IMMUNE MODULATION

Field of the Invention

The present invention relates to microorganisms that are able to positively modulate inflammatory disorders and which may be used in therapy or preventative medicine.

Background of the Invention

Bacteroides thetaiotaomicron has potent anti-inflammatory effects *in vitro* and *in vivo* (Kelly et al. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol. 2004 Jan;5(1):104-12). It modulates molecular signalling pathways of NF-κB (Kelly et al, Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol. 2004 Jan;5(1):104-12). In particular, it stops binding of the active component (RelA) of NF-κB to key genes in the nucleus, thereby preventing the activation of pro-inflammatory pathways (Kelly et al, Supra 2004). The full genome of *B. thetaiotaomicron* was sequenced and annotated by the Gordon Group (Washington University School of Medicine, USA) in 2003 [Xu *et al*, A genomic view of the human-Bacteroides thetaiotaomicron symbiosis. Science. 2003 Mar 28;299(5615):2074-6].

Summary of the Invention

The present invention is based on the discovery of a strain of *Bacteroides thetaiotaomicron* (BT) that has surprising efficacy against inflammatory disorders. Accordingly the strain of BT is useful as a therapeutic or in preventative medicine against inflammatory disorders and/or autoimmune disorders and /or allergic disorders.

According to a first aspect of the invention, there is a *Bacteroides thetaiotaomicron* deposited as NCIMB Accession Number 42341, or a derivative thereof.

According to a second aspect of the invention, there is a nutritional supplement comprising a *Bacteroides thetaiotaomicron* as defined in claim 1, and a nutritionally acceptable excipient, carrier or diluent.

According to a third aspect of the invention, there is a feedstuff, food product, dietary supplement, or food additive comprising a *Bacteroides thetaiotaomicron* as defined in claim 1.

According to a fourth aspect of the invention, a *Bacteroides thetaiotaomicron* as defined in claim 1, a composition as defined in claim 2 or 3, a pharmaceutical composition as defined in claim 4 or 5, a nutritional supplement as defined in claim 6 or 7 or a feedstuff, a food product, a dietary supplement, or a food additive as defined in claim 8 or 9, for use in modulating the inflammation of a tissue or an organ in a subject.

According to a fifth aspect of the invention, a *Bacteroides thetaiotaomicron* as defined in claim 1, a composition as defined in claim 2 or 3, a pharmaceutical composition as defined in claim 4 or 5, a nutritional supplement as defined in claim 6 or 7 or a feedstuff, a food product, a dietary supplement, or a food additive as defined in claim 8 or 9, for use in the treatment and/or prevention of a disorder in a subject; wherein said disorder is an inflammatory disorder and/or an autoimmune disorder.

According to a sixth aspect of the invention, a *Bacteroides thetaiotaomicron* as defined in claim 1, a composition as defined in claim 2 or 3, a pharmaceutical composition as defined in claim 4 or 5, a nutritional supplement as defined in claim 6 or 7 or a feedstuff, a food product, a dietary supplement, or a food additive as defined in claim 8 or 9 for use in reducing disruption to the colon of a subject, preferably said subject has IBD.

According to a seventh aspect of the invention, a *Bacteroides thetaiotaomicron* as defined in claim 1, a composition as defined in claim 2 or 3, a pharmaceutical composition as defined in claim 4 or 5, a nutritional supplement as defined in claim 6 or 7 or a feedstuff, a food product, a dietary supplement, or a food additive as defined in claim 8 or 9 for use in reducing the expression of one or more pro-inflammatory genes in a cell or cells of a subject.

According to an eighth aspect of the invention, a *Bacteroides thetaiotaomicron* as defined in claim 1, a composition as defined in claim 2 or 3, a pharmaceutical composition as defined in claim 4 or 5, a nutritional supplement as defined in claim 6 or 7 or a feedstuff, a food product, a dietary supplement, or a food additive as defined in claim 8 or 9 for use in increasing the percentage of

regulatory T cells (Tregs) in the alimentary canal or a section of the alimentary canal.

According to a ninth aspect of the invention, a process for producing a pharmaceutical composition according to claim 4 or 5, said process comprising admixing said *Bacteroides thetaiotaomicron* with a pharmaceutically acceptable excipient, carrier or diluent, wherein said *Bacteroides thetaiotaomicron* is optionally encapsulated in said process.

According to a tenth aspect of the invention, a method for modulating the inflammation of a tissue or an organ in a subject wherein said method comprises administering to the subject a *Bacteroides thetaiotaomicron* as defined in claim 1, a composition as defined in claim 2 or 3, a pharmaceutical composition as defined in claim 4 or 5, a nutritional supplement as defined in claim 6 or 7 or a feedstuff, a food product, a dietary supplement, or a food additive as defined in claim 8 or 9.

According to an eleventh aspect of the invention, a method for treating and/or preventing of an inflammatory disorder and/or an autoimmune disorder in a subject wherein said method comprises administering to the subject a *Bacteroides thetaiotaomicron* as defined in claim 1, a composition as defined in claim 2 or 3, a pharmaceutical composition as defined in claim 4 or 5, a nutritional supplement as defined in claim 6 or 7 or a feedstuff, a food product, a dietary supplement, or a food additive as defined in claim 8 or 9.

According to a twelfth aspect of the invention, a method for reducing disruption to the colon of a subject wherein said method comprises administering to the subject a *Bacteroides thetaiotaomicron* as defined in claim 1, a composition as defined in claim 2 or 3, a pharmaceutical composition as defined in claim 4 or 5, a nutritional supplement as defined in claim 6 or 7 or a feedstuff, a food product, a dietary supplement, or a food additive as defined in claim 8 or 9, preferably wherein the subject has IBD.

According to a thirteenth aspect of the invention, a method for reducing the expression of one or more pro-inflammatory genes in a cell or cells of a subject wherein said method comprises administering to the subject a *Bacteroides thetaiotaomicron* as defined in claim 1, a composition as defined in claim 2 or 3, a pharmaceutical composition as defined in claim 4 or 5, a nutritional supplement as defined in claim 6 or 7 or a feedstuff, a food product, a dietary supplement, or a food additive as defined in claim 8 or 9.

According to a fourteenth aspect of the invention, a method for increasing the percentage of Regulatory T cells (Tregs) in the alimentary canal or a section of the alimentary canal wherein said method comprises administering to the subject a *Bacteroides thetaiotaomicron* as defined in claim 1, a composition as defined in claim 2 or 3, a pharmaceutical composition as defined in claim 4 or 5, a nutritional supplement as defined in claim 6 or 7 or a feedstuff, a food product, a dietary supplement, or a food additive as defined in claim 8 or 9.

According to a fifteenth aspect of the invention, a *Bacteroides thetaiotaomicron* as defined in claim 1, a composition as defined in claim 2 or 3, a pharmaceutical composition as defined in claim 4 or 5, a nutritional supplement as defined in claim 6 or 7 or a feedstuff, a food product, a dietary supplement, or a food additive as defined in claim 8 or 9, for the manufacture of a medicament for modulating the inflammation of a tissue or an organ in a subject.

According to a sixteenth aspect of the invention, a *Bacteroides thetaiotaomicron* as defined in claim 1, a composition as defined in claim 2 or 3, a pharmaceutical composition as defined in claim 4 or 5, a nutritional supplement as defined in claim 6 or 7 or a feedstuff, a food product, a dietary supplement, or a food additive as defined in claim 8 or 9, for the manufacture of a medicament for the treatment and/or prevention of an inflammatory disorder and/or an autoimmune disorder in a subject.

According to a seventeenth aspect of the invention, a *Bacteroides thetaiotaomicron* as defined in claim 1, a composition as defined in claim 2 or 3, a pharmaceutical composition as defined in claim 4 or 5, a nutritional supplement as defined in claim 6 or 7 or a feedstuff, a food product, a dietary supplement, or a food additive as defined in claim 8 or 9 for the manufacture of a medicament for reducing disruption to the colon of a subject, preferably wherein the subject has IBD.

According to an eighteenth aspect of the invention, a *Bacteroides thetaiotaomicron* as defined in claim 1, a composition as defined in claim 2 or 3, a pharmaceutical composition as defined in claim 4 or 5, a nutritional supplement as defined in claim 6 or 7 or a feedstuff, a food product, a dietary supplement, or a food additive as defined in claim 8 or 9 for the manufacture of a medicament for reducing the expression of one or more pro-inflammatory genes in a cell or cells of a subject.

According to a nineteenth aspect of the invention, a *Bacteroides thetaiotaomicron* as defined in claim 1, a composition as defined in claim 2 or 3, a pharmaceutical composition as defined in claim 4 or 5, a nutritional supplement as defined in claim 6 or 7 or a feedstuff, a food product, a dietary supplement, or a food additive as defined in claim 8 or 9 for the manufacture of a medicament for increasing the percentage of Regulatory T cells (Tregs) in the alimentary canal or a section of the alimentary canal.

Brief Description of the Figures

The invention is described with reference to the accompanying figures, wherein:

Figure 1 illustrates the attenuation of colitis via expansion of Treg cells with *B. thetaiotaomicron* strain BT2013 in a DSS induced colitis model.

Figure 2 illustrates that *B. thetaiotaomicron* strain BT2013 does not influence Treg cells, but influences Teff cells, in conventional mice;

Figure 3: illustrates the myeloperoxidase (MPO) activity in ileum (a) and caecum (b) of mice dosed with DSS with or without a daily intake of *B. thetaiotaomicron*

Figure 4: illustrates histopathology in ascending colon of female C57Bl/6 mice dosed with DSS (a) or DSS and *B. thetaiotaomicron* (b)

Figure 5: illustrates the mean histopathological tissue scores for the ascending colon from mice colonised with *B. thetaiotaomicron* strains E1 and BT2013 during DSS-induced colitis;

Figure 6: illustrates the expression of proinflammatory genes (IL-1β and IL-6) and anti-inflammatory gene (IL-10) in the ascending colon of mice treated with *B. thetaiotaomicron* strains E1, E2 and BT2013;

Figure 7: illustrates the expression of IL-8 in Caco-2 cells incubated with PMA and medium or bacterial cells E1, E2 and BT2013.

Detailed Description of the Invention

The present invention is based on the finding that BT strain BT2013 has more potent anti-inflammatory effects compared to control BT strains.

BT strain BT2013 has been deposited under Accession number 42341 on 3 December 2014 at National Collections of Industrial, Food and Marine Bacteria (NCIMB) at NCIMB Ltd, Ferguson Building, Craibstone Estate, Bucksburn,

Aberdeen, UK, AB21 9YA. The deposit was made under the terms of the Budapest Treaty. The deposit was made by GT Biologics Ltd. (Life Sciences Innovation Building, Aberdeen, AB25 2ZS, Scotland). GT Biologics Ltd. has subsequently changed its name to 4D Pharma Research Limited.

Derivative

The present invention encompasses derivatives of the deposited strain. The term "derivative" includes daughter strains (progeny) or stains cultured (sub-cloned) from the original but modified in some way (including at the genetic level), without altering negatively the biological activity, i.e. the derivative strain will have at least the same immune modulatory activity as the original BT2013 strain.

Biotypes

A genome sequence for strain BT2013 is provided in SEQ ID NO:1.

Bacterial strains that are biotypes of the bacterium deposited under accession number NCIMB 42341 are also expected to be effective for treating or preventing inflammatory disorders and/or autoimmune disorders and/or allergic disorders. A biotype is a closely related strain that has the same or very similar physiological and biochemical characteristics.

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 the bacterium deposited under accession number NCIMB 42341.

Alternatively, strains that are biotypes of the bacterium deposited under accession number NCIMB 42341 and that are suitable for use in the invention may be identified by sequencing other nucleotide sequences for the bacterium deposited under accession number NCIMB 42341. 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). Other suitable sequences for use in identifying biotype strains may include hsp60 or repetitive sequences such as BOX, ERIC, $(GTG)_5$, or REP (Masco *et al.* (2003) *Systematic and Applied Microbiology,* 26:557-563). 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 the bacterium deposited under accession number NCIMB 42341.

In certain embodiments, the bacterial strain for use in the invention has a genome with sequence identity to SEQ ID NO:1. In preferred embodiments, the bacterial strain for use in the invention has a genome with at least 90% sequence identity (e.g. at least 92%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity) to SEQ ID NO:1 across at least 60% (e.g. at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of SEQ ID NO:1. For example, the bacterial strain for use in the invention may have a genome with at least 90% sequence identity to SEQ ID NO:1 across 70% of SEQ ID NO:1, or at least 90% sequence identity to SEQ ID NO:1 across 80% of SEQ ID NO:1, or at least 90% sequence identity to SEQ ID NO:1 across 90% of SEQ ID NO:1, or at least 90% sequence identity to SEQ ID NO:1 across 100% of SEQ ID NO:1, or at least 95% sequence identity to SEQ ID NO:1 across 70% of SEQ ID NO:1, or at least 95% sequence identity to SEQ ID NO:1 across 80% of SEQ ID NO:1, or at least 95% sequence identity to SEQ ID NO:1 across 90% of SEQ ID NO:1, or at least 95% sequence identity to SEQ ID NO:1 across 100% of SEQ ID NO:1, or at least 98% sequence identity to SEQ ID NO:1 across 70% of SEQ ID NO:1, or at least 98% sequence identity to SEQ ID NO:1 across 80% of SEQ ID NO:1, or at least 98% sequence identity to SEQ ID NO:1 across 90% of SEQ ID NO:1, or at least 98% sequence identity to SEQ ID NO:1 across 100% of SEQ ID NO:1.

Alternatively, strains that are biotypes of the bacterium deposited under accession number NCIMB 42341 and that are suitable for use in the invention may be identified by using the accession number NCIMB 42341 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 certain embodiments, strains that are biotypes of the bacterium deposited under accession number NCIMB 42341 and that are suitable for use in the invention are strains that provide the same pattern as the bacterium deposited under accession number NCIMB 42341 when analysed by amplified ribosomal DNA restriction analysis (ARDRA), for example when using Sau3AI restriction enzyme (for exemplary methods and guidance see, for example, Srůtková *et al*.

(2011) *J. Microbiol. Methods*, 87(1):10-6). Alternatively, biotype strains are identified as strains that have the same carbohydrate fermentation patterns as the bacterium deposited under accession number NCIMB 42341.

Bacterial strains that are biotypes of the bacterium deposited under accession number NCIMB 42341 and that are useful in the compositions and methods of the invention may be identified using any appropriate method or strategy. For example, bacterial strains that have similar growth patterns, metabolic type and/or surface antigens to the bacterium deposited under accession number NCIMB 42341 may be useful in the invention. A biotype strain will have comparable immune modulatory activity to the NCIMB 42341 strain. For example, a biotype strain will elicit comparable effects on the DSS-induced colitis models and comparable effects on Treg levels, MPO enzymatic activity, inflammation-associated gene expression and colon histopathology to the effects shown in the Functional Assays, which may be identified by using the protocols described in the Functional Assays.

Disorders

The *Bacteroides thetaiotaomicron* strain BT2013 may be used for the treatment and/or prevention of a disorder in a subject, wherein said disorder is an inflammatory disorder and/or an autoimmune disorder.

In one embodiment, the disorder affects the alimentary canal, a section of the alimentary canal, the liver, liver cells, immune cells, epithelial cells, epidermal cells, neuronal cells, endothelial cells, fibroblasts, the pancreas, and/or pancreatic cells (such as the islets of Langerhans).

Examples of sections (i.e. parts) of the alimentary canal include the oesophagus, the stomach and the intestine (such as the small intestine (e.g. the duodenum, the jejunum and the ileum) and/or the large intestine (e.g. the caecum, ascending colon, transverse colon, descending colon, and sigmoid colon)).

Examples of epithelial cells include intestinal epithelial cells. Examples of immune cells include dendritic cells, monocytes/macrophages, Tcells and neutrophils.

In one embodiment, the disorder is selected from the group consisting of:

1. Organ associated disorders such as irritable bowel syndrome (IBS), inflammatory bowel disease including Crohn's disease and ulcerative colitis, necrotising enterocolitis, pouchitis, coeliac disease, multiple sclerosis (brain), type I diabetes, Goodpasture's syndrome, Hashimoto thyroiditis, chronic active hepatitis, cardiomyopathy, uveitis and rhinitis.

2. Systemic disorders such as rheumatoid arthritis, systemic lupus erythematosus, scleroderma, psoriasis, atopic dermatitis, vitiligo, multiple sclerosis, alopecia areata, sarcoidosis, polymyositis and combinations thereof.

In one aspect, the disorder affects the intestine.

In one aspect, the disorder is an inflammatory disorder. For example, the disorder is an inflammatory bowel disorder (IBD) such as Crohn's disease.

In one aspect, the disorder is an autoimmune disorder. For example, the autoimmune disorder is selected from the group consisting of ulcerative colitis, pouchitis, rheumatoid arthritis, psoriasis, multiple sclerosis, type I diabetes, allergies (including coeliac disease), atopic dermatitis and rhinitis.

Subject

In one embodiment, the subject is a monogastric animal.

Examples of monogastric animals include poultry, humans, rats, pigs, dogs, cats, horses and rabbits.

In another embodiment, the subject is a mammal such as a monogastric mammal.

Examples of monogastric mammals include omnivores (such as humans, rats, and pigs), carnivores (such as dogs and cats), and herbivores (such as horses and rabbits).

Preferably, the subject is a human.

In one aspect, the subject has a disorder is selected from the group consisting of inflammatory bowel disorder (IBD), colitis, rheumatoid arthritis, psoriasis, multiple sclerosis, type I diabetes, coeliac disease, atopic dermatitis, rhinitis, irritable bowel syndrome (IBS), ulcerative colitis, pouchitis, Crohn's disease, functional dyspepsia, atopic diseases, necrotising enterocolitis, non alcoholic fatty liver disease, gastrointestinal infection and combinations thereof. For example, the subject has IBD.

Modulation/regulation

The terms "modulation" and "regulation" may be used interchangeably herein.

In one embodiment The *B. thetaiotaomicron* strain BT2013 is used to modulate the inflammation of a cell, a tissue or an organ in a subject.

In one embodiment, the term "modulation" refers to an increase and/or induction and/or promotion and/or activation. In an alternative embodiment, the term "modulation" refers to a decrease and/or reduction and/or inhibition.

In one embodiment, the term "regulation" refers to an upregulation. In an alternative embodiment, the term "regulation" refers to a downregulation.

In one embodiment, the *B. thetaiotaomicron* strain BT2013 as described herein reduces the inflammation of a cell, a tissue or an organ. For example, inflammation of the alimentary canal, a section (i.e. part) of the alimentary canal (such as the intestine), the liver, liver cells, epithelial cells, epidermal cells, neuronal cells, endothelial cells, fibroblasts, the pancreas, and/or pancreatic cells (such as the islets of Langerhans) is reduced.

In one example, inflammation of the alimentary canal or part thereof (such as the intestine) is reduced.

In another example, inflammation by immune cells of the tissue or the organ is reduced.

In another example, inflammation by epithelial cells of the tissue or the organ is reduced.

The term "inflammation" as used herein refers to one or more of the following: redness, swelling, pain, tenderness, heat, and disturbed function of a cell, a tissue or organ due to an inflammatory process triggered by over-reaction of the immune system.

In one embodiment, the numbers of cells which are inflamed in a subject is at least 10%, 20%, 30%, 40% or 50% lower after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the numbers of cells which are inflamed in a subject before the strain BT2013 as described herein is administered to the subject.

In one embodiment, the amount of a tissue or organ which is inflamed in a subject is at least 10%, 20%, 30%, 40% or 50% lower after administration of strain

BT2013 when compared to the amount of tissue or organ which is inflamed in a subject before the strain BT2013 is administered to the subject.

In one embodiment, the strain BT2013 reduces the inflammation by epithelial cells of the tissue or the organ.

For example, the epithelial cells are epithelial cells of the alimentary canal or part thereof (such as the intestine).

Without wishing to be bound by theory, the strain BT2013 increases the production of T cells (such as regulatory T cells which may also be referred to as Tregs) in a subject. This increase in Treg numbers may combat the effects of other effector T cells (also referred to as Teffs), such as Th1, Th17 and Th2 which drive inflammation, autoimmunity and allergic/atopic conditions. In Crohn's disease and ulcerative colitis the Teff/Treg cell balance is lost.

In one embodiment, the production of T cells in a subject is increased such that there are at least 10%, 20%, 30%, 40% or 50% more T cells, or greater than 100% more T cells after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the number of T cells in the subject before the strain BT2013 is administered to the subject.

Intestine barrier integrity

In one embodiment, the strain BT2013 is used to improve intestine barrier integrity in a subject.

The term "improving intestine barrier integrity" as used herein refers to a reduction in the numbers and/or types of microorganisms which spread from the intestine into other cells in a subject after administration of the strain BT2013 when compared to the numbers and/or types of microorganisms which spread from the intestine into other cells in a subject before administration of the strain BT2013 as described herein.

In one embodiment, the numbers of microorganisms which spread from the intestine into other cells in a subject are at least 10%, 20%, 30%, 40% or 50% lower after administration of the strain BT2013 when compared to the numbers of microorganisms which spread from the intestine into other cells in a subject administration.

In one embodiment, there are at least 5%, 10%, 15% or 20% fewer types of microorganisms which spread from the intestine into other cells in a subject

after administration of the strain BT2013 when compared to the types of microorganisms which spread from the intestine into other cells in a subject before the administration.

Intestine disruption

In one embodiment strain BT2013, is used to reduce disruption to the intestine (e.g. large intestine) of a subject (such as a subject with IBD).

The term "disruption to the intestine of a subject" as used herein refers to an affect on the integrity of the mucosal epithelium and/or an affect on the number of goblet cells in the epithelium and/or an affect on the number of immune cells infiltrating the lamina propria.

In one embodiment, strain BT2013 reduces or prevents disruption to the integrity of the mucosal epithelium and/or reduces or prevents a reduction in the number of goblet cells in the epithelium and/or reduces or prevents the infiltration of immune cells into the lamina propria.

In one embodiment, a reduction in disruption to the integrity of the mucosal epithelium is a reduction of at least 5%, 10%, 15% or 20% in the numbers of bacteria crossing from the intestinal lumen into intestinal cells after administration of strain BT2013 when compared to the numbers of bacteria crossing from the intestinal lumen into intestinal cells in a subject before administration.

In one embodiment, a reduction in the number of goblet cells in the epithelium is a reduction of at least 2%, 5%, 10%, 15% or 20% in the numbers of goblet cells in the epithelium of a subject after administration of strain BT2013 when compared to the number of goblet cells in the epithelium of a subject before administration.

In one embodiment, the reduction in the infiltration of immune cells into the lamina propria is such that over a fixed time period (such as 24 hours) there is a reduction of at least 5%, 10%, 15%, 20% or 30% in the numbers of immune cells (e.g. T cells) crossing into lamina propria cells after administration of strain BT2013 when compared to the numbers of immune cells (e.g. T cells) crossing into the lamina propria in a subject before administration.

Pro-inflammatory genes and barrier integrity genes

In one embodiment, strain BT2013 is used to regulate the expression of one or more pro-inflammatory genes and/or one or more barrier integrity genes in a cell or cells of a subject.

In one embodiment, the term "regulate" refers to an upregulation in the expression of one or more pro-inflammatory genes. In an alternative embodiment, the term "regulate" refers to a downregulation in the expression of one or more pro-inflammatory genes.

In one embodiment, strain BT2013 downregulates the expression of one or more pro-inflammatory genes in a cell or cells of a subject.

The term "pro-inflammatory gene" as used herein refers to a gene which, when expressed, promotes inflammation. Examples of pro-inflammatory genes include genes encoding but not limited to IL1-β, IL4, IL5, IL6, IL8, IL12, IL13, IL17, IL21, IL22, IL23, IL27, IFN, CCL2, CCL3, CCL5, CCL20, CXCL5, CXCL10, CXCL12, CXCL13, and TNF-α.

In one embodiment, the pro-inflammatory gene is selected from the group consisting of IL1-β, IL6 and IL8.

In one embodiment, the expression level (e.g. mRNA level) of one or more pro-inflammatory genes is decreased (i.e. downregulated) such that the level is at least 10%, 20%, 30%, 40% or 50% lower after administration of the strain BT2013 when compared to the level in the subject before administration.

The term "barrier integrity genes" as used herein refers to a gene which, when expressed, has a role in the function of the barrier of the intestine such as the repair of the barrier and the prevention of microorganisms crossing the barrier. Examples of barrier integrity genes include genes encoding Retnlg|Retnlb, Si, Defa24, Hsd11b2, Hsd17b2, and Nr1d1|Thra.

In one embodiment, the term "regulate" refers to an upregulation in the expression of one or more barrier integrity genes. In an alternative embodiment, the term "regulate" refers to a downregulation in the expression of one or more barrier integrity genes.

In one embodiment, strain BT2013 upregulates the expression of barrier integrity genes in a cell or cells of a subject

In one embodiment, the barrier integrity gene is selected from the group consisting of Retnlg|Retnlb, Si, Defa24, Hsd11b2, Hsd17b2, and Nr1d1|Thra.

In one embodiment, the expression level (e.g. mRNA level) of one or more barrier integrity genes is increased (i.e. upregulated) such that the level is at least 10%, 20%, 30%, 40% or 50% higher after administration of strain BT2013 when compared to the level in the subject before administration.

Alimentary canal

Parts of the alimentary canal include the oesophagus, the stomach and the intestine (such as the small intestine (e.g. the duodenum, the jejunum and the ileum) and/or the large intestine (e.g. the caecum, ascending colon, transverse colon, descending colon, and sigmoid colon)).

Herein, the term "large intestine" may be used interchangeably with the term "colon".

In one embodiment, strain BT2013 is used for improving alimentary canal health in a subject.

The term "improving alimentary canal health" as used herein refers to reducing the level of inflammation in the alimentary canal or part thereof and/or improving intestinal microbiota.

In one embodiment, the level of inflammation in the alimentary canal is at least 10%, 20%, 30%, 40% or 50% lower after administration of strain BT2013 when compared to the level of inflammation in the alimentary canal of a subject before administration.

In one embodiment, strain BT2013 is used for improving intestinal microbiota in a subject.

The term "intestinal microbiota" as used herein refers to microorganisms that live in the digestive tract of the host animals. These microorganisms perform a wide variety of metabolic, structural, protective and other beneficiary functions.

As used herein, the term "improving intestinal microbiota" refers to increasing the number and/or type of desirable microorganisms present in the intestine of a subject (e.g. the host), and/or increasing the activity of said desirable microorganisms in terms of their metabolic, structural, protective and other beneficiary functions. The term "improving intestinal microbiota" may also refer to decreasing the number and/or type of undesirable microorganisms present in the intestine of a subject (e.g. the host), and/or decreasing the activity of said

undesirable microorganisms in terms of their metabolic, structural, protective and other beneficiary functions.

Microorganisms which are desirable in the intestine of a host are those microorganisms which have a protective and beneficiary function. Firmicutes and bacteroidetes bacteria are examples of desirable microorganisms in the intestine of a host.

Microorganisms which are undesirable in the intestine of a host are those microorganisms which can interfere with the metabolic, structural, protective and other beneficiary functions of desirable microorganisms in the intestine have a protective and beneficiary function. In addition or alternatively, undesirable microorganisms are those which cause, for example, inflammation and/or diarrhoea. *E. coli* is an example of an undesirable microorganism in the intestine of a host.

For example, a change in the microbiota balance between desirable microorganism (such as firmicutes and bacteroidetes bacteria) and undesirable microorganisms (such as *E.coli*: ETEC, EPEC, EIEC, EHEC and EAEC) within the intestine may occur in subjects with inflammatory bowel disease (IBD) once strain BT2013 has been administered to the subject.

In one embodiment, the number of desirable microorganisms (such as firmicutes and bacteroidetes bacteria) present in the intestine of a subject (e.g. the host), is increased such that the number of microorganisms is at least 10%, 20%, 30%, 40% or 50% higher, or greater than 100% higher after administration of the strain BT2013 compared to the level in the subject before administration. In addition, or alternatively, the types of desirable microorganisms (such as firmicutes and bacteroidetes) present in the intestine of a subject (e.g. the host), are increased such that there are at least 2%, 5%, 10%, or 15% more types of microorganisms after administration of strain BT2013 when compared to the types in the subject before administration.

In one embodiment, the number of undesirable microorganisms (such as *E. coli* ETEC, EPEC, EIEC, EHEC and EAEC) present in the intestine of a subject (e.g. the host), is decreased such that the number of microorganisms is at least 10%, 20%, 30%, 40% or 50% lower after administration strain BT2013 when compared to the level in the subject before administration. In addition, or alternatively, the types of undesirable microorganisms (such as *E. coli* ETEC,

EPEC, EIEC, EHEC and EAEC) present in the intestine of a subject (e.g. the host), are decreased such that there are at least 1%, 2%, 5%, or 10%, fewer types of undesirable microorganisms after administration of strain BT2013 when compared to the types in the subject before administration.

Encapsulation

In one embodiment, the *B. thetaiotaomicron* strain BT2013 is encapsulated.

In a further embodiment, a pharmaceutical composition comprising the strain BT2013 is encapsulated.

In another embodiment, a nutritional supplement comprising the strain BT2013 is encapsulated.

In a further embodiment, a feedstuff, food product, dietary supplement, or food additive as described herein is encapsulated.

The term “encapsulated” as used herein refers to a means for protecting the strain BT2013 from an incompatible environment by physical separation so that it can be delivered to the target site (e.g. the intestine) without degradation or significant degradation in order that the strain BT2013 can have an effect on the target site. An example is an enteric coated capsule or an enterically-resistant capsule.

Even when the objective of the encapsulation is the isolation of the strain from its surroundings, the protective coating or shell must be ruptured at the time of desired action. The rupturing of the protective coating or shell is typically brought about through the application of chemical and physical stimuli such as pressure, enzyme attack, chemical reaction and physical disintegration.

For example, encapsulation ensures that the strain can be ingested so that the microorganisms can be delivered to the target site (e.g. the intestine) in an amount which is effective to produce an effect at the target site.

Pharmaceutical composition

In one embodiment, a pharmaceutical composition comprises microorganisms of the strain BT2013 and optionally a pharmaceutically acceptable excipient, carrier or diluent.

The pharmaceutical composition may be any pharmaceutical composition. In one aspect, the pharmaceutical composition is to be administered orally, enterally or rectally. For example, the composition may be an edible composition. "Edible" means a material that is approved for human or animal consumption.

The pharmaceutical compositions may be for human or animal usage in human and veterinary medicine.

Examples of such suitable excipients for the various different forms of pharmaceutical compositions described herein may be found in the “Handbook of Pharmaceutical Excipients, 2nd Edition, (1994), Edited by A Wade and PJ Weller.

Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985).

Examples of suitable carriers include lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol and the like.

Examples of suitable diluents include one or more of: water, ethanol, glycerol, propylene glycol and glycerin, and combinations thereof.

The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as, or in addition to, the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s).

Examples of suitable binders include starch, gelatin, natural sugars such as glucose, anhydrous lactose, free-flow lactose, beta-lactose, corn sweeteners, natural and synthetic gums, such as acacia, tragacanth or sodium alginate, carboxymethyl cellulose and polyethylene glycol.

Examples of suitable lubricants include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like.

Preservatives, stabilizers, dyes and even flavouring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

In one aspect, the microorganisms of strain BT2013 pharmaceutical composition are encapsulated.

The pharmaceutical may be in the form of a solution or as a solid – depending on the use and/or the mode of application and/or the mode of administration.

As used herein, the term "medicament" encompasses medicaments for both human and animal usage in human and veterinary medicine. In addition, the term "medicament" as used herein means any substance, which provides a therapeutic and/or beneficial effect. The term "medicament" as used herein is not necessarily limited to substances, which need Marketing Approval, but may include substances which, can be used in cosmetics, nutraceuticals, food (including feeds and beverages for example), probiotic cultures, nutritional supplements and natural remedies. In addition, the term "medicament" as used herein encompasses a product designed for incorporation in animal feed, for example livestock feed and/or pet food.

Nutritional supplements

Nutritionally acceptable carriers, diluents and excipients include those suitable for human or animal consumption and that are used as standard in the food industry. Typical nutritionally acceptable carriers, diluents and excipients will be familiar to the skilled person in the art.

In one embodiment, a nutritional supplement comprises microorganisms of strain BT2013 or a host cell comprising an expression vector comprising said polynucleotide sequence, and a nutritional acceptable excipient, carrier or diluent.

In one example, the microorganisms of strain BT2013 are encapsulated.

Feedstuff/products

A further aspect of the invention relates to feedstuffs, food products, dietary supplements and food additives comprising microorganisms of strain BT2013.

The terms “feedstuff”, "food product" “food additive” and “dietary supplement” as used herein are intended to cover all consumable products that can be solid, jellied or liquid.

The term “food product” is used in a broad sense – and covers food for humans as well as food for animals (i.e. a feed). In one aspect, the food product is for human consumption. Examples of food products include diary products (such as milk, cheese, beverages comprising whey protein, milk drinks, lactic acid

bacteria drinks, yoghurt, drinking yoghurt), bakery products, beverages and beverage powders.

The “feedstuff”, "food product" “food additive” and “dietary supplement” may be in the form of a solution or as a solid – depending on the use and/or the mode of application and/or the mode of administration.

As used herein the term “dietary supplement” includes a formulation which is or can be added to a food product or feedstuff as a nutritional supplement. The term “dietary supplement” as used here also refers to formulations which can be used at low levels in a wide variety of products that require gelling, texturising, stabilising, suspending, film-forming and structuring, retention of juiciness and improved mouthfeel, without adding viscosity.

Suitable food products may include, for example, functional food products, food compositions, pet food, livestock feed, health foods, feedstuffs and the like. In one aspect, the food product is a health food.

As used herein, the term "functional food product" means food that is capable of providing not only a nutritional effect, but is also capable of delivering a further beneficial effect to the consumer. Accordingly, functional foods are ordinary foods that have components or ingredients (such as those described herein) incorporated into them that impart to the food a specific functional - e.g. medical or physiological benefit - other than a purely nutritional effect.

Examples of specific food products that are applicable to the present invention include milk-based products, ready to eat desserts, powders for re-constitution with, e.g., milk or water, chocolate milk drinks, malt drinks, ready-to-eat dishes, instant dishes or drinks for humans or food compositions representing a complete or a partial diet intended for pets or livestock.

In one aspect, the feedstuff, food product, dietary supplement or food additive according to the present invention are intended for humans, pets or livestock such as monogastric animals. The feedstuff, food product, dietary supplement or food additive may be intended for animals selected from the group consisting of dogs, cats, pigs, horses, or poultry. In a further embodiment, the food product, dietary supplement or food additive is intended for adult species, in particular human adults.

The term "milk-based product" as used herein means any liquid or semi-solid milk or whey based product having a varying fat content. The milk-based

product can be, e.g., cow's milk, goat's milk, sheep's milk, skimmed milk, whole milk, milk recombined from powdered milk and whey without any processing, or a processed product, such as yoghurt, curdled milk, curd, sour milk, sour whole milk, butter milk and other sour milk products. Another important group includes milk beverages, such as whey beverages, fermented milks, condensed milks, infant or baby milks; flavoured milks, ice cream; milk-containing food such as sweets.

The feedstuffs, food products, dietary supplements or food additives of the present invention may be - or may be added to - food supplements, also referred to herein as dietary or nutritional supplements or food additives.

The feedstuffs, food products, dietary supplements or food additives according to the invention may also be used in animal nutrition (e.g. in pig nutrition), particularly in the early-weaned period and growing fattening period. The feedstuffs, food products, dietary supplements or food additives are expected to enhance immune function reduce and prevent infectious diseases, beneficially alter the microbiota composition, and improve growth and performance of animals, for example, through increased feed conversion efficiency.

In one embodiment the feedstuff, food product, dietary supplement, or food additive is encapsulated.

Live biotherapeutic product

The microorganisms of strain BT2013 may be used in or as a live biotherapeutic product (LBP).

In one aspect, the LBP is an orally administrable composition of metabolically active, i.e., live and/or lyophilized, or non-viable heat-killed, irradiated or lysed bacteria. The LBP may contain other ingredients. The LBP can be administered orally, i.e., in the form of a tablet, capsule or powder. The LBP may additionally comprise other bacterial species, for example, the bacterial species *R. hominis.* Encapsulated products are favoured for *R. hominis* as it is an anaerobe. Other ingredients (such as vitamin C, for example), may be included as oxygen scavengers and substrates (such as these improve the colonisation and survival *in vivo*). Alternatively, the LBP 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.

A suitable daily dose of the bacteria in the LBP is from about 1 x 10^3 to about 1 x 10^{12} colony forming units (CFU); for example, from about 1 x 10^7 to about 1 x 10^{10} CFU; in another example from about 1 x 10^6 to about 1 x 10^{10} CFU.

In one aspect, the LBP contains the bacterial species and/or cellular components thereof, as active ingredients, in an amount of from about 1 x 10^6 to about 1 x 10^{12} CFU/g, respect to the weight of the composition; for example, from about 1 x 10^8 to about 1 x 10^{10} CFU/g. Typically, a LBP is optionally combined with at least one suitable prebiotic compound. A prebiotic 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 one aspect, the LBP of the present description includes a prebiotic 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 hereinbelow 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.

Administration

The pharmaceutical compositions, the nutritional supplements, feedstuffs, food products, dietary supplements or food additives of the present invention may be adapted for oral, rectal, vaginal, parenteral, intramuscular, intraperitoneal, intraarterial, intrathecal, intrabronchial, subcutaneous, intradermal, intravenous, nasal, buccal or sublingual routes of administration.

In one aspect, the pharmaceutical compositions, the nutritional supplements, feedstuffs, food products, dietary supplements or food additives of the present invention are adapted for oral, rectal, vaginal, parenteral, nasal, buccal or sublingual routes of administration.

In a further aspect, the pharmaceutical compositions, the nutritional supplements, feedstuffs, food products, dietary supplements or food additives of the present invention are adapted for oral administration.

For oral administration, particular use is made of compressed tablets, pills, tablets, gellules, drops, and capsules.

Other forms of administration comprise solutions or emulsions which may be injected intravenously, intraarterially, intrathecally, subcutaneously, intradermally, intraperitoneally or intramuscularly, and which are prepared from sterile or sterilisable solutions. The pharmaceutical compositions of the present invention may also be in form of suppositories, pessaries and suspensions. Pharmaceutical compositions, the nutritional supplements, feedstuffs, food products, dietary supplements or food additives may be formulated in unit dosage form, i.e., in the form of discrete portions containing a unit dose, or a multiple or sub-unit of a unit dose.

Dosage

A person of ordinary skill in the art can easily determine an appropriate dosage amount of the strain BT2013 to administer to a subject without undue experimentation. Typically, a physician will determine the actual dosage which will be most suitable for an individual patient and it will depend on a variety of factors including the activity of the strain employed, the metabolic stability and length of action of that strain, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the individual undergoing therapy. The dosages disclosed herein are exemplary of the average case. There can of course be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

Combinations

In one aspect, microorganisms of strain BT2013 are administered in combination with one or more other active agents. In such cases, the microorganisms of strain BT2013 may be administered consecutively, simultaneously or sequentially with the one or more other active agents.

Functional Assays:

In Vivo Model

C57BL/6 mice (6 weeks old) were used to evaluate the therapeutic effect of *B. thetaiotaomicron* strains E1, E2 and BT2013 during DSS-induced colitis. The mice were colonised with one of the *B. thetaiotaomicron* strains prior to treatment with DSS. The animals were euthanized and intestinal tissue sampling was performed. Small intestine was collected for immunological analysis by flow cytometry and enzymatic activity measurements of the enzyme myeloperoxidase (MPO). Ascending colon were divided into equal parts and transferred to neutral buffered formalin (NBF; Sigma-Aldrich) for histological analysis or RNAlater (Ambion) for molecular analysis..

Flow cytometry analysis of T cell populations in small intestine lamina propria was carried out (figure 1 and figure 2). DSS alone and *B. thetaiotaomicron* treatment did not affect the total percentage of the CD3+CD4+CD8- population. The populations influenced by DSS alone and *B. thetaiotaomicron* were the Tregs (CD25+FoxP3+* and $FR4^{hi}CD25+*$) and Teff cells ($FR4^{lo}CD25+*$) (figure 1 and 2). The percentage of Tregs was increased in mice treated with *B. thetaiotaomicron* strain BT2013 compared to DSS alone. The strain E1W did not appear to have any effect on Tregs. (figure 1). The effects of BT2013 in Tregs were only apparent in mice co-treatment with DSS. The strain had no effect on Tregs in untreated mice but did influence the Teff cell population (figure 2).

The enzymatic activity of MPO in the ileum and caecum was determined (Figure 3 a and 3b). MPO is a proinflammatory enzyme stored in the azurophilic granules of neutrophilic granulocytes. MPO is used as an indicator of inflammation, specifically neutrophil recruitment and accumulation. The lower levels of MPO activity detected in ileal or caecal tissue samples from the B. thetaiotaomicron/DSS treated mice compared to DSS alone indicates a reduction in neutrophil recruitment and therefore a reduction in inflammation.

Histological analysis of ascending colon was carried out (Figures 4 and 5 and Table 1). The histopathology grading scheme was based on the criteria of Berg et al 1996, as summarised:

0 = Shallow crypts, no or few infiltrating inflammatory cells, intact epithelium, goblet cells appear full of mucin. ie no pathology

1 = Crypts may exhibit slight epithelial cell hyperplasia, some diffuse

infiltrating inflammatory cells may be seen between crypts, luminal epithelium

appears intact, goblet cells may appear slightly depleted of mucin.

2 = Crypts appear deeper with distinct evidence of epithelial hyperplasia, depletion of mucin from goblet cells, infiltrating inflammatory cells evident and

may be multifocal in nature ,though the infiltrates are not seen in the submucosa.

3 = Lesions involved a larger area of the mucosa and /or were more frequent than that seen in grade 2. The lesions did not involve the submucosa.

The luminal epithelial cells exhibited small erosions. The lesions are not transmural.

4 = Crypt epithelium appears eroded. Abscesses may be present. Luminal epithelial cells appear irregular, sometimes with complete loss.

Transmural infiltrate is observed - this was often associated with complete loss

of epithelial cells into the lumen.

The disruption to the colon as a result of DSS induced colitis was significantly reduced by treatment of mice with *B. thetaiotaomicron* strains E1, E2 and BT2013. The expression of inflammation–associated genes in the ascending colon was reduced in mice colonised with *B. thetaiotaomicron* compared to mice treated with DSS alone. The strains E1 and BT2013 greatly reduced IL1B and IL6 inflammatory gene expression compared to strain E2. (Figure 6)

Table 1

TTEST	CONTROL	BT E1	BT 2013	BT E2
DSS	0.000	0.032	0.041	0.089

In Vitro Model

The expression of the inflammatory gene interleukin-8 induced in intestinal epithelial cells after PMA exposure was modulated in the presence of *B. thetaiotaomicron* strains E1, E2 and BT2013 (Figure 7).

Sequencing of strain BT2013 genome

A DNA sample from strain BT2013 was subjected to sequencing on MiSeq (v2 nano 2x250bp) using a Nextera XT library for fast fragmentation and tagging with sequencing adaptors, to give a total of 4605120 reads (1115615927 bases).

The data analysis is summarised below:

a. Mapping to reference sequence (NC_004663 and NC_004703) using bowtie2 (2.2.2)

b. SNV and small InDel calling using VarScan (2.3.7) and SNVer (0.5.3) performing a consensus call to avoid false positives

c. Annotation of variations using reference gff

d. Large InDel calling using pindel (0.2.5a3)

e. De-novo assembly of unmapped reads using SOAPdenovo (2.04)

f. Blast of assembled contigs against NCBI nt database

g. Subsampling of all reads of the sample to 50%

h. De-novo assembly on the subsampled reads using SOAPdenovo (2.04)

The sequences were mapped to the reference sequence (NC_004663 and NC_004703) using bowtie2 (2.2.2). Nucleotide variations and small insertions and/or deletions were identified using VarScan (2.3.7) and SNVer (0.5.3) to avoid false positives during sequencing and variations were annotated using a reference sequence. Large insertions and deletions were identified using pindel (0.2.5a3). Unmapped reads were assembled *de novo* using SOAPdenovo (2.04). The sequencing fragments were reassembled into contigs which were blasted against the NCBI nucleotide database. All the reads of the sample were subsampled to 50% and were then assembled *de novo* using SOAPdenovo (2.04) to provide a concatenated version of the *de novo* sequence assembly of BT2013.

Sequences

SEQ ID NO:1 (concatenated version of the *de novo* sequence assembly of BT2013) – see electronic sequence listing.