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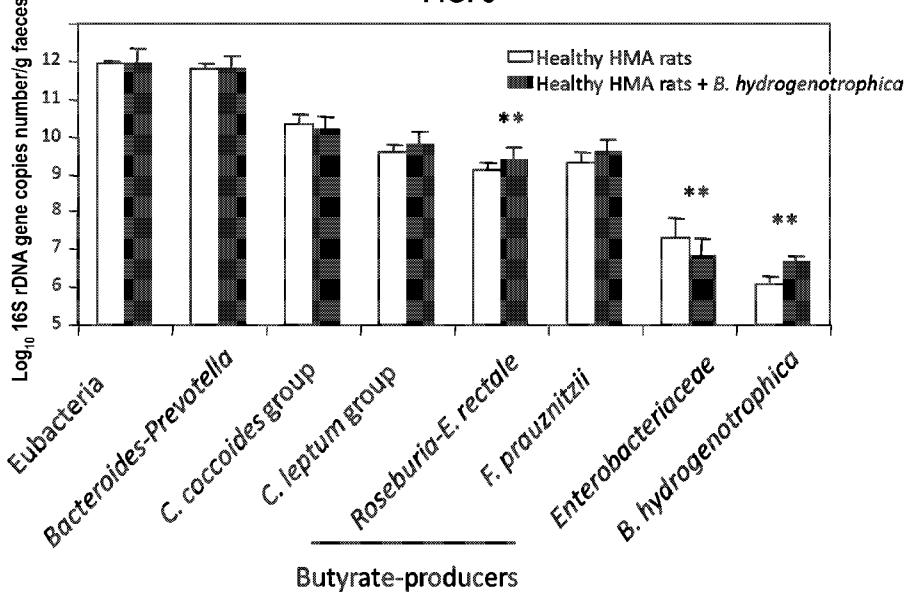
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(54) Title: COMPOSITIONS COMPRISING BACTERIAL STRAINS

FIG. 5



(57) Abstract: The invention provides compositions comprising one or more bacterial strains for use in a method of reducing the level of Enterobacteriaceae in the gastrointestinal tract.

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- *as to the identity of the inventor (Rule 4.17(i))*
- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
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COMPOSITIONS COMPRISING BACTERIAL STRAINS

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.

5 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].

20 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].

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

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

US 2010/0247489 describes the use of mineral nutrients to treat digestive disorders. US'789 proposes optionally also using numerous different genera of bacteria, including *Enterobacteriaceae*, to prevent and reduce gas formation in the colon, so this document suggests that increasing *Enterobacteriaceae* spp. in the gastrointestinal tract may be employed to treat certain diseases. US'789 does not discuss any methods for reducing *Enterobacteriaceae* spp.

WO 2016/086206 suggests that bacteria in the order Clostridiales, including *Enterbacteriaceace* spp., can be used to treat or prevent dysbiosis. There is no suggestion in WO'206 that reducing the level of *Enterobacteriaceace* spp. in the gastrointestinal tract can be used to treat diseases, nor any suggestion regarding how a reduction in the level of *Enterbacteriaceace* spp. might be achieved.

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

SUMMARY OF THE INVENTION

The inventors have developed new therapies for treating and preventing diseases associated with *Enterobacteriaceae*, in particular *E. coli*. In particular, the inventors have identified that bacterial strains from the genus *Blautia* can be effective for reducing the level of *Enterobacteriaceae* in the gastrointestinal tract. As described in the examples, oral administration of compositions comprising *Blautia hydrogenotrophica* may reduce the level of *Enterobacteriaceae* in the gastrointestinal tract, including *E. coli*, and may treat or prevent diseases associated with *Enterobacteriaceae* or *E. coli*. Therefore, in a first embodiment, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in a method of reducing the level of *Enterobacteriaceae* in the gastrointestinal tract.

In preferred embodiments of all aspects of the invention, the *Enterobacteriaceae* is *E. coli*. In preferred embodiments of all aspects of the invention, the bacterial strain is of *Blautia hydrogenotrophica* and is preferably the bacterium deposited under accession number DSM 10507/14294.

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 a disease associated with *Enterobacteriaceae* infection, such as *E. coli* infection. In certain embodiments, the compositions of the invention are for use in treating or preventing diarrhoea, gastroenteritis, urinary tract infection or neonatal meningitis. In certain embodiments, the compositions of the invention are for use in treating or preventing *Enterobacteriaceae* infection, such as *E. coli* infection.

In further preferred embodiments, the compositions of the invention are for use in reducing the level of *Enterobacteriaceae* in the gastrointestinal tract, preferably the level of *E. coli*, in the treatment or

prevention of IBS, Crohn's disease, ulcerative colitis, functional dyspepsia, diarrhoea, gastroenteritis, urinary tract infection or neonatal meningitis.

The inventors have developed new therapies for treating and preventing diarrhoea. In particular, the inventors have identified that bacterial strains from the genus *Blautia* can be effective for reducing diarrhoea. As described in the examples, oral administration of compositions comprising *Blautia hydrogenotrophica* may reduce diarrhoea in patients having irritable bowel syndrome (IBS).

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 reducing the level of *Enterobacteriaceae* in the gastrointestinal tract. 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 reducing the level of *Enterobacteriaceae* in the gastrointestinal tract and thereby treating or preventing diseases associated with *Enterobacteriaceae*, 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 IBS HMI rats receiving the BH lyophilisate.

Figure 2: 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 3: Transit time of *B. hydrogenotrophica* after oral administration (10⁹/day) to healthy HIM rats.

Figure 4: 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 10¹⁰ /day/rat.

Figure 5: Effect of *B. hydrogenotrophica* administration on faecal microbiota composition of healthy-human microbiota-associated rats. Administration of 10¹⁰ *B. hydrogenotrophica* / day for 14 days (results from 2 healthy human faecal microbiota (20 HIM rats)- qPCR analysis).

Figure 6: Impact of *B. hydrogenotrophica* administration on the microbial populations in IBS-HIM rats.

Figure 7a: Change in levels of patient microbiota *Blautia hydrogenotrophica* during and following Blautix treatment.

Figure 7b: Change in levels of patient microbiota *Enterobacteria* during and following Blautix treatment.

Figure 8: Hydrogen breath test Cmax results for day 1, day 2, day 15 and day 16 for IBS patients treated with Blautix (**Figure 8a**) and IBS patients treated with placebo (**Figure 8b**). **Figure 8c** is a

graph comparing the percentage of Blautix treated patients with a reduction in hydrogen between the mean of days 15 and 16 and the mean of days 1 and 2 to the percentage of placebo treated patients with a reduction in hydrogen between these time points.

Figure 9a: Hydrogen uncorrected and hydrogen corrected breath test paired data for day 1 and day 15 for Blautix treated IBS patients; **Figure 9b:** graph comparing mean hydrogen uncorrected breath test results for day 1 and day 15 for the Blautix treatment group; **Figure 9c:** graph comparing mean hydrogen corrected breath test results for day 1 and day 15 for the Blautix treatment group.

Figure 10a: Hydrogen uncorrected and hydrogen corrected breath test paired data for day 1 and day 15 for placebo treated IBS patients; **Figure 10b:** graph comparing mean hydrogen uncorrected breath test results for day 1 and day 15 for the placebo group; **Figure 10c:** graph comparing mean hydrogen corrected breath test results for day 1 and day 15 for the placebo group.

Figure 11: Graphs comparing the mean hydrogen breath test results from day 1 and day 15 for the Bautix treatment group (Verum) and placebo group (**Figure 11a:** uncorrected hydrogen; **Figure 11b:** corrected hydrogen).

Figure 12: 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 13: Bacteria enumeration in IBS HMA-rat faecal samples after *B. hydrogenotrophica* (BlautiX) administration versus control solution..

Figure 14: Changes in patient symptoms during dosing period (days 1-16) of Phase I clinical trial.

Figure 15: Changes in patient symptoms during washout period of Phase I clinical trial.

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 reducing the level of *Enterobacteriaceae* in the gastrointestinal tract, in particular *E. coli*. 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*. Most preferred is *Blautia hydrogenotrophica*, particularly the bacterium deposited under accession number DSM 10507/14294.

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 H₂/CO₂ to acetate, which 5 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. 10 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 15 BH. Strain BH was deposited with the Deutsche Sammlung von Mikroorganismen [German Microorganism Collection] (Mascheroder Weg 1b, 38124 Braunschweig, Germany) in 26th January 1996 as “*Ruminococcus hydrogenotrophicus*” under accession number DSM 10507 and also under accession number DSM 14294 as “S5a33” on 14th May 2001. The depositor was INRA Laboratoire de Microbiologie CR de Clermont-Ferrand/Theix 63122 Saint Genès Champanelle, France. Ownership 20 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* 25 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. 30 (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, 35 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 “*Blautia/Ruminococcus MRx0008*” and was assigned accession number NCIMB 42486.

5 Bacterial strains closely related to the strain tested in the examples are also expected to be effective for reducing the level of *Enterobacteriaceae* in the gastrointestinal tract, in particular *E. coli*. 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 15 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 20 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 25 NCIMB 42486 are also expected to be effective for reducing the level of *Enterobacteriaceae* in the gastrointestinal tract, in particular *E. coli*. 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 30 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 35 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 5 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 10 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 15 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.

20 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 25 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 30 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 35 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 *Enterobacteriaceae* 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

5 accession number DSM 10507/14294. This is the exemplary BH strain tested in the examples and shown to be effective for reducing the level of *Enterobacteriaceae* in the gastrointestinal tract, in particular *E. coli*. 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.

10 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

15 10507/14294, NCIMB 42381 or NCIMB 42486 strain. In particular, a derivative strain will elicit comparable effects on *Enterobacteriaceae* 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.

20 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.

30 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 certain embodiments, the compositions of the invention are for use in reducing the level of *Enterobacteriaceae* in the gastrointestinal tract. Increased levels of *Enterobacteriaceae* in the gastrointestinal tract are associated with numerous pathological conditions and diseases, and the examples demonstrate that the compositions of the invention may be effective for reducing the level of *Enterobacteriaceae* in the gastrointestinal tract.

In preferred embodiments of all aspects of the invention, the *Enterobacteriaceae* is *E. coli*. Therefore, preferably the compositions of the invention are for use in reducing the level of *E. coli* in the gastrointestinal tract.

10 In certain embodiments, the *Enterobacteriaceae* is a pathogenic strain, such as *E. coli* O157:H7, O104:H4, O121, O26, O103, O111, O145, O104:H21, or O104:H4.

In alternative embodiments, the *Enterobacteriaceae* is a commensal or non-pathogenic strain. Increased levels of such *Enterobacteriaceae* may contribute to conditions such as IBS and Crohn's disease, and the examples demonstrate that the compositions of the invention may have a reducing effect on *Enterobacteriaceae* in the context of IBS.

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

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

25 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.

In certain embodiments, the compositions of the invention are for use in treating or preventing *Enterobacteriaceae* infection, such as *E. coli* infection. In preferred embodiments, the compositions of

the invention are for use in treating or preventing infection of the gastrointestinal tract, and in particular the caecum. In such embodiments the *Enterobacteriaceae* may be a pathogenic strain.

In some embodiments, the level of *Enterobacteriaceae* is reduced in stool in the subject. In some embodiments, the level of *Enterobacteriaceae* is reduced in a stool sample from the subject. In some 5 embodiments, the level of *Enterobacteriaceae* is reduced in the distal gut of the subject. In some embodiments, the level of *Enterobacteriaceae* is reduced in the caecum. In some embodiments, the level of *Enterobacteriaceae* is reduced in the colon. In some embodiments, the level of *Enterobacteriaceae* is reduced in the rectum. In some embodiments, the level of *Enterobacteriaceae* is reduced in the small intestine.

10 In preferred embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in the treatment of infections caused by pathogenic *Enterobacteriaceae*.

In preferred embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in the treatment of *E. coli* infection.

15 In preferred embodiments, the compositions of the invention are for use in reducing the level of *Enterobacteriaceae* in the gastrointestinal tract, preferably the level of *E. coli*, in the treatment or prevention of a disease associated with increased levels of *Enterobacteriaceae*, such as IBS, Crohn's disease, ulcerative colitis, functional dyspepsia, diarrhoea, gastroenteritis, urinary tract infection or neonatal meningitis. *Enterobacteriaceae* and in particular *E. coli* are known to be potential triggers for, or known to exacerbate, Crohn's disease and ulcerative colitis [22-24], so the effect shown in the 20 examples for the compositions of the invention may be beneficial in the treatment of these conditions.

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

25 In preferred embodiments, the compositions of the invention comprise the bacterium deposited under accession number DSM 10507/14294 and are for use in reducing the level of *Enterobacteriaceae* in the gastrointestinal tract in the treatment of Crohn's disease, ulcerative colitis, functional dyspepsia, or most preferably IBS. In further preferred embodiments, the compositions of the invention comprise the bacterium deposited under accession number DSM 10507/14294 and are for use in treating or 30 preventing Crohn's disease, ulcerative colitis, functional dyspepsia, or most preferably IBS by reducing the level of *Enterobacteriaceae* in the gastrointestinal tract.

In certain embodiments, the composition is administered in combination with an antibiotic, such as a fluoroquinolone, azithromycin, ciprofloxacin or rifaximin. In certain embodiments, the composition is administered in combination with a dehydration treatment such as oral rehydration solution. The

composition of the invention may be administered at the same time as the antibiotic or dehydration treatment, or sequentially with the antibiotic or dehydration treatment.

In certain embodiments, the compositions are for use in patients that exhibit, or are expected to exhibit, increased levels of *Enterobacteriaceae* in the 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 a method of treating, preventing or reducing colonisation of the gastrointestinal tract by *Enterobacteriaceae*.

In some embodiments, the composition of the invention is for use in a method of reducing the level of *Enterobacteriaceae* in the gastrointestinal tract in a subject having an increased level of hydrogen in their breath relative to a healthy subject. In some embodiments, the composition of the invention is for use in reducing the hydrogen level in the breath of a subject exhibiting or who is expected to exhibit an increased level of *Enterobacteriaceae* in the gastrointestinal tract. The subject is preferably a subject diagnosed as having IBS, Crohn's disease, ulcerative colitis, functional dyspepsia, diarrhoea, gastroenteritis, urinary tract infection or neonatal meningitis and/or an infection caused by pathogenic *Enterobacteriaceae*, for example, *E. coli*. The examples show that treatment with a composition of the invention reduces the level of hydrogen detected in hydrogen breath tests. Accordingly, the hydrogen levels are preferably assessed using a hydrogen breath test. The hydrogen breath test is well known in the art and so the skilled person will know how to conduct such a test. In some embodiments, the patient is administered lactulose as the substrate for the test.

The hydrogen breath test is also a useful tool for monitoring the effectiveness or likely effectiveness of reducing the level of *Enterobacteriaceae* and of treatment or prevention using a composition of the invention. For example, a reduction in the level of hydrogen detected in a subject's breath following treatment with a composition of the invention may indicate that the treatment is having a reducing, therapeutic or preventative effect. Accordingly, in some embodiments the methods and uses of the invention further comprise monitoring the hydrogen level in a subject's breath during and/or following treatment with a composition of the invention and thereby assessing the effectiveness or likely effectiveness of reducing, treatment or prevention. For example, hydrogen levels may be monitored at one or more (e.g. 1, 2, 3, 4 or more than 4) times, for example, including before treatment, at the start of treatment, during treatment, at the end of treatment and/or following treatment, as desired. In some embodiments, the level of hydrogen in the subject's breath at the end and/or following the dosing period (during which the composition is administered to the subject) is compared to the level at the start and/or before the dosing period and a reduction in the level indicates the effectiveness or likely effectiveness of the reducing, treatment or prevention. For example, in embodiments in which the dosing period is 16 days, it may be desirable to take measurements at day 1 and day 16, or for example at day 1, day 2, day 15 and day 16. In some embodiments, multiple measurements are taken and the

mean of those measurements obtained (for example, the mean of day 1 and day 2 and the mean of day 15 and day 16). In some embodiments, a reduction in at least 40 ppm in the hydrogen level Cmax indicates that the reducing, treatment or prevention is effective or likely to be effective. In some embodiments, the hydrogen level in the subject's breath is measured only once, for example, at the end of or following treatment, and the finding that the level is at or close to a predetermined level is indicative that the reducing, treatment or prevention is likely to have been effective. The hydrogen breath test is a standard assay and so predetermined levels are known in the art.

Treatment or prevention 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.

10 Levels of *Enterobacteriaceae* may be detected in faeces from a patient, using standard techniques, such as the qPCR techniques used in the examples.

Modes of administration

15 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.

20 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.

25 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.

30 In certain embodiments, the compositions of the invention are administered regularly, such as daily, every two days, or weekly, for an extended period of time, such as for at least one week, two weeks, one month, two months, six months, or one year. The examples demonstrate that BH administration

may not result in permanent colonisation of the intestines, so regular administration for extended periods of time may provide greater therapeutic benefits.

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 5 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 10 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 elevated levels of *Enterobacteriaceae* 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 15 elevated levels of *Enterobacteriaceae* or a disease or condition associated with elevated levels of *Enterobacteriaceae*, or that has been identified as being at risk of elevated levels of *Enterobacteriaceae*. The compositions may also be administered as a prophylactic measure to prevent the development of elevated levels of *Enterobacteriaceae* in a healthy patient.

The compositions of the invention may be administered to a patient that has been identified as having 20 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 25 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.

30 **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 [25-5 27]. The examples demonstrate that lyophilisate compositions are particularly effective.

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 [28-29].

30 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 1×10^3 to about 1×10^{11} colony forming units (CFU); for example, from about 1×10^7 to about 1×10^{10} CFU; in another example from about 1×10^6 to about 1×10^{10} CFU; in another example from about 1×10^7 to about 1×10^{11} CFU; in another example from about 1×10^8 to about 1×10^{10} CFU; in another example from about 1×10^8 to about 1×10^{11} CFU.

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 1×10^6 to about 1×10^{11} CFU/g, respect to the weight of the composition; for example, from about 1×10^8 to about 1×10^{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 [30]. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art and are described, for example, in reference [31]. Examples of suitable carriers include lactose, starch, glucose, methyl cellulose, 5 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 10 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 15 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.

20 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, 25 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. 30 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.

35 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 5 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*, 10 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 15 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 20 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, 25 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 30 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.

10 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.

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

20 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 reduce levels of *Enterobacteriaceae* and/or treat a disorder associated with elevated levels of *Enterobacteriaceae* when administered to a subject in need thereof; and wherein the disorder is associated with elevated levels of *Enterobacteriaceae*, such as Crohn's disease, ulcerative colitis, functional dyspepsia or, more preferably, IBS, or diarrhoea, gastroenteritis, urinary tract infection or neonatal meningitis .

25 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.

30 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.

10 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.

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

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

20 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.

25 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.

30 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 5 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 10 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, 15 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.

20 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 25 does not contain any film forming polymer. In some embodiments, the capsule comprises a shell and the shell comprises hydroxypropylmethylcellulose and does not comprise any film forming polymer (e.g. see [32]). In some embodiments, the formulation is an intrinsically enteric capsule (for example, Vcaps® from Capsugel).

30 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 35 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 [33-35].

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), NaHCO₃ (0.4 g), cysteine (0.1 g), K₂HPO₄ (0.045 g), KH₂PO₄ (0.045 g), NaCl (0.09 g), (NH₄)₂SO₄ (0.09 g), MgSO₄ · 7H₂O (0.009 g), CaCl₂ (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 [36- 43], *etc.*

15 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*±10%.

20 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. [44]. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in ref. [45].

30 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 – Administration of bacterial inocula to rats harbouring IBS gut microbiota

5 Summary

Rats were inoculated with the faecal microbiota from a human IBS subject. The rats were then administered with compositions comprising bacterial strains according to the invention and colonisation by *Blautia hydrogenotrophica* was analysed.

Strain

10 *Blautia hydrogenotrophica* (BH) strain DSM 10507/14294.

Compositions and administration

BH lyophilisate - administered by oral gavage

Control solution administered by oral gavage

Rats

15 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 lyophilisate, or control solution

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

20 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.

25 Conclusions

Administration of compositions comprising *Blautia hydrogenotrophica* led to successful colonisation.

Example 2 – Administration of bacterial lyophilisate to healthy rats and effect on Enterobacteriaceae

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 2-5. Further details regarding the experiments are provided above in the descriptions of the figures. Figure 2 shows that an appropriate dose for BH in rats is 10^9 cells per day or greater. Figure 3 shows that in these experiments BH did not permanently colonise the rat digestive tract. Figure 4 shows that BH is primarily found in the caecum. Strikingly, Figure 5 shows that administration of BH induces a statistically-significant reduction in *Enterobacteriaceae* in rat faeces as detected by qPCR.

Example 3 - Administration of bacterial lyophilisate in a rat model of IBS

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. The results are reported in Figure 6 and further details regarding the experiments are provided above in the descriptions of the figures. Figure 6 confirms that administration of BH lyophilisate causes a statistically-significant reduction in *Enterobacteriaceae*, which is mainly *E. coli*. As expected, an increase in BH is also observed.

Example 4 –Comparison of patient microbiota Blautia hydrogenotrophica

A Phase I clinical trial was conducted in which *Blautia hydrogenotrophica* (“Blautix”, strain deposited under accession number DSM 10507/14294) was administered to human patients having irritable bowel syndrome (IBS). Patients were administered Blautix during a dosing period (day 1-16) with the washout period being day 19-23. Blautix was found to be both safe and well tolerated. Patient microbiota *Blautia hydrogenotrophica* (Blautix) were measured at day 1, day 16 and at the end of the study (EOS) with results as follows:

25 Table 1:

Patient	Treatment	Blautix Day 1		Blautix Day 16		Blautix EOS	
		Mean	SD	Mean	SD	Mean	SD
Placebo	Healthy	4.30E+07	8.40E+07	4.79E+07	7.63E+07	3.59E+07	6.23E+07
Blautix	Healthy	2.92E+07	2.25E+07	6.35E+07	1.02E+08	2.58E+07	3.34E+07
Placebo	IBS	3.58E+07	3.45E+07	2.44E+07	3.13E+07	2.09E+07	2.47E+07
Blautix	IBS	3.17E+07	6.28E+07	8.53E+07	1.57E+08	3.71E+07	5.56E+07

Analysis shows trends in *Blautia hydrogenotropica* levels in patient stool samples (see Figure 7a). Participants receiving Blautix have increased levels of the bacteria in their stool samples following the dosing period (day 16). This demonstrates the successful delivery of Blautix to the gut. The levels return to normal following the washout period (End-of study; EOS). Thus Blautix does not permanently colonise the gut once dosing ceases. Both observations are consistent with the pre-clinical model.

Example 5 - Comparison of patient microbiota Enterobacteria.

Patient microbiota Enterobacteria were also measured during the Phase I clinical trial at day 1, day 16 and at the end of the study with results as follows:

Patient	Treatment	Enterobacteria Day 1		Enterobacteria Day 16		Enterobacteria EOS	
		Mean	SD	Mean	SD	Mean	SD
Placebo	Healthy	5.89E+08	1.00E+09	3.32E+08	6.26E+08	2.85E+08	4.28E+08
Blautix	Healthy	9.96E+08	1.06E+09	4.87E+08	6.64E+08	2.70E+08	3.76E+08
Placebo	IBS	7.85E+08	1.28E+09	3.01E+08	4.92E+08	2.45E+09	2.45E+09
Blautix	IBS	8.38E+09	1.96E+10	1.12E+09	1.45E+09	6.17E+08	9.91E+08

10

Analysis shows trends in Enterobacteria levels in patients stool samples (see Figure 7b). IBS patients treated with Blautix had a reduction in Enterobacteria. This is consistent with observations from the preclinical model.

Example 6 – Hydrogen breath test results

Breath hydrogen levels are a biomarker of Blautix activity – MoA involves metabolism of endogenous H₂ to produce acetate. Human subjects were administered lactulose and hydrogen (H₂) levels (Cmax) were sampled at four time points: day 1, day 2, day 15 and day 16. Hydrogen uncorrected results were converted to hydrogen corrected results.

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

25

mentioned as excluded above, was also a methane producer). Subject 4.09 (Placebo) was excluded due to an aberrant value.

The results of the corrected hydrogen analysis from the end of the dosing period (day 15/16) were compared to those from the baseline (day 1/2). 10 out of 12 patients (83%) receiving Blautix had a reduction in hydrogen levels over this period (Figure 8a and 8c). In contrast, 3 out of 6 (50%) patients receiving placebo had reduced hydrogen levels (Figure 8b and 8c).

Figure 9 shows the uncorrected and corrected hydrogen results for the Blautix (Verum) treatment group together with a statistical analysis of the results. The mean values for both uncorrected and corrected H₂ were found to differ between day 1 and day 15. After 13.5 days of treatment, a statistically significant (p < 0.05) decrease of H₂ in Cmax breath test was detected after lactulose stimulation. In contrast, for the placebo group, the mean was found to be equivalent for day 1 and day 15 (p > 0.05) (Figure 10). Thus, the mean for the treatment group (also referred to as the VERUM group), decreases between day 1 and day 15 whereas the mean for the placebo group is equivalent between day 1 and day 15 for both the uncorrected hydrogen results and the corrected hydrogen results (Figure 11).

15 **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.

Example 8 – The ability of *B. hydrogenotrophica* to alter the microbiota in human Microbiota Associated rat (HMA rat) model

Summary

25 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 a 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 a composition comprising the bacterial strain of *B. hydrogenotrophica* according to the invention, while the other half animals received a control solution. Day 28 after administration, the levels of microorganisms from faecal microbiota, previously found to be affected in IBS patients was analysed.

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.

5 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

10 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).

15 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

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

Results

25 **Figure 12** 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 13 reports on effect of administration of *B. hydrogenotrophica* on certain microorganisms in faecal microbiota, previously found to be affected in IBS patients. The level of *Enterobacteriaceae* species decreased after *B. hydrogenotrophica* administration.

30 Conclusions

Administration of *Blautia hydrogenotrophica* resulted in a reduction in *Enterobacteriaceae*.

Example 9 – 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 diarrhoea. 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 14 and 15.

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 14). Improvement of symptoms, of which one was diarrhoea, supports the use of Blautix for treating or preventing diarrhoea.

50% of patients receiving placebo reported an improvement in symptoms (Figure 14). High placebo response rates are an established phenomenon in IBS clinical studies. Xifaxan was recently approved to treat IBS based on much smaller improvements over placebo (see: <http://www.accessdata.fda.gov/spl/data/5ab6fceb-4d22-4480-81fc-8bc28c16770d/5ab6fceb-4d22-4480-81fc-8bc28c16770d.xml>).

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 15). The worsening of symptoms, one of which is diarrhoea, following cessation of Blautix dosing therefore also supports the use of Blautix in treating or preventing diarrhoea.

Sequences

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

```
1 tgcaagtcga gcgaaagcgct tacgacagaa cttcgaaaa aagatgttaag ggactgagcg
61 gcggacgggt gagtaacgcg tggtaacct gcctcataca gggggataac agttggaaac
121 ggctgctaat accgcataag cgcacggtat cgcatacatac agtgtaaaa actccgggtgg
181 tatgagatgg acccgcgatct gattagctat ttggaggggt aacggccac caaggcgacg
```

241 atcagtagcc ggcctgagag ggtgaacggc cacattggga ctgagacacg gcccagactc
 301 ctacgggagg cagcagtggg gaatattgca caatggggg aaccctgatg cagcgacgcc
 361 gcgtgaagga agaagtatct cggtatgtaa acttctatca gcagggaga aatgacggt
 421 acctgactaa gaagccccgg ctaactacgt gccagcagcc gcggtaatac gtagggggca
 481 agcgttatcc ggatttactg ggtgtaaagg gagcgtagac ggaagagcaa gtctgatgtg
 541 aaaggctggg gcttaacccc aggactgcat tggaaactgt ttttctttag tgccggagag
 601 gtaagcggaa ttccctagtgt agcggtgaaa tgcgtagata ttaggaggaa caccagtggc
 661 gaaggcggct tactggacgg taactgacgt tgaggctcga aagcgtgggg agcaaacagg
 721 attagataacc ctggtagtcc acgcccgtaaa cgatgaatac taggtgttgg ggagcaaagc
 781 tcttcggtgc cgcaagaaac gcaataagta ttccacctgg ggagtacgtt cgcaagaatg
 841 aaactcaaag gaattgacgg ggaccgcac aagcggtgga gcatgtggtt taattcgaag
 901 caacgcgaag aaccttacca agtcttgaca tcgatctgac cggttcgtaa tggAACCTT
 961 ctttcgggac agagaagaca ggtggtgcat gtttgcgtc agctcgtgtc gtgagatgtt
 1021 gggtaagtc ccgcaacgag cgcaacccct atcctcagta gccagcaggt gaagctgggg
 1081 actctgtgga gactgccagg gataacctgg aggaaggcgg ggacgcgtc aaatcatcat
 1141 gccccttatg atttgggcta cacacgtgct acaatggcgt aaacaaaggg aagcgagccc
 1201 gcgagggggga gcaaatccc aaaataacgt cccagttcgg actgcagtct gcaactcgac
 1261 tgcacgaagc tggaatcgct agtaatcgcg aatcagaatg tcgcggtaa tacgttcccg
 1321 ggtcttgcac acaccggcccg tcacaccatg ggagtcaagta acgcccgaag tc

20

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

1 caagtcaac gggattttt ttattgaaac ttccgtcgat ttaattttat tctagtggcg
 61 gacgggtgag taacgcgtgg gtaacctgcc ttatacaggg ggataacagt cagaaatggc
 121 tgctaataacc gcataagcgc acagagctgc atggctcagt gtaaaaact ccgggtgtat
 181 aagatggacc cgcgttggat tagcttggat gtggggtaac ggccaccaa ggacgcgtac
 241 catagccggc ctgagagggt gaacggccac attggactg agacacggcc cagactccta
 301 cgggaggcag cagtggggaa tattgcacaa tgggggaaac cctgatgcag cgacgcccgc
 361 tgaaggaaga agtatctcgg tatgtaaact tctatcagca gggaaatgt tgacggtacc
 421 tgactaagaa gccccggcta actacgtgcc agcagccgc gtaatacgtt gggggcaagc
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 781 ttccggtgcgg tcgcaaacgc agtaagtatt ccacctgggg agtacgttgc caagaatgaa
 841 actcaaaaggaa attgacgggg acccgacaaa gcgggtggagc atgtggttt attcgaagca
 901 acgcgaagaa ccttaccaag tcttgacatc cgcctgaccg atccttaacc ggatcttcc
 961 ttccggacag gcgagacagg tggtgcatgg ttgtcgttag ctcgtgtcgt gagatgttgg
 1021 gttaagtccc gcaacgagcg caaccctat cctcagtagc cagcattaa ggtgggcact
 1081 ctggggagac tgccaggat aacctggagg aaggcggggtaa tgacgtcaaa tcatacatgccc

40

1141 ccttatgatt tgggctacac acgtgctaca atggcgtaaa caaaggaaag cgagattgtg
 1201 agatggagca aatcccaaaa ataacgtccc agttcggact gtagtctgca acccgactac
 1261 acgaagctgg aatcgctagt aatcgccggat cagaatgccg cggtgaatac gttcccggt
 1321 cttgtacaca cccgcccgtca caccatggga gtcagtaacg cccgaagtca gtgaccta
 5 1381 tgcaaagaag gagctgccga aggccggacc gatgactggg gtgaagtcgt aacaaggt

SEQ ID NO:3 (consensus 16S rRNA sequence for *Blautia stercoris* strain 830)

TTKGTCGGCTCAGGATGAACGCTGGCGCGTCTAACACATGCAAGTCGAGCGAACGCTTACGACAGAACCTT
 CGGGGGAAAGATGTAAGGGACTGAGCGCGGACGGGTGAGTAACCGCTGGTAACCTGCCTCATACAGGGGGATAACA
 10 GTTGGAAACGGCTGCTAATACCGCATAAGCGCACAGTATCGCATGATACTAGTGTGAAAAACTCCGGTGGTATGAGAT
 GGACCCCGCTGATTAGCTAGTGGAGGGTAACGGCCACCAAGGCAGCATCGTAGCCGGCTGAGAGGGTGA
 ACGGCCACATTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGAATATTGACAATGGGGAAA
 CCCTGATGCGACGCCGCTGAAGGAAGAAGTATCTCGTATGTAAACTCTATCAGCAGGGAAAGAAAATGACGG
 15 TACCTGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGGTAATACGTAGGGGCAAGCGTTATCCGGATT
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 20 ACCCTGGTAGTCCACGCCGTAAACGATGAATACTAGGTGTTGGGAGCAAAGCTCTCGGTGCCGAGCAAACGCAA
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 CATGTGGTTATTGAAAGCAACCGAAGAACCTTACCAAGTCTTGACATCGATCTGACCGGTTCGTAATGGAACCTT
 25 TCCTCGGGACAGAGAAGACAGGTGGTGCATGGTGTGTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAA
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 AAGGCGGGGACGCTCAAATCATGCCCCTTATGATTGGCTACACACGTGCTACAATGGCGTAAACAAAGGG
 AAGCGAGCCCGGAGGGGGAGCAAATCCAAAAATAACGTCCCAGTTCGGACTGCACTGCAACTCGACTGCACGA
 AGCTGGAATCGTAGTAACTCGGAATCAGAATGTCGGGTGAATACGTTCCGGGTCTGTACACACCGCCCGTCAC
 30 ACCATGGGAGTCAGTAACGCCGAAGTCAGTGACCCAACCTAGGGAGGGAGCTGCCGAAGGCAGGGATTGATAACTG
 GGGTAGGTCTAGGGGGT

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

30 TTCATTGAGACTTCGGTGGATTAGATTCTATTCTAGTGGCGGACGGGTGAGTAACCGCTGGTAACCTGCCTTAT
 ACAGGGGGATAACAGTCAGAAATGGCTGCTAACACCGCATAAGCGCACAGAGCTGCATGGCTCAGTGTGAAAAACTC
 CGGTGGTATAAGATGGACCCCGCTGGATTAGCTTGGTGGGTAAACGGCCACCAAGGCAGCATAGCCG
 GCCTGAGAGGGTGAACGGCCACATTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGAATATTG
 CACAATGGGGAAACCTGATGCACCGACGCCCGTGAAGGAAGAAGTATCTCGTATGTAAACTCTATCAGCAGG
 35 GAAGATAGTGCAGGTACCTGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGGTAATACGTAGGGGCAAG
 CGTTATCCGGATTACTGGGTGTAAGGGAGCGTAGACGGTGTGGCAAGTCTGATGTGAAAGGCATGGCTAACCT
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 AAACAGGATTAGATACCCCTGGTAGTCCACGCCGTAAACGATGAATACTAGGTGTCNGGGAGCATGGCTCTCGGTG
 40 CCGTCGCAAACGCAGTAAGTATTCCACCTGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGGACCC
 GCACAAGCGGTGGAGCATGTGGTTAATTGAAAGCAACCGCAAGAACCTTACCAAGTCTTGACATCCGCCTGACCGA

TCCTTAACCGGATCTTCCTCGGGACAGGCAGACAGGTGGTGCATGGTGTGTCAGCTCGTGTGAGATGTT
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 GGGATAACCTGGAGGAAGGCGGGATGACGTCAAATCATCATGCCCTTATGATTGGGCTACACACGTGCTACAAT
 5 GGCATGAAACAAAGGGAAAGCGAGATCGTGAGATGGAGCAAATCCAAAAATAACGTCCCAGTCGGACTGTAGTCTGC
 AACCCGACTACACGAAGCTGGAATCGCTAGTAATCGCGGATCAGAATGCCGGTGAATACGTTCCGGGTCTGTA
 CACACCGCCCGTCACACCATGGGAGTCAGTAACGCCGAAGTCAGTGCACCTAACTGCAAAGAAGGAGCTGCCGAA

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

10 1 gatgaacgct ggcggcgtgc ttaacacatg caagtcgaac gaagcgatacg agaacggaga
 61 tttcggttga agttttctat tgactgagtg gcggacgggt gagtaacgcg tggtaaccc
 121 gccctataca gggggataac agtttagaaat gactgctaatt accgcataag cgcacagctt
 181 cgcacatgaaac ggtgtgaaaaa actgaggtgg tataggatgg acccgcgttg gattagctag
 241 ttgggtgaggt aacggccac caaggcgacg atccatagcc ggcctgagag ggtgaacggc
 15 301 cacattggga ctgagacacg gcccaaactc ctacgggagg cagcagtggg gaatattgca
 361 caatggggga aaccctgtatc cagcgcacgc gcgtgaagga agaagtatct cggtatgtaa
 421 acttctatca gcagggaaaga aagtgacggt acctgactaa gaagccccgg ctaattacgt
 481 gccagcagcc gcggtaatac gtaagggca agcgttatcc ggatttactg ggtgtaaagg
 541 gagcgttagac ggtttggcaa gtctgatgtg aaaggcatgg gctcaacctg tggactgcat
 20 601 tggaaactgt cagacttgag tgccggagag gcaagcggaa ttcctagtgt agcggtgaaa
 661 tgcgtagata ttaggaggaa caccagtggc gaaggcggcc tgctggacgg taactgacgt
 721 tgaggctcga aagcgtgggg agcaaaacagg attagatacc ctggtagtcc acgctgtaaa
 781 cgatgaatac taggtgtcgg gtggcaaagc cattcggtgc cgacgaaac gcaataagta
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 25 901 caagcggtgg agcatgtggg ttaattcgaa gcaacgcgaa gaaccttacc aaatcttgc
 961 atccctctga ccgggaagta atgttccctt ttcttcggaa cagaggagac aggtgggtca
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 30 1201 tacaatggcg taaacaaagg gaagcgaagg ggtgacctgg agcaaatttc aaaaataaacg
 1261 tctcagttcg gattgttagtc tgcaactcga ctacatgaag ctggaaatcgc tagtaatcgc
 1321 gaatcagaat gtcgcggtga atacgttccc gggcttgta cacaccgccc gtcacaccat
 1381 gggagtcagt aacgccccgaa gtcagtgacc caaccnaaag gagggagctg ccgaagggtgg
 1441 gactgataac tgggtga

35

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CLAIMS

1. A composition comprising a bacterial strain of the genus *Blautia*, for use in a method of reducing the level of *Enterobacteriaceae* in the gastrointestinal tract.
2. The composition of claim 1, wherein the *Enterobacteriaceae* is *E. coli*.
5. The composition of claim 1 or claim 2, wherein the composition is for use in a method of reducing the level of *Enterobacteriaceae* in the gastrointestinal tract in the treatment or prevention of IBS, Crohn's disease, ulcerative colitis, functional dyspepsia, diarrhoea, gastroenteritis, urinary tract infection or neonatal meningitis.
10. The composition of claim 1 or claim 2, wherein the composition is for use in treating or preventing diarrhoea, gastroenteritis, urinary tract infection or neonatal meningitis.
15. A composition comprising a bacterial strain of the genus *Blautia*, for use in the treatment of infections caused by pathogenic *Enterobacteriaceae*.
20. A composition comprising a bacterial strain of the genus *Blautia*, for use in the treatment of *E. coli* infection.
25. The composition of any preceding claim, wherein the bacterial strain is of *Blautia hydrogenotrophica*.
30. The composition of any of claims 1-6, wherein the bacterial strain is of *Blautia stercoris*.
35. The composition of any of claims 1-6, wherein the bacterial strain is of *Blautia wexlerae*.
40. The composition of any of claims 1-6, 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*.
45. The composition of claim 10, 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.
50. The composition of any of claims 1-6, 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*.
55. The composition of any of claims 1-6, 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*.
60. The composition of claim 1, wherein the composition comprises a bacterial strain of the species *Blautia hydrogenotrophica*, for use in a method of reducing the level of *Enterobacteriaceae* in the gastrointestinal tract in a subject diagnosed with IBS.
65. The composition of claim 1, wherein the composition comprises a bacterial strain of the species *Blautia stercoris*, for use in a method of reducing the level of *Enterobacteriaceae* in the gastrointestinal tract in a subject diagnosed with IBS.

16. The composition of claim 1, wherein the composition comprises a bacterial strain of the species *Blautia wexlerae*, for use in a method of reducing the level of *Enterobacteriaceae* in the gastrointestinal tract in a subject diagnosed with IBS.
17. The composition of any preceding claim, wherein the composition is for oral administration.
- 5 18. The composition of any preceding claim, wherein the composition comprises one or more pharmaceutically acceptable excipients or carriers.
19. The composition of any preceding claim, wherein the bacterial strain is lyophilised.
20. The composition of any preceding claim, wherein the bacterial strain is viable.
- 10 21. The composition of any preceding claim, wherein the composition comprises a single strain of the genus *Blautia*.
22. The composition of any preceding claim, which comprises the *Blautia* bacterial strain as part of a microbial consortium.
23. A food product comprising the composition of any preceding claim, for the use of any preceding claim.
- 15 24. A vaccine composition comprising the composition of any preceding claim, for the use of any preceding claim.
25. A method of reducing the level of *Enterobacteriaceae* in the gastrointestinal tract, comprising administering a composition comprising a bacterial strain of the genus *Blautia* to a patient in need thereof.
- 20 26. A method according to claim 25, wherein the *Blautia* is *B. hydrogenotrophica*.

FIG. 1
Measurement of BH population by q-PCR

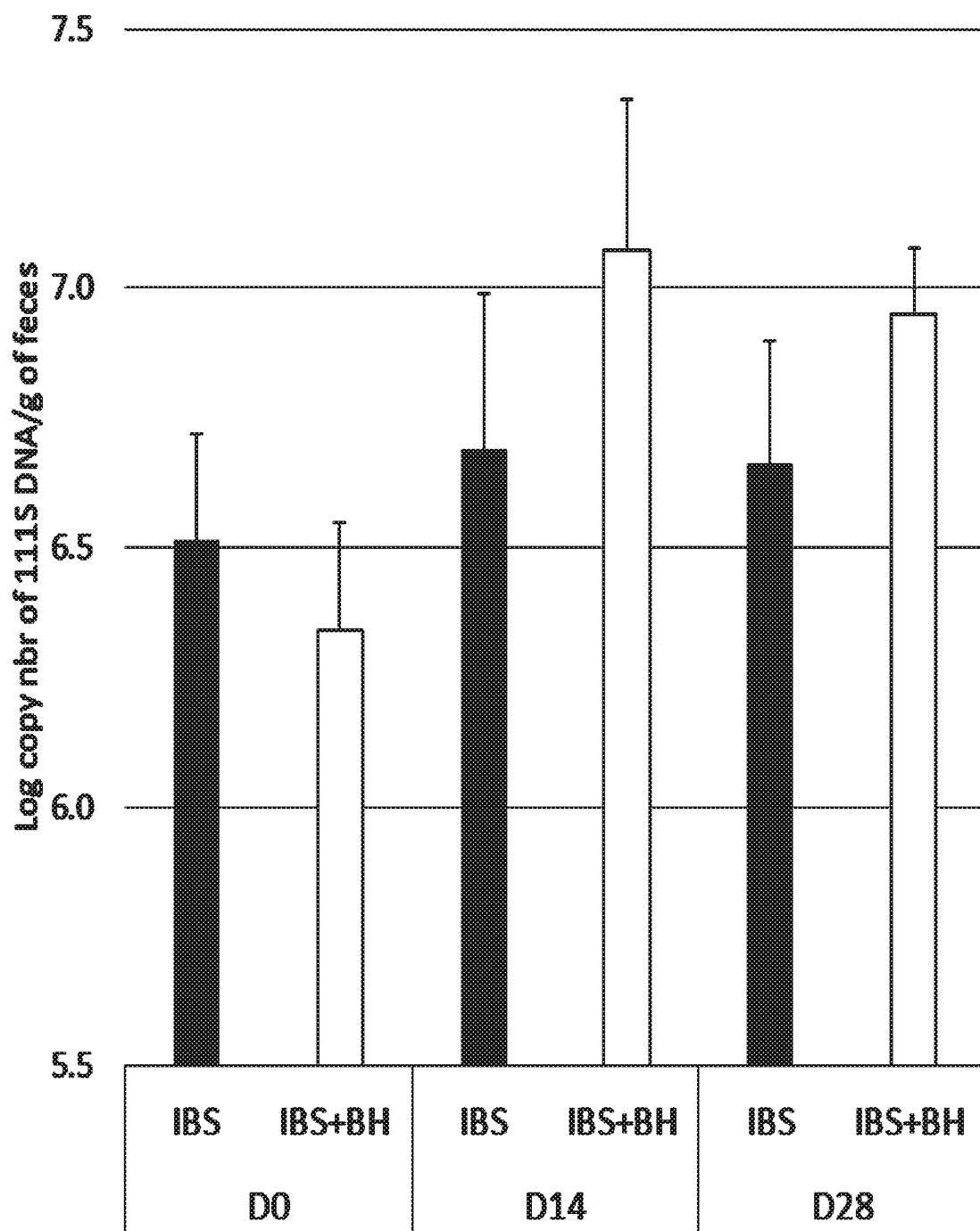
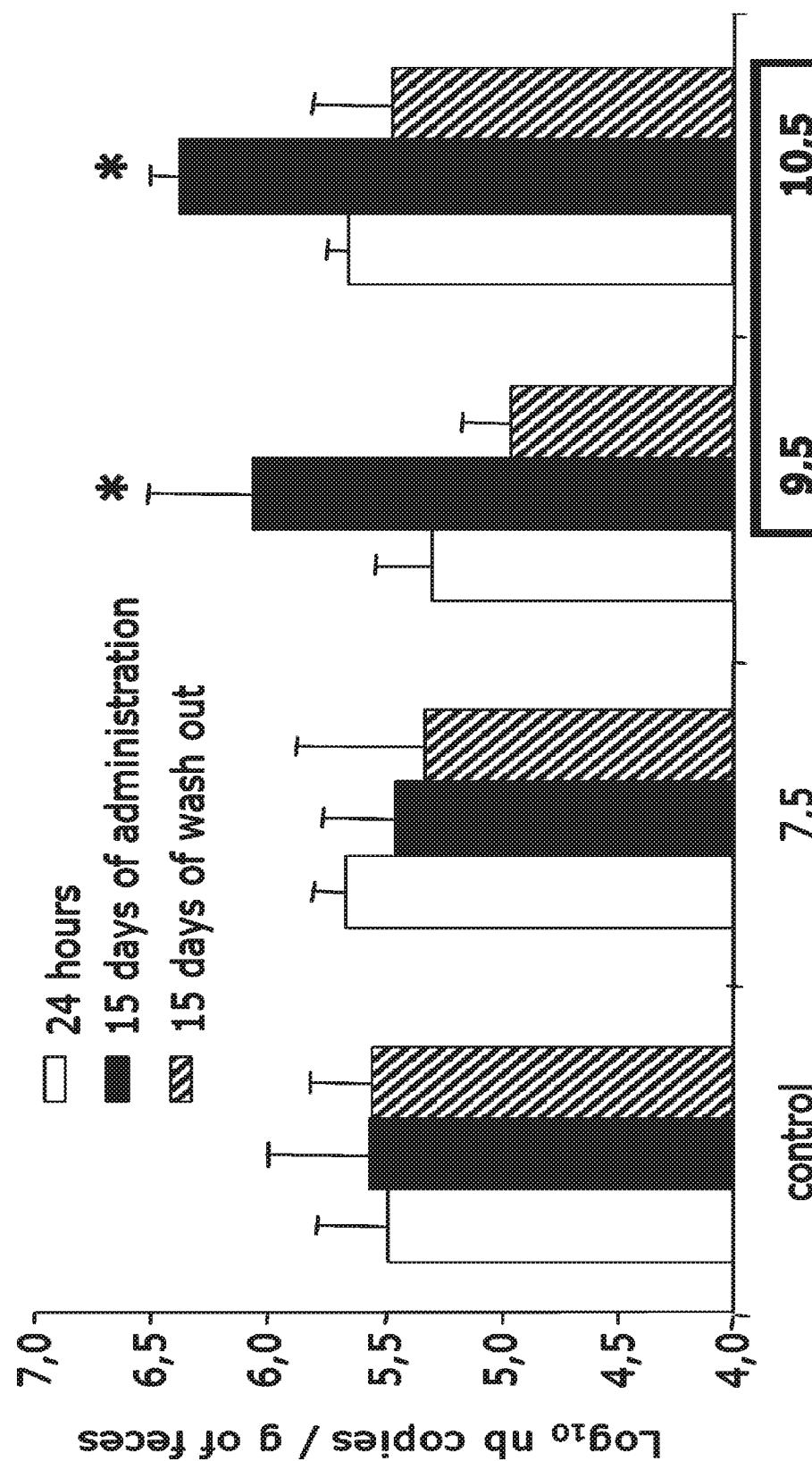


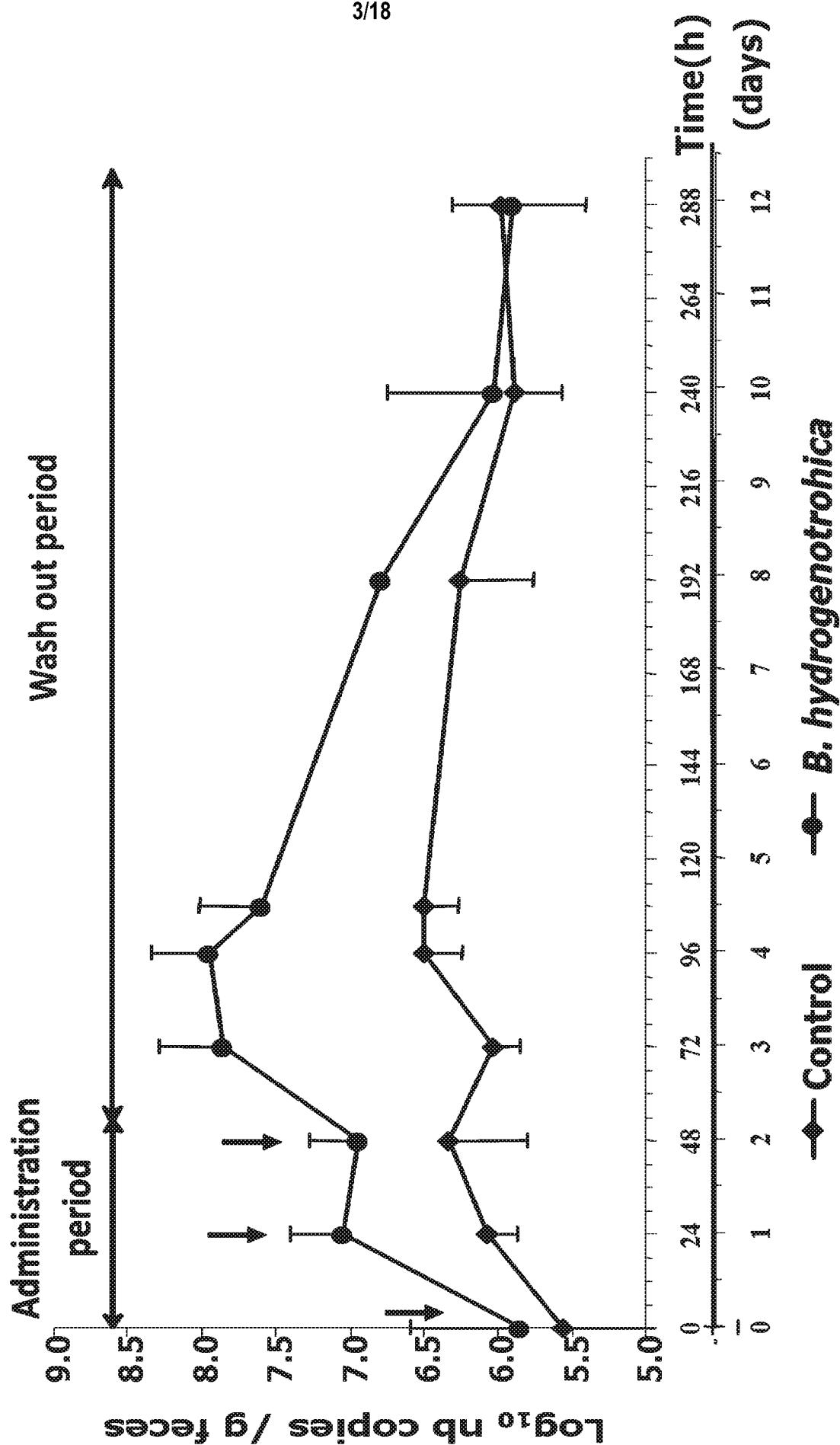
FIG. 2



B. hydrogenotrophica administrated (\log_{10} per ml)

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FIG. 3
Wash out period



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FIG. 4

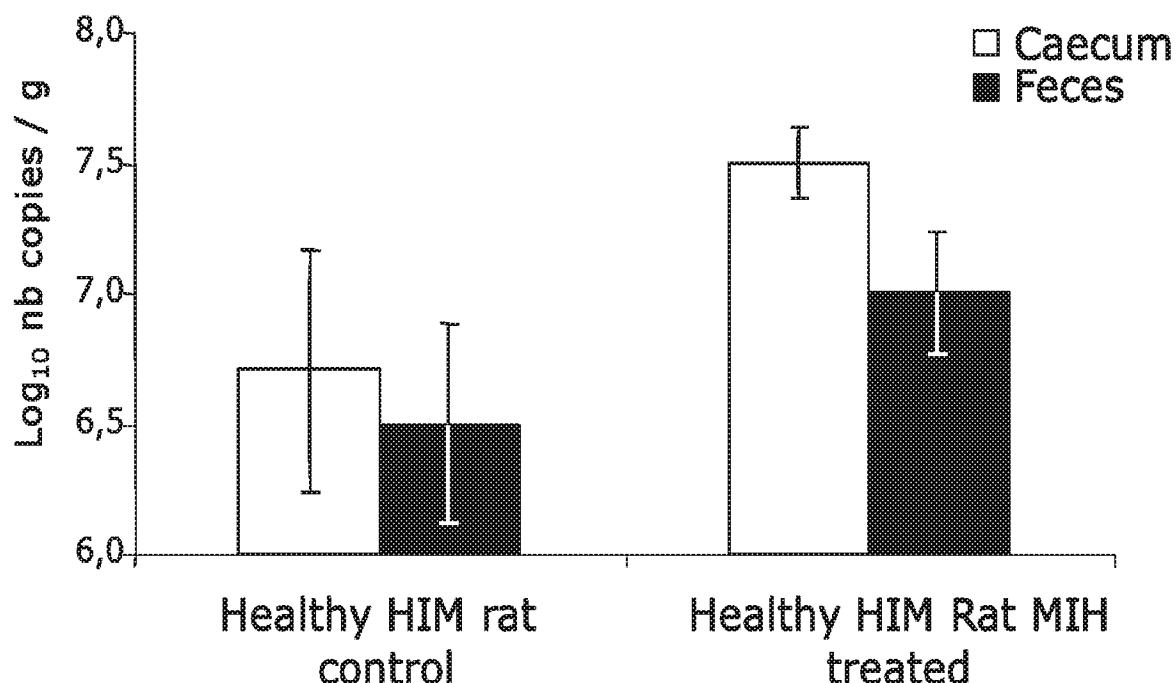


FIG. 6

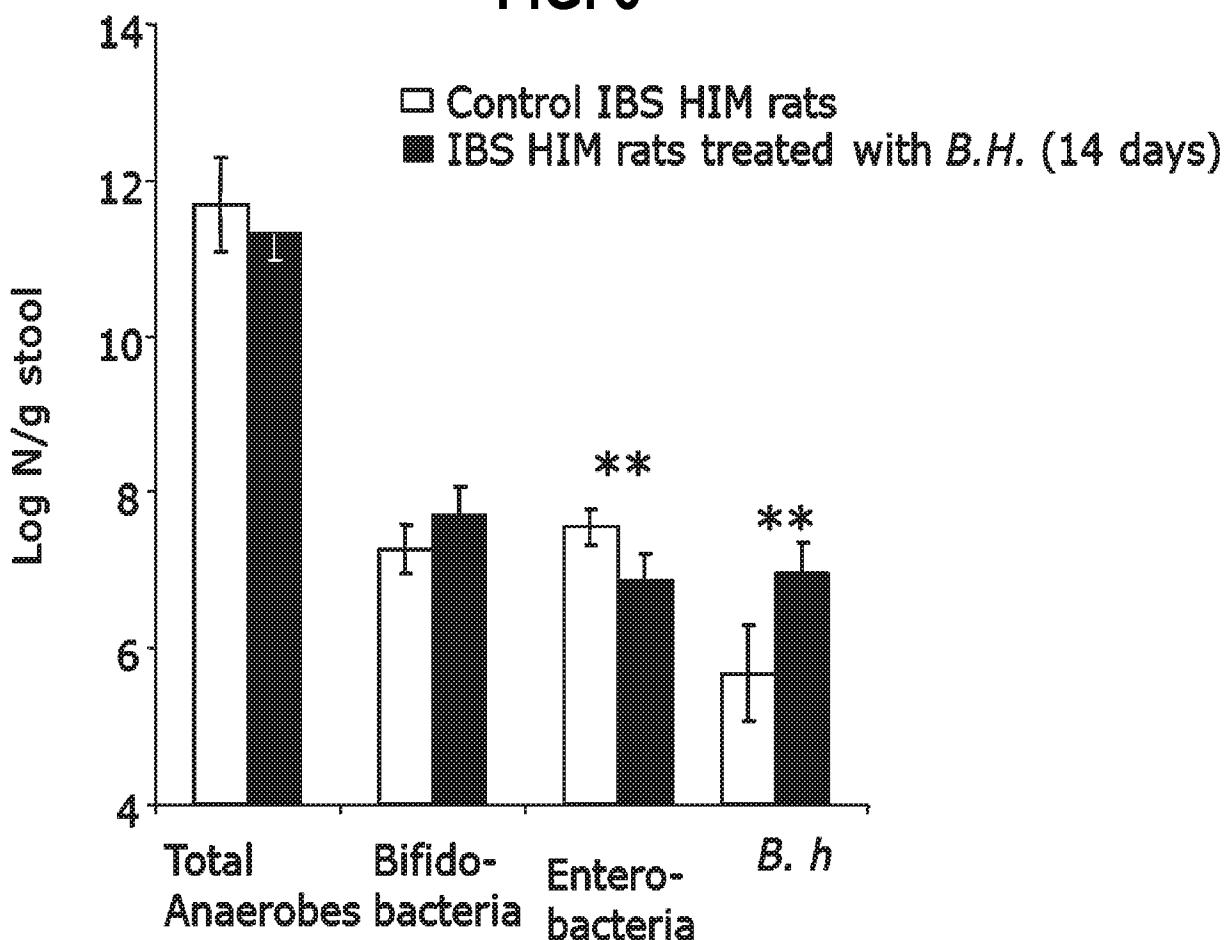
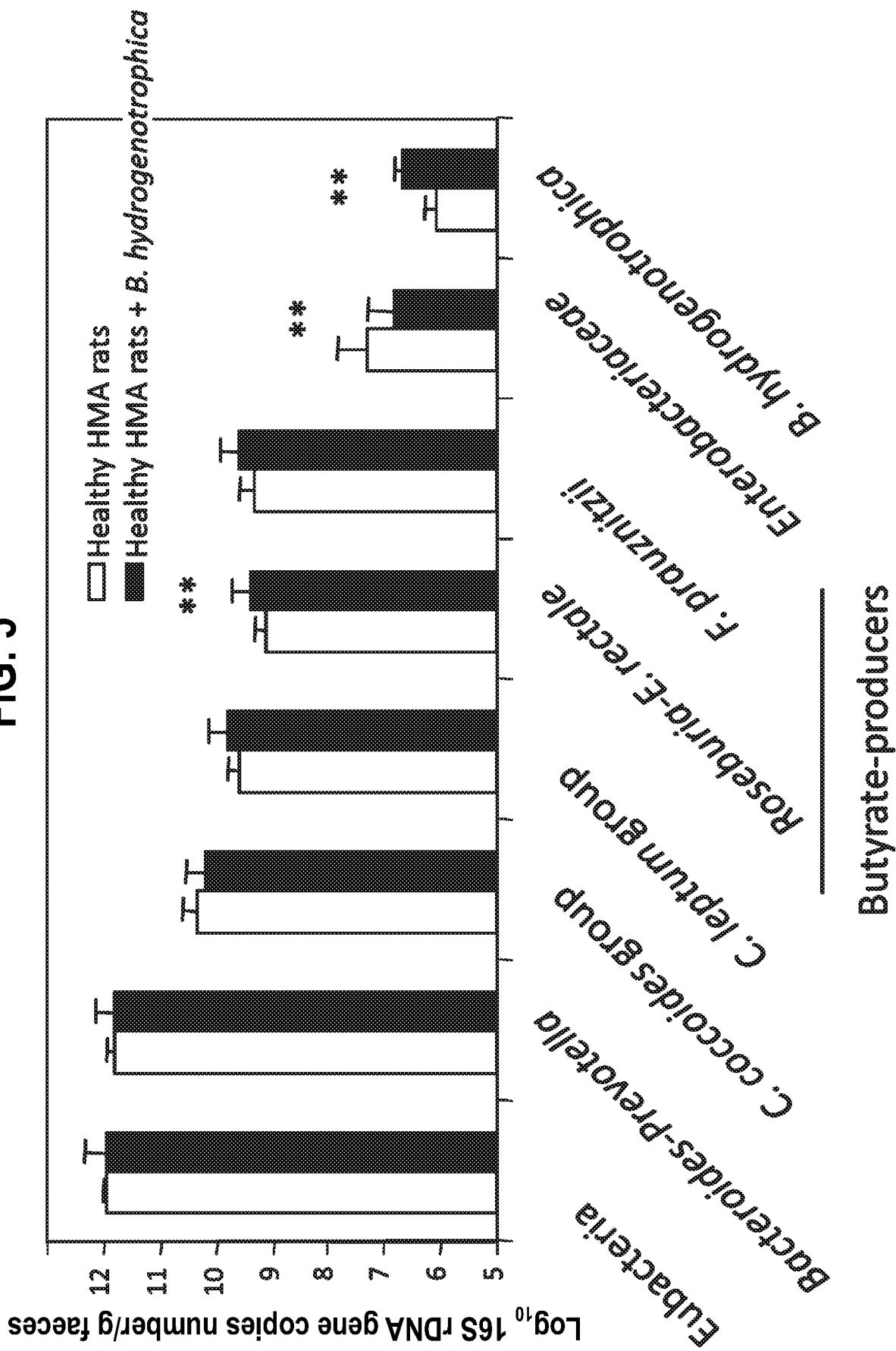
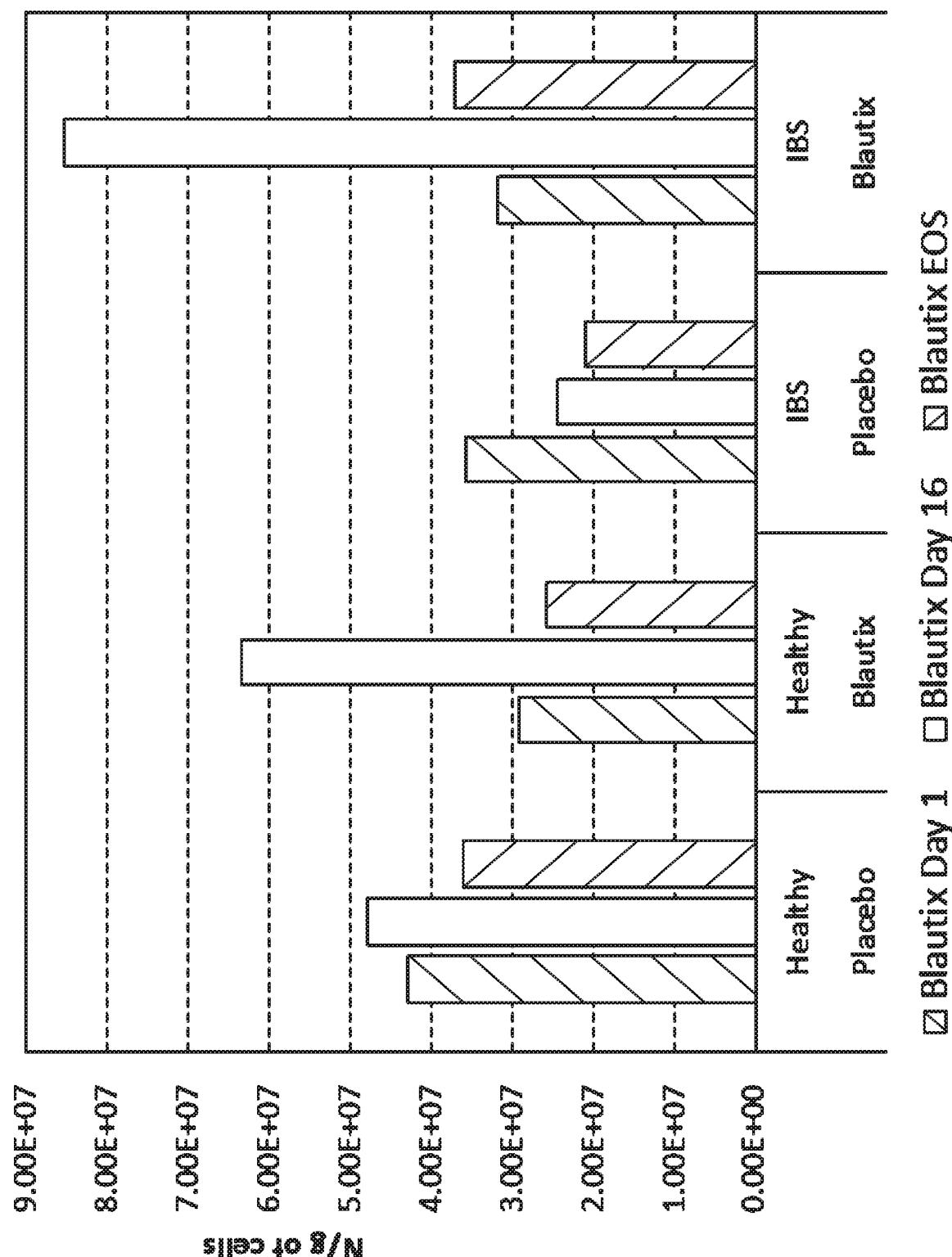


FIG. 5





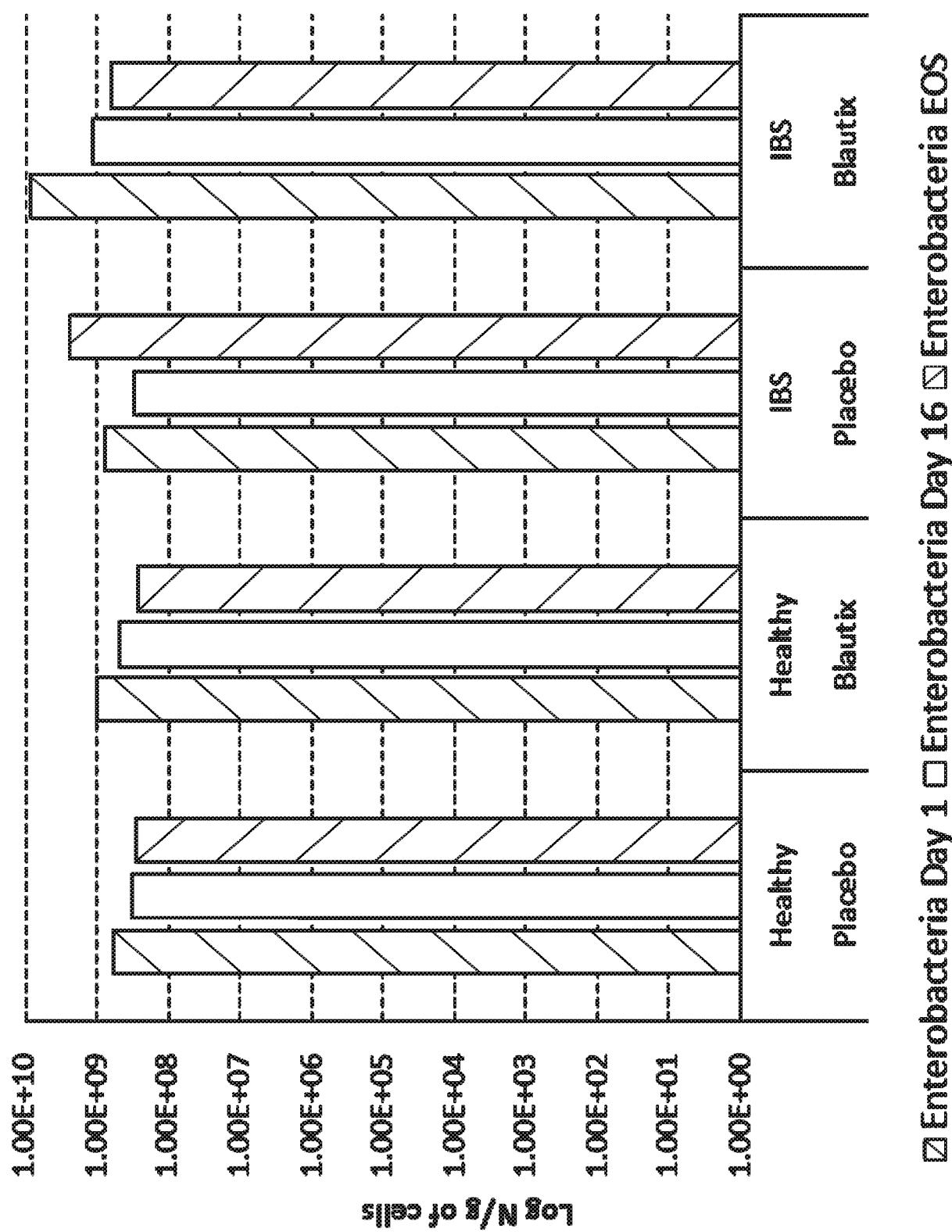


FIG. 7b

█ Enterobacteria Day 1 □ Enterobacteria Day 16 □ Enterobacteria EOS

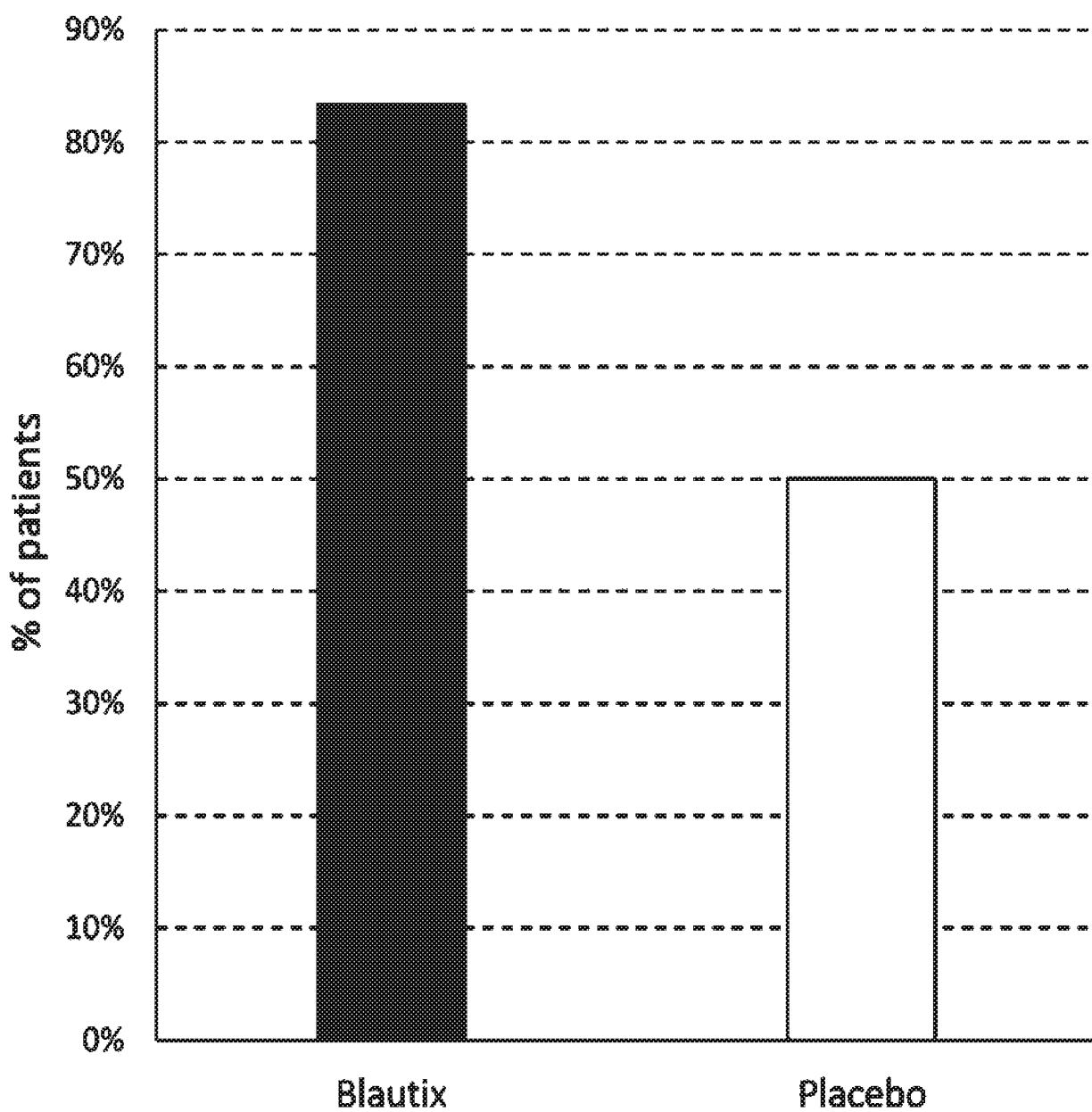
FIG. 8a Blautix 10/12 show reduction in hydrogen (83%)

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

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FIG. 8b Placebo 3/6 show reduction in hydrogen (50%)

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

FIG. 8c

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FIG. 9a

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FIG. 9b
Hydrogen uncorrected

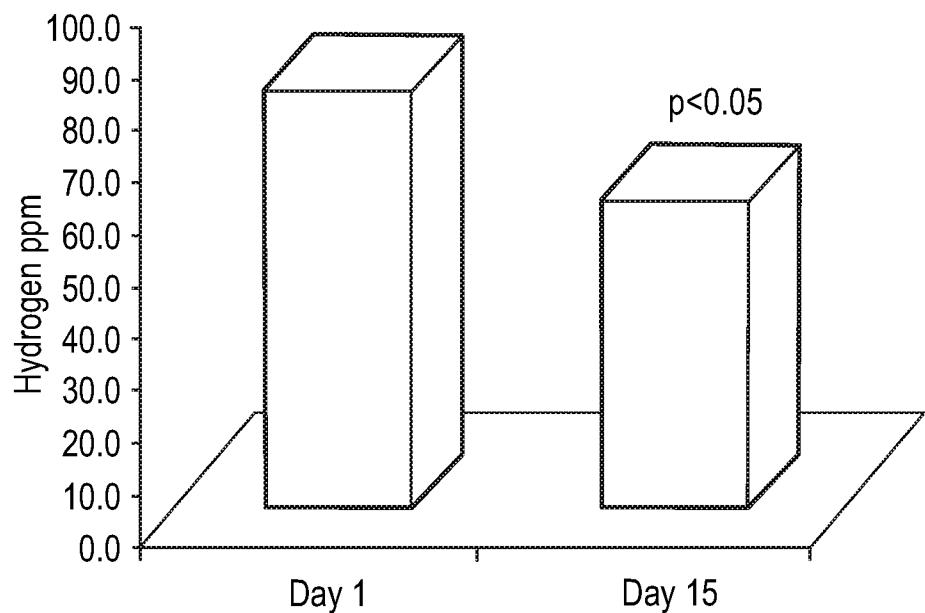
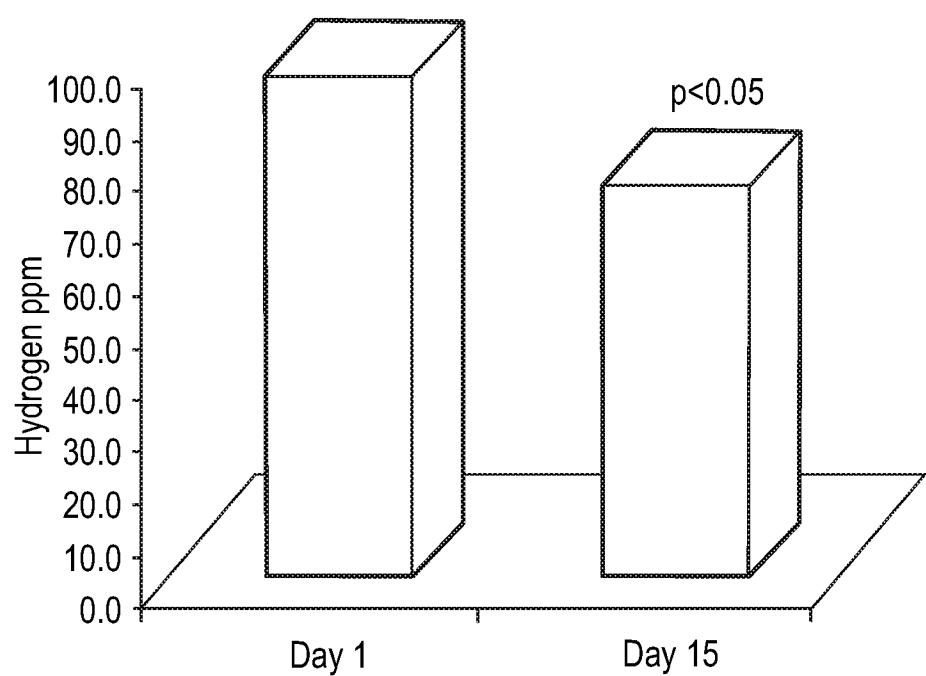


FIG. 9c
Hydrogen corrected



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PLACEBO Group

NUMBER	DAY1		DAY15		H2 corrected	RANDOM	DAY1	DAY15
	NUMBER	CMAX	CMAX	CMAX				
306	103.3	81.9			3.06	126.0	86.0	
308	42.3	43.3			3.08	55.0	58.0	
310	97.6	84.3			3.10	124.0	107.0	
314	35.0	42.4			3.14	55.0	53.0	
316	59.0	80.9			3.16	98.0	123.0	
323	41.1	62.2			3.23	58.0	79.0	
413	53.2	25.0			4.13	58.0	25.0	
	T Test unpaired		0.41620609			T Test unpaired		0.27096549
DAY1		DAY15			DAY1		DAY15	
MEAN	61.7	60.0			MEAN	62.0	75.9	
SD	27.7	23.5			SD	33.1	33.5	
N	7	7			N	7	7	
MEDIAN	53.2	62.2			MEDIAN	58.0	79.0	
MIN	35.0	25.0			MIN	55.0	25.0	
MAX	103.3	84.3			MAX	126.0	123.0	

FIG. 10a

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FIG. 10b
Hydrogen uncorrected placebo

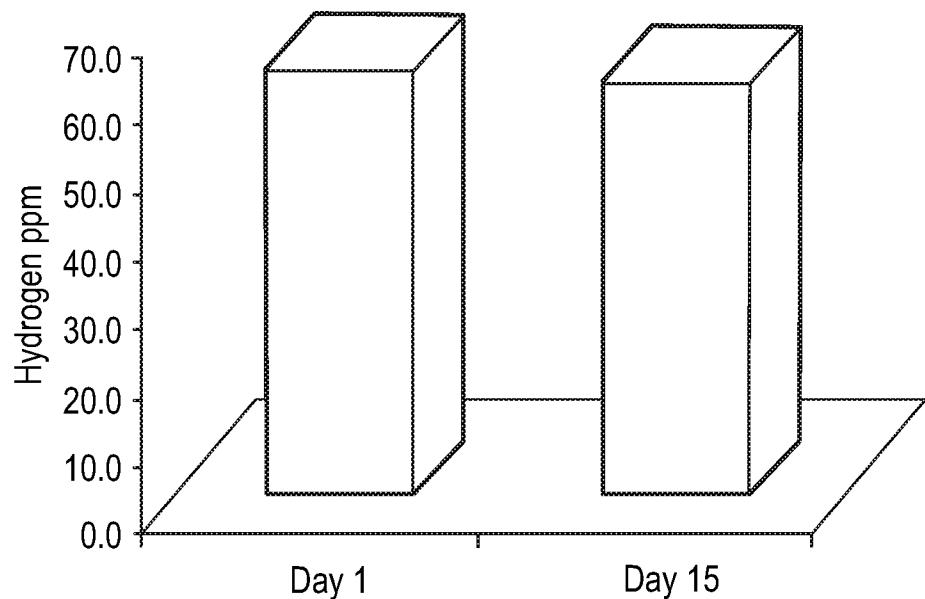
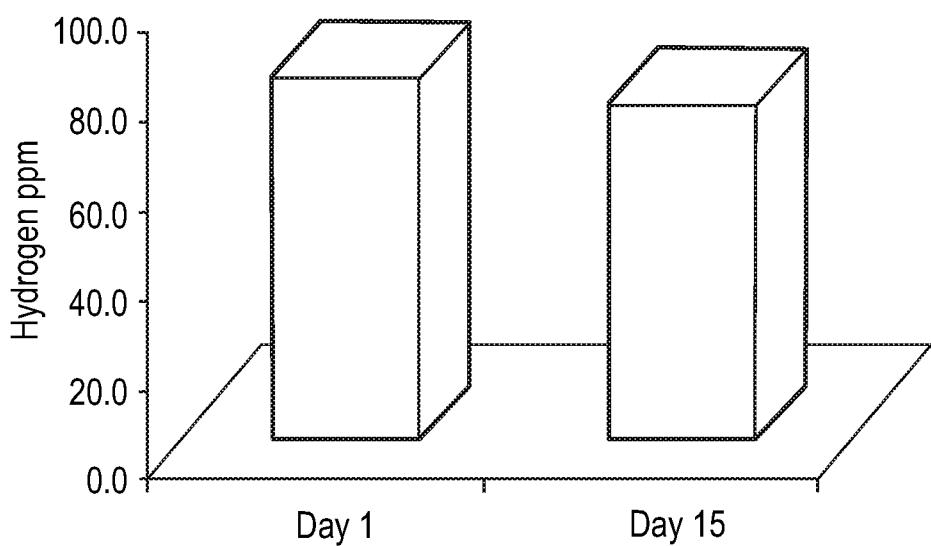


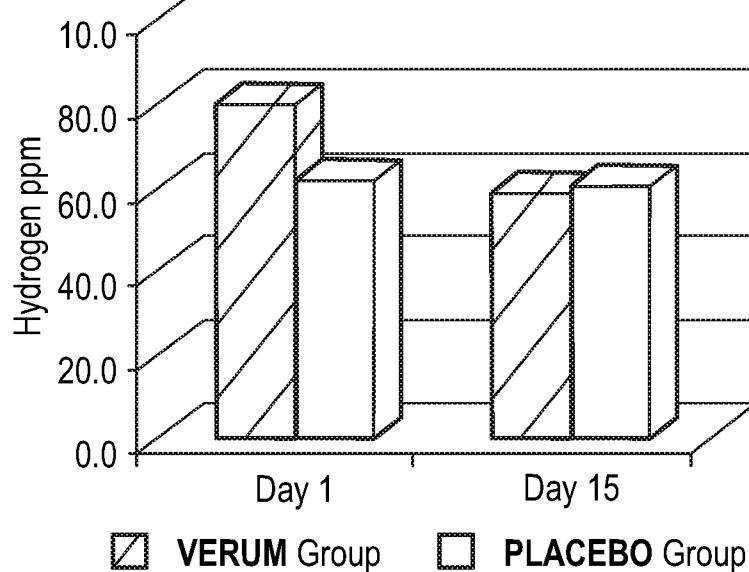
FIG. 10c
Hydrogen corrected placebo



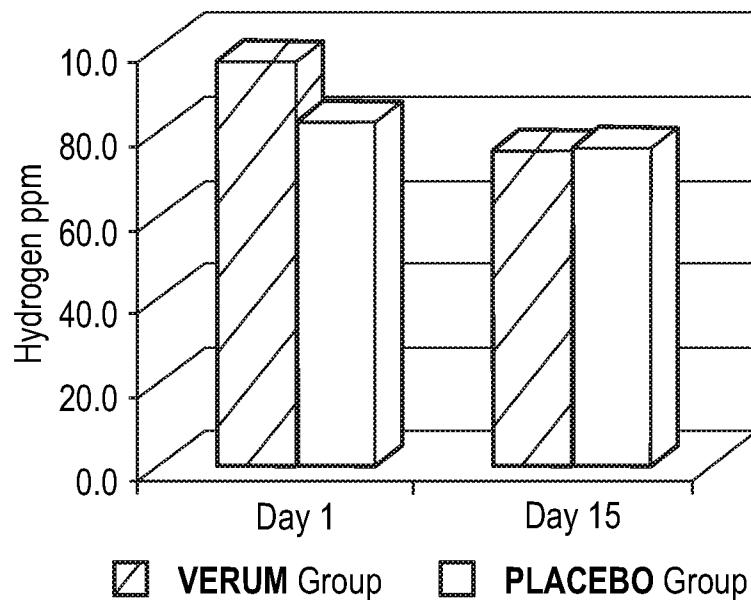
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FIG. 11a

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

Uncorrected Hydrogen**FIG. 11b**

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

Corrected Hydrogen

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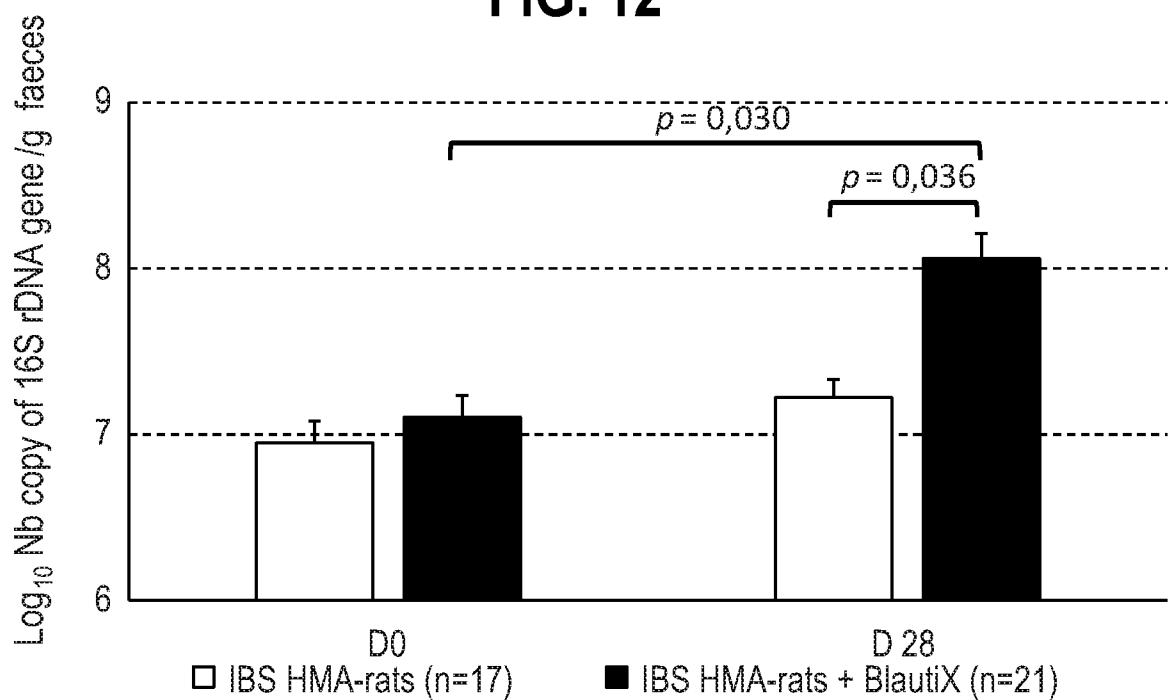
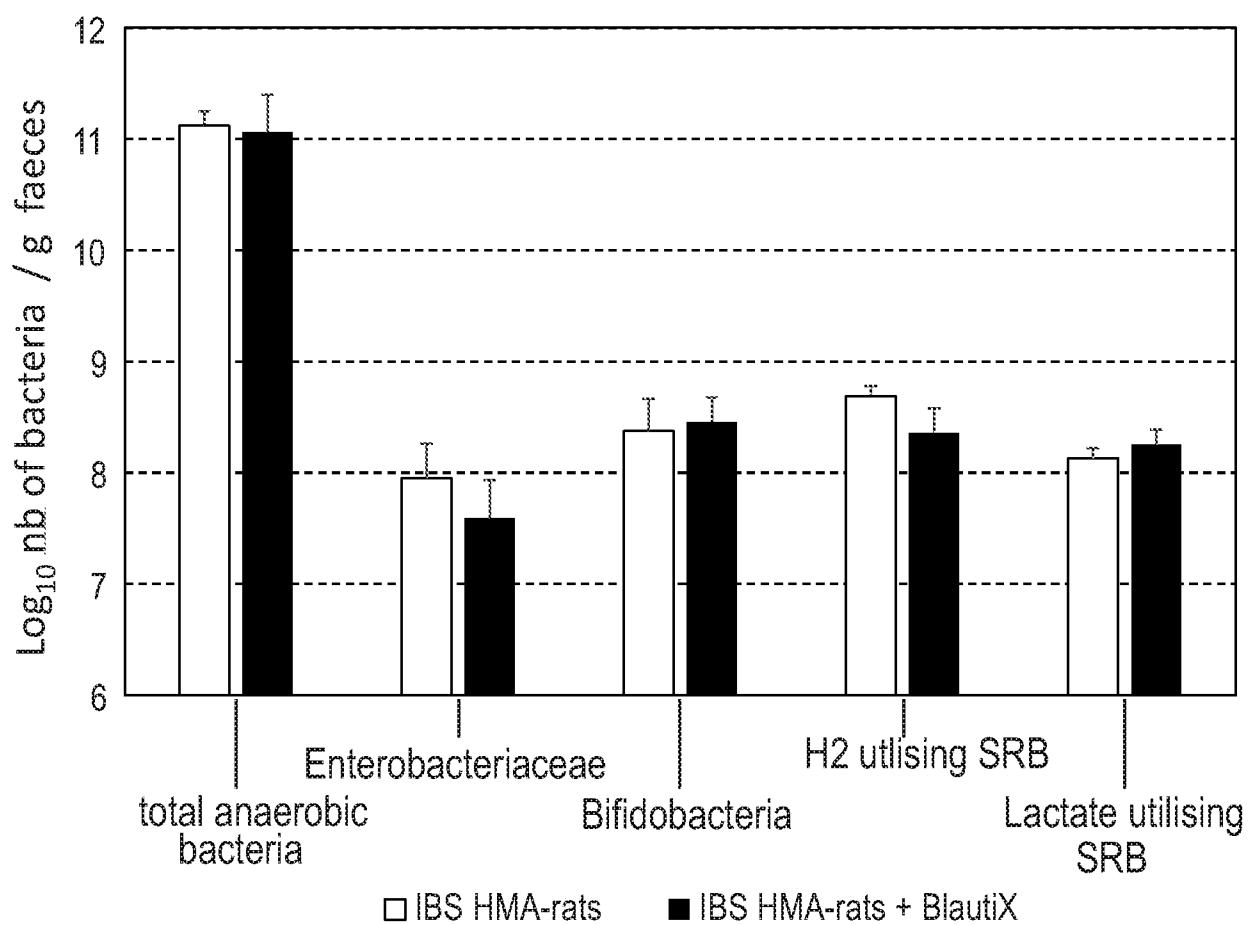
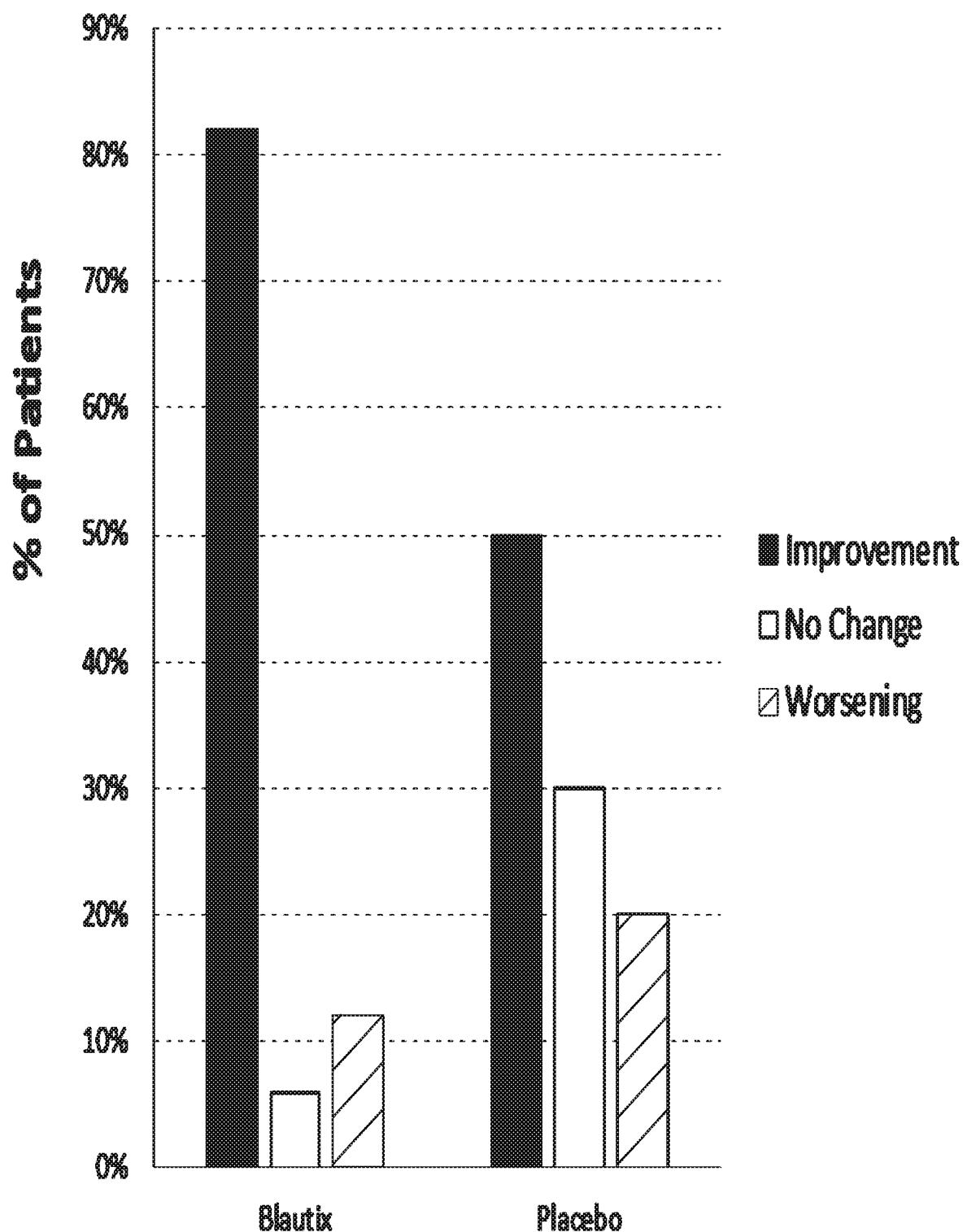
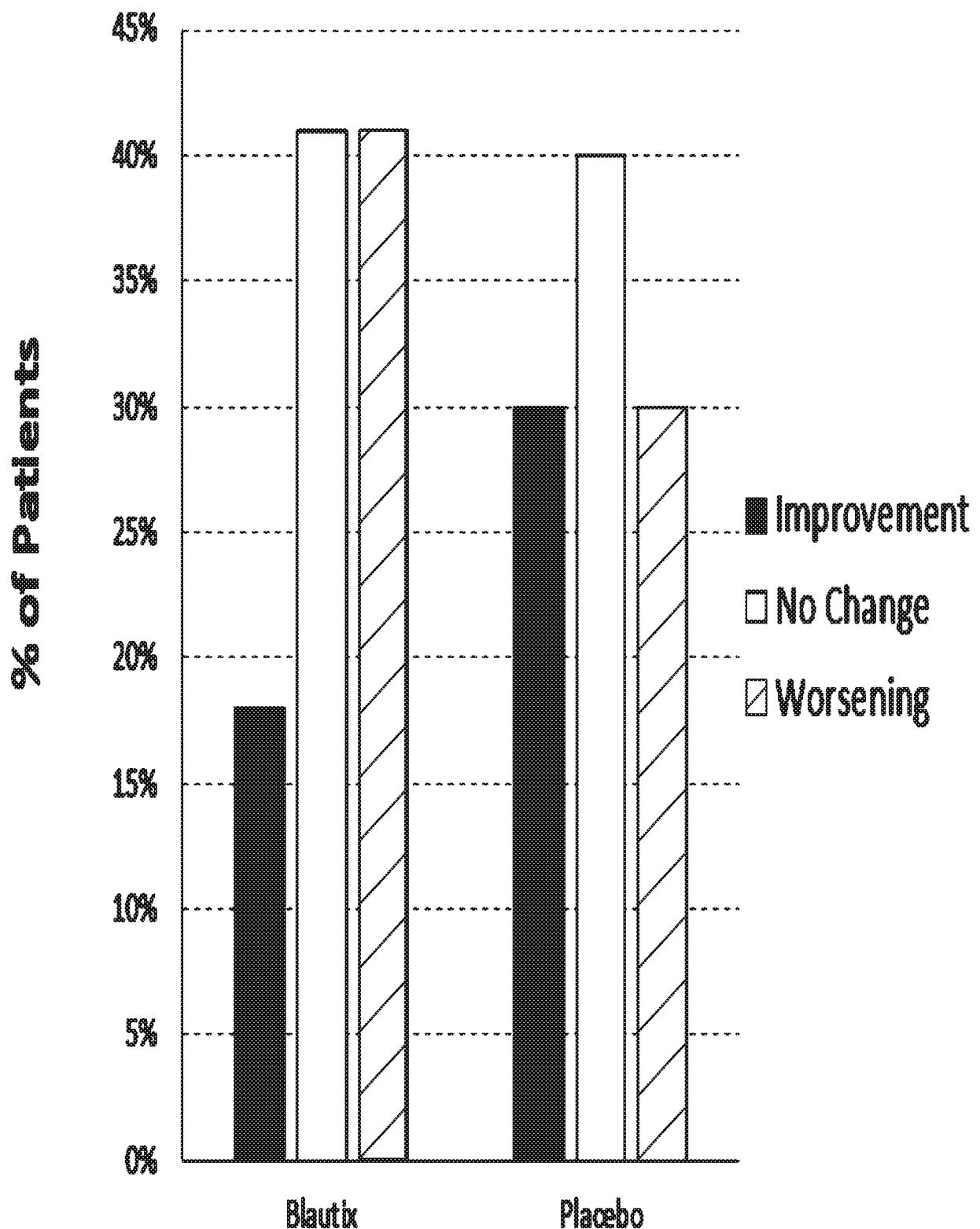
FIG. 12**FIG. 13**

FIG. 14

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FIG. 15



INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2017/052076

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K35/74 A61K9/19
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2016/184370 A1 (MCKENZIE GREGORY [US] ET AL) 30 June 2016 (2016-06-30) paragraphs [0058], [0109], [0121], [0132], [0140], [0144], [0149], [0165] - [0167], [0304], [0309] paragraphs [0436], [0440], [0490] examples 15,25; tables 15,19 ----- US 2010/247489 A1 (SAUR-BROSCH ROLAND [DE]) 30 September 2010 (2010-09-30) paragraph [0115]; claims 1-9 ----- WO 2012/142605 A1 (SAMARITAN HEALTH SERVICES [US]; CUNNINGHAM GLEN [US]) 18 October 2012 (2012-10-18) claims 1-6 ----- ----- -----	1-26 1-7, 14, 17-26 1-6, 17-25 -/-
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26 September 2017	16/10/2017
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Böhmerova, Eva

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2017/052076

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/07741 A1 (BORODY THOMAS J [AU]) 31 January 2002 (2002-01-31) claims 29-70 -----	1-26
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A	WO 2016/057671 A1 (UNIV VIRGINIA PATENT FOUND [US]) 14 April 2016 (2016-04-14) claims 31-37 -----	1-26
A	C. LIU ET AL: "Reclassification of Clostridium coccoides, Ruminococcus hansenii, Ruminococcus hydrogenotrophicus, Ruminococcus luti, Ruminococcus productus and Ruminococcus schinkii as Blautia coccoides gen. nov., comb. nov., Blautia hansenii comb. nov., Blautia hydrogenotrophica comb. nov., Blautia luti comb. nov", INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, vol. 58, no. 8, 1 August 2008 (2008-08-01), pages 1896-1902, XP055005236, ISSN: 1466-5026, DOI: 10.1099/ij.s.0.65208-0 the whole document -----	1-26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2017/052076

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
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		CA 2899926	A1	07-08-2014	
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		EP 2953474	A2	16-12-2015	
		EP 2956006	A2	23-12-2015	
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		HK 1218837	A1	17-03-2017	
		HK 1219022	A1	24-03-2017	
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		JP 2016509003	A	24-03-2016	
		KR 20150115888	A	14-10-2015	
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		US 2016184370	A1	30-06-2016	
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