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DESCRIPTION

TECHNICAL FIELD

[0001] This invention is in the field of compositions comprising bacterial strains of the species *Enterococcus faecium* and the use of such compositions in the treatment of disease, particularly inflammatory or autoimmune disease.

BACKGROUND TO THE INVENTION

[0002] The human intestine is thought to be sterile *in utero*, but it is exposed to a large variety of maternal and environmental microbes immediately after birth. Thereafter, a dynamic period of microbial colonization and succession occurs, which is influenced by factors such as delivery mode, environment, diet and host genotype, all of which impact upon the composition of the gut microbiota, particularly during early life. Subsequently, the microbiota stabilizes and becomes adult-like [1]. The human gut microbiota contains more than 500-1000 different phylotypes belonging essentially to two major bacterial divisions, the Bacteroidetes and the Firmicutes [2]. 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. 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 [3-5].

[0003] Dramatic changes in microbiota composition have been documented in gastrointestinal disorders such as inflammatory bowel disease (IBD). For example, the levels of *Clostridium* cluster XIVa 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 [6-9]. Interestingly, this microbial dysbiosis is also associated with imbalances in T effector cell populations.

[0004] In recognition of the potential positive effect that certain bacterial strains may have on the animal gut, various strains have been proposed for use in the treatment of various diseases (see, for example, [10-13]). Also, certain strains, including mostly *Lactobacillus* and *Bifidobacterium* strains, have been proposed for use in treating various inflammatory and autoimmune diseases that are not directly linked to the intestines (see [14] and [15] for reviews). However, the relationship between different diseases and different bacterial strains, and the precise effects of particular bacterial strains on the gut and at a systemic level and on any particular types of diseases, are poorly characterised.

[0005] There is a requirement in the art for new methods of treating inflammatory and autoimmune diseases. There is also a requirement for the potential effects of gut bacteria to be characterised so that new therapies using gut bacteria can be developed.

[0006] S. Chen et al. (2014) Int J Inflam. 878054 discloses a composition comprising *Bacillus subtilis* and *Enterococcus faecium* for use in treating experimental colitis.

SUMMARY OF THE INVENTION

[0007] The inventors have developed new therapies for treating and preventing inflammatory and autoimmune diseases. In particular, the inventors have developed new therapies for treating and preventing diseases and conditions mediated by IL-17 or the Th17 pathway. In particular, the inventors have identified that bacterial strains of the species *Enterococcus faecium* can be effective for treating and preventing diseases and conditions mediated by IL-17 or the Th17 pathway. As described in the examples, oral administration of compositions comprising *Enterococcus faecium* may reduce the severity of the inflammatory response, including the Th17 inflammatory response, in mouse models of uveitis.

[0008] Therefore, in a first aspect, the invention provides a composition comprising a bacterial strain of the species *Enterococcus faecium* having a 16s rRNA sequence that is at least 99% identical to SEQ ID NO: 2, for use in a method of treating or preventing an inflammatory or autoimmune disease, wherein the composition does not comprise a bacterial strain of the species *Bacillus subtilis*, and wherein the amount of the bacterial strain is from about 1×10^6 to about 1×10^{11} colony forming units per gram with respect to a weight of the composition. The inventors have identified that treatment with bacterial strains from this species can provide clinical benefits in mouse models of inflammatory and autoimmune diseases mediated by IL-17 and the Th17 pathway, may reduce levels of cytokines that are part of the Th17 pathway, including IL-17, and may alleviate the Th17 inflammatory response.

[0009] In particular embodiments, the invention provides a composition comprising a bacterial strain of the species *Enterococcus faecium*, for use in a method of treating or preventing an inflammatory or autoimmune disease selected from the group consisting of: uveitis; multiple sclerosis; arthritis, such as rheumatoid arthritis, osteoarthritis, psoriatic arthritis, or juvenile idiopathic arthritis; neuromyelitis optica (Devic's disease); ankylosing spondylitis; spondyloarthritis; psoriasis; systemic lupus erythematosus; inflammatory bowel disease, such as Crohn's disease or ulcerative colitis; celiac disease; asthma, such as allergic asthma or neutrophilic asthma; chronic obstructive pulmonary disease (COPD); scleritis; vasculitis; Behcet's disease; atherosclerosis; atopic dermatitis; emphysema; periodontitis; allergic rhinitis; and allograft rejection. The effect shown for the bacterial strains from the species *Enterococcus faecium* on the Th17 inflammatory response and on diseases mediated by IL-17 and the Th17 pathway may provide therapeutic benefits for other diseases and conditions mediated by IL-17 and the Th17 pathway, such as those listed above.

[0010] In particularly preferred embodiments, the invention provides a composition comprising a bacterial strain of the species *Enterococcus faecium*, for use in a method of treating or preventing uveitis, such as posterior uveitis. The inventors have identified that treatment with *Enterococcus faecium* strains can reduce disease incidence and disease severity in a mouse model of uveitis and can prevent or reduce retinal damage. In preferred embodiments, the invention provides a composition comprising a bacterial strain of the species *Enterococcus faecium*, for use in the treatment of uveitis. Compositions using *Enterococcus faecium* may be particularly effective for treating uveitis.

[0011] In further preferred embodiments, the invention provides a composition comprising a bacterial strain of the species *Enterococcus faecium*, for use in a method of treating or preventing asthma, such as neutrophilic asthma or allergic asthma. Treatment with *Enterococcus faecium* strains may reduce recruitment of neutrophils and eosinophils into the lungs, which can help treat or prevent asthma. In certain embodiments, the composition is for use in a method of treating or preventing neutrophilic asthma or eosinophilic asthma. The compositions of the invention may be particularly effective for treating or preventing neutrophilic asthma and eosinophilic asthma. Indeed, in certain embodiments, the composition is for use in a method of reducing a neutrophilic inflammatory response in the treatment or prevention of asthma, or the composition is for use in a method of reducing an eosinophilic inflammatory response in the treatment or prevention of asthma. In preferred embodiments, the invention provides a composition comprising a bacterial strain of the species *Enterococcus faecium* for use in the treatment of asthma, and in particular eosinophilic or allergic asthma. Also, *Enterococcus faecium* may have a particularly pronounced effect on neutrophils in asthma models and treatment with *Enterococcus faecium* may be particularly effective for treating neutrophilic asthma.

[0012] In further preferred embodiments, the invention provides a composition comprising a bacterial strain of the species *Enterococcus faecium*, for use in a method of treating or preventing rheumatoid arthritis. Treatment with *Enterococcus faecium* strains may provide clinical benefits in a mouse model of rheumatoid arthritis and reduce joint swelling. In preferred embodiments, the invention provides a composition comprising a bacterial strain of the species *Enterococcus faecium*, for use in the treatment of rheumatoid arthritis. Compositions using *Enterococcus faecium* may be particularly effective for treating rheumatoid arthritis.

[0013] In further preferred embodiments, the invention provides a composition comprising a bacterial strain of the species *Enterococcus faecium*, for use in a method of treating or preventing multiple sclerosis. Treatment with *Enterococcus faecium* strains may reduce disease incidence and disease severity in a mouse model of multiple sclerosis. In preferred embodiments, the invention provides a composition comprising a bacterial strain of the species *Enterococcus faecium*, for use in the treatment of multiple sclerosis. Compositions using *Enterococcus faecium* may be particularly effective for treating multiple sclerosis.

[0014] In certain embodiments, the compositions of the invention are for use in a method of

reducing IL-17 production or reducing Th17 cell differentiation in the treatment or prevention of a disease or condition mediated by IL-17 or the Th17 pathway. In particular, the compositions of the invention may be used in reducing IL-17 production or reducing Th17 cell differentiation in the treatment or prevention of asthma, rheumatoid arthritis, multiple sclerosis, or uveitis. Preferably, the invention provides compositions comprising a bacterial strain of the species *Enterococcus faecium*, for use in reducing IL-17 production or reducing Th17 cell differentiation in the treatment or prevention of asthma, rheumatoid arthritis, multiple sclerosis, or uveitis.

[0015] In certain embodiments, the composition is for use in a patient with elevated IL-17 levels or Th17 cells. The effect shown for *Enterococcus faecium* on uveitis, which is strongly associated with the Th17 inflammatory response, means that *Enterococcus faecium* strains may be particularly beneficial for such patients.

[0016] The bacterial strain in the composition has a 16s rRNA sequence that is at least 99%, or preferably 99.5% or 99.9% identical to SEQ ID NO: 2. Preferably, the bacterial strain for use in the invention has the 16s rRNA sequence represented by SEQ ID NO:2.

[0017] In certain embodiments, the composition of the invention is for oral administration. Oral administration of the strains of the invention can be effective for treating IL-17- or Th17 pathway-mediated diseases and conditions. Also, oral administration is convenient for patients and practitioners and allows delivery to and / or partial or total colonisation of the intestine.

[0018] In certain embodiments, the composition of the invention comprises one or more pharmaceutically acceptable excipients or carriers.

[0019] 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.

[0020] In another aspect, the invention provides a food product comprising the composition as described above.

[0021] In another aspect, the invention provides a vaccine composition comprising the composition as described above.

[0022] In developing the above invention, the inventors have identified and characterised a bacterial strain that is particularly useful for therapy. The *Enterococcus faecium* strain of the invention is shown to be effective for treating the diseases described herein, such as uveitis. Therefore, in another aspect, the invention provides a cell of the *Enterococcus faecium* strain deposited under accession number NCIMB 42487, or a derivative thereof. In another aspect, the invention provides compositions comprising such cells, or biologically pure cultures of such cells. In another aspect, the invention provides a cell of the *Enterococcus faecium* strain deposited under accession number NCIMB 42487, or a derivative thereof, for use in therapy, in

particular for the diseases described herein.

BRIEF DESCRIPTION OF DRAWINGS

[0023]

Figure 1: Mouse model of uveitis - Lymph node proliferative response to IRBP peptide. Media background subtracted [IRBP peptide stimulated - media background] counts per minute based on 3H-thymidine incorporation. All data are presented as Mean + SEM (n=3).

Figure 2: Mouse model of uveitis - TEFI Scores in the control group. Data are presented as Mean ± SEM.

Figure 3: Mouse model of uveitis - TEFI Scores on Day 21. Data are presented as Mean ± SEM.

Figure 4: Rapid ID 32 A analysis of MRx0010

DISCLOSURE OF THE INVENTION

Bacterial strains

[0024] The compositions of the invention comprise a bacterial strain of the species *Enterococcus faecium*. The examples demonstrate that bacteria of this species are useful for treating or preventing uveitis and diseases and conditions mediated by IL-17 or the Th17 pathway.

[0025] The invention provides a composition comprising a bacterial strain of the species *Enterococcus faecium* for use in treating or preventing an inflammatory and/or autoimmune disease. In certain embodiments, the compositions of the invention comprise *Enterococcus faecium* and do not contain any other bacterial species. In certain embodiments, the compositions of the invention comprise a single strain of *Enterococcus faecium* and do not contain any other bacterial strains or species.

[0026] *Enterococcus faecium* is a Gram-positive, alpha-hemolytic or nonhemolytic bacterium in the genus *Enterococcus* that often occurs in pairs (diplococci) or short chains. The type strain of *Enterococcus faecium* is ATCC 19434 = CCUG 542 = CIP 103014 = CFBP 4248 = DSM 20477 = HAMBI 1710 = JCM 5804 = JCM 8727 = LMG 11423 = NBRC 100486 = NBRC 100485 = NCIMB 11508 (formerly NCDO 942) = NCTC 7171 [16]. The GenBank accession number for the 16S rRNA gene sequence of *Enterococcus faecium* strain LMG 11423 is

AJ301830 (disclosed herein as SEQ ID NO:1). This exemplary *Enterococcus faecium* strain is described in [16].

[0027] Other *Enterococcus faecium* strains for use in the invention include: R13 [17], CFR 3003 [18], AL41 [19], DSM 10663 NCIMB 10415 [20], NCIMB 10415 E1707 [21], NM113 and NM213 [22]. In certain embodiments, the compositions of the invention comprise one of these strains, or a derivative or biotype thereof. A further example of an *Enterococcus faecium* for use in the invention is the DO strain. The genomic sequence of this bacterium consists of a chromosome and three plasmids. The sequence of the chromosome is disclosed herein as SEQ ID NO:3 and the sequence of the three plasmids is disclosed as SEQ ID NOs:4, 5 and 6. The genomic sequence was obtained using whole shotgun sequences and is available using GenBank accession number NC_017960.1.

[0028] The *Enterococcus faecium* bacterium deposited under accession number NCIMB 42487 was tested in the Examples and is also referred to herein as strain MRX010. The terms "MRX010" and "MRx0010" are used interchangeably herein. A 16S rRNA sequence for the MRX010 strain that was tested is provided in SEQ ID NO:2. Strain MRX010 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 "*Enterococcus faecium*" and was assigned accession number NCIMB 42487.

[0029] Bacterial strains closely related to the strain tested in the examples are also expected to be effective for treating or preventing uveitis and diseases and conditions mediated by IL-17 or the Th17 pathway. The bacterial strain for use in the invention has a 16s rRNA sequence that is at least 99%, or preferably 99.5% or 99.9% identical to SEQ ID NO: 2. Preferably, the bacterial strain for use in the invention has the 16s rRNA sequence represented by SEQ ID NO:2.

[0030] Bacterial strains that are biotypes of the bacterium deposited under accession number 42487 are also expected to be effective for treating or preventing uveitis and diseases and conditions mediated by IL-17 or the Th17 pathway. A biotype is a closely related strain that has the same or very similar physiological and biochemical characteristics.

[0031] Strains that are biotypes of the bacterium deposited under accession number NCIMB 42487 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 42487. For example, substantially the whole genome may be sequenced and a biotype strain for use in the invention may have at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity across at least 80% of its whole genome (e.g. across at least 85%, 90%, 95% or 99%, or across its whole genome). For example, in some embodiments, a biotype strain has at least 98% sequence identity across at least 98% of its genome or at least 99% sequence identity across 99% of its genome. Other suitable sequences for use in identifying biotype strains may include hsp60 or repetitive sequences such as BOX, ERIC, (GTG)₅, or REP or [23]. 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 42487. In some embodiments, a biotype strain has a sequence with at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of strain MRX010 deposited as NCIMB 42487 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:2. In some embodiments, a biotype strain has a sequence with at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of strain MRX010 deposited as NCIMB 42487 and has the 16S rRNA sequence of SEQ ID NO:2.

[0032] Alternatively, strains that are biotypes of the bacterium deposited under accession number NCIMB 42487 and that are suitable for use in the invention may be identified by using the accession number NCIMB 42487 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 *Enterococcus faecium* strains.

[0033] In certain embodiments, strains that are biotypes of the bacterium deposited under accession number NCIMB 42487 and that are suitable for use in the invention are strains that provide the same pattern as the bacterium deposited under accession number NCIMB 42487 when analysed by amplified ribosomal DNA restriction analysis (ARDRA), for example when using Sau3AI restriction enzyme (for exemplary methods and guidance see, for example, [24]). Alternatively, biotype strains are identified as strains that have the same carbohydrate fermentation patterns as the bacterium deposited under accession number NCIMB 42487.

[0034] In some embodiments, the bacterial strain used in the invention is:

1. (i) Positive for at least one of (e.g. at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or all of): arginine dihydrolase, β -glucosidase, mannose fermentation, glutamic acid decarboxylase, arginine arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase and serine arylamidase; and/or
2. (ii) Intermediate for N-acetyl- β -glucosaminidase;

preferably as determined by an assay of carbohydrate, amino acid and nitrate metabolism, and optionally an assay of alkaline phosphatase activity, more preferably as determined by Rapid ID 32A analysis (preferably using the Rapid ID 32A system from bioMérieux).

[0035] Other *Enterococcus faecium* strains that are useful in the compositions and methods of the invention, such as biotypes of the bacterium deposited under accession number NCIMB 42487, 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 in anaerobic YCFA and/or administering the bacteria to the type II collagen-induced arthritis mouse model and then assessing cytokine levels. In particular, bacterial strains that

have similar growth patterns, metabolic type and/or surface antigens to the bacterium deposited under accession number NCIMB 42487 may be useful in the invention. A useful strain will have comparable immune modulatory activity to the NCIMB 42487 strain. In particular, a biotype strain will elicit comparable effects on the uveitis disease models to the effects shown in the Examples, which may be identified by using the culturing and administration protocols described in the Examples.

[0036] A particularly preferred strain of the invention is the *Enterococcus faecium* strain deposited under accession number NCIMB 42487. This is the exemplary MRX010 strain tested in the examples and shown to be effective for treating disease. Therefore, the invention provides a cell, such as an isolated cell, of the *Enterococcus faecium* strain deposited under accession number NCIMB 42487, or a derivative thereof. The invention also provides a composition comprising a cell of the *Enterococcus faecium* strain deposited under accession number NCIMB 42487, or a derivative thereof. The invention also provides a biologically pure culture of the *Enterococcus faecium* strain deposited under accession number NCIMB 42487. The invention also provides a cell of the *Enterococcus faecium* strain deposited under accession number NCIMB 42487, or a derivative thereof, for use in therapy, in particular for the diseases described herein. A derivative of the strain deposited under accession number NCIMB 42487 may be a daughter strain (progeny) or a strain cultured (subcloned) from the original.

[0037] 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 immune modulatory activity to the original NCIMB 42487 strain. In particular, a derivative strain will elicit comparable effects on the uveitis disease models 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 NCIMB 42487 strain will generally be a biotype of the NCIMB 42487 strain.

[0038] References to cells of the *Enterococcus faecium* strain deposited under accession number NCIMB 42487 encompass any cells that have the same safety and therapeutic efficacy characteristics as the strains deposited under accession number NCIMB 42487, and such cells are encompassed by the invention. Thus, in some embodiments, reference to cells of the *Enterococcus faecium* strain deposited under accession number NCIMB 42487 refers only to the MRX010 strain deposited under NCIMB 42487 and does not refer to a bacterial strain that was not deposited under NCIMB 42487. In some embodiments, reference to cells of the *Enterococcus faecium* strain deposited under accession number NCIMB 42487 refers to cells that have the same safety and therapeutic efficacy characteristics as the strains deposited under accession number NCIMB 42487, but which are not the strain deposited under NCIMB 42487.

[0039] In certain embodiments, the bacterial strain for use in the invention has a chromosome with sequence identity to SEQ ID NO:3. In some embodiments, the bacterial strain for use in the invention has a chromosome 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:3 across at least 60% (e.g. across at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of SEQ ID NO:3. For example, the bacterial strain for use in the invention may have a chromosome with at least 90% sequence identity to SEQ ID NO:3 across 70% of SEQ ID NO:3, or at least 90% sequence identity to SEQ ID NO:3 across 80% of SEQ ID NO:3, or at least 90% sequence identity to SEQ ID NO:3 across 90% of SEQ ID NO:3, or at least 90% sequence identity to SEQ ID NO:3 across 100% of SEQ ID NO:3, or at least 95% sequence identity to SEQ ID NO:3 across 70% of SEQ ID NO:3, or at least 95% sequence identity to SEQ ID NO:3 across 80% of SEQ ID NO:3, or at least 95% sequence identity to SEQ ID NO:3 across 90% of SEQ ID NO:3, or at least 95% sequence identity to SEQ ID NO:3 across 100% of SEQ ID NO:3, or at least 98% sequence identity to SEQ ID NO:3 across 70% of SEQ ID NO:3, or at least 98% sequence identity to SEQ ID NO:3 across 80% of SEQ ID NO:3, or at least 98% sequence identity to SEQ ID NO:3 across 90% of SEQ ID NO:3, or at least 98% identity across 95% of SEQ ID NO:3, or at least 98% sequence identity to SEQ ID NO:3 across 100% of SEQ ID NO:3, or at least 99.5% sequence identity to SEQ ID NO:3 across 90% of SEQ ID NO:3, or at least 99.5% identity across 95% of SEQ ID NO:3, or at least 99.5% identity across 98% of SEQ ID NO:3, or at least 99.5% sequence identity to SEQ ID NO:3 across 100% of SEQ ID NO:3.

[0040] In certain embodiments, the bacterial strain for use in the invention has a chromosome with sequence identity to SEQ ID NO:3, for example as described above, and a 16S rRNA sequence with sequence identity to SEQ ID NO: 1 or 2, for example as described above, and with a 16S rRNA sequence that is at least 99% identical to SEQ ID NO: 2, preferably which comprises the 16S rRNA sequence of SEQ ID NO:2.

[0041] In certain embodiments, the bacterial strain for use in the invention has a chromosome with sequence identity to SEQ ID NO:3, for example as described above, and is effective for treating or preventing diseases and conditions mediated by IL-17 or the Th17 pathway.

[0042] In certain embodiments, the bacterial strain for use in the invention has a chromosome with sequence identity to SEQ ID NO:3, for example as described above, and a 16S rRNA sequence with sequence identity to SEQ ID NO:1 or 2, for example as described above, and is effective for treating or preventing diseases and conditions mediated by IL-17 or the Th17 pathway.

[0043] In certain embodiments, the bacterial strain for use in the invention has a 16S rRNA sequence that is at least 99%, 99.5% or 99.9% identical to the 16S rRNA sequence represented by SEQ ID NO: 2 (for example, which comprises the 16S rRNA sequence of SEQ ID NO:2) and a chromosome with at least 95% sequence identity to SEQ ID NO:3 across at least 90% of SEQ ID NO:3, and which is effective for treating or preventing diseases and conditions mediated by IL-17 or the Th17 pathway.

[0044] In certain embodiments, the bacterial strain for use in the invention is a *Enterococcus faecium* and has a 16S rRNA sequence that is at least 99%, 99.5% or 99.9% identical to the 16S rRNA sequence represented by SEQ ID NO: 2 (for example, which comprises the 16S

rRNA sequence of SEQ ID NO:2) and a chromosome with at least 98% sequence identity (e.g. at least 99% or at least 99.5% sequence identity) to SEQ ID NO:3 across at least 98% (e.g. across at least 99% or at least 99.5%) of SEQ ID NO:3, and which is effective for treating or preventing diseases and conditions mediated by IL-17 or the Th17 pathway.

[0045] 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

[0046] As demonstrated in the examples, the bacterial compositions of the invention are effective for reducing the Th17 inflammatory response. In particular, treatment with compositions of the invention achieves clinical improvements in animal models of conditions mediated by IL-17 and the Th17 pathway and may achieve a reduction in IL-17A levels and other Th17 pathway cytokines. Therefore, the compositions of the invention may be useful for treating or preventing inflammatory and autoimmune diseases, and in particular diseases or conditions mediated by IL-17. In particular, the compositions of the invention may be useful for reducing or preventing elevation of the IL-17 inflammatory response.

[0047] Th17 cells are a subset of T helper cells that produce, for example, IL-17A, IL-17F, IL-21 and IL-22. Th17 cell differentiation and IL-17 expression may be driven by IL-23. These cytokines and others form important parts of the Th17 pathway, which is a well-established inflammatory signalling pathway that contributes to and underlies a number of inflammatory and autoimmune diseases (as described in, for example, [25-30]). Diseases wherein the Th17 pathway is activated are Th17 pathway-mediated diseases. Th17 pathway-mediated diseases can be ameliorated or alleviated by repressing the Th17 pathway, which may be through a reduction in the differentiation of Th17 cells or a reduction in their activity or a reduction in the level of Th17 pathway cytokines. Diseases mediated by the Th17 pathway may be characterised by increased levels of cytokines produced by Th17 cells, such as IL-17A, IL-17F, IL-21, IL-22, IL-26, IL-9 (reviewed in [31]). Diseases mediated by the Th17 pathway may be characterised by increased expression of Th-17-related genes, such as Stat3 or IL-23R. Diseases mediated by the Th17 pathway may be associated with increased levels of Th17 cells.

[0048] IL-17 is a pro-inflammatory cytokine that contributes to the pathogenesis of several inflammatory and autoimmune diseases and conditions. IL-17 as used herein may refer to any member of the IL-17 family, including IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F. IL-17-mediated diseases and conditions are characterised by high expression of IL-17 and/or the accumulation or presence of IL-17-positive cells in a tissue affected by the disease or condition. Similarly, IL-17-mediated diseases and conditions are diseases and conditions that are exacerbated by high IL-17 levels or an increase in IL-17 levels, and that are alleviated by low IL-17 levels or a reduction in IL-17 levels. The IL-17 inflammatory response may be local or systemic.

[0049] Examples of diseases and conditions that may be mediated by IL-17 or the Th17 pathway include uveitis; multiple sclerosis; arthritis, such as rheumatoid arthritis, osteoarthritis, psoriatic arthritis, or juvenile idiopathic arthritis; neuromyelitis optica (Devic's disease); ankylosing spondylitis; spondyloarthritis; psoriasis; systemic lupus erythematosus; inflammatory bowel disease, such as Crohn's disease or ulcerative colitis; celiac disease; asthma, such as allergic asthma or neutrophilic asthma; chronic obstructive pulmonary disease (COPD); scleritis; vasculitis; Behcet's disease; atherosclerosis; atopic dermatitis; emphysema; periodontitis; allergic rhinitis; and allograft rejection. In preferred embodiments, the compositions of the invention are used for treating or preventing one or more of these conditions or diseases. In further preferred embodiments, these conditions or diseases are mediated by IL-17 or the Th17 pathway.

[0050] 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.

[0051] In certain embodiments, the compositions of the invention are for use in a method of reducing IL-17 production or reducing Th17 cell differentiation in the treatment or prevention of a disease or condition mediated by IL-17 or the Th17 pathway. In certain embodiments, the compositions of the invention are for use in treating or preventing an inflammatory or autoimmune disease, wherein said treatment or prevention is achieved by reducing or preventing elevation of the Th17 inflammatory response. In certain embodiments, the compositions of the invention are for use in treating a patient with an inflammatory or autoimmune disease, wherein the patient has elevated IL-17 levels or elevated Th17 cells or is exhibiting a Th17 inflammatory response. In certain embodiments, the patient may have been diagnosed with a chronic inflammatory or autoimmune disease or condition, or the composition of the invention may be for use in preventing an inflammatory or autoimmune disease or condition developing into a chronic inflammatory or autoimmune disease or condition. In certain embodiments, the disease or condition may not be responsive to treatment with TNF- α inhibitors. These uses of the invention may be applied to any of the specific disease or conditions listed in the preceding paragraph.

[0052] IL-17 and the Th17 pathway are often associated with chronic inflammatory and autoimmune diseases, so the compositions of the invention may be particularly useful for treating or preventing chronic diseases or conditions as listed above. In certain embodiments, the compositions are for use in patients with chronic disease. In certain embodiments, the compositions are for use in preventing the development of chronic disease.

[0053] The compositions of the invention may be useful for treating diseases and conditions

mediated by IL-17 or the Th17 pathway and for addressing the Th17 inflammatory response, so the compositions of the invention may be particularly useful for treating or preventing chronic disease, treating or preventing disease in patients that have not responded to other therapies (such as treatment with TNF- α inhibitors), and/or treating or preventing the tissue damage and symptoms associated with IL-17 and Th17 cells. For example, IL-17 is known to activate matrix destruction in cartilage and bone tissue and IL-17 has an inhibitory effect on matrix production in chondrocytes and osteoblasts, so the compositions of the invention may be useful for treating or preventing bone erosion or cartilage damage.

[0054] In certain embodiments, treatment with compositions of the invention provides a reduction or prevents an elevation in IL-17 levels, in particular IL-17A levels. In certain embodiments, treatment with compositions of the invention provides a reduction or prevents an elevation in TNF α , IFN- γ or IL-6 levels. Such reduction or prevention of elevated levels of these cytokines may be useful for treating or preventing inflammatory and autoimmune diseases and conditions, in particular those mediated by IL-17 or the Th17 pathway.

Uveitis

[0055] In preferred embodiments, the compositions of the invention are for use in treating or preventing uveitis. The examples demonstrate that the compositions of the invention achieve a reduction in disease incidence and disease severity in an animal model of uveitis and so they may be useful in the treatment or prevention of uveitis. Uveitis is inflammation of the uvea and can result in retinal tissue destruction. It can present in different anatomical forms (anterior, intermediate, posterior or diffuse) and result from different, but related, causes, including systemic autoimmune disorders. IL-17 and the Th17 pathway are centrally involved in uveitis, so the efficacy of the compositions of the invention for treating uveitis indicates that the compositions of the invention may be particularly effective for treating and preventing diseases and conditions mediated by IL-17 or the Th17 pathway. References [32-39] describe elevated serum levels of interleukin-17A in uveitis patients, specific association of IL17A genetic variants with panuveitis, the role of Th17-associated cytokines in the pathogenesis of experimental autoimmune uveitis, the imbalance between Th17 Cells and regulatory T Cells during monophasic experimental autoimmune uveitis, the up-regulation of IL-17A in patients with uveitis and active Adamantiades-Behçet and Vogt-Koyanagi-Harada (VKH) diseases, the treatment of non-infectious uveitis with secukinumab (anti-IL-17A antibody), and Th17 in uveitic eyes.

[0056] In certain embodiments, the uveitis is posterior uveitis. Posterior uveitis presents primarily with inflammation of the retina and choroid and the examples demonstrate that the compositions of the invention are effective for reducing retinal inflammation and damage.

[0057] In certain embodiments, treatment with the compositions of the invention results in a reduction in retinal damage. In certain embodiments, the compositions of the invention are for use in reducing or preventing retinal damage in the treatment of uveitis. In certain

embodiments, the compositions are for use in treating patients with severe uveitis that are at risk of retinal damage. In certain embodiments, treatment with the compositions of the invention results in a reduction in optic disc inflammation. In certain embodiments, the compositions of the invention are for use in reducing or preventing optic disc inflammation. In certain embodiments, treatment with the compositions of the invention results in a reduction in retinal tissue infiltration by inflammatory cells. In certain embodiments, the compositions of the invention are for use in reducing retinal tissue infiltration by inflammatory cells. In certain embodiments, treatment with the compositions of the invention results in vision being maintained or improved. In certain embodiments, the compositions of the invention are for use in maintaining or improving vision.

[0058] In certain embodiments, the compositions are for use in treating or preventing uveitis associated with a non-infectious or autoimmune disease, such as Behçet disease, Crohn's disease, Fuchs heterochromic iridocyclitis, granulomatosis with polyangiitis, HLA-B27 related uveitis, juvenile idiopathic arthritis, sarcoidosis, spondyloarthritis, sympathetic ophthalmia, tubulointerstitial nephritis and uveitis syndrome or Vogt-Koyanagi-Harada syndrome. IL-17A has been shown to be involved in, for example, Behçet and Vogt-Koyanagi-Harada diseases.

[0059] Treatment or prevention of uveitis may refer to, for example, an alleviation of the severity of symptoms or a prevention of relapse.

Asthma

[0060] In preferred embodiments, the compositions of the invention are for use in treating or preventing asthma. The compositions of the invention may achieve a reduction in the recruitment of neutrophils and/or eosinophils into the airways following sensitisation and challenge with house dust mite extract and so they may be useful in the treatment or prevention of asthma. Asthma is a chronic disease characterised by inflammation and restriction of the airways. The inflammation in asthma may be mediated by IL-17 and/or Th17 cells, and so the compositions of the invention may be particularly effective for preventing or treating asthma. The inflammation in asthma may be mediated by eosinophils and/or neutrophils.

[0061] In certain embodiments, the asthma is eosinophilic or allergic asthma. Eosinophilic and allergic asthma are characterised by increased numbers of eosinophils in peripheral blood and in airway secretions and is associated pathologically with thickening of the basement membrane zone and pharmacologically by corticosteroid responsiveness [46]. Compositions that reduce or inhibit eosinophil recruitment or activation may be useful for treating or preventing eosinophilic and allergic asthma.

[0062] In additional embodiments, the compositions of the invention are for use in treating or preventing neutrophilic asthma (or non-eosinophilic asthma). High neutrophil numbers are associated with severe asthma that may be insensitive to corticosteroid treatment.

Compositions that reduce or inhibit neutrophil recruitment or activation may be useful for treating or preventing neutrophilic asthma.

[0063] Eosinophilic and neutrophilic asthma are not mutually exclusive conditions and treatments that help address either the eosinophil and neutrophil responses may be useful for treating asthma in general.

[0064] Increased IL-17 levels and activation of the Th17 pathway are associated with severe asthma, so the compositions of the invention may be useful for preventing the development of severe asthma or for treating severe asthma.

[0065] In certain embodiments, the compositions of the invention are for use in methods reducing an eosinophilic inflammatory response in the treatment or prevention of asthma, or for use in methods of reducing a neutrophilic inflammatory response in the treatment or prevention of asthma. As noted above, high levels of eosinophils in asthma is associated pathologically with thickening of the basement membrane zone, so reducing eosinophilic inflammatory response in the treatment or prevention of asthma may be able to specifically address this feature of the disease. Also, elevated neutrophils, either in combination with elevated eosinophils or in their absence, is associated with severe asthma and chronic airway narrowing. Therefore, reducing the neutrophilic inflammatory response may be particularly useful for addressing severe asthma.

[0066] In certain embodiments, the compositions reduce peribronchiolar infiltration in allergic asthma, or are for use in reducing peribronchiolar infiltration in the treatment of allergic asthma. In certain embodiments, the compositions reduce peribronchiolar and/or perivascular infiltration in neutrophilic asthma, or are for use in reducing peribronchiolar and/or perivascular infiltration in the treatment of allergic neutrophilic asthma.

[0067] In certain embodiments, treatment with compositions of the invention provides a reduction or prevents an elevation in TNF α levels.

[0068] In certain embodiments, the compositions of the invention are for use in a method of treating asthma that results in a reduction of the eosinophilic and/or neutrophilic inflammatory response. In certain embodiments, the patient to be treated has, or has previously been identified as having, elevated neutrophil or eosinophil levels, for example as identified through blood sampling or sputum analysis.

[0069] The compositions of the invention may be useful for preventing the development of asthma in a new-born when administered to the new-born, or to a pregnant woman. The compositions may be useful for preventing the development of asthma in children. The compositions of the invention may be useful for treating or preventing adult-onset asthma. The compositions of the invention may be useful for managing or alleviating asthma. The compositions of the invention may be particularly useful for reducing symptoms associated with asthma that is aggravated by allergens, such as house dust mites.

[0070] Treatment or prevention of asthma 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.

Arthritis

[0071] In preferred embodiments, the compositions of the invention are for use in treating or preventing rheumatoid arthritis (RA). The compositions of the invention may achieve a reduction in the clinical signs of RA in a mouse model, reduce cartilage and bone damage, and reduce the IL-17 inflammatory response, and so they may be useful in the treatment or prevention of RA. RA is a systemic inflammatory disorder that primarily affects joints. RA is associated with an inflammatory response that results in swelling of joints, synovial hyperplasia, and destruction of cartilage and bone. IL-17 and Th17 cells may have a key role in RA, for example because IL-17 inhibits matrix production in chondrocytes and osteoblasts and activates the production and function of matrix metalloproteinases and because RA disease activity is correlated to IL-17 levels and Th-17 cell numbers [47,48], so the compositions of the invention may be particularly effective for preventing or treating RA.

[0072] In certain embodiments, the compositions of the invention are for use in lowering IL-17 levels or preventing elevation of IL-17 levels in the treatment or prevention of RA. In certain embodiments, treatment with compositions of the invention provides a reduction or prevents an elevation in IL-17 levels, in particular IL-17A levels. In certain embodiments, treatment with compositions of the invention provides a reduction or prevents an elevation in IFN- γ or IL-6 levels.

[0073] In certain embodiments, treatment with the compositions of the invention results in a reduction in the swelling of joints. In certain embodiments, the compositions of the invention are for use in patients with swollen joints or patients identified as at risk of having swollen joints. In certain embodiments, the compositions of the invention are for use in a method of reducing joint swelling in RA.

[0074] In certain embodiments, treatment with the compositions of the invention results in a reduction in cartilage damage or bone damage. In certain embodiments, the compositions of the invention are for use in reducing or preventing cartilage or bone damage in the treatment of RA. In certain embodiments, the compositions are for use in treating patient with severe RA that are at risk of cartilage or bone damage.

[0075] Increased IL-17 levels and Th17 cell numbers are associated with cartilage and bone destruction in RA [47,48]. IL-17 is known to activate matrix destruction in cartilage and bone tissue and IL-17 has an inhibitory effect on matrix production in chondrocytes and osteoblasts. Therefore, in certain embodiments, the compositions of the invention are for use in preventing bone erosion or cartilage damage in the treatment of RA. In certain embodiments, the

compositions are for use in treating patients that exhibit bone erosion or cartilage damage or patients identified as at risk of bone erosion or cartilage damage.

[0076] TNF- α is also associated with RA, but TNF- α is not involved in the pathogenesis of the later stages of the disease. In contrast, IL-17 has a role throughout all stages of chronic disease [49]. Therefore, in certain embodiments the compositions of the invention are for use in treating chronic RA or late-stage RA, such as disease that includes joint destruction and loss of cartilage. In certain embodiments, the compositions of the invention are for treating patients that have previously received anti-TNF- α therapy. In certain embodiments, the patients to be treated do not respond or no longer respond to anti-TNF- α therapy.

[0077] The compositions of the invention may be useful for modulating a patient's immune system, so in certain embodiments the compositions of the invention are for use in preventing RA in a patient that has been identified as at risk of RA, or that has been diagnosed with early-stage RA. The compositions of the invention may be useful for preventing the development of RA.

[0078] The compositions of the invention may be useful for managing or alleviating RA. The compositions of the invention may be particularly useful for reducing symptoms associated with joint swelling or bone destruction. Treatment or prevention of RA 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.

Multiple sclerosis

[0079] In preferred embodiments, the compositions of the invention are for use in treating or preventing multiple sclerosis. The compositions of the invention may achieve a reduction in the disease incidence and disease severity in a mouse model of multiple sclerosis (the EAE model), and so they may be useful in the treatment or prevention of multiple sclerosis. Multiple sclerosis is an inflammatory disorder associated with damage to the myelin sheaths of neurons, particularly in the brain and spinal column. Multiple sclerosis is a chronic disease, which is progressively incapacitating and which evolves in episodes. IL-17 and Th17 cells may have a key role in multiple sclerosis, for example because IL-17 levels may correlate with multiple sclerosis lesions, IL-17 can disrupt blood brain barrier endothelial cell tight junctions, and Th17 cells can migrate into the central nervous system and cause neuronal loss [50,51]. Therefore, the compositions of the invention may be particularly effective for preventing or treating multiple sclerosis.

[0080] In certain embodiments, treatment with the compositions of the invention results in a reduction in disease incidence or disease severity. In certain embodiments, the compositions of the invention are for use in reducing disease incidence or disease severity. In certain embodiments, treatment with the compositions of the invention prevents a decline in motor function or results in improved motor function. In certain embodiments, the compositions of the

invention are for use in preventing a decline in motor function or for use in improving motor function. In certain embodiments, treatment with the compositions of the invention prevents the development of paralysis. In certain embodiments, the compositions of the invention are for use in preventing paralysis in the treatment of multiple sclerosis.

[0081] The compositions of the invention may be useful for modulating a patient's immune system, so in certain embodiments the compositions of the invention are for use in preventing multiple sclerosis in a patient that has been identified as at risk of multiple sclerosis, or that has been diagnosed with early-stage multiple sclerosis or "relapsing-remitting" multiple sclerosis. The compositions of the invention may be useful for preventing the development of sclerosis.

[0082] The compositions of the invention may be useful for managing or alleviating multiple sclerosis. The compositions of the invention may be particularly useful for reducing symptoms associated with multiple sclerosis. Treatment or prevention of multiple sclerosis 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.

Modes of administration

[0083] 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.

[0084] In certain embodiments, the compositions of the invention may be administered as a foam, as a spray or a gel.

[0085] 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. suppcire, witepsol), glycerol-gelatin, polyethylene glycol, or soap glycerin composition.

[0086] 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.

[0087] 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.

[0088] In certain embodiments of the invention, treatment according to the invention is

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

[0089] 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 an inflammatory or autoimmune disease developing in her child *in utero* and / or after it is born.

[0090] The compositions of the invention may be administered to a patient that has been diagnosed with a disease or condition mediated by IL-17 or the Th17 pathway, or that has been identified as being at risk of a disease or condition mediated by IL-17 or the Th17 pathway. The compositions may also be administered as a prophylactic measure to prevent the development of diseases or conditions mediated by IL-17 or the Th17 pathway in a healthy patient.

[0091] The compositions of the invention may be administered to a patient that has been identified as having an abnormal gut microbiota. For example, the patient may have reduced or absent colonisation by *Enterococcus faecium*.

[0092] The compositions of the invention may be administered as a food product, such as a nutritional supplement.

[0093] Generally, the compositions of the invention are for the treatment of humans, although they may be used to treat animals including monogastric mammals such as poultry, pigs, cats, dogs, horses or rabbits. The compositions of the invention may be useful for enhancing the growth and performance of animals. If administered to animals, oral gavage may be used.

Compositions

[0094] 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.

[0095] 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 [52-54].

[0096] Alternatively, the composition of the invention may comprise a live, active bacterial culture.

[0097] 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.

[0098] In some embodiments, the composition comprises a mixture of live bacterial strains and bacterial strains that have been killed.

[0099] 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 [55] and [56].

[0100] The composition may be administered orally and may be in the form of a tablet, capsule or powder. Encapsulated products are preferred because *Enterococcus faecium* 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.

[0101] The composition may be formulated as a probiotic.

[0102] 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.

[0103] 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.

[0104] 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.

[0105] 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.

[0106] 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 embodiment, 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.

[0107] The compositions of the invention may comprise pharmaceutically acceptable excipients or carriers. Examples of such suitable excipients may be found in the reference [57]. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art and are described, for example, in reference [58]. Examples of suitable carriers include lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol and the like. Examples of suitable diluents include ethanol, glycerol and water. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as, or in addition to, the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s). Examples of suitable binders include starch, gelatin, natural sugars such as glucose, anhydrous lactose, free-flow lactose, beta-lactose, corn sweeteners, natural and synthetic gums, such as acacia, tragacanth or sodium alginate, carboxymethyl cellulose and polyethylene glycol. Examples of suitable lubricants include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Preservatives, stabilizers, dyes and even flavouring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

[0108] The compositions of the invention may be formulated as a food product. For example, a food product may provide nutritional benefit in addition to the therapeutic effect of the invention, such as in a nutritional supplement. Similarly, a food product may be formulated to enhance the taste of the composition of the invention or to make the composition more attractive to consume by being more similar to a common food item, rather than to a pharmaceutical composition. In certain embodiments, the composition of the invention is formulated as a milk-based product. The term "milk-based product" means any liquid or semi-solid milk- or whey- based product having a varying fat content. The milk-based product can be, e.g., cow's milk, goat's milk, sheep's milk, skimmed milk, whole milk, milk recombined from powdered milk and whey without any processing, or a processed product, such as yoghurt, curdled milk, curd, sour milk, sour whole milk, butter milk and other sour milk products. Another important group includes milk beverages, such as whey beverages, fermented milks, condensed milks, infant or baby milks; flavoured milks, ice cream; milk-containing food such as sweets.

[0109] In some embodiments, the compositions of the invention comprise one or more bacterial strains of the species *Enterococcus faecium* and do not contain bacteria from any other genus, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another genus. Thus, in some embodiments, the invention provides a composition comprising one or more bacterial strains of the species *Enterococcus faecium*, which does not contain bacteria from any other genus or which comprises only *de minimis* or biologically irrelevant amounts of bacteria from another genus, for use in therapy.

[0110] In some embodiments, the compositions of the invention comprise one or more bacterial strains of the species *Enterococcus faecium* and do not contain bacteria from any other *Enterococcus* species, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another *Enterococcus* species. Thus, in some embodiments, the invention provides a composition comprising one or more bacterial strains of the species *Enterococcus faecium*, which does not contain bacteria from any other *Enterococcus* species or which comprises only *de minimis* or biologically irrelevant amounts of bacteria from another *Enterococcus* species, for use in therapy.

[0111] 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.

[0112] In some embodiments, the invention provides a composition comprising a single bacterial strain of the species *Enterococcus faecium*, which does not contain bacteria from any other strains or which comprises only *de minimis* or biologically irrelevant amounts of bacteria from another strain for use in therapy.

[0113] In some embodiments, the compositions of the invention comprise more than one

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

[0114] In some embodiments, the composition comprises a microbial consortium. For example, in some embodiments, the composition comprises the *Enterococcus faecium* bacterial strain as part of a microbial consortium. For example, in some embodiments, the *Enterococcus faecium* 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 *Enterococcus faecium* 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.

[0115] In some embodiments, the composition of the invention additionally comprises a bacterial strain that has the same safety and therapeutic efficacy characteristics as strain MRX010, but which is not MRX010 deposited as NCIMB 42487, or which is not a *Enterococcus faecium*.

[0116] The composition of the invention does not comprise *Bacillus subtilis*. In some embodiments, the composition of the invention does not additionally comprise *Bacillus coagulans*.

[0117] 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.

[0118] 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.

[0119] As mentioned above, in some embodiments, the one or more *Enterococcus faecium* 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.

[0120] The compositions for use in accordance with the invention may or may not require marketing approval.

[0121] 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.

[0122] 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.

[0123] The compositions of the invention can comprise pharmaceutically acceptable excipients, diluents or carriers.

[0124] In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain as used in the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat a disorder when administered to a subject in need thereof; and wherein the disorder is selected from the group consisting of uveitis; multiple sclerosis; arthritis, such as rheumatoid arthritis, osteoarthritis, psoriatic arthritis, or juvenile idiopathic arthritis; neuromyelitis optica (Devic's disease); ankylosing spondylitis; spondyloarthritis; psoriasis; systemic lupus erythematosus; inflammatory bowel disease, such as Crohn's disease or ulcerative colitis; celiac disease; asthma, such as allergic asthma or neutrophilic asthma; chronic obstructive pulmonary disease (COPD); scleritis; vasculitis; Behcet's disease; atherosclerosis; atopic dermatitis; emphysema;

periodontitis; allergic rhinitis; and allograft rejection.

[0125] In certain embodiments, the invention provides pharmaceutical composition comprising: a bacterial strain as used in the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat or prevent a disease or condition mediated by IL-17 or the Th17 pathway. In preferred embodiments, said disease or condition is selected from the group consisting of uveitis; multiple sclerosis; arthritis, such as rheumatoid arthritis, osteoarthritis, psoriatic arthritis, or juvenile idiopathic arthritis; neuromyelitis optica (Devic's disease); ankylosing spondylitis; spondyloarthritis; psoriasis; systemic lupus erythematosus; inflammatory bowel disease, such as Crohn's disease or ulcerative colitis; celiac disease; asthma, such as allergic asthma or neutrophilic asthma; chronic obstructive pulmonary disease (COPD); scleritis; vasculitis; Behcet's disease; atherosclerosis; atopic dermatitis; emphysema; periodontitis; allergic rhinitis; and allograft rejection.

[0126] The invention provides the above pharmaceutical composition, wherein the amount of the bacterial strain is from about 1×10^6 to about 1×10^{11} colony forming units per gram with respect to a weight of the composition.

[0127] 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.

[0128] 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.

[0129] 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.

[0130] In certain embodiments, the invention provides the above pharmaceutical composition, comprising a diluent selected from the group consisting of ethanol, glycerol and water.

[0131] 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.

[0132] In certain embodiments, the invention provides the above pharmaceutical composition, further comprising at least one of a preservative, an antioxidant and a stabilizer.

[0133] 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.

[0134] 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.

[0135] 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.

[0136] 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").

[0137] 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.

[0138] Pharmaceutical formulations suitable for oral administration include solid plugs, solid microparticulates, semi-solid and liquid (including multiple phases or dispersed systems) such as tablets; soft or hard capsules containing multi- or nano-particulates, liquids (e.g. aqueous solutions), emulsions or powders; lozenges (including liquid-filled); chews; gels; fast dispersing dosage forms; films; ovules; sprays; and buccal/mucoadhesive patches.

[0139] In some embodiments the pharmaceutical formulation is an enteric formulation, i.e. a gastro-resistant formulation (for example, resistant to gastric pH) that is suitable for delivery of the composition of the invention to the intestine by oral administration. Enteric formulations may be particularly useful when the bacteria or another component of the composition is acid-sensitive, e.g. prone to degradation under gastric conditions.

[0140] In some embodiments, the enteric formulation comprises an enteric coating. In some embodiments, the formulation is an enteric-coated dosage form. For example, the formulation may be an enteric-coated tablet or an enteric-coated capsule, or the like. The enteric coating may be a conventional enteric coating, for example, a conventional coating for a tablet, capsule, or the like for oral delivery. The formulation may comprise a film coating, for example, a thin film layer of an enteric polymer, e.g. an acid-insoluble polymer.

[0141] In some embodiments, the enteric formulation is intrinsically enteric, for example, gastro-resistant without the need for an enteric coating. Thus, in some embodiments, the formulation is an enteric formulation that does not comprise an enteric coating. In some

embodiments, the formulation is a capsule made from a thermogelling material. In some embodiments, the thermogelling material is a cellulosic material, such as methylcellulose, hydroxymethylcellulose or hydroxypropylmethylcellulose (HPMC). In some embodiments, the capsule comprises a shell that does not contain any film forming polymer. In some embodiments, the capsule comprises a shell and the shell comprises hydroxypropylmethylcellulose and does not comprise any film forming polymer (e.g. see [59]). In some embodiments, the formulation is an intrinsically enteric capsule (for example, Vcaps[®] from Capsugel).

[0142] In some embodiments, the formulation is a soft capsule. Soft capsules are capsules which may, owing to additions of softeners, such as, for example, glycerol, sorbitol, maltitol and polyethylene glycols, present in the capsule shell, have a certain elasticity and softness. Soft capsules can be produced, for example, on the basis of gelatine or starch. Gelatine-based soft capsules are commercially available from various suppliers. Depending on the method of administration, such as, for example, orally or rectally, soft capsules can have various shapes, they can be, for example, round, oval, oblong or torpedo-shaped. Soft capsules can be produced by conventional processes, such as, for example, by the Scherer process, the Accogel process or the droplet or blowing process.

Culturing methods

[0143] The bacterial strains for use in the present invention can be cultured using standard microbiology techniques as detailed in, for example, references [60-62].

[0144] The solid or liquid medium used for culture may 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).

Bacterial strains for use in vaccine compositions

[0145] The inventors have identified that the bacterial strains of the invention are useful for treating or preventing diseases or conditions mediated by IL-17 or the Th17 pathway. This is likely to be a result of the effect that the bacterial strains of the invention have on the host immune system. Therefore, the compositions of the invention may also be useful for preventing diseases or conditions mediated by IL-17 or the Th17 pathway, when administered as vaccine compositions. In certain such embodiments, the bacterial strains of the invention are viable. In certain such embodiments, the bacterial strains of the invention are capable of partially or totally colonising the intestine. In certain such embodiments, the bacterial strains of

the invention are viable and capable of partially or totally colonising the intestine. In other certain such embodiments, the bacterial strains of the invention may be killed, inactivated or attenuated. In certain such embodiments, the compositions may comprise a vaccine adjuvant. In certain embodiments, the compositions are for administration via injection, such as via subcutaneous injection.

General

[0146] 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 [63] and [64-70], *etc.*

[0147] 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.

[0148] The term "about" in relation to a numerical value x is optional and means, for example, x+10%.

[0149] 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.

[0150] 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. [71]. 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. [72].

[0151] 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.

[0152] Various embodiments of the invention are described herein. It will be appreciated that the features specified in each embodiment may be combined with other specified features, to provide further embodiments. In particular, embodiments highlighted herein as being suitable, typical or preferred may be combined with each other (except when they are mutually exclusive).

MODES FOR CARRYING OUT THE INVENTION

Example 1 - Efficacy of bacterial inocula in a mouse model of uveitis

Summary

[0153] This study used a mouse model of interphotoreceptor retinoid-binding protein (IRBP)-induced uveitis to test the effects of bacterial administration on uveitis. Uveitis is a sight-threatening condition resulting from intraocular inflammation and retinal tissue destruction. This disease can be studied in rodents in a model of experimental autoimmune uveoretinitis (EAU) [73]. EAU is an organ-specific disorder where Th1/Th17 cells are directed toward retinal antigens and produce cytokines that activate resident and infiltrating mononuclear cells leading to tissue destruction. EAU can be induced in mice by challenge with retinal antigens including interphotoreceptor retinoid binding protein peptide (IRBPp). Disease onset normally occurs from day 8-9 and peaks after days 14-15. Signs of clinical disease can be monitored using topical endoscopic fundal imaging (TEFI).

Strain

[0154] MRX010: *Enterococcus faecium*, bacteria deposited under accession number NCIMB 42487.

[0155] Biotherapeutic was provided in glycerol stock. Microbiological growth media (YCFA) was used for the culture of the agent.

Mice

[0156] The mice were strain C57BL/6 and were over 6 weeks old at the beginning of the study. 72 mice were used (+ 36 Satellite animals). Unhealthy animals were excluded from the study. Animals were housed in specific pathogen free (spf) conditions, in a thermostatically monitored holding room (22 ± 4°C). Animals were allowed to acclimatise under standard animal house conditions for a minimum of one week prior to use. The health status of the animals was monitored throughout this period and the suitability of each animal for experimental use was assessed prior to study start. Mice were housed in groups of up to 10 animals per cage for the duration of the study. Irradiated pellet diet (Lab diet, EU Rodent diet 22%, 5LF5) and water were available ad libitum throughout the acclimatisation and study periods. It is unlikely that

any constituent of the diet or water interfered with the study.

Experimental Outline

[0157] Adult female C57BL/6 mice were randomly allocated to experimental groups and allowed to acclimatise for one week. Treatments were administered according to the schedule below. On Day 0, animals were administered with an emulsion containing 200 µg of interphotoreceptor retinoid binding protein peptide 1-20 (IRBP p1-20) in complete Freund's adjuvant (CFA) supplemented with 2.5 mg/ml *Mycobacterium Tuberculosis* H37 Ra by subcutaneous injection. Also on Day 0, animals were administered with 1.5 µg *Bordetella Pertussis* toxin by intra-peritoneal injection. From Day -14, animals are weighed three times per week. From Day -1 until the end of the experiment on Day 42, animals are monitored twice per week for clinical signs of uveitis using topical endoscopic fundal imaging (TEFI).

Administration schedule

[0158]

All Groups are n=12

Vehicle for oral administration is YCFA medium.

[0159] Administration volume for twice daily oral administration is 5 ml/kg.

Group	Treatment	Dose	Route	Frequency	Disease Induction
1	Vehicle	5 ml/kg	PO	BID	Day 0: IRBP/CFA, SC
2	MRX010	5 ml/kg		Day -14-End	Day 0: PTx, IP

PO: oral administration, BID: twice daily, SC: subcutaneous injection, IP: intra-peritoneal injection,
 IRBP: interphotoreceptor binding protein, CFA: complete Freund's adjuvant, PTx: Pertussis toxin

[0160] A positive control group was also tested using treatment with the drug cyclosporin A.

Readouts

[0161] Bodyweights. From Day -14, animals are weighed three times a week. Animals with a

bodyweight loss equal to or greater than 15% of their initial (Day 0) bodyweight on two consecutive occasions are culled.

[0162] Non-specific clinical observations. From Day -14 until the end of the experiment, animals are checked daily for non-specific clinical signs to include abnormal posture (hunched), abnormal coat condition (piloerection) and abnormal activity levels (reduced or increased activity).

[0163] Clinical Scores: Retinal imaging by topical endoscopic fundal imaging (TEFI). From Day -1 until the end of the experiment, animals are scored twice per week for clinical signs of uveitis. Retinal images are captured using TEFI in non-anaesthetised but restrained animals following pupil dilatation using Tropicamide 1% then Phenylephrine hydrochloride 2.5%. Retinal images are scored using the following system. The maximum cumulative score is 20.

Score	Optic disc Inflammation	Retinal vessels	Retinal tissue Infiltration	Structural damage
1	Minimal	1-4 mild cuffings	1-4 small lesions or 1 linear lesion	Retinal lesions or atrophy involving $\frac{1}{4}$ to $\frac{3}{4}$ of retinal area
2	Mild	>4 mild cuffings or 1-3 moderate cuffings	5-10 small lesions or 2-3 linear lesions	Panretinal atrophy with multiple small lesions (scars) or ≤ 3 linear lesions (scars)
3	Moderate	>3 moderate cuffings	>10 small lesions or >3 linear lesions	Panretinal atrophy with >3 linear lesions or confluent lesions (scars)
4	Severe	>1 severe cuffings	Linear lesion confluent	Retinal detachment with folding
5	Not visible (white-out or severe detachment)			

Results

[0164] The results of the study are shown in Figures 1-3.

[0165] Cell Proliferation (satellite animals, day 21). Draining lymph nodes (DLN) were removed and cells were isolated. After counting, cells were cultured for 72h in the presence or absence of IRBP peptide. Supernatants for cytokine analysis were removed prior to pulsing with 3H-Thymidine. Cells were then cultured for a further 18h prior to harvesting and determination of proliferation by incorporation of 3H-thymidine using a beta-counter.

[0166] The groups showed cell proliferation to IRBP stimulus above that seen in the media control wells (these background values were subtracted from the IRBP result to provide data as Δ cpm). Both groups gave strong proliferative responses to a positive control stimulus (anti-CD3/CD28) showing cells in culture were viable and able to proliferate (data not shown).

[0167] Proliferative responses to IRBP peptide were analysed by one-way ANOVA followed by Dunnett's post-test for comparison of stimulated responses in the experimental group with the control group.

[0168] Proliferative responses to IRBP peptide in disease control animals were of the magnitude expected for this model. The positive control drug, Cyclosporin A, reduced proliferation, although this reduction did not achieve statistical significance (Figure 1).

[0169] The treatment groups (including vehicle alone) showed non-significantly increased proliferation above that seen in control animals.

[0170] Proliferative responses to IRBP peptide were analysed by one-way ANOVA followed by Dunnett's post-test for comparison of stimulated responses in treatment groups with the vehicle group. No statistical differences were seen.

[0171] Clinical Scores: Retinal imaging by topical endoscopic fundal imaging (TEFI). TEFI scores data measured in the Control group from Day 0 until Day 21 were analysed by Kruskal-Wallis test for non-parametric data followed by Dunn's post-test for multiple comparisons between experimental days.

[0172] IRBP administration induced a significant increase in the TEFI scores measured from Day 14 ($p < 0.01$) and on Day 21 ($p < 0.0001$) when compared to Day 0 in the Control group (Figure 2).

[0173] TEFI scores data measured in all experimental groups on Day 21 were analysed by Kruskal-Wallis test for non-parametric data followed by Dunn's post-test for multiple comparisons between experimental groups.

[0174] At this stage in the experiment, there was no significant difference between experimental groups, but TEFI scores were lower in the MRX010 treated group than in the negative control group. Indeed, TEFI scores in the MRX010 treated group were comparable to the positive control, cyclosporin A treated group.

[0175] Conclusions. Proliferative responses to IRBP peptide were seen in lymph node cultures from all experimental groups, excluding naive animals, indicating successful disease induction. Clinical scores determined by TEFI increased from Day 14, as expected in this model of IRBP-induced uveitis. By Day 21, significant differences between experimental groups are not yet visible, but a striking reduction in disease incidence and disease severity was

observed in the MRX010 treated group, which was a comparable reduction to that seen for the positive control group. In particular, these data indicate that treatment with the strain MRX010 reduced retinal damage, optic disc inflammation and/or retinal tissue infiltration by inflammatory cells (see TEFI retinal image scoring system above). These data indicate the strain MRX010 may be useful for treating or preventing uveitis.

Example 2 - Stability testing

[0176] 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 3 - Characterisation of enzymatic activity

Summary

[0177] The Analytical Profile Index (API®) test system consists of strips that contain miniaturised biochemical tests that assay for enzymatic activity in bacterial species. MRX010 (the bacterium deposited under accession number NCIMB 42487) was characterised using Rapid ID 32A - This system is designed specifically for anaerobic species and encompasses tests for carbohydrate, amino acid and nitrate metabolism as well as alkaline phosphatase activity.

[0178] Rapid ID 32A testing was carried out on bacterial colonies as per manufacturer's instructions. Briefly, bacteria were cultured on YCFA agar for 24 hours at 37 °C in an anaerobic workstation. Colonies were removed from plates using a sterile 5 µl inoculating loop and resuspended in a 2 ml ampoule of API® Suspension Medium until a density roughly equivalent to that of McFarland standard No. 4 was achieved. Fifty-five microlitres of bacterial suspension was added to each cupule on a Rapid ID 32A strip, and the urease test was overlaid with two drops of mineral oil. Strips were covered with a plastic lid and incubated aerobically at 37 °C for 4 hours, following which the bottom row of cupules were developed using the following reagents: NIT: 1 drop each of NIT1 and NIT2; IND: 1 drop of James reagent; all remaining cupules: 1 drop of FastBlue reagent. Strips were incubated at room temperature for 5 minutes, following which the colour of each cupule was recorded and assigned a value of negative, intermediate positive or positive.

Results

[0179] The results of the Rapid ID 32A analysis are shown in Figure 4. MRX010 tested positive for arginine dihydrolase, β -glucosidase, mannose fermentation, glutamic acid decarboxylase, arginine arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase and serine arylamidase. MRX010 showed an intermediate reaction for N-acetyl- β -glucosaminidase.

Sequences

[0180]

SEQ ID NO: 1 (*Enterococcus faecium* 16S rRNA gene, strain LMG 11423 - AJ301830)

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1 agagttttgat cctgggctcag gacgaacgct ggcgggcgtgc ctatacatgc aagtcgaacg
61 cttcttttttc caccggagct tgctccaccg gaaaaagagg agtggcgaac gggtagtaaa
121 cacgtgggta acctgcccac cagaaaggga taacacttgg aaacaggtgc taataccgta
181 taacaaatca aaaccgcatg gttttgattt gaaaggcgct ttcgggtgtc gctgatggat
241 ggacccgcgg tgcattagct agttggtgag gtaacggctc accaaggcca cgatgcatag
301 ccgcacctga gagggtgatc ggccacattg ggactgagac acggcccaaa ctctacggga
361 ggcagcagta gggaatcttc ggcaatggac gaaagtctga ccgagcaacg ccgcgtgagt
421 gaagaagggt ttcggatcgt aaaactctgt tgttagagaa gaacaaggat gagagtaact
481 gttcatccct tgacggtatc taaccagaaa gccacggcta actacgtgcc agcagccgcg
541 gtaatacgtg ggtggcaagc gttgtccgga tttattgggc gtaaagcgag cgcaggcggg
601 tcttaagtct gatgtgaaag ccccgggctc aaccggggag ggtcattgga aactgggaga
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Patentkrav

1. Sammensætning, der omfatter en bakteriestamme af arten *Enterococcus faecium*
5 med en 16s-rRNA-sekvens, som er mindst 99 % identisk med SEQ ID NO: 2, til
anvendelse i en fremgangsmåde til behandling eller forebyggelse af en inflammatorisk
eller autoimmun sygdom, hvor sammensætningen ikke omfatter en bakteriestamme
af arten *Bacillus subtilis*, og hvor mængden af bakteriestammen er fra ca. 1×10^6 til
ca. 1×10^{11} kolonidannende enheder pr. gram i forhold til en vægt af
10 sammensætningen.
2. Sammensætning til anvendelse ifølge krav 1, hvor sammensætningen er til
anvendelse i en fremgangsmåde til behandling eller forebyggelse af en inflammatorisk
eller autoimmun sygdom valgt fra gruppen bestående af: uveitis; multipel sklerose;
15 arthritis, såsom reumatoid arthritis, osteoarthritis, psoriasisarthritis eller juvenil idiopatisk
arthritis; neuromyelitis optica (Devics sygdom); ankyloserende spondylitis;
spondyloarthritis; psoriasis; systemisk lupus erythematosus; inflammatorisk
tarmsygdom, såsom Crohns sygdom eller ulcerativ colitis; cøliaki; astma, såsom
allergisk astma eller neutrofil astma; kronisk obstruktiv lungesygdom (KOL); skleritis;
20 vaskulitis; Behçets sygdom; aterosklerose; atopisk dermatitis; emfysem; periodontitis;
allergisk rinitis; og allograft-afstødning.
3. Sammensætning til anvendelse ifølge krav 2, hvor sammensætningen er til
anvendelse i en fremgangsmåde til behandling eller forebyggelse af uveitis, eventuelt
25 hvor sammensætningen er til anvendelse i en fremgangsmåde til reduktion eller
forebyggelse af nethindeskade i uveitis.
4. Sammensætning til anvendelse ifølge krav 2, hvor sammensætningen er til
anvendelse i en fremgangsmåde til behandling eller forebyggelse af astma, såsom
30 neutrofil astma eller allergisk astma, eventuelt hvor sammensætningen er til
anvendelse i en fremgangsmåde til reduktion af neutrofili eller eosinofili ved
behandling af astma.

5. Sammensætning til anvendelse ifølge krav 2, hvor sammensætningen er til anvendelse i en fremgangsmåde til behandling eller forebyggelse af reumatoid arthritis, eventuelt hvor sammensætningen er til anvendelse i en fremgangsmåde til reduktion af ledhævelse ved reumatoid arthritis.

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6. Sammensætning til anvendelse ifølge krav 2, hvor sammensætningen er til anvendelse i en fremgangsmåde til behandling eller forebyggelse af multipel sklerose, eventuelt hvor sammensætningen er til anvendelse i en fremgangsmåde til reduktion af sygdomsforekomst eller sygdomssværhedsgrad.

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7. Sammensætning til anvendelse ifølge et hvilket som helst af de foregående krav, hvor sammensætningen er til anvendelse i en fremgangsmåde til reduktion af IL-17-produktion eller reduktion af Th17-celledifferentiering ved behandling eller forebyggelse af den inflammatoriske eller autoimmune sygdom; eller hvor sammensætningen er til anvendelse ved behandling af en patient med en inflammatorisk eller autoimmun sygdom, hvor patienten har forhøjede IL-17-niveauer eller Th17-celler.

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8. Sammensætning til anvendelse ifølge et hvilket som helst af kravene 1-7, hvor bakteriestammen har en 16s-rRNA-sekvens, der er mindst 99,5 % eller 99,9 % identisk med SEQ ID NO: 2, eller hvor bakteriestammen har den 16s-rRNA-sekvens, der er angivet ved SEQ ID NO:2.

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9. Sammensætning til anvendelse ifølge et hvilket som helst af de foregående krav, hvor sammensætningen er til oral administration; hvor sammensætningen omfatter ét eller flere farmaceutisk acceptable hjælpe- eller bærestoffer; hvor bakteriestammen er lyofiliseret; eller hvor bakteriestammen er levedygtig og i stand til helt eller delvist at kolonisere tarmen.

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10. Sammensætning til anvendelse ifølge et hvilket som helst af de foregående krav, hvor sammensætningen omfatter en enkelt stamme af *Enterococcus faecium* og ikke indeholder nogen andre bakteriestammer eller -arter eller kun omfatter *de minimis* eller biologisk irrelevante mængder af andre bakteriestammer eller -arter; eller hvor

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sammensætningen omfatter bakteriestammen *Enterococcus faecium* som en del af et mikrobielt konsortium.

5 **11.** Fødevareprodukt eller vaccinesammensætning, der omfatter sammensætningen ifølge et hvilket som helst foregående krav, til anvendelse ifølge et hvilket som helst foregående krav.

12. Celle eller biologisk ren kultur af *Enterococcus faecium*-stammen, der er deponeret under deponeringsnummer NCIMB 42487.

10

13. Sammensætning, der omfatter cellen ifølge krav 12 og eventuelt omfatter et farmaceutisk acceptabelt bære- eller hjælpestof.

15 **14.** Celle af *Enterococcus faecium*-stammen, der er deponeret under deponeringsnummer NCIMB 42487, til anvendelse i terapi, eventuelt hvor cellen er til anvendelse i en fremgangsmåde, der er defineret i et hvilket som helst af kravene 1-7.

DRAWINGS

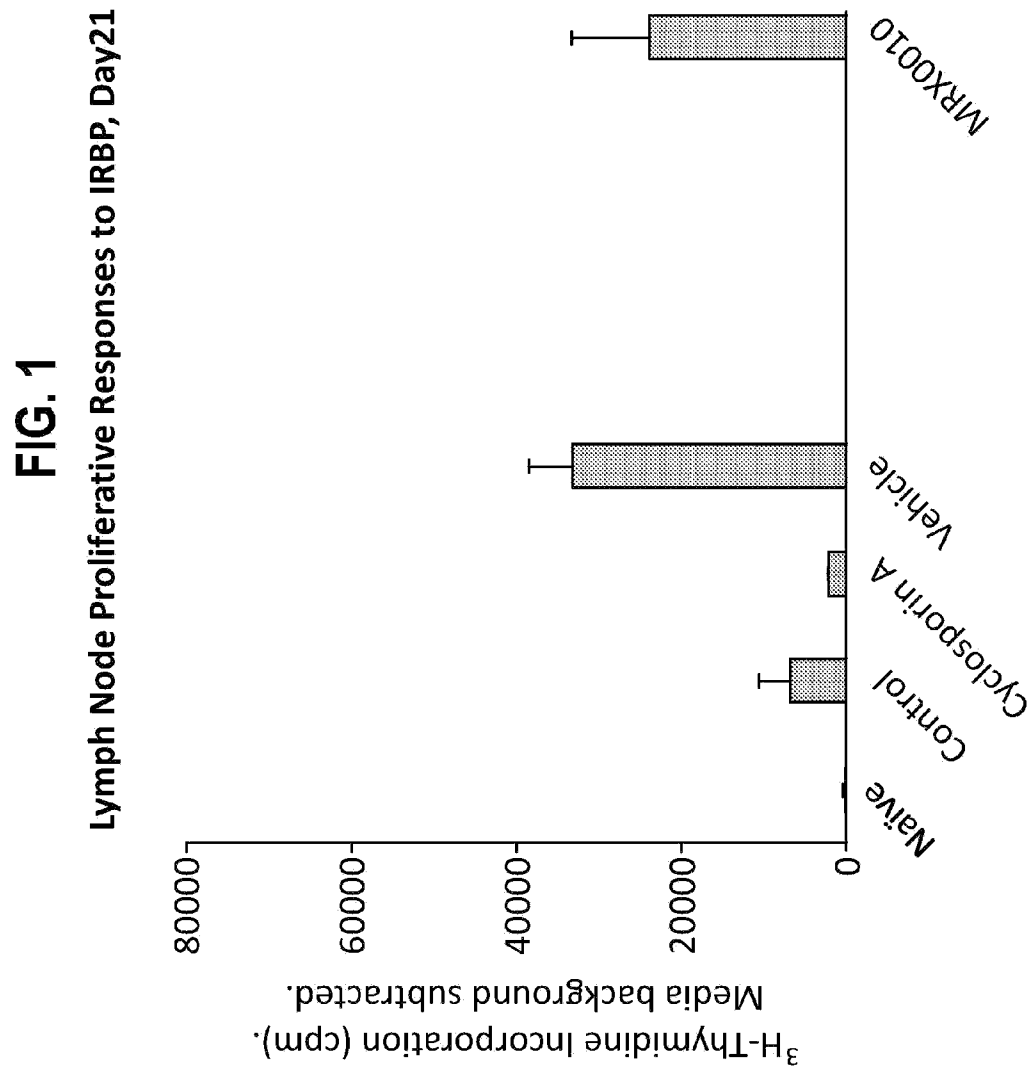


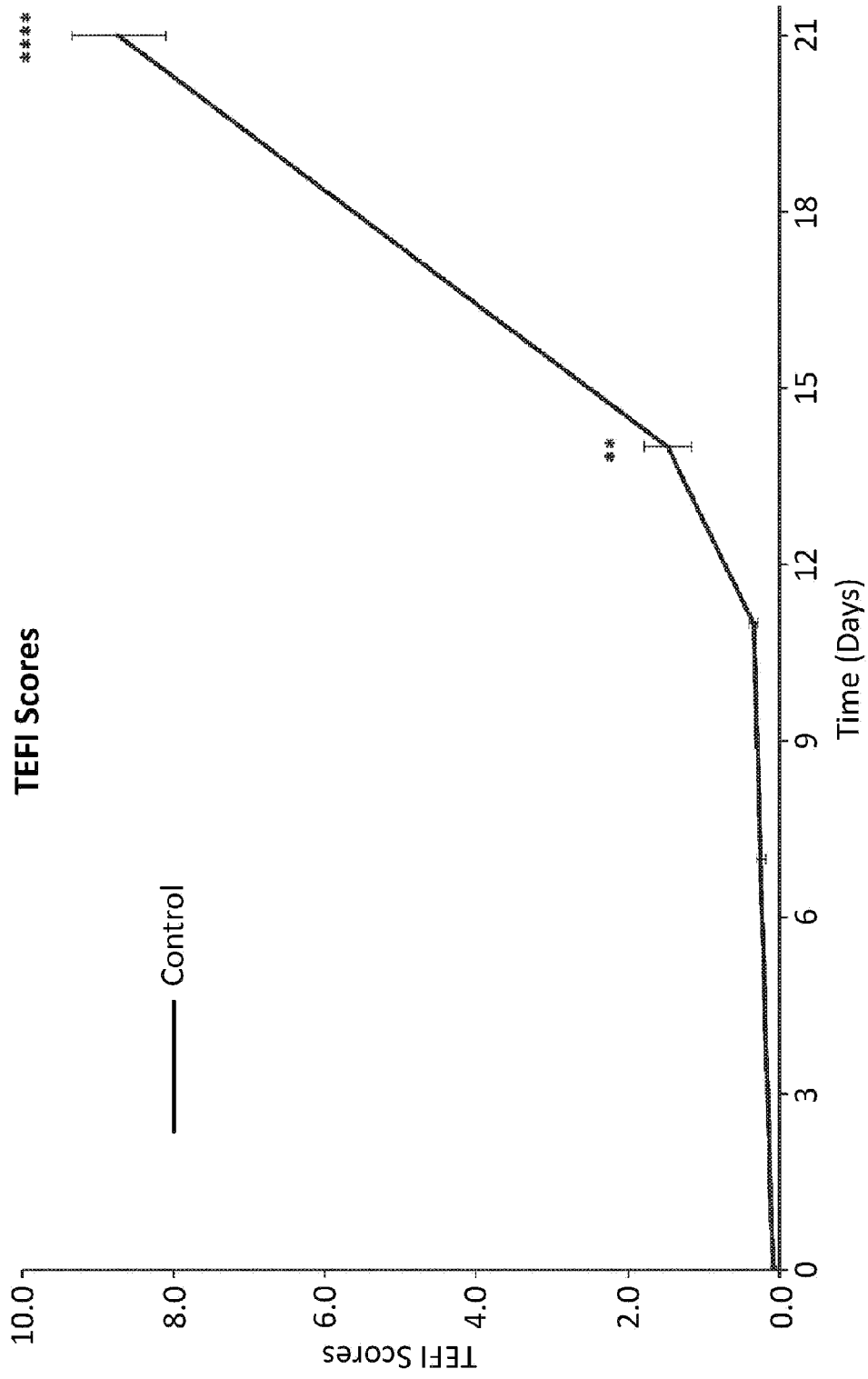
FIG. 2

FIG. 3
TEFI Scores

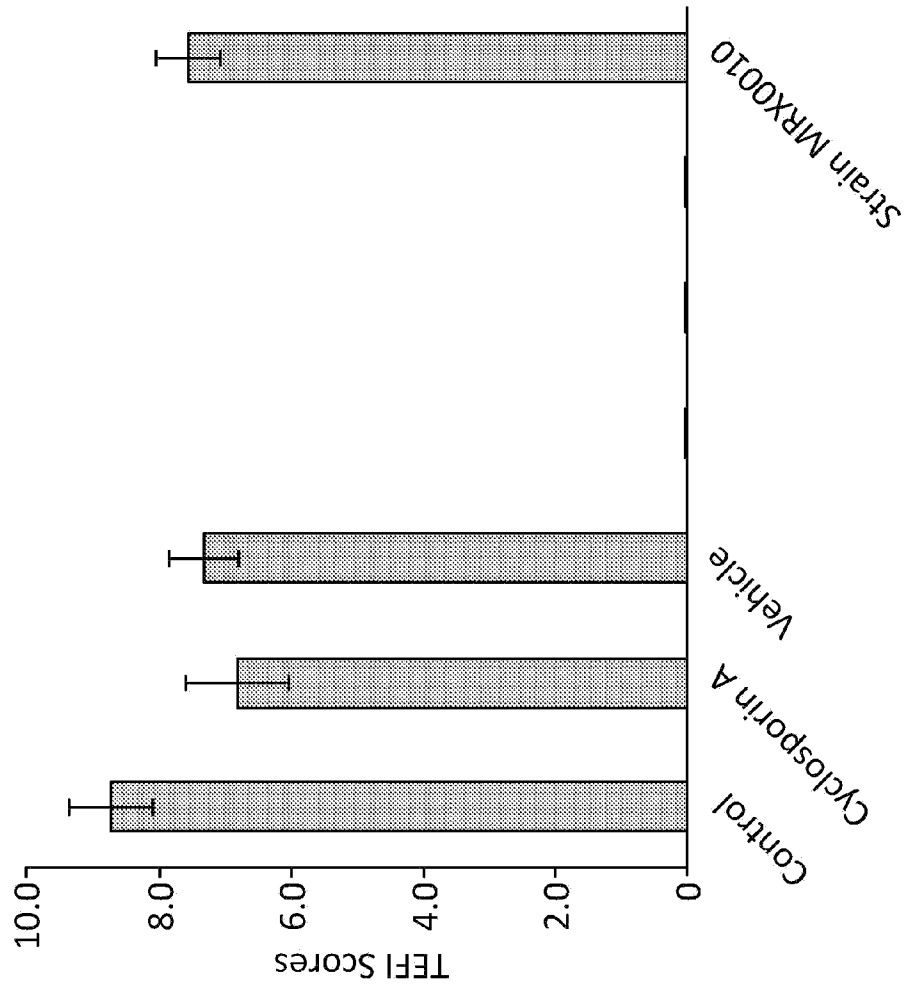
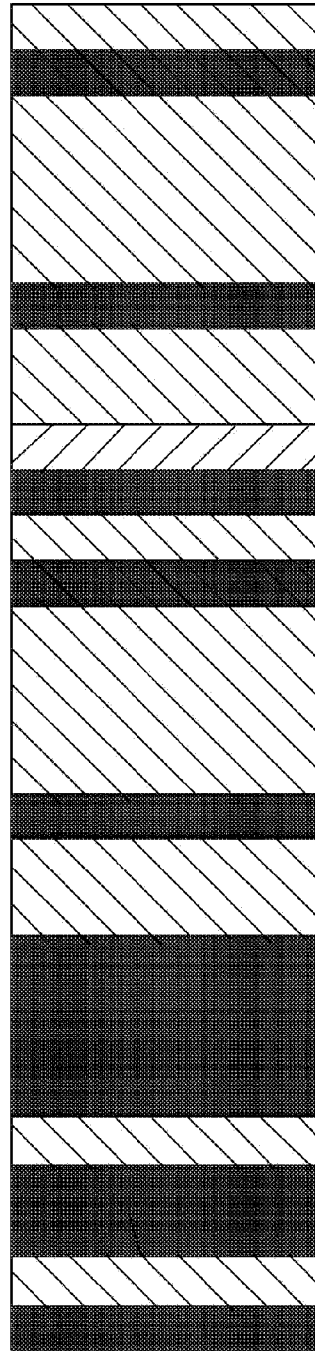
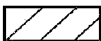


FIG. 4**Test****MRx0010**

Urease
 Arginine dihydrolase
 α -galactosidase
 β -galactosidase
 β -galactosidase 6 phosphate
 α -glucosidase
 β -glucosidase
 α -arabinose
 β -glucuronidase
 N-acetyl- β -glucosaminidase
 Mannose fermentation
 Raffinose fermentation
 Glutamic acid decarboxylase
 α -fucosidase
 Nitrate reduction
 Indole production
 Alkaline phosphatase
 Arginine arylamidase
 Proline arylamidase
 Leucyl glycine arylamidase
 Phenylalanine arylamidase
 Leucine arylamidase
 Pyroglutamic acid arylamidase
 Tyrosine arylamidase
 Alanine arylamidase
 Glycine arylamidase
 Histidine arylamidase
 Glutamyl glutamic acid arylamidase
 Serine arylamidase



Positive 
 Intermediate positive 
 Negative 