAL HYPERSENSITIVITY

TECHNICAL FIELD

This invention is in the field of compositions comprising bacterial strains isolated from the mammalian digestive tract and the use of such compositions in the treatment of disease.

BACKGROUND TO THE INVENTION

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].

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].

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 (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.

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

SUMMARY OF THE INVENTION

The inventors have developed new therapies for treating and preventing visceral hypersensitivity. In particular, the inventors have identified that bacterial strains from the genus *Blautia* can be effective for reducing visceral hypersensitivity. As described in the examples, oral administration of compositions comprising *Blautia hydrogenotrophica* may reduce visceral hypersensitivity in rat models of visceral hypersensitivity and irritable bowel syndrome (IBS). Therefore, in a first embodiment, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in a method of treating or preventing visceral hypersensitivity.

In preferred embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in a method of treating or preventing visceral hypersensitivity in a subject diagnosed with Crohn's disease, ulcerative colitis, functional dyspepsia or, more preferably, IBS. In other preferred embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in a method of treating or preventing visceral hypersensitivity in a subject diagnosed with Crohn's disease, ulcerative colitis, functional dyspepsia, infantile colic or, more preferably, IBS.

In further preferred embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia,* for use in treating or preventing visceral hypersensitivity in the abdomen, preferably in the gastrointestinal tract, and most preferably in the lower gastrointestinal tract. In further embodiments, the compositions of the invention are for use in treating or preventing visceral hypersensitivity in the caecum, colon or rectum.

In preferred embodiments of the invention, the bacterial strain in the composition is of *Blautia hydrogenotrophica*. 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.

In further embodiments of the invention, the bacterial strain in the composition is of *Blautia stercoris*. 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 stercoris*. 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:1 or 3. Preferably, the sequence identity is to SEQ ID NO:3. Preferably, the bacterial strain for use in the invention has the 16s rRNA sequence represented by SEQ ID NO:3.

In further embodiments of the invention, the bacterial strain in the composition is of *Blautia wexlerae*. 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 wexlerae*. 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:2 or 4. Preferably, the sequence identity is to SEQ ID NO:4. Preferably, the bacterial strain for use in the invention has the 16s rRNA sequence represented by SEQ ID NO:4.

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 visceral hypersensitivity. Also, oral administration is convenient for patients and practitioners and allows delivery to and / or partial or total colonisation of the intestine.

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

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.

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

In certain embodiments, the invention provides a vaccine composition comprising the composition as described above.

Additionally, the invention provides a method of treating or preventing visceral hypersensitivity, comprising administering a composition comprising a bacterial strain of the genus *Blautia*.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1: Measurement of BH population by qPCR, showing an increase in BH at days 14 and 28 for animals receiving the BH lyophilisate.

Figure 2: Impact of BH culture and lypophilisate on animals' response to distension, showing a reduction in contractions for animals receiving BH compositions.

Figure 3: Impact of BH culture on microbiota, showing a 1 log decrease in SRB in rats receiving a daily dose of BH culture.

Figure 4: Impact of BH lyophilisate on microbiota.

Figure 5: Impact of BH lyophilisate on microbiota fermentation – short chain fatty acids, showing an increase in acetate production in rats treated with BH.

Figure 6: Impact of BH lyophilisate on microbiota fermentation – sulphides, showing a decrease in sulphides production.

Figure 7: Impact of BH lyophilisate on animals' response to distension. Rats MIH IBS + BH: CRD test.

Figure 8: Impact of BH lyophilisate on animals' response to distension. Rats MIH IBS + BH: All data - CRD test.

Figure 9: Impact of BH lyophilisate on sulphides. Rats MIH IBS + BH: Sulphides concentrations (mg/L). Figure 9a shows the caecal sulphides concentration for each rat after treatment with BH lyophilisate or a control solution. Figure 9b shows the mean +/- SEM of caecal sulphides concentration measured in IBS-microbiota associated rats after treatment with BH lyophilisate or a control solution.

Figure 10: Impact of BH lyophilisate on sulphides. Rats MIH IBS + BH: All data - Sulphides concentrations (mg/L). Figure 10a shows the caecal sulphides concentration for each rat after treatment with BH lyophilisate or a control solution. Figure 10b shows the mean +/- SEM caecal sulphides concentration measured in IBS-microbiota associated rats after treatment with BH lyophilisate or a control solution.

Figure 11: Dosing study in HIM rats - RT-PCR quantification of *B. hydrogenotrophica* in fecal samples of Healthy HIM rats receiving different concentration of the bacterial species.

Figure 12: Transit time of *B. hydrogenotrophica* after oral administration (10^9/day) to healthy HIM rats.

Figure 13: Comparison of *B. hydrogenotrophica* levels found in fecal and caecal samples of healthy HIM rats (RT-PCR quantification) after 14 days administration - *B. hydrogenotrophica* administrated at 10^{10} /day/rat.

Figure 14: Effect of *B. hydrogenotrophica* (10^{10} / day for 14 days) on short chain fatty acids production (RMN ^{1}H) in caecal contents of healthy HIM rats.

Figure 15: Impact of *B. hydrogenotrophica* administration on the microbial populations in IBS-HIM rats.

Figure 16: Sulphides production in IBS-HIM Rats treated with *B. hydrogenotrophica* (10^{10}/day for 14 days). Control rats were not treated.

Figure 17: Changes in patient symptoms during dosing period (days 1-16) of Phase I clinical trial.

Figure 18: Changes in patient symptoms during washout period of Phase I clinical trial.

Figure 19: qPCR evaluation of *B. hydrogenotrophica* population in faecal samples of IBS-HMA rats treated or not with a composition comprising *B. hydrogenotrophica* (BlautiX) for 28 days.

Figure 20: Abdominal response to colorectal distension in IBS-HMA rats treated or not with *B. hydrogenotrophica* (BlautiX) for 28 days and in untreated healthy HMA rats.

Figure 21: Bacteria enumeration in IBS HMA-rat faecal samples after *B. hydrogenotrophica* (BlautiX) administration versus control solution.

Figure 22: Sulphide concentration in caecal samples of IBS HMA-rats treated or not with *B. hydrogenotrophica* (Blautix) for 28 days.

Figure 23: Short chain fatty acids (SCFA) concentrations in caecal samples of IBS-HMA rats treated or not with *B. hydrogenotrophica* (Blautix) for 28 days. **Figure 23a** shows concentration of total SCFA. **Figure 23b** shows concentration of Acetic acid, Propionic acid and Butyric acid.

DISCLOSURE OF THE INVENTION

Bacterial strains

The compositions of the invention comprise a bacterial strain of the genus *Blautia*. The examples demonstrate that bacteria of this genus are useful for treating or preventing visceral hypersensitivity. The preferred bacterial strains are of the species *Blautia hydrogenotrophica, Blautia stercoris* and *Blautia wexlerae.* Other preferred bacterial strains for use in the invention are *Blautia producta, Blautia coccoides* and *Blautia hansenii.*

Examples of *Blautia* strains for use in the invention 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.

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

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 January 1996

as "*Ruminococcus hydrogenotrophicus*" under accession number DSM 10507 and also under accession number DSM 14294 as "S5a33" on 10th May 2001. The depositor was INRA Laboratoire de Microbiologie CR de Clermont-Ferrand/Theix 63122 Saint Genès Champanelle, France. Ownership of the deposits has passed to 4D Pharma Plc by way of assignment.

The GenBank accession number for the 16S rRNA gene sequence of *Blautia stercoris* strain GAM6-1^T is HM626177 (disclosed herein as SEQ ID NO:1). An exemplary *Blautia stercoris* strain is described in [19]. The type strain of *Blautia wexlerae* is WAL 14507 = ATCC BAA-1564 = DSM 19850 [17]. The GenBank accession number for the 16S rRNA gene sequence of *Blautia wexlerae* strain WAL 14507 T is EF036467 (disclosed herein as SEQ ID NO:2). This exemplary *Blautia wexlerae* strain is described in [17].

A preferred *Blautia stercoris* strain is the strain deposited under accession number NCIMB 42381, which is also referred to herein as strain 830. A 16S rRNA sequence for the 830 strain is provided in SEQ ID NO:3. Strain 830 was deposited with the international depositary authority NCIMB, Ltd. (Ferguson Building, Aberdeen, AB21 9YA, Scotland) by GT Biologics Ltd. (Life Sciences Innovation Building, Aberdeen, AB25 2ZS, Scotland) on 12th March 2015 as "*Blautia stercoris* 830" and was assigned accession number NCIMB 42381. GT Biologics Ltd. subsequently changed its name to 4D Pharma Research Limited.

A preferred *Blautia wexlerae* strain is the strain deposited under accession number NCIMB 42486, which is also referred to herein as strain MRX008. A 16S rRNA sequence for the MRX008 strain is provided in SEQ ID NO:4. Strain MRX008 was deposited with the international depositary authority NCIMB, Ltd. (Ferguson Building, Aberdeen, AB21 9YA, Scotland) by 4D Pharma Research Ltd. (Life Sciences Innovation Building, Aberdeen, AB25 2ZS, Scotland) on 16th November 2015 as "*Blaqutia/Ruminococcus MRx0008*" and was assigned accession number NCIMB 42486.

Bacterial strains closely related to the strain tested in the examples are also expected to be effective for treating or preventing visceral hypersensitivity. 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.

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 stercoris*. 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:1 or SEQ ID NO:3. Preferably, the sequence identity is to SEQ ID NO:3. Preferably, the bacterial strain for use in the invention has the 16s rRNA sequence represented by SEQ ID NO:3. 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 wexlerae*. 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:2 or SEQ ID NO:4. Preferably, the sequence identity is to SEQ ID NO:4. Preferably, the bacterial strain for use in the invention has the 16s rRNA sequence represented by SEQ ID NO:4.

Bacterial strains that are biotypes of the bacterium deposited under accession number DSM 10507/14294 or biotypes of the bacteria deposited under accession numbers NCIMB 42381 and NCIMB 42486 are also expected to be effective for treating or preventing visceral hypersensitivity. A biotype is a closely related strain that has the same or very similar physiological and biochemical characteristics.

Strains that are biotypes of a bacterium deposited under accession number DSM 10507/14294, NCIMB 42381 or NCIMB 42486 and 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, NCIMB 42381 or NCIMB 42486. 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.

Alternatively, strains that are biotypes of a bacterium deposited under accession number DSM 10507/14294, NCIMB 42381 or NCIMB 42486 and that are suitable for use in the invention may be identified by using the accession number DSM 10507/14294 deposit, the accession number NCIMB 42381 deposit, or the accession number NCIMB 42486 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, Blautia stercoris* or *Blautia wexlerae* strains.

In certain embodiments, strains that are biotypes of a bacterium deposited under accession number DSM 10507/14294, NCIMB 42381 or NCIMB 42486 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, NCIMB 42381 or NCIMB 42486 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, NCIMB 42381 or NCIMB 42486.

Other *Blautia* strains that are useful in the compositions and methods of the invention, such as biotypes of a bacterium deposited under accession number DSM 10507/14294, NCIMB 42381 or NCIMB 42486, 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, NCIMB 42381 or NCIMB 42486 may be useful in the invention. A useful strain will have comparable microbiota modulatory activity to the DSM 10507/14294, NCIMB 42381 or NCIMB 42486 strain. In particular, a biotype strain will elicit comparable effects on the visceral hypersensitivity model to the effects shown in the Examples, which may be identified by using the culturing and administration protocols described in the Examples.

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.

A derivative of the strain deposited under accession number DSM 10507/14294, NCIMB 42381 or NCIMB 42486 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. 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, NCIMB 42381 or NCIMB 42486 strain. In particular, a derivative strain will elicit comparable effects on the visceral hypersensitivity model 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. A derivative of the NCIMB 42381 strain will generally be a biotype of the NCIMB 42381 strain. A derivative of the NCIMB 42486 strain will generally be a biotype of the NCIMB 42486 strain.

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. References to cells of the *Blautia stercoris* strain deposited under accession number NCIMB 42381 encompass any cells that have the same safety and therapeutic efficacy characteristics as the strains deposited under accession number NCIMB 42381, and such cells are encompassed by the invention. References to cells of the *Blautia wexlerae* strain deposited under accession number NCIMB 42486 encompass any cells that have the same safety and therapeutic efficacy characteristics as the strains deposited under accession number NCIMB 42486, and such cells are encompassed by the invention.

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

In preferred embodiments, the compositions of the invention are for use in treating visceral hypersensitivity. Visceral hypersensitivity is a specific type of pain characterized by a subjectively painful perception located in the abdominal area resulting from activation of nociceptors of the thoracic, pelvic, or abdominal viscera (organs). Visceral hypersensitivity is generally diffuse and difficult to localise, and therefore contrasts with somatic pain, which is generally sharper and more localised. Also, visceral hypersensitivity generally is not associated with specific structural lesions, unlike somatic pain. Visceral nociceptors are intrinsically different from cutaneous and most other non-visceral nociceptors [22].

Visceral hypersensitivity is generally experienced in the abdomen, but not all abdominal pain is visceral hypersensitivity. In contrast, there are many potential causes of abdominal pain and abdominal pain may be somatic, referred or visceral pain. In the abdomen, somatic pain may be caused by an inflamed organ and is generally sharp and localised. Abdominal pain may be caused by fibromyalgia, which is a condition of somatic (skin and muscle) hypersensitivity. Referred pain is felt in a cutaneous site distant from the diseased organ.

Visceral hypersensitivity is often associated with functional dyspepsia and irritable bowel syndrome (IBS). However, not all pain associated with functional dyspepsia and IBS is visceral hypersensitivity. Indeed, many patients with IBS also exhibit a wide variety of somatic symptoms in abdominal regions (back pain, heartburn) and non-abdominal regions (migraine headaches, dyspareunia, muscle pain in body regions somatotopically distinct from the gut) [23].

In some embodiments, the pathogenesis of the disease or condition affects the intestine. In some embodiments, the pathogenesis of the disease or condition does not affect the intestine. In some embodiments, the pathogenesis of the disease or condition is not localised at the intestine. In some embodiments, the treating or preventing occurs at a site other than at the intestine. In some

embodiments, the treating or preventing occurs at the intestine and also at a site other than at the intestine. In certain embodiments, the disease or condition is systemic.

Visceral hypersensitivity is also known as visceral pain, and these two terms are used interchangeably herein.

As demonstrated in the examples, bacterial compositions of the invention may be effective for reducing visceral hypersensitivity. In particular, bacterial compositions of the invention can reduce the response to colorectal distension, which is a manifestation of visceral hypersensitivity that affects many patients. In preferred embodiments, the compositions of the invention are for use in treating or preventing visceral hypersensitivity in the abdomen, preferably in the gastrointestinal tract, and most preferably in the lower gastrointestinal tract. In further embodiments, the compositions of the invention are for use in treating or preventing visceral hypersensitivity in the caecum, colon or rectum.

In preferred embodiments, the compositions of the invention are for use in treating or preventing visceral hypersensitivity associated with Crohn's disease, ulcerative colitis, functional dyspepsia, infantile colic or, more preferably, IBS. In preferred embodiments, the compositions of the invention are for use in treating or preventing visceral hypersensitivity in a subject diagnosed with Crohn's disease, ulcerative colitis, functional dyspepsia, infantile colic, or, more preferably, IBS. In preferred embodiments the compositions of the invention are for use in treating or preventing visceral hypersensitivity in the treatment of Crohn's disease, ulcerative colitis, functional dyspepsia, infantile colic, or, more preferably, IBS.

In preferred embodiments, the compositions of the invention are for use in treating or preventing visceral hypersensitivity associated with Crohn's disease, ulcerative colitis, functional dyspepsia or, more preferably, IBS. In preferred embodiments, the compositions of the invention are for use in treating or preventing visceral hypersensitivity in a subject diagnosed with Crohn's disease, ulcerative colitis, functional dyspepsia or, more preferably, IBS. In preferred embodiments the compositions of the invention are for use in treating or preventing visceral hypersensitivity in the treatment of Crohn's disease, ulcerative colitis, functional dyspepsia or, more preferably, IBS. In certain embodiments, the compositions of the invention are for use in treating visceral hypersensitivity in a patient suffering from painful distension of the gastrointestinal tract, in particular in the colon or rectum.

Certain aspects of the discomfort and suffering associated with IBS and other bowel conditions may be caused by the excess production of gases in the gastrointestinal tract and the bulk volume of these accumulated gases. The increased volume of different gases may result in flatulence, for example. As shown in the examples, the bacterial compositions of the invention may be effective for treating a specific aspect of IBS and other bowel conditions - visceral hypersensitivity. Without wishing to be bound by any theory, the observed effect of the bacterial compositions of the invention on visceral hypersensitivity may be associated with an effect of the bacteria on a specific gas – H_2S, and an effect on sulphate reducing bacteria (SRB), which synthesise H_2S in the gut. H_2S may have important roles

as a pain signalling molecule and the effect of the compositions of the invention on visceral hypersensitivity observed in the examples may be related to a reduction in the production of H_2S in the bowel, which may contribute to visceral hypersensitivity by affecting pain signalling, independently from any bloating effects related to gas volume. The examples demonstrate that the bacterial compositions of the invention can be effective for reducing SRB and reducing H_2S. In some embodiments, the bacterial compositions of the invention reduce SRB and/or reduce H_2S in the caecum. SRB are anaerobic bacteria that use sulphate reduction for the generation of energy and examples of SRB include members of the genus *Desulfovibrio,* and in particular *Desulfovibrio piger,* which is the most abundant species, and also the genera *Desulfobacter, Desulfobulbus* and *Desulfotomaculum.*

In certain embodiments, the compositions of the invention are for use in reducing colonisation of the gastrointestinal tract by SRB in the treatment of visceral hypersensitivity. In such embodiments, the composition may preferably be in the form of a bacterial culture. In such embodiments, the composition may preferably be a lyophilisate. In certain embodiments, the compositions of the invention are for use in lowering H_2S levels or preventing elevated H_2S levels in the gastrointestinal tract in the treatment of visceral hypersensitivity. In such embodiments, the composition may preferably be a lyophilisate.

In certain embodiments, the compositions of the invention are for use in reducing colonisation, community and / or population levels of the gastrointestinal tract by SRB in the treatment of visceral hypersensitivity. In certain embodiments, the compositions of the invention are for use in reducing colonisation, community and / or population levels of the caecum by SRB in the treatment of visceral hypersensitivity.

In preferred embodiments, the compositions of the invention are for use in reducing colonisation of the gastrointestinal tract by SRB, lowering H_2S levels, or preventing elevated H_2S levels in the treatment of visceral hypersensitivity associated with IBS. In further embodiments, the compositions of the invention are for use in reducing colonisation of the gastrointestinal tract by SRB, lowering H_2S levels, or preventing elevated H_2S levels in the treatment of visceral hypersensitivity associated with Crohn's Disease, ulcerative colitis, functional dyspepsia or infantile colic, for example in the treatment of visceral hypersensitivity associated with Crohn's Disease, ulcerative colitis or functional dyspepsia.

In preferred embodiments, the compositions of the invention are for use in reducing colonisation, community and / or population levels of the gastrointestinal tract by SRB, lowering H2S levels, or preventing elevated H2S levels in the treatment of visceral hypersensitivity associated with IBS. In further embodiments, the compositions of the invention are for use in reducing colonisation, community and / or population levels of the gastrointestinal tract by SRB, lowering H2S levels, or preventing elevated H2S levels in the treatment of visceral hypersensitivity associated with Crohn's

Disease, ulcerative colitis, functional dyspepsia or infantile colic, for example, in the treatment of visceral hypersensitivity associated with Crohn's Disease, ulcerative colitis or functional dyspepsia.

In preferred embodiments, the compositions of the invention are for use in reducing colonisation of the gastrointestinal tract by SRB, lowering H_2S levels, or preventing elevated H_2S levels in the treatment of visceral hypersensitivity in the abdomen, preferably in the gastrointestinal tract, more preferably in the lower gastrointestinal tract, in the caecum, in the colon or in the rectum. In preferred embodiments, the compositions of the invention are for use in reducing colonisation, community and / or population levels of the gastrointestinal tract by SRB, lowering H2S levels, or preventing elevated H2S levels in the treatment of visceral hypersensitivity in the abdomen, preferably in the gastrointestinal tract, more preferably in the lower gastrointestinal tract, in the caecum, in the colon or in the rectum.

In certain embodiments, the compositions of the invention are for use in a method of treating, preventing or reducing colonisation of the gastrointestinal tract by SRB. In certain embodiments, the compositions of the invention are for use in a method of treating, preventing or reducing colonisation, community and / or population levels of the gastrointestinal tract by SRB. In certain embodiments, the compositions of the invention are for use in a method of lowering H_2S levels or preventing elevated H_2S levels in the gastrointestinal tract.

In certain embodiments, the compositions of the invention are for use in treating patients that exhibit, or are expected to exhibit, increased levels of SRB and/or H_2S in their gastrointestinal tract, for example, when compared to a healthy subject, or a population of healthy subjects.

In certain embodiments, the compositions of the invention are for use in preventing visceral hypersensitivity in a subject that is receiving or has received 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 visceral hypersensitivity and that may be prevented by the compositions of the invention. The compositions of the invention may be administered concurrently with an antibiotic treatment.

In preferred embodiments, treatment with compositions of the invention results in a reduction in visceral hypersensitivity, a reduction in colonisation by SRB, and/or a reduction in H_2S levels.

Treatment or prevention of visceral hypersensitivity 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. For example, in some embodiments the composition of the invention is for use in treating or preventing severe visceral hypersensitivity. In some embodiments the subject having severe visceral hypersensitivity is a subject diagnosed with Crohn's disease, ulcerative colitis, functional dyspepsia, infantile colic, or, more preferably, IBS. In some embodiments the subject having severe visceral hypersensitivity is a subject diagnosed with Crohn's disease, ulcerative colitis, functional dyspepsia or, more preferably, IBS.

Modes of administration

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.

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

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.

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.

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 daily administration provides successfully colonisation and clinical benefits in the rat model of visceral hypersensitivity.

The examples also 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. Thus, the examples show successful delivery of the bacterial strain of the invention to the colon following daily administration.

Accordingly, 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.

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.

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.

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 visceral hypersensitivity developing in her child *in utero* and / or after it is born.

The compositions of the invention may be administered to a patient that has been diagnosed with visceral hypersensitivity or a disease or condition associated with visceral hypersensitivity, or that has been identified as being at risk of visceral hypersensitivity. The compositions may also be administered as a prophylactic measure to prevent the development of visceral hypersensitivity in a healthy patient.

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.*

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

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.

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

Generally, the composition of the invention comprises bacteria. 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.

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 [24-26].The examples demonstrate that lyophilisate compositions are particularly effective. In preferred embodiments, the compositions of the invention comprises lyophilised bacteria and is for the treatment of visceral hypersensitivity associated with IBS, preferably for the lowering H_2S levels or preventing elevated H_2S levels in the treatment of visceral hypersensitivity associated with IBS. In further preferred embodiments, the compositions of the invention comprises lyophilised bacteria and is for the treatment of visceral hypersensitivity associated with IBS, preferably for use in reducing colonisation of the gastrointestinal tract by SRB in the treatment of visceral hypersensitivity. In further preferred embodiments, the composition of the invention comprises lyophilised bacteria and is for the treatment of visceral hypersensitivity associated with IBS, preferably for use in reducing colonisation,

community and / or population levels of the gastrointestinal tract by SRB in the treatment of visceral hypersensitivity.

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.

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.

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

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 [27-28].

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.

The composition may be formulated as a probiotic.

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.

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

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.

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.

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.

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.

The compositions of the invention may comprise pharmaceutically acceptable excipients or carriers. Examples of such suitable excipients may be found in the reference [29]. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art and are described, for example, in reference [30]. 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.

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.

In some embodiments, the compositions of the invention 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.

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.

In certain embodiments, the compositions of the invention 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 a single species of *Blautia,* for example, a *Blautia hydrogenotrophica,* and do not contain any other bacterial species, or which

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,* for example, of *Blautia hydrogenotrophica,* 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.

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.

In some embodiments, the composition comprises a microbial consortium. For example, in some embodiments, the composition comprises the *Blautia* bacterial strain 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.

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*.

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.

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.

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

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

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.

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.

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

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 visceral hypersensitivity, such as visceral hypersensitivity associated with Crohn's disease, ulcerative colitis, functional dyspepsia, infantile colic or, more preferably, IBS.

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 visceral hypersensitivity, such as visceral hypersensitivity associated with Crohn's disease, ulcerative colitis, functional dyspepsia or, more preferably, IBS.

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.

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.

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.

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.

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

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.

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

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.

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 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.

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.

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").

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.

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.

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.

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.

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

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

The bacterial strains for use in the present invention can be cultured using standard microbiology techniques as detailed in, for example, references [32-34].

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

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 [35-42], *etc.*

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.

The term "about" in relation to a numerical value *x* is optional and means, for example, $x \pm 10\%$.

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.

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. [43]. 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. [44].

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.

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 – Efficacy of bacterial inocula in a rat model of visceral hypersensitivity

Summary

Rats were inoculated with the faecal microbiota from a human IBS subject exhibiting visceral hypersensitivity. The rats were then administered with compositions comprising bacterial strains according to the invention and were then tested using a distension assay to measure visceral hypersensitivity. The compositions of the invention were found to reduce the rats' response to distension, indicating a reduction in visceral hypersensitivity.

Strain

Blautia hydrogenotrophica (BH) strain DSM 10507/14294.

Compositions and administration

BH culture (16H) or lyophilisate - administered by oral gavage

Control solution administered by oral gavage

Rats

Inoculated with human intestinal microbiota from an IBS subject.

Study design

Day -14 – rats inoculated with human intestinal microbiota from an IBS subject

Days 0 to 28 – daily dose of BH culture or lyophilisate, or control solution

Days 0, 14 and 28 – qPCR of BH population in faecal samples

Between days 14 and 28 – operation to implant electrode into the abdomen (for distension assay)

Day 28 – distension assay, caecal samples collected for sulphides and short chain fatty acid (SCFA) analysis, enumeration of microbiota in faecal samples on selective media

Results

Figure 1 presents the results of a qPCR analysis of the BH population in faecal samples from rats administered control solution (IBS) or BH lyophilisate (IBS+BH). An increase in the BH population was seen at days 14 and 28 in rats receiving the BH lyophilisate, which confirms successful colonisation.

Figure 2 presents the results of the distension assay. Rats were subjected to colorectal distension and the number of contractions per minute were recorded as a specific measure of visceral hypersensitivity. The rats treated with the compositions of the invention exhibited reduced contractions and reduced visceral hypersensitivity.

Figures 3 and 4 report on the effects of administration of BH culture and lyophilisate on the microbiota in faecal samples. Administration of BH culture resulted in a notable reduction (1 log) in sulphate reducing bacteria (SRB).

Figure 5 reports on the impact of administration of BH lyophilisate on microbiota fermentation as measured by short chain fatty acid concentrations in caecal samples. Administration of BH lyophilisate resulted in an increase in acetate production.

Figure 6 reports on the impact of administration of BH lyophilisate on microbiota fermentation as measured by sulphide concentration in caecal samples (H_2S). Administration of BH resulted in a decrease in sulphide production.

Conclusions

Administration of compositions comprising *Blautia hydrogenotrophica* led to successful colonisation and a notable reduction in visceral hypersensitivity, as measured using the distension assay. This effect was observed when *Blautia hydrogenotrophica* was administered as a culture and as a lyophilisate. Administration of *Blautia hydrogenotrophica* also had a notable effect on microbiota constitution and fermentation, with observed reductions in SRB and sulphide production. These data indicate that *Blautia hydrogenotrophica* may be useful for reducing visceral hypersensitivity, and in particular

visceral hypersensitivity associated with IBS. The reductions in visceral hypersensitivity may be associated with the observed reductions in SRB and sulphide production.

Example 2 – Efficacy of bacterial lyophilisate in a rat model of visceral hypersensitivity

The observations of Example 1 were confirmed in further experiments using a lyophilisate of Blautia hydrogenotrophica (BH) strain DSM 10507/14294 and a rat model of IBS. As shown in Figures 7 and 8, administration of BH lyophilisate provided a statistically-significant reduction in the number of abdominal contractions in response to distension, indicating a reduction in visceral hypersensitivity. In Figure 7 a fecal sample from one IBS subject was used to inoculate the rats. In Figure 8 fecal samples from three IBS subjects were used to inoculate the rats, with one of these IBS subjects being the same as the IBS subject used in Figure 7. Furthermore, as shown in Figures 9 and 10, administration of BH lyophilisate provided a statistically-significant reduction in sulphides. In Figure 9 a fecal sample from one IBS subject was used to inoculate the rats. In Figure 10, fecal samples from three IBS subjects were used to inoculate the rats, with one of these IBS subjects being the same as the IBS subject used in Figure 9.

Example 3 – Effects of bacterial lyophilisate on healthy rats

The effects of administration of a lyophilisate of *Blautia hydrogenotrophica* (BH) strain DSM 10507/14294 on healthy HIM rats were studied and the results are reported in Figures 11-14. Further details regarding the experiments are provided above in the descriptions of the figures. Figure 11 shows that an appropriate dose for BH in rats is 10^9 cells per day or greater. Figure 12 shows that in these experiments BH did not permanently colonise the rat digestive tract. Figure 13 shows that BH is primarily found in the caecum. Figure 14 shows that administration of BH induces an increase in acetate as well as in butyrate production.

Example 4 - Efficacy of bacterial lyophilisate in a rat model of visceral hypersensitivity

The effects of administration of a lyophilisate of *Blautia hydrogenotrophica* (BH) strain DSM 10507/14294 on a rat model of IBS were further investigated. Germ-free rats were inoculated with faecal samples from C-IBS (with constipation) or U-IBS (unsubtyped) patients. Most of the experiments were carried out with faecal samples from IBS patients showing visceral hypersensitivity (VH measured with barostat). The results are reported in Figures 15 and 16 and further details regarding the experiments are provided above in the descriptions of the figures. Figure 15 confirms that administration of BH lyophilisate causes a statistically-significant reduction in sulphate-reducing bacteria. As expected, an increase in BH is also observed. Figure 16 shows that BH administration induced a statistically-significant decrease in the amount of H_2S produced by IBS HIM rats. Over-production of caecal H_2S by gut microbiota is associated with visceral hypersensitivity.

Example 5 – Changes in patient symptoms during Phase I clinical trial

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 one was abdominal pain. 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 17 and 18.

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 17). Improvement of symptoms, one of which is abdominal pain, supports the use of Blautix for treating or preventing visceral hypersensitivity. Notably, patients 3.02, 3.17 and 3.24, who all had severe abdominal pain at the beginning of the study, had mild, mild, and no abdominal pain, respectively at day 15/16.

50% of patients receiving placebo reported an improvement in symptoms (Figure 17). 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 [45].

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 18). The worsening of symptoms, one of which is abdominal pain, following cessation of Blautix dosing therefore also supports the use of Blautix in treating or preventing visceral hypersensitivity.

Example 6 – Efficacy of B. hydrogenotrophica on visceral hypersensitivity studied in human Microbiota Associated rat (HMA rat) model

Summary

Groups of 16 germ-free rats (comprising 8 rats in the control group and 8 rats in the treatment group) were inoculated with the faecal microbiota from a human IBS subject (IBS-HMA rats). Three successive experiments were carried out using faecal samples from 3 different IBS patients. Two other groups of rats (n = 10) were inoculated with faecal samples of healthy subject (n=2 subjects; 2 groups of healthy-HMA rats) as visceral sensitivity control. Thus, there were 24 IBS-microbiota associated rats (control), 24 IBS microbiota associated rats treated with Blautix and 20 healthy-microbiota associated rats. Half of the IBS-HMA rats were then administered for 28 days with composition comprising the bacterial strain of *B. hydrogenotrophica* according to the invention while the other half animals received a control solution. After 28 days of administration, all HMA-rats were tested using a colonic distension assay to measure visceral sensitivity. The composition of the invention were found to reduce the IBS-HMA rats' response to distension, indicating a reduction in visceral hypersensitivity that reached a normo-sensitivity as observed in healthy-HMA rats.

Strain

Blautia hydrogenotrophica (BH) strain DSM 10507^T/14294.

Composition and administration

BH lyophilisate was suspended in sterile mineral solution to a concentration of 10^{10} bacteria per ml. Two ml of this suspension was administered daily per IBS-HMA rat, by oral gavage, for a 28 days period.

The control solution was the sterile mineral solution that was administered daily (2 ml per rat) by oral gavage to the control group of IBS-HMA rats.

Rats

Germ-Free male Fisher rats (aged 10 weeks) were inoculated with human faecal microbiota from an IBS subject (IBS-HMA rats). Sixteen rats were inoculated with the same human faecal inoculum. Three successive experiments were performed with faecal samples from three different IBS subjects. Two other groups of ten rats were inoculated with faecal sample from 2 healthy subjects (normo-sensitivity control groups).

Study design

Day -14 – Inoculation of Germ-free rats with human faecal microbiota.

Days 0 to 28 – Daily dose of BH lyophilisate (assay group), or control solution (control group) by oral gavage

Between days 14 and 22 – operation to implant electrode into the abdomen (for distension assay)

Days 22-28 – Adaptation of the rats to avoid stress associated with distension test.

Day 28 – distension assay and euthanasia of animals to collect the caecal samples for sulphides and short chain fatty acid (SCFA) analysis.

Days 0, 14 and 28 – Collection of faecal samples for microbial analysis: qPCR for evaluating BH population and other commensal groups of miccroorganisms and enumeration of functional groups of microorganisms using selective media and strictly anaerobic method.

Results

Figure 19 presents the results of qPCR analysis of the *B. hydrogenotrophica* population in faecal samples from IBS-HMA rats receiving control solution or BH lyophilisate. A significant increase in the BH population was observed at the end of the administration period (D 28) in rats receiving the BH lyophilisate, which confirms successful delivery of BH in the colon.

Figure 20 presents the results of the distension assay. Rats were subjected to colorectal distension and the number of contractions per 5 minutes were recorded as a specific measure of visceral

hypersensitivity. The IBS-HMA rats treated with the composition of the invention exhibited reduced contractions reflecting a reduction in visceral hypersensitivity. After *B. hydrogenotrophica* treatment, the IBS-HMA rats showed a normo-visceral sensitivity, comparing with that measured in healthy HMA rats.

Figure 21 reports on the effects of administration of *B. hydrogenotrophica* on some groups of microorganisms from faecal microbiota, previously found to be affected in IBS patients. Administration of BH resulted in a significant reduction in sulphate-reducing bacteria (SRB).

Figure 22 reports on the impact of administration of BH on sulphide (H_2S) concentration in caecal samples of IBS-HMA rats. Administration of BH resulted in a significant decrease in sulphide production. The data in Figure 22 are the same as those in Figure 10 but the scale is different.

Figure 23 reports on the impact of administration of BH on the main fermentative metabolites, short chain fatty acids, in caecal samples of IBS-HMA rats. Administration of BH-resulted in a significant increase in acetate concentration as well as in a significant increase in butyrate concentration (Figure 23b).

Conclusions

Administration of a composition comprising *Blautia hydrogenotrophica* led a significant reduction in visceral hypersensitivity, as measured using the distension assay. After treatment, the visceral sensitivity of IBS-HMA rats was found to be similar to that measured in healthy-HMA rats. Administration of the composition comprising *B. hydrogenotrophica* can restore visceral sensitivity of IBS-HMA animals to a normal one. Administration of *Blautia hydrogenotrophica* also had a significant effect on microbiota constitution and fermentation, and especially induced important reductions in SRB and sulphide production. These data indicate that *Blautia hydrogenotrophica* may be useful for reducing visceral hypersensitivity, and in particular visceral hypersensitivity associated with IBS. The reductions in visceral hypersensitivity may be associated with the observed reductions in SRB and sulphide production.

Example 7 – Stability testing

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

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

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SEQ ID NO:2 (*Blautia wexlerae* strain WAL 14507 16S ribosomal RNA gene, partial sequence - EF036467)

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1321 cttgtacaca ccgcccgtca caccatggga gtcagtaacg cccgaagtca gtgacctaac
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SEQ ID NO:3 (consensus 16S rRNA sequence for *Blautia stercoris* strain 830)

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SEQ ID NO:4 (consensus 16S rRNA sequence for *Blautia wexlerae* strain MRX008)

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SEQ ID NO:5 (*Blautia hydrogenotrophica* strain S5a36 16S ribosomal RNA gene, partial sequence - X95624.1)

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1441 gactgataac tggggtga
```

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1-3-1	Name of depositary institution	NCIMB National Collections of Industrial, Food and Marine Bacteria (NCIMB)
1-3-2	Address of depositary institution	NCIMB Ltd, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen AB21 9YA, United Kingdom
1-3-3	Date of deposit	12 March 2015 (12.03.2015)
1-3-4	Accession Number	NCIMB 42381
1-5	**Designated States for Which Indications are Made**	All designations

2	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
2-1	**page**	6
2-2	**line**	18-23
2-3	**Identification of deposit**	
2-3-1	Name of depositary institution	NCIMB National Collections of Industrial, Food and Marine Bacteria (NCIMB)
2-3-2	Address of depositary institution	NCIMB Ltd, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen AB21 9YA, United Kingdom
2-3-3	Date of deposit	16 November 2015 (16.11.2015)
2-3-4	Accession Number	NCIMB 42486
2-5	**Designated States for Which Indications are Made**	All designations

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3	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
3-1	**page**	5-6
3-2	**line**	33-4
3-3	**Identification of deposit**	
3-3-1	Name of depositary institution	DSMZ Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ)
3-3-2	Address of depositary institution	Inhoffenstr. 7B, 38124 Braunschweig, Germany
3-3-3	Date of deposit	10 May 2001 (10.05.2001)
3-3-4	Accession Number	DSMZ 14294
3-5	**Designated States for Which Indications are Made**	All designations
4	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
4-1	**page**	5-6
4-2	**line**	33-4
4-3	**Identification of deposit**	
4-3-1	Name of depositary institution	DSMZ Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ)
4-3-2	Address of depositary institution	Inhoffenstr. 7B, 38124 Braunschweig, Germany
4-3-4	Accession Number	DSMZ 10507
4-5	**Designated States for Which Indications are Made**	All designations

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0-4	**This form was received with the international application:** (yes or no)	Yes
0-4-1	Authorized officer	Aoustin, Isabelle

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CLAIMS

1. A composition comprising a bacterial strain of the genus *Blautia*, for use in a method of treating or preventing visceral hypersensitivity.

2. The composition of claim 1, wherein the visceral hypersensitivity is associated with IBS, Crohn's disease, ulcerative colitis, functional dyspepsia or infantile colic.

3. The composition of claim 2, wherein the visceral hypersensitivity is associated with IBS, Crohn's disease, ulcerative colitis or functional dyspepsia.

4. The composition of claim 1, wherein the composition is for use in treating or preventing visceral hypersensitivity in a subject diagnosed with IBS, Crohn's disease, ulcerative colitis, functional dyspepsia or infantile colic.

5. The composition of claim 4, wherein the composition is for use in treating or preventing visceral hypersensitivity in a subject diagnosed with IBS, Crohn's disease, ulcerative colitis or functional dyspepsia.

6. The composition of any preceding claim, wherein the composition is for use in reducing colonisation of the gastrointestinal tract by sulphate reducing bacteria (SRB) in the treatment or prevention of visceral hypersensitivity.

7. The composition of any preceding claim, wherein the composition is for use in lowering H_2S levels or preventing elevated H_2S levels in the gastrointestinal tract in the treatment or prevention of visceral hypersensitivity.

8. The composition of any preceding claim, wherein the composition is for use in treating or preventing visceral hypersensitivity in a patient suffering from painful distension of the gastrointestinal tract, in particular in the colon or rectum.

9. The composition of any preceding claim, wherein the bacterial strain is of *Blautia hydrogenotrophica*.

10. The composition of any of claims 1-8, wherein the bacterial strain is of *Blautia stercoris*.

11. The composition of any of claims 1-8, wherein the bacterial strain is of *Blautia wexlerae*.

12. The composition of any of claims 1-8, wherein the bacterial strain 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*.

13. The composition of claim 12, wherein the bacterial strain has a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:5 or which has the 16s rRNA sequence of SEQ ID NO:5.

14. The composition of any of claims 1-8, wherein the bacterial strain 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 stercoris*.

15. The composition of any of claims 1-8, wherein the bacterial strain 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 wexlerae*.

16. The composition of claim 1, wherein the composition comprises a bacterial strain of the species *Blautia hydrogenotrophica*, for use in a method of treating or preventing visceral hypersensitivity in a subject diagnosed with IBS.

17. The composition of claim 1, wherein the composition comprises a bacterial strain of the species *Blautia hydrogenotrophica*, for use in reducing colonisation of the gastrointestinal tract by sulphate reducing bacteria (SRB), lowering H_2S levels or preventing elevated H_2S levels in the gastrointestinal tract in the treatment or prevention of visceral hypersensitivity.

18. The composition of claim 1, wherein the composition comprises a bacterial strain of the species *Blautia stercoris*, for use in a method of treating or preventing visceral hypersensitivity in a subject diagnosed with IBS.

19. The composition of claim 1, wherein the composition comprises a bacterial strain of the species *Blautia stercoris*, for use in reducing colonisation of the gastrointestinal tract by sulphate reducing bacteria (SRB), lowering H_2S levels or preventing elevated H_2S levels in the gastrointestinal tract in the treatment or prevention of visceral hypersensitivity.

20. The composition of claim 1, wherein the composition comprises a bacterial strain of the species *Blautia wexlerae*, for use in a method of treating or preventing visceral hypersensitivity in a subject diagnosed with IBS.

21. The composition of claim 1, wherein the composition comprises a bacterial strain of the species *Blautia wexlerae*, for use in reducing colonisation of the gastrointestinal tract by sulphate reducing bacteria (SRB), lowering H_2S levels or preventing elevated H_2S levels in the gastrointestinal tract in the treatment or prevention of visceral hypersensitivity.

22. The composition of any preceding claim, wherein the composition is for oral administration.

23. The composition of any preceding claim, wherein the composition comprises one or more pharmaceutically acceptable excipients or carriers.

24. The composition of any preceding claim, wherein the bacterial strain is lyophilised.

25. The composition of any preceding claim, wherein the bacterial strain is viable.

26. The composition of any preceding claim, wherein the composition comprises a single strain of the genus *Blautia.*

27. The composition of any preceding claim, which comprises the *Blautia* bacterial strain as part of a microbial consortium.

28. A food product comprising the composition of any preceding claim, for the use of any preceding claim.

29. A vaccine composition comprising the composition of any preceding claim, for the use of any preceding claim.

30. A method of treating or preventing visceral hypersensitivity, comprising administering a composition comprising a bacterial strain of the genus *Blautia* to a patient in need thereof.

31. A method according to claim 30, wherein the *Blautia* is *B. hydrogenotrophica*.