

Combination therapy for inflammatory bowel disease

Field of the Art

[0001] The present disclosure relates generally to methods for the treatment or prevention of inflammatory bowel disease.

Background

[0002] Inflammatory bowel disease is a complex idiopathic condition of the gastrointestinal tract that is increasingly prevalent in industrialized countries, with an estimated more than one million sufferers in the United States alone. There are two main subtypes of inflammatory bowel disease, Crohn's disease and ulcerative colitis. Crohn's disease can occur at any point along the lower gastrointestinal tract, although it primarily affects the ileum and the large intestine. Ulcerative colitis is mostly limited to the colon and can predispose individuals to colitis-associated cancer, typically colorectal cancer.

[0003] The etiologies of Crohn's disease and ulcerative colitis remain to be fully elucidated, however they are considered to be inflammatory conditions of the intestinal mucosa, characterized by the alteration and dysregulation of immune response towards commensal microbiota of the gastrointestinal tract. Symptomatically, the diseases are associated with abdominal pain, diarrhea (often bloody), and varied other clinical manifestations such as arthritis and uveitis.

[0004] For many years steroids (in particular corticosteroids such as prednisone) were the primary therapeutic option for inflammatory bowel disease. However long-term steroid use is associated with significant unwanted side effects, and the disease can become refractory to steroid treatment. Current non-steroid based therapeutic options for treating both Crohn's disease and ulcerative colitis include aminosalicylates (such as 5-aminosalicylic acid, sulfasalazine and mesalamine), antibiotics (such as ciprofloxacin and metronidazole), immunosuppressants (such as cyclosporin A, tacrolimus, azathioprine and methotrexate), tumor necrosis factor (TNF) antagonists (such as infliximab and adalimumab) and Janus kinase (JAK) inhibitors (such as tofacitinib). However patient response to these therapies varies with disease severity and can also vary over cycles of active inflammation and remission.

[0005] There is a continuing need for the development of new and improved

therapeutic options for the treatment of inflammatory bowel disease.

Summary of the Disclosure

[0006] A first aspect of the present disclosure provides a method for treating an inflammatory bowel disease or at least one symptom thereof, comprising administering to a subject in need thereof an effective amount of:

- (i) a compound selected from an immunosuppressant and an aminosalicylate; and
- (ii) one or more *Lactobacillus* species selected from *Lactobacillus buchneri*, *Lactobacillus paracasei*, *Lactobacillus zeae*, *Lactobacillus rapi*, *Lactobacillus parafarraginis*, and *Lactobacillus diolivorans*, and/or a culture supernatant or cell free culture filtrate derived from culture media in which said one or more *Lactobacillus* species has been cultured.

[0007] The inflammatory bowel disease may be an acute condition or a chronic condition. Typically, the inflammatory bowel disease is ulcerative colitis or Crohn's disease. In an exemplary embodiment the inflammatory bowel disease is ulcerative colitis. The ulcerative colitis may be chronic ulcerative colitis.

[0008] The symptom of the inflammatory bowel disease may be a clinical symptom, for example, poor stool consistency, faecal blood presence, abdominal pain or diarrhoea, or may be a physiological symptom including the elevated expression of a pro-inflammatory cytokine.

[0009] In an embodiment, the immunosuppressant is a calcineurin inhibitor. In an exemplary embodiment the calcineurin inhibitor is cyclosporin A.

[00010] In an embodiment, the immunosuppressant is a TNF inhibitor. In an exemplary embodiment the TNF inhibitor is adalimumab.

[00011] In an embodiment, the immunosuppressant is a JAK inhibitor. In an exemplary embodiment the JAK inhibitor is tofacitinib.

[00012] In an exemplary embodiment the aminosalicylate is 5-aminosalicylic acid.

[00013] The one or more *Lactobacillus* species may comprise a combination of at least three of said *Lactobacillus* species, optionally comprising *L. buchneri*, *L. paracasei* and *L. zeae*. Thus, in an embodiment, the method comprises administering to the subject a combination of *L. buchneri*, *L. paracasei* and *L. zeae* or culture supernatant(s) or cell free culture filtrate(s) therefrom.

[00014] In an embodiment, the method comprises administering to the subject an effective amount of cyclosporin A and a combination of *L. buchneri*, *L. paracasei* and *L. zae* or culture supernatant(s) or cell free culture filtrate(s) therefrom.

[00015] In an embodiment, the method comprises administering to the subject an effective amount of a TNF inhibitor, optionally adalimumab, and a combination of *L. buchneri*, *L. paracasei* and *L. zae* or culture supernatant(s) or cell free culture filtrate(s) therefrom.

[00016] In an embodiment, the method comprises administering to the subject an effective amount of a JAK inhibitor, optionally tofacitinib, and a combination of *L. buchneri*, *L. paracasei* and *L. zae* or culture supernatant(s) or cell free culture filtrate(s) therefrom.

[00017] In an embodiment, the method comprises administering to the subject an effective amount of 5-aminosalicylic acid and a combination of *L. buchneri*, *L. paracasei* and *L. zae* or culture supernatant(s) or cell free culture filtrate(s) therefrom.

[00018] The immunosuppressant or aminosalicilate and the one or more *Lactobacillus* species, culture supernatant(s) or cell free culture filtrate(s) therefrom, may be formulated in the same composition for administration. Alternatively, the immunosuppressant or aminosalicilate and the one or more *Lactobacillus* species may be administered in separate compositions. Such separate administration may be sequential or simultaneous.

[00019] The immunosuppressant or aminosalicilate and the one or more *Lactobacillus* species, culture supernatant(s) or cell free culture filtrate(s) therefrom, may be administered by the same or different routes, for example, orally, sublingually, topically or parenterally.

[00020] A second aspect of the present disclosure provides a method for reducing the expression of one or more pro-inflammatory cytokines in a subject suffering from inflammatory bowel disease, comprising administering to the subject an effective amount of:

- (i) a compound selected from an immunosuppressant and an aminosalicilate; and
- (ii) one or more *Lactobacillus* species selected from *Lactobacillus buchneri*, *Lactobacillus paracasei*, *Lactobacillus zae*, *Lactobacillus rari*, *Lactobacillus parafarraginis*, and *Lactobacillus diolivorans*, and/or a culture supernatant or cell free culture filtrate derived from culture media in which said one or more *Lactobacillus* species has been cultured.

[00021] In an embodiment, the pro-inflammatory cytokines are selected from IL-6, KC-GRO, TNF α and IL-1 β .

[00022] In a particular embodiment, the compound is selected from: a calcineurin inhibitor, optionally cyclosporin A; a TNF inhibitor, optionally adalimumab; a JAK inhibitor, optionally tofacitinib; and 5-aminosalicylic acid.

[00023] The one or more *Lactobacillus* species may comprise a combination of at least three of said *Lactobacillus* species, optionally comprising *L. buchneri*, *L. paracasei* and *L. zae*. Thus, in an embodiment, the method comprises administering to the subject a combination of *L. buchneri*, *L. paracasei* and *L. zae* or culture supernatant(s) or cell free culture filtrate(s) therefrom.

[00024] In a particular embodiment, the method comprises administering to the subject an effective amount of cyclosporin A and a combination of *L. buchneri*, *L. paracasei* and *L. zae* or culture supernatant(s) or cell free culture filtrate(s) therefrom.

[00025] A third aspect of the present disclosure provides the use of:

- (i) a compound selected from an immunosuppressant and an aminosalicylate; and
- (ii) one or more *Lactobacillus* species selected from *Lactobacillus buchneri*, *Lactobacillus paracasei*, *Lactobacillus zae*, *Lactobacillus rami*, *Lactobacillus parafarraginis*, and *Lactobacillus diolivorans*, and/or a culture supernatant or cell free culture filtrate derived from culture media in which said one or more *Lactobacillus* species has been cultured, in the manufacture of a medicament for the treatment of an inflammatory bowel disease or at least one symptom thereof.

[00026] In a particular embodiment, the compound is selected from: a calcineurin inhibitor, optionally cyclosporin A; a TNF inhibitor, optionally adalimumab; a JAK inhibitor, optionally tofacitinib; and 5-aminosalicylic acid.

[00027] In a particular embodiment, the one or more *Lactobacillus* species comprise a combination of *L. buchneri*, *L. paracasei* and *L. zae*. Thus, in an embodiment, the medicament comprises a combination of *L. buchneri*, *L. paracasei* and *L. zae* or culture supernatant(s) or cell free culture filtrate(s) therefrom.

[00028] In an embodiment, the medicament comprises cyclosporin A and a combination of *L. buchneri*, *L. paracasei* and *L. zae* or culture supernatant(s) or cell free culture filtrate(s) therefrom.

[00029] In an embodiment, the medicament comprises a TNF inhibitor, optionally adalimumab, and a combination of *L. buchneri*, *L. paracasei* and *L. zae* or culture supernatant(s) or cell free culture filtrate(s) therefrom.

[00030] In an embodiment, the medicament comprises a JAK inhibitor, optionally tofacitinib, and a combination of *L. buchneri*, *L. paracasei* and *L. zeae* or culture supernatant(s) or cell free culture filtrate(s) therefrom.

[00031] In an embodiment, the medicament comprises 5-aminosalicylic acid and a combination of *L. buchneri*, *L. paracasei* and *L. zeae* or culture supernatant(s) or cell free culture filtrate(s) therefrom.

[00032] In accordance with the above aspects and embodiments, and as described and exemplified herein, typically the combination of the immunosuppressant or aminosalicylate and the one or more *Lactobacillus* species is a synergistic combination.

Brief Description of the Drawings

[00033] Exemplary embodiments of the present disclosure are described herein, by way of non-limiting example only, with reference to the following drawings.

[00034] **Figure 1.** Faecal blood (A), stool consistency (B) and disease activity index (C) scores in mice of a 3% DSS-induced model of colitis, following treatment as described in Example 1. From left to right: Groups 1 to 5, respectively, as described in Example 1. *, $p < 0.05$, Dunnett's test compared to Group 2. **, $p < 0.01$, Dunnett's test compared to Group 2. ***, $p < 0.001$, Dunnett's test compared to Group 2.

[00035] **Figure 2.** Colonic mucosal cytokine expression analysis (A, IL-6; B, KC-GRO; C, TNF α ; D, IL-1 β) in mice of a 3% DSS-induced model of colitis, following treatment as described in Example 1. From left to right: Groups 1 to 5, respectively, as described in Example 1. For A-C: **, $p < 0.01$, Dunnett's test compared to Group 2; ***, $p < 0.001$, Dunnett's test compared to Group 2. For D: **, $p < 0.005$, Dunnett's test compared to Group 2.

[00036] **Figure 3.** Total, proximate, middle and distal composite scores for ulceration and inflammation in the colon of mice of a 3% DSS-induced model of colitis, following treatment as described in Example 1. From left to right: Groups 1 to 5 as described in Example 1. *, $p < 0.05$, Dunnett's test compared to Group 2. ***, $p < 0.001$ Dunnett's test compared to Group 2.

[00037] **Figure 4.** Colon length (mm) in mice of a 3% DSS-induced model of colitis, following treatment as described in Example 1. From left to right: Groups 1 to 5 as described in Example 1. *, $p < 0.05$, Dunnett's test compared to Group 2. ***, $p < 0.001$ Dunnett's test compared to Group 2.

[00038] **Figure 5.** Faecal blood (A), stool consistency (B) and disease activity index (C) scores in mice of a 3% DSS-induced model of colitis, on days 1 to 11 following treatment as described in Example 2: circle, naïve mice; square, 3% DSS + vehicle (control); triangle, 3% DSS + *Lactobacillus* SVT strains; inverted triangle, 3% DSS + 5-ASA; diamond, 3% DSS + *Lactobacillus* SVT strains + 5-ASA.

[00039] **Figure 6.** Faecal blood (A), stool consistency (B) and disease activity index (C) scores in mice of a 3% DSS-induced model of colitis, on days 1 to 11 following treatment as described in Example 3. Numbers 1 to 5 (indicated by dashed lines) represent treatment groups 1 to 5, respectively, of Example 3: circle (1), naïve mice; square (2), 3% DSS + vehicle (control); triangle (3), 3% DSS + *Lactobacillus* SVT strains; circle (4), 3% DSS + tofacitinib; square (5), 3% DSS + *Lactobacillus* SVT strains + tofacitinib.

[00040] **Figure 7.** Faecal blood (A), stool consistency (B) and disease activity index (C) scores in mice of a 3% DSS-induced model of colitis, on days 1 to 11 following treatment as described in Example 4. Numbers 1 to 5 (indicated by dashed lines) represent treatment groups 1 to 5, respectively, of Example 4: circle (1), naïve mice; square (2), 3% DSS + vehicle (control); triangle (3), 3% DSS + *Lactobacillus* SVT strains; triangle (4), 3% DSS + adalimumab; inverted triangle (5), 3% DSS + *Lactobacillus* SVT strains + adalimumab.

Detailed Description

[00041] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the disclosure belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, typical methods and materials are described.

[00042] The articles “a” and “an” are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

[00043] In the context of this specification, the term “about,” is understood to refer to a range of numbers that a person of skill in the art would consider equivalent to the recited value in the context of achieving the same function or result.

[00044] Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” or “comprising”,

will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[00045] As used herein the term "effective amount" includes within its meaning a non-toxic but sufficient amount of composition to provide the desired therapeutic effect. The exact amount required will vary from subject to subject depending on factors such as the species being treated, the age and general condition of the subject, the severity of the condition being treated, the particular agent being administered and the mode of administration and so forth. For any given case, an appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine experimentation.

[00046] The term "subject" as used herein refers to mammals and includes humans, primates, livestock animals (e.g. cattle, dairy cows, horses, sheep, pigs), laboratory test animals (e.g. mice, rabbits, rats, guinea pigs), companion animals (e.g. dogs, cats), performance animals (e.g. racehorses), and captive wild animals. In exemplary embodiments, the mammal is human.

[00047] As used herein the terms "treating", "treatment" and the like refer to any and all applications which remedy, or otherwise hinder, retard, or reverse the progression of, an inflammatory bowel disease, or at least one symptom of such a disease, including reducing the severity of the disease. Thus, treatment does not necessarily imply that a subject is treated until complete elimination of, or recovery from, the disease.

[00048] The term "optionally" is used herein to mean that the subsequently described feature may or may not be present or that the subsequently described event or circumstance may or may not occur. Hence the specification will be understood to include and encompass embodiments in which the feature is present and embodiments in which the feature is not present, and embodiments in which the event or circumstance occurs as well as embodiments in which it does not.

[00049] In the context of this specification, the term "microbial biotherapeutic" is to be given its broadest construction and is understood to refer to a microbial cell population or preparation, or component of a microbial cell population or preparation, which when administered to a subject in an effective amount promotes a health benefit in the subject.

[00050] In the context of this specification, the term "prebiotic" is to be given its broadest construction and is understood to refer to any non-digestible substance that stimulates the growth and/or activity of commensal beneficial bacteria in the digestive system.

[00051] In the context of this specification, the terms "food", "foods", "beverage" or "beverages" include but are not limited to health foods and beverages, functional foods and beverages, and foods and beverages for specified health use. When such foods or beverages of the present invention are used for subjects other than humans, the terms can be used to include a feedstuff.

[00052] Provided herein are methods for treating an inflammatory bowel disease or at least one symptom thereof, comprising administering to a subject in need thereof an effective amount of:

- (i) a compound selected from an immunosuppressant and an aminosalicilate; and
- (ii) one or more *Lactobacillus* species selected from *Lactobacillus buchneri*, *Lactobacillus paracasei*, *Lactobacillus zeae*, *Lactobacillus rapi*, *Lactobacillus parafarraginis*, and *Lactobacillus diolivorans*, and/or a culture supernatant or cell free culture filtrate derived from culture media in which said one or more *Lactobacillus* species has been cultured.

[00053] Also provided herein are methods reducing the expression of one or more pro-inflammatory cytokines in a subject suffering from inflammatory bowel disease, comprising administering to the subject an effective amount of:

- (i) a compound selected from an immunosuppressant and an aminosalicilate; and
- (ii) one or more *Lactobacillus* species selected from *Lactobacillus buchneri*, *Lactobacillus paracasei*, *Lactobacillus zeae*, *Lactobacillus rapi*, *Lactobacillus parafarraginis*, and *Lactobacillus diolivorans*, and/or a culture supernatant or cell free culture filtrate derived from culture media in which said one or more *Lactobacillus* species has been cultured.

[00054] The inflammatory bowel disease to which methods of the present disclosure relate may be selected from, for example, ulcerative colitis, Crohn's disease, ischemic colitis, enterocolitis, antibiotic-associated hemorrhagic colitis (AAHC), microscopic colitis and pouchitis. In particular embodiments, the inflammatory bowel disease is selected from ulcerative colitis and Crohn's disease. The ulcerative colitis may be acute or chronic ulcerative colitis.

[00055] The at least one symptom associated with the inflammatory bowel disease may be a clinical or physiological symptom including, for example, diarrhoea, poor stool consistency, faecal blood presence, abdominal pain, ulceration of the epithelial lining of the small intestine, large intestine or colon, or elevated expression of one or more pro-inflammatory cytokines relative to the level of expression observed in individuals not suffering from the inflammatory bowel disease. Those skilled in the art will readily appreciate that the scope of the present

disclosure should not be limited by reference these exemplary symptoms, and there are other symptoms of inflammatory bowel disease that will be encompassed by the present disclosure.

[00056] The pro-inflammatory cytokines, the expression of which may be reduced in accordance with embodiments of the present disclosure, include but are not limited to interleukins such as IL-6 and IL-1 β , KC-GRO (keratinocyte chemoattractant/human growth-regulated oncogene) and TNF α .

[00057] In embodiments of the present disclosure in which methods reduce the expression of one or more pro-inflammatory cytokines in a subject suffering from inflammatory bowel disease, the reduction observed is relative to the level of expression of the pro-inflammatory cytokines observed in the subject in the absence of said treatment. Such reduction may comprise normalization of the level of expression of the pro-inflammatory cytokines.

[00058] The methods of the present disclosure may inhibit inflammation associated with the inflammatory bowel disease. The term "inhibit" and variations thereof such as "inhibition", "inhibits", "reduces", "reducing" and the like, are used interchangeably herein to denote an improvement (*i.e.*, reduction) in the severity of inflammation associated with the inflammatory bowel disease.

[00059] Methods of the present disclosure employ the administration of an immunosuppressant or aminosalicylate in combination with one or more *Lactobacillus* species selected from *Lactobacillus buchneri*, *Lactobacillus paracasei*, *Lactobacillus zeae*, *Lactobacillus rami*, *Lactobacillus parafarraginis*, and *Lactobacillus diolivorans*, and/or a culture supernatant or cell free culture filtrate derived from culture media in which said one or more *Lactobacillus* species has been cultured. As exemplified herein, typically the combination of the immunosuppressant or aminosalicylate and the one or more *Lactobacillus* species is a synergistic combination.

[00060] In particular embodiments the immunosuppressant may be a calcineurin inhibitor, a thiopurine, a TNF inhibitor or a JAK inhibitor. Exemplary calcineurin inhibitors include but are not limited to cyclosporin A, tacrolimus and analogues thereof. Exemplary thiopurines include but are not limited to azathiopurine, 6-mercaptopurine and analogues thereof. Exemplary TNF inhibitors include but are not limited to monoclonal antibodies such as adalimumab, infliximab, natalizumab, certolizumab, golimumab and biosimilars thereof, but does not include etanercept. In particular embodiments the aminosalicylate is 5-aminosalicylic acid (mesalazine) or sulfasalazine, or an analogue thereof. The JAK inhibitor may be a selective or non-selective inhibitor and may be, for example, a JAK1 inhibitor, a JAK2 inhibitor, a

JAK1/JAK2 inhibitor, a JAK3 inhibitor or a TYK2 inhibitor. Exemplary JAK inhibitors include but are not limited to tofacitinib, baracitinib, upadacitinib, ruxolitinib, oclacitinib, peficitinib, filgotinib, fedracitinib, deucravacitinib and abrocitinib.

[00061] In some embodiments, the immunosuppressant or aminosalicylate is one that is known to have at least partial efficacy, when used as a sole therapeutic agent, in the treatment of inflammatory bowel disease.

[00062] In an exemplary embodiment the compound is selected from: a calcineurin inhibitor, optionally cyclosporin A; a TNF inhibitor, optionally adalimumab; a JAK inhibitor, optionally tofacitinib; and 5-aminosalicylic acid.

[00063] Methods of the present disclosure employ the administration of one or more *Lactobacillus* species selected from *Lactobacillus buchneri*, *Lactobacillus paracasei*, *Lactobacillus zeae*, *Lactobacillus rafi*, *Lactobacillus parafarraginis*, and *Lactobacillus diolivorans* and/or a culture supernatant or cell free culture filtrate derived from culture media in which said one or more *Lactobacillus* species has been cultured. In view of some taxonomic discrepancies and uncertainties, *Lactobacillus zeae* may also be referred to elsewhere as *Lactobacillus casei*. However for the purposes of the present disclosure the *Lactobacillus zeae* nomenclature is retained.

[00064] In the following discussion, in the context of administration of the *Lactobacillus* species or culture supernatants or cell free culture filtrates derived from culture media in which the *Lactobacillus* has been cultured, and in the context of compositions comprising the same, the term "*Lactobacillus*" may be used to refer not only to the specific *Lactobacillus* species defined herein *per se*, but also more broadly to refer to culture supernatants or cell free culture filtrates derived from culture media in which the specific *Lactobacillus* species defined herein have been cultured. Where *Lactobacillus* species *per se* are administered, in particular embodiments, the cells are reproductively viable.

[00065] The term "culture" as used herein refers to both liquid and plate cultures. "Culturing", as used herein, refers to the propagation of organisms on or in media of various kinds. A "pure" culture is a population of organisms growing in the absence of other species or types. A "substantially pure culture" of a species or strain(s) refers to a culture which contains substantially no other microbes than the desired species or strain(s). In other words, a substantially pure culture is substantially free of other contaminants, which can include microbial contaminants as well as undesirable chemical contaminants. A culture to be administered may comprise cells cultured to, for example, stationary phase or to log phase, optionally early, mid

or late log phase. Similarly, a culture supernatant or cell free culture filtrate to be administered may be prepared from cells cultured to, for example, stationary phase or log phase, optionally early, mid or late log phase.

[00066] A cell free culture filtrate may be prepared using any method known to the person skilled in the art. By way of example only, one or more *Lactobacillus* species disclosed herein may be inoculated from an agar slant to a suitable nutrient medium and grown to late log phase. The bacterial cells may be harvested by passing the liquid culture through a filter and the supernatant ("culture filtrate") may be filter-sterilized, e.g. through 0.2µm filter, to remove any remaining cells. The culture filtrate may thereafter be lyophilized or freeze-dried and reconstituted, in concentrate form, in deionized water as needed. The culture filtrate can then be filter-sterilized and diluted to an appropriate concentration for use. In another example, the cell free filtrate can be diluted (e.g. 1 in 10) in sterile water without freeze drying or lyophilization. Those skilled in the art will appreciate that to be regarded as a cell-free culture filtrate or culture supernatant it may not be necessary to remove every viable cell from the culture, but rather it is sufficient that the filtrate or supernatant is substantially or predominantly free of cultured cells. In some embodiments the terms cell free culture filtrate and culture supernatant may be used interchangeably herein. Optionally, a culture supernatant may differ from a cell free culture filtrate by the absence of one or more filtration steps. That is, a culture supernatant may be prepared by centrifugation of a culture grown to, for example, stationary phase or log phase, optionally early, mid or late log phase.

[00067] Methods of the present disclosure may comprise the administration of any two, three, four, five or all six of the *Lactobacillus* species *Lactobacillus buchneri*, *Lactobacillus paracasei*, *Lactobacillus zeae*, *Lactobacillus rami*, *Lactobacillus parafarraginis*, and *Lactobacillus diolivorans*, or culture supernatants or cell free culture filtrates derived from culture media in which two, three, four, five or all six of said *Lactobacillus* have been cultured. In such embodiments the bacteria may be cultured separately or together. Accordingly, the administration may comprise administration of a composition comprising a combination of two, three, four, five or all six of the *Lactobacillus* species described herein. Similarly, where culture supernatants or cell free culture filtrates derived from culture media in which two, three, four, five or all six of said *Lactobacillus* have been cultured are administered, the culture supernatants or cell free culture filtrates may be derived from the culturing of *Lactobacillus* species individually, said supernatants or cell free culture filtrates being combined prior to administration, or may be derived from a combined culture of two, three, four, five or all six of the *Lactobacillus* species described herein.

[00068] In an exemplary embodiment, the methods of the present disclosure comprise the administration of a combination of *Lactobacillus buchneri*, *Lactobacillus paracasei*, *Lactobacillus zeae*, or culture supernatants or cell free culture filtrates thereof.

[00069] The *Lactobacillus buchneri* may be *Lactobacillus buchneri* Lb23 available under Accession Number V11/022946, previously described in WO2013/063658. The *L. buchneri* may be *L. buchneri* SVT 06B1 (which may be elsewhere referred to by the alternate designation SVT-23) deposited pursuant to the Budapest Treaty with the Belgian Co-Ordinated Collections of Micro-organisms (BCCM) on 27 February 2019 under Accession Number LMG P-31293.

[00070] The *Lactobacillus paracasei* may be *Lactobacillus paracasei* Lp9 available under Accession Number V12/022849, previously described in WO2014/172758 (designated as strain 'T9' therein). The *Lactobacillus paracasei* may be *Lactobacillus paracasei* SVT 04P1 (which may be elsewhere referred to by the alternate designation SVT-09) deposited pursuant to the Budapest Treaty with the Belgian Co-Ordinated Collections of Micro-organisms (BCCM) on 27 February 2019 under Accession Number LMG P-31290.

[00071] The *Lactobacillus zeae* may be *Lactobacillus zeae* Lz26 available under Accession Number V11/022948, previously described in WO2013/063658. The *Lactobacillus zeae* may be *Lactobacillus zeae* SVT 08Z1 (which may be elsewhere referred to by the alternate designation SVT-26) deposited pursuant to the Budapest Treaty with the Belgian Co-Ordinated Collections of Micro-organisms (BCCM) on 27 February 2019 under Accession Number LMG P-31295.

[00072] The *Lactobacillus rami* may be *Lactobacillus rami* Lr24 available under Accession Number V11/022947, previously described in WO2013/063658. The *Lactobacillus rami* may be *Lactobacillus rami* SVT 07R1 (which may be elsewhere referred to by the alternate designation SVT-24) deposited pursuant to the Budapest Treaty with the Belgian Co-Ordinated Collections of Micro-organisms (BCCM) on 27 February 2019 under Accession Number LMG P-31294.

[00073] The *Lactobacillus parafarraginis* may be *Lactobacillus parafarraginis* Lp18 available under Accession Number V11/022945, previously described in WO2013/063658. The *Lactobacillus parafarraginis* may be *Lactobacillus parafarraginis* SVT 05P2 (which may be elsewhere referred to by the alternate designation SVT-18) deposited pursuant to the Budapest Treaty with the Belgian Co-Ordinated Collections of Micro-organisms (BCCM) on 27 February 2019 under Accession Number LMG P-31292.

[00074] The *Lactobacillus diolivorans* may be *Lactobacillus diolivorans* Ld3 available under Accession Number V12/022847, previously described in WO2014/172758 (designated as strain 'N3' therein). The *Lactobacillus diolivorans* may be *Lactobacillus diolivorans* SVT 01D1 (which may be elsewhere referred to by the alternate designation SVT-03) deposited pursuant to the Budapest Treaty with the Belgian Co-Ordinated Collections of Micro-organisms (BCCM) on 27 February 2019 under Accession Number LMG P-31287.

[00075] Where *Lactobacillus* organisms per se are administered, the concentrations of individual *Lactobacillus* species to be administered in accordance with methods of the present disclosure will depend on a variety of factors including the identity and number of individual species employed, the exact nature and severity of the inflammatory bowel disease to be treated, the form in which a composition is applied and the means by which it is applied. For any given case, appropriate concentrations may be determined by one of ordinary skill in the art using only routine experimentation. By way of example only, the concentration of the *Lactobacillus* species, or each species present in the case of a combination, may be from about 1×10^2 cfu/ml to about 1×10^{12} cfu/ml, and may be about 1×10^3 cfu/ml, about 2.5×10^3 cfu/ml, about 5×10^3 cfu/ml, 1×10^4 cfu/ml, about 2.5×10^4 cfu/ml, about 5×10^4 cfu/ml, 1×10^5 cfu/ml, about 2.5×10^5 cfu/ml, about 5×10^5 cfu/ml, 1×10^6 cfu/ml, about 2.5×10^6 cfu/ml, about 5×10^6 cfu/ml, 1×10^7 cfu/ml, about 2.5×10^7 cfu/ml, about 5×10^7 cfu/ml, 1×10^8 cfu/ml, about 2.5×10^8 cfu/ml, about 5×10^8 cfu/ml, 1×10^9 cfu/ml, about 2.5×10^9 cfu/ml, or about 5×10^9 cfu/ml, about 1×10^{10} cfu/ml, about 1.5×10^{10} cfu/ml, about 2.5×10^{10} cfu/ml, about 5×10^{10} cfu/ml, about 1×10^{11} cfu/ml, about 1.5×10^{11} cfu/ml or about 1×10^{12} cfu/ml.

[00076] Also contemplated by the present disclosure is the use of variants of the *Lactobacillus* species described herein. As used herein, the term "variant" refers to both naturally occurring and specifically developed variants or mutants of the species disclosed and exemplified herein. Variants may or may not have the same identifying biological characteristics of the specific species exemplified herein, provided they share similar advantageous properties in terms of treating or preventing inflammatory conditions. Illustrative examples of suitable methods for preparing variants exemplified herein include, but are not limited to, gene integration techniques such as those mediated by insertional elements or transposons or by homologous recombination, other recombinant DNA techniques for modifying, inserting, deleting, activating or silencing genes, intraspecific protoplast fusion, mutagenesis by irradiation with ultraviolet light or X-rays, or by treatment with a chemical mutagen such as nitrosoguanidine, methylmethane sulfonate, nitrogen mustard and the like, and bacteriophage-mediated transduction. Suitable and applicable methods are well known in the art and are

described, for example, in J. H. Miller, *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1972); J. H. Miller, *A Short Course in Bacterial Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1992); and J. Sambrook, D. Russell, *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2001), *inter alia*.

[00077] Also encompassed by the term “variant” as used herein are microbial strains phylogenetically closely related to the *Lactobacillus* species described herein and strains possessing substantial sequence identity with the species described herein at one or more phylogenetically informative markers such as rRNA genes, elongation and initiation factor genes, RNA polymerase subunit genes, DNA gyrase genes, heat shock protein genes and *recA* genes. For example, the 16S rRNA genes of a “variant” strain as contemplated herein may share about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with a strain disclosed herein.

[00078] The *Lactobacillus* species described herein, and combinations thereof, or culture supernatants or cell free culture filtrates derived from culture media are typically administered in accordance with the present disclosure in the form of a composition. In embodiments in which combinations of species, or culture supernatants or cell free culture filtrates derived from culturing multiple species, those skilled in the art will appreciate that each of the species, supernatants or culture filtrates to be administered need not be contained in the same composition. Where administration is separate, administration may be sequential or simultaneous.

[00079] Similarly, the immunosuppressant or aminosalicylate may be administered in the same composition as the one or more *Lactobacillus* species or culture supernatant(s) or cell free culture filtrate(s), or may be administered in a different composition. Where the immunosuppressant or aminosalicylate is present in a different composition, the compositions may be administered sequentially or simultaneously.

[00080] Compositions for use in accordance with the present disclosure may be prepared by admixing the relevant components and formulating the resulting mixture into a dosage form that is suitable for administration to a subject. Accordingly, the compositions may comprise pharmaceutically acceptable carriers, diluents, excipients and/or adjuvants. The carriers, diluents, excipients and adjuvants must be “acceptable” in terms of being compatible with other components of the composition, and not deleterious to the subject who is to receive the composition. Methods for preparing suitable compositions for administration, and carriers,

diluents, excipients and adjuvants suitable for use in compositions formulated for topical, oral or sublingual administration are well known to those skilled in the art. In exemplary embodiments, the composition may comprise one or more microbial biotherapeutic strains concentrated (e.g. by centrifugation and/or filtration) following cell culture to remove excess media. As such, the composition may comprise one or more microbial biotherapeutic strains in residual food grade media. Alternatively, the composition may be formulated with a carrier comprising sterile isotonic saline or 3% sucrose.

[00081] Compositions may be administered via any convenient or suitable route, variety of routes including, but not limited to, oral, sublingual, buccal, rectal, topical, intranasal, intraocular, transmucosal, intestinal, enteral, intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intracerebral, intravesical, intravenous or intraperitoneal. The appropriate route may depend, for example, on the nature and severity of the inflammatory bowel disease to be treated. Where the immunosuppressant or aminosalicylate is administered in a different composition to the one or more *Lactobacillus* species or culture supernatant(s) or cell free culture filtrate(s), the route of administration of the compositions may be the same or different.

[00082] By way of example only: compositions comprising the one or more *Lactobacillus* species or a culture supernatant or cell free culture filtrate derived from culture media in which said one or more *Lactobacillus* species has been cultured may be administered orally; compositions comprising the aminosalicylate may be administered orally; and compositions comprising the immunosuppressant may be administered orally or by injection. For example, compositions comprising tofacitinib may be administered orally, and compositions comprising adalimumab may be administered by subcutaneous injection.

[00083] Accordingly, methods of the present disclosure contemplate the administration of components of the combinations described in the same or different compositions, and via the same or different routes. Exemplary embodiments of methods of the disclosure comprise the oral administration of one or more *Lactobacillus* strains and an immunosuppressant such as CsA or tofacitinib, wherein the *Lactobacillus* strains and the CsA or tofacitinib are in the same or different compositions. Exemplary embodiments of methods of the disclosure comprise the oral administration of one or more *Lactobacillus* strains and ASA, wherein the *Lactobacillus* strains and the ASA are in the same or different compositions. Exemplary embodiments of methods of the disclosure comprise the oral administration of one or more *Lactobacillus* strains and the administration of an immunosuppressant such as adalimumab by injection, optionally subcutaneous injection.

[00084] Compositions may be administered in accordance with the present disclosure in any suitable form, typically in solid or liquid form. For example, the compositions may be formulated using methods and techniques well known to those skilled in the art, into tablets, troches, capsules, caplets, elixirs, suspensions, syrups, wafers, granules, powders, gels, pastes, solutions, creams, sprays, suspensions, soluble sachets, lozenges, effervescent tablets, chewable tablets, multi-layer tablets, and the like. For oral administration, the *Lactobacillus* or compositions may be conveniently incorporated in a variety of beverages, food products, nutraceutical products, nutritional supplements, food additives, pharmaceuticals, over-the-counter formulations and animal feed supplements. For topical application, suitable vehicles include, but are not limited to, lotions, liniments, gels, creams, ointments, foams, sprays, oils, powders and the like. Compositions may also be impregnated into transdermal patches, plasters, and wound dressings such as bandages or hydrocolloid dressings, typically in liquid or semi-liquid form.

[00085] As will be appreciated by those skilled in the art, the choice of pharmaceutically acceptable carrier or diluent will be dependent on the route of administration and on the nature and severity of the condition and the subject to be treated. The particular carrier or delivery system and route of administration may be readily determined by a person skilled in the art. A person skilled in the art will readily be able to determine appropriate formulations useful in the methods of the disclosure using conventional approaches.

[00086] For example, compositions of the present disclosure may be formulated for administration in the form of liquids, containing acceptable diluents (such as saline and sterile water), or may be in the form of lotions, creams or gels containing acceptable diluents or carriers to impart the desired texture, consistency, viscosity and appearance. Acceptable diluents and carriers are familiar to those skilled in the art and include, but are not restricted to, ethoxylated and nonethoxylated surfactants, fatty alcohols, fatty acids, hydrocarbon oils (such as palm oil, coconut oil, and mineral oil), cocoa butter waxes, silicon oils, pH balancers, cellulose derivatives, emulsifying agents such as non-ionic organic and inorganic bases, preserving agents, wax esters, steroid alcohols, triglyceride esters, phospholipids such as lecithin and cephalin, polyhydric alcohol esters, fatty alcohol esters, hydrophilic lanolin derivatives and hydrophilic beeswax derivatives.

[00087] The *Lactobacillus* can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. These carriers may be selected from sugars, starches, cellulose and its derivatives, malt, gelatine, talc,

calcium sulfate, vegetable oils, synthetic oils, polyols, alginic acid, phosphate buffered solutions, emulsifiers, isotonic saline and pyrogen-free water.

[00088] Some examples of suitable carriers, diluents, excipients and adjuvants for oral use in accordance with the present disclosure include liquid paraffin, sodium carboxymethylcellulose, methylcellulose, sodium alginate, gum acacia, gum tragacanth, dextrose, sucrose, sorbitol, mannitol, gelatine and lecithin. In addition these oral formulations may contain suitable flavouring and colourings agents. When used in capsule form the capsules may be coated with compounds such as glyceryl monostearate or glyceryl distearate which delay disintegration. Adjuvants typically include emollients, emulsifiers, thickening agents, preservatives, bactericides and buffering agents. For administration as an injectable solution or suspension, non-toxic parenterally acceptable diluents or carriers can include, Ringer's solution, isotonic saline, phosphate buffered saline, ethanol and 1,2 propylene glycol.

[00089] Solid forms for oral administration may contain binders acceptable in human and veterinary pharmaceutical practice, sweeteners, disintegrating agents, diluents, flavourings, coating agents, preservatives, lubricants and/or time delay agents. Suitable binders include gum acacia, gelatine, corn starch, gum tragacanth, sodium alginate, carboxymethylcellulose or polyethylene glycol. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, guar gum, xanthan gum, bentonite, alginic acid or agar. Suitable diluents include lactose, sorbitol, mannitol, dextrose, kaolin, cellulose, calcium carbonate, calcium silicate or dicalcium phosphate. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

[00090] Liquid forms for oral administration may contain, in addition to the above agents, a liquid carrier. Suitable liquid carriers include water, oils such as olive oil, peanut oil, sesame oil, sunflower oil, safflower oil, arachis oil, coconut oil, liquid paraffin, ethylene glycol, propylene glycol, polyethylene glycol, ethanol, propanol, isopropanol, glycerol, fatty alcohols, triglycerides or mixtures thereof. Suspensions for oral administration may further comprise dispersing agents and/or suspending agents. Suitable suspending agents include sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, poly-vinyl-

pyrrolidone, sodium alginate or acetyl alcohol. Suitable dispersing agents include lecithin, polyoxyethylene esters of fatty acids such as stearic acid, polyoxyethylene sorbitol mono- or di-oleate, -stearate or -laurate, polyoxyethylene sorbitan mono- or di-oleate, -stearate or -laurate and the like. Emulsions for oral administration may further comprise one or more emulsifying agents. Suitable emulsifying agents include dispersing agents as exemplified above or natural gums such as guar gum, gum acacia or gum tragacanth.

[00091] Methods for preparing suitable parenterally administrable compositions will be well known to those skilled in the art, and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa., hereby incorporated by reference herein.

[00092] For compositions formulated for topical administration, examples of pharmaceutically acceptable diluents are demineralised or distilled water; saline solution; vegetable based oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oil, arachis oil or coconut oil; silicone oils, including polysiloxanes, such as methyl polysiloxane, phenyl polysiloxane and methylphenylpolysiloxane; volatile silicones; mineral oils such as liquid paraffin, soft paraffin or squalane; cellulose derivatives such as methyl cellulose, ethyl cellulose, carboxymethylcellulose, sodium carboxymethylcellulose or hydroxypropylmethylcellulose; lower alkanols, for example ethanol or iso-propanol; lower alkanols; lower polyalkylene glycols or lower alkylene glycols, for example polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, 1,3-butylene glycol or glycerin; fatty acid esters such as isopropyl palmitate, isopropyl myristate or ethyl oleate; polyvinylpyrrolidone; agar; carrageenan; gum tragacanth or gum acacia, and petroleum jelly.

[00093] In further embodiments, the composition may further comprise suspending agents and/or humectants, such as povidone or propylene glycol, and neutralising agents for adjusting the viscosity of the composition, such as sodium hydroxide, triethanolamine (TEA) or ethylenediamine tetraacetic acid (EDTA).

[00094] Compositions of the present disclosure may be administered, for example one or more times a week, optionally for example once a week, once every second day, once a day, twice a day or three times a day, depending on the condition to be treated or prevented, the severity of the condition and the desired outcome. The duration of administration by a subject will also vary depending on the condition to be treated or prevented, the severity of the condition and the desired outcome. The amount of composition to be administered by a subject will vary

depending on a range of factors including the identity of the microorganisms administered, the nature and severity of the condition to be treated or prevented, the age and general wellbeing of the subject, and the desired outcome. Suitable dosage regimes can readily be determined by the skilled addressee.

[00095] In exemplary embodiments, about 1 ml to about 25 ml liquid formulation of a *Lactobacillus* species at a final concentration of between about 10^5 and 10^{12} cfu/ml may be administered to a subject on a once-a-day, twice-a-day or more frequent basis. The volume of the liquid formulation may be, for example, about 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml, 7 ml, 8ml, 9 ml, 10 ml, 11 ml, 12 ml, 13 ml, 14 ml, 15 ml, 16 ml, 17 ml, 18 ml, 19 ml, 20 ml, 21 ml, 22 ml, 23 ml, 24 ml, or 25 ml.

[00096] The combination of immunosuppressant or aminosalicylate and one or more *Lactobacillus* species or culture supernatant(s) or cell free culture filtrate(s) may be administered in conjunction with one or more other therapeutic agents for example, but not limited to, antibiotics, antimicrobial agents, antiseptics, anaesthetics, anti-inflammatory agents, immunosuppressive agents and other therapeutic agents indicated for the treatment of inflammatory conditions such as steroids, and NSAIDs. Administration of such additional agents may be at the same time or at different times, *i.e.* simultaneous or sequential, and may be administered by the same or different routes, with respect to compositions described herein and the subject of the present disclosure.

[00097] Non-limiting examples of additional anti-inflammatory agents that may be employed include steroidal and non-steroidal compounds such as clobetasol propionate, betamethasone dipropionate, halobetasol propionate, diflorasone diacetate, fluocinonide, halcinonide, amcinonide, desoximetasone, triamcinolone acetonide, mometasone furoate, fluticasone propionate, betamethasone dipropionate, fluocinolone acetonide, hydrocortisone valerate, hydrocortisone butyrate, flurandrenolide, triamcinolone acetonide, mometasone furoate, triamcinolone acetonide, fluticasone propionate, desonide, fluocinolone acetonide, hydrocortisone valerate, prednicarbate, triamcinolone acetonide, desonide, hydrocortisone, hydrocortisone aceponate, hydrocortisone buteprate, methylprednisolone aceponate, mometasone furoate and prednicarbate. Non-limiting examples of suitable non-steroidal anti-inflammatory compounds include indomethacin, ketoprofen, felbinac, diclofenac, ibuprofen, piroxicam, benzydamin, acetylsalicylic acid, diflunisal, salsalate, naproxen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, loxoprofen, indomethacin, sulindac, etodolac, ketorolac, diclo-fenac, nabumetone, piroxicam, meloxicam, tenoxicam, droxicam, lornoxicam, isoxicam, mefenamic acid, meclofenamic acid, flufenamic acid, tolfenamic acid, firocoxib, and licofelone,

semi-synthetic glycosaminoglycosan ethers, flavanols, flavonoids, isoflavones and derivatives. The anti-inflammatory agent may be a suppressor of cytokine signalling such as, for example, cyclosporin A, 6-thioguanine, sulfasalazine, mesalamine (5-aminosalicylic acid), etanercept, prednisolone, or balsalazide.

[00098] The anti-infective agent may be any agent which treats an infection in a subject. In particular embodiments, the anti-infective agent is able to kill or inhibit the growth of an infectious organism which is capable of being transferred, in entirety or in part, between cells via an apoptotic body. Suitable anti-infective agents include, but are not limited to, an anti-viral agent, an anti-bacterial agent, an anti-protozoal agent, or a combination thereof.

[00099] Illustrative anti-viral agents include, but are not limited to, abacavir sulfate, acyclovir especially acyclovir sodium, adefovir, amantadine especially amantadine hydrochloride, amprenavir, amplitgen, atazanavir, cidofovir, darunavir, delavirdine especially delavirdine mesylate, didanosine, docosanol, dolutegravir, edoxudine, efavirenz, emtricitabine, elvitegravir, enfuvirtide, entecavir, famciclovir, fomivirsen especially fomivirsen sodium, foscarnet especially foscarnet sodium, ganciclovir, ibacitabine, idoxuridine, imiquimod, indinavir especially indinavir sulfate, inosine pranobex, lamivudine, lopinavir, maraviroc, metisazone, moroxydine, nelfinavir especially nelfinavir mesylate, nevirapine, nitazoxanide, oseltamivir particularly oseltamivir phosphate, penciclovir, peramivir, pleconaril, podophyllotoxin, raltegravir, ribavirin, rimantadine especially rimantadine hydrochloride, ritonavir, saquinavir especially saquinavir mesylate, sofosbuvir, stavudine, telaprevir, tenofovir, tipranavir, trifluridine, tromantadine, umifenovir, valacyclovir especially valacyclovir hydrochloride, valganciclovir, vicriviroc, vidarabine, viramidine, zalcitabine, zanamivir, zidovudine and pharmaceutically acceptable salts and combinations thereof.

[000100] Illustrative anti-bacterial agents include, but are not limited to, quinolones (e.g. amifloxacin, cinoxacin, ciprofloxacin, enoxacin, fleroxacin, flumequine, lomefloxacin, nalidixic acid, norfloxacin, ofloxacin, levofloxacin, lomefloxacin, oxolinic acid, pefloxacin, rosoxacin, temafloxacin, tosufloxacin, sparfloxacin, clinafloxacin, gatifloxacin, moxifloxacin, gemifloxacin, and garenoxacin), tetracyclines, glycyclines and oxazolidinones (e.g. chlortetracycline, demeclocycline, doxycycline, lymecycline, methacycline, minocycline, oxytetracycline, tetracycline, tigecycline; linezolid, eperezolid), glycopeptides, aminoglycosides (e.g. amikacin, arbekacin, butirosin, dibekacin, fortimicins, gentamicin, kanamycin, menomycin, netilmicin, ribostamycin, sisomicin, spectinomycin, streptomycin, tobramycin), β -lactams (e.g. imipenem, meropenem, biapenem, cefaclor, cefadroxil, cefamandole, cefatrizine, cefazidone, cefazolin, cefixime, cefmenoxime, cefodizime, cefonicid,

cefoperazone, ceforanide, cefotaxime, cefotiam, cefpimizole, cefpiramide, cefpodoxime, cefsulodin, ceftazidime, cefteteram, ceftazole, ceftibuten, ceftizoxime, ceftriaxone, cefuroxime, cefuzonam, cephacetrile, cephalixin, cephaloglycin, cephaloridine, cephalothin, cephapirin, cephradine, cefinetazole, ceftioxin, cefotetan, azthreonam, carumonam, flomoxef, moxalactam, amdinocillin, amoxicillin, ampicillin, azlocillin, carbenicillin, benzylpenicillin, carfecillin, cloxacillin, dicloxacillin, methicillin, mezlocillin, nafcillin, oxacillin, penicillin G, piperacillin, sulbenicillin, temocillin, ticarcillin, cefditoren, SC004, KY-020, cefdinir, ceftibuten, FK-312, S-1090, CP-0467, BK-218, FK-037, DQ-2556, FK-518, cefozopran, ME1228, KP-736, CP-6232, Ro 09-1227, OPC-20000, LY206763), rifamycins, macrolides (e.g. azithromycin, clarithromycin, erythromycin, oleandomycin, rokitamycin, rosaramicin, roxithromycin, troleandomycin), ketolides (e.g. telithromycin, cethromycin), coumermycins, lincosamides (e.g. clindamycin, lincomycin), chloramphenicol, clofazimine, cycloserine, dapsone, ethambutol hydrochloride, isoniazid, pyrazinamide, rifabutin, rifampin, rifapentine and streptomycin sulfate.

[000101] Illustrative anti-protozoal agents include, but are not limited to, atovaquone, metronidazole including metronidazole hydrochloride, pentamidine including pentamidine isethionate, chloroquine including chloroquine hydrochloride and chloroquine phosphate, doxycycline, hydroxychloroquine sulfate, mefloquine including mefloquine hydrochloride, primaquine including primaquine phosphate, pyrimethamine, pyrimethamine with sulfadoxine, trimethoprim, sulfamethoxazole, clindamycin, quinine, quinidine, sulfadiazine, artemether, lumefantrine, artesunate, nitazoxanide, suramin, melarsoprol, eflornithine, nifurtimox, stibogluconate including sodium stibogluconate, amphotericin B including liposomal amphotericin B, miltefosine, paromomycin, ketoconazole, itraconazole, fluconazole, and pharmaceutically acceptable salts and combinations thereof.

[000102] Illustrative immunosuppressive agents include, but are not limited to: corticosteroids such as, for example, budesonide, prednisone and prednisolone; mTOR inhibitors such as, for example, sirolimus and everolimus; and monoclonal antibodies such as, for example, certolizumab, ustekinumab and vedolizumab, and biosimilars thereof.

[000103] In exemplary embodiments the one or more *Lactobacillus* species described herein are provided and administered in the form of microbial biotherapeutic compositions. Such compositions may further comprise one or more additional microorganisms such as, for example, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactococcus lactis*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *Bifidobacterium bifidum*, *Bifidobacterium lactis*, *Bifidobacterium animalis* and *Saccharomyces boulardii*.

[000104] Microbial biotherapeutic compositions may comprise one or more prebiotic components. Suitable prebiotics include, for example, polydextrose, inulin, fructooligosaccharides (FOS), xylooligosaccharides (XOS), galactooligosaccharides (GOS), mannan oligosaccharides, protein-based green lipped mussel extract, and various prebiotic-containing foods such as raw onion, raw leek, raw chickory root and raw artichoke. In certain embodiments the prebiotic is a fructooligosaccharide.

[000105] Compositions comprising *Lactobacillus* species as described herein may be administered in any suitable form, including any of the dosage forms described above. The microbial biotherapeutic compositions may be provided to the user in a powder form, suitable for mixing by the user into any type of drink or food product (for example water, fruit juice or yoghurt) or for consumption as a powder in the absence of a drink or additional food product. The microbial biotherapeutic compositions may therefore be conveniently incorporated in a variety of food and/or beverage products, nutraceutical products, supplements, food additives, and over-the-counter formulations. The food or food additive may be a solid form such as a powder, or a liquid form. Specific examples of the types of beverages or foods include, but are not limited to water-based, milk-based, yoghurt-based, other dairy-based, milk-substitute based such as soy milk or oat milk, or juice-based beverages, water, soft drinks, carbonated drinks, and nutritional beverages, (including a concentrated stock solution of a beverage and a dry powder for preparation of such a beverage); baked products such as crackers, breads, muffins, rolls, bagels, biscuits, cereals, bars such as muesli bars, health food bars and the like, dressings, sauces, custards, yoghurts, puddings, pre-packaged frozen meals, soups and confectioneries.

[000106] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

[000107] The present disclosure will now be described with reference to the following specific examples, which should not be construed as in any way limiting the scope of the invention.

Examples

[000108] The following examples are illustrative of the invention and should not be construed as limiting in any way the general nature of the disclosure of the description throughout this specification.

Example 1 – DSS-induced colitis model – cyclosporin A and microbial biotherapeutics

[000109] Cyclosporin A (CsA) is an immunosuppressant clinically employed in the treatment of ulcerative colitis and Crohn's disease. In the present study the inventors used a dextran sodium sulfate (DSS)-induced model of acute colitis in mice to compare the efficacy of CsA with: (i) a combination of microbial biotherapeutic bacterial strains *Lactobacillus paracasei* (SVT 04P1), *Lactobacillus buchneri* (SVT 06B1) and *Lactobacillus zeae* (SVT 08Z1); and (ii) combination therapy comprising CsA and the microbial biotherapeutics *L. paracasei* (SVT 04P1), *L. buchneri* (SVT 06B1) and *L. zeae* (SVT 08Z1).

[000110] DSS was from MP BioMedicals, stored at room temperature. The model used in this study is a particularly effective model of acute colitis, based on the concentration of DSS employed (3%) and the administration of DSS daily for eight days with no washout period in between administrations. Mice administered 3% DSS according to this regime show clear histopathological signs of ulceration, edema, inflammation and crypt loss in the colon (data not shown).

[000111] 50 female C57BL/6NTac mice were divided into five treatment groups:

- Group 1 – non-treatment (negative control) group. n = 10.
- Group 2 – 3% DSS + vehicle (0.9% sterile saline + 2.5% sucrose). n = 10.
- Group 3 – 3% DSS + combination of SVT 04P1, SVT 06B1 and SVT 08Z1, at a concentration of 1.5×10^{10} cfu/ml. n = 10.
- Group 4 – 3% DSS + CsA at dose of 40 mg/kg + vehicle (0.9% sterile saline + 2.5% sucrose). n = 10.
- Group 5 – 3% DSS + CsA at dose of 40 mg/kg + combination of SVT 04P1, SVT 06B1 and SVT 08Z1, at a concentration of 1.5×10^{10} cfu/ml. n = 10.

[000112] Animals of Groups 2 to 5 received 3% DSS *ad libitum* via sterile drinking water daily from days 1 to 8, while Group 1 animals continued to receive only sterile water as drinking water. In addition to DSS, animals of Group 2 received vehicle (0.9% sterile saline + 2.5% sucrose) by oral gavage daily from days 1 to 7 in a dose volume of 1 mL. In addition to

DSS, animals of Group 3 received *Lactobacillus* strains (1.5×10^{10} cfu/ml) by oral gavage daily from days 1 to 7 in a dose volume of 1 mL. In addition to DSS, animals of Group 4 received CsA (40 mg/kg) by oral gavage daily from days 1 to 7 in a dose volume of 10 mL/kg at a dose concentration of 4 mg/mL, 1 to 2 hours after administration of vehicle (0.9% sterile saline + 2.5% sucrose) by oral gavage in a dose volume of 1 mL. In addition to DSS, animals of Group 5 received CsA (40 mg/kg) by oral gavage daily from days 1 to 7 in a dose volume of 10 mL/kg at a dose concentration of 4 mg/mL, 1 to 2 hours after administration of the *Lactobacillus* strains (1.5×10^{10} cfu/ml) by oral gavage in a dose volume of 1 mL.

[000113] Symptoms/characteristics of DSS-induced colitis (stool consistency and faecal blood occurrence) were assessed by measuring in-life endpoints from days 1 to 8. Evaluations were performed 1 to 2 hours after dosing.

[000114] Stool was collected from day 1 for each mouse and examined for consistency. Stool consistency was graded as follows: normal = 0; soft, but still formed = 1; very soft = 2; diarrhoea = 4. Faecal blood in the stool was detected using the Hemoccult Tape Test Kit (Beckman Coulter, according to manufacturer's instructions). Scoring for faecal blood was as follows: negative hemoccult = 0; positive hemoccult (slight colour on strip) = 1; positive hemoccult (darker colour on strip) = 2; visible traces of blood = 3; gross rectal bleeding = 4. Percent body weight loss was also measured from day 1, graded as 0 (none), 1 (1-5%), 2 (>5-10%), 3 (>10-20%) and 4 (>20%).

[000115] Stool consistency scores, faecal occurrence scores and body weight loss scores were pooled to give a weighted in-life score of overall disease state, the disease activity index (DAI).

[000116] At completion of the study mice were euthanized, prior to determination of cytokine expression in the colonic mucosa and a histological examination of the colon of each animal.

[000117] As shown in Figure 1A, faecal blood occurrence was significantly reduced ($p < 0.001$) in mice of treatment Groups 3 to 5 compared to Group 2. Faecal blood occurrence was markedly reduced in Group 5 (CsA plus the combination of *Lactobacillus* strains) relative to Groups 3 (combination of *Lactobacillus* strains alone) and 4 (CsA alone). As shown in Figure 1B, stool consistency in Groups 3 and 4, while improved relative to Group 2, did not differ significantly from Group 2, whereas stool consistency was significantly improved ($p < 0.01$) in

Group 5. As shown in Figure 1C, the DAI was also significantly improved ($p<0.05$) in treatment Groups 3 to 5 compared to Group 2.

[000118] Analysis of colonic mucosal cytokine expression revealed a significant reduction in expression of IL-6 ($p<0.001$), KC-GRO (keratinocyte chemoattractant/human growth-regulated oncogene) ($p<0.001$), TNF α ($p<0.001$) and IL-1 β ($p<0.005$) in Group 5 (CsA plus the combination of *Lactobacillus* strains) compared to Group 2, and greater reductions in the expression of these cytokines in Group 5 relative to Groups 3 (combination of *Lactobacillus* strains alone) and 4 (CsA alone) (see Figure 2).

[000119] Colon samples were analysed for overall ulcer extent, percent of section affected by any inflammatory changes, percent of section affected by severe inflammatory changes with obliteration of normal architecture, erosion/ulceration and/or crypt abscesses and a total composite score calculated by the sum of the three individual scores for each colon segment. Scoring was calculated for each of the proximal, middle and distal sections of the colon samples with an overall total composite score demonstrating a statistically significant reduction in ulceration and inflammation for Groups 4 and 5 ($p<0.001$) compared to Group 2. A statistically significant reduction in scoring was observed for the proximal segment in Groups 3 ($p<0.05$) and Groups 4 and 5 ($p<0.001$); and for the middle and distal sections in Groups 4 and 5 ($p<0.001$) (Figure 3). Notably, Group 5 (CsA plus the combination of *Lactobacillus* strains) had the most profound effect on DSS-induced microscopic changes as it remarkably resulted in near complete resolution of DSS-induced changes in six out of ten mice. There was also a statistically significant increase in colon length in Groups 4 and 5 compared to Group 2 (Figure 4), that correlates with the total composite scores for inflammation and ulceration, with Group 5 showing the greatest statistically significant increase in length ($p<0.001$).

[000120] A number of therapeutic agents (including cyclosporin A, sulfasalazine and prednisolone) clinically employed in the treatment of inflammatory bowel diseases such as ulcerative colitis, have previously been tested in the same DSS-induced colitis model as used in the present study, by the same testing laboratory (Charles River Laboratories (CRL)). None of these drugs statistically improved the in-life disease score to the extent observed with the Group 5 treatment of the present study. CsA at 40 and 80 mg/kg showed some decrease in DAI score in a less severe 2% DSS model, however not as efficacious as present Group 5 (data not shown) in a 3% DSS model. The results obtained in the present study represent a significant advance in prospective treatment for ulcerative colitis when compared to existing therapies.

**Example 2 – DSS-induced colitis model – 5-aminosalicylic acid and microbial
biotherapeutics**

[000121] 5-aminosalicylic acid (ASA) is an immunosuppressant clinically employed in the treatment of ulcerative colitis and Crohn's disease. In the present study the inventors used a dextran sodium sulfate (DSS)-induced model of acute colitis in mice (as described in Example 1) to compare the efficacy of ASA with: (i) a combination of microbial biotherapeutic bacterial strains *Lactobacillus paracasei* (SVT 04P1), *Lactobacillus buchneri* (SVT 06B1) and *Lactobacillus zeae* (SVT 08Z1); and (ii) combination therapy comprising ASA and the microbial biotherapeutics *L. paracasei* (SVT 04P1), *L. buchneri* (SVT 06B1) and *L. zeae* (SVT 08Z1).

[000122] 50 female C57BL/6NTac mice were divided into five treatment groups:

- Group 1 – non-treatment (negative control) group. n = 10.
- Group 2 – 3% DSS + vehicle (0.9% sterile saline + 2.5% sucrose). n = 10.
- Group 3 – 3% DSS + combination of SVT 04P1, SVT 06B1 and SVT 08Z1, at a concentration of 3.0×10^{10} cfu/ml. n = 10.
- Group 4 – 3% DSS + ASA at dose of 75 mg/kg + vehicle (0.9% sterile saline + 2.5% sucrose). n = 10.
- Group 5 – 3% DSS + ASA at dose of 75 mg/kg + combination of SVT 04P1, SVT 06B1 and SVT 08Z1, at a concentration of 3.0×10^{10} cfu/ml. n = 10.

[000123] Animals of Groups 2 to 5 received 3% DSS *ad libitum* via sterile drinking water daily from days 1 to 8, while Group 1 animals continued to receive only sterile water as drinking water. In addition to DSS, animals of Group 2 received vehicle (0.9% sterile saline + 2.5% sucrose) by oral gavage daily from days 1 to 7 in a dose volume of 1 mL. In addition to DSS, animals of Group 3 received *Lactobacillus* strains (3.0×10^{10} cfu/ml) by oral gavage daily from days 1 to 7 in a dose volume of 0.5 mL. In addition to DSS, animals of Group 4 received ASA (75 mg/kg) by oral gavage daily from days 1 to 7 in a dose volume of 10 mL/kg at a dose concentration of 7.5 mg/mL, 1 to 2 hours after administration of vehicle (0.9% sterile saline + 2.5% sucrose) by oral gavage in a dose volume of 1 mL. In addition to DSS, animals of Group 5 received ASA (75 mg/kg) by oral gavage daily from days 1 to 7 in a dose volume of 10 mL/kg at a dose concentration of 7.5 mg/mL, 1 to 2 hours after administration of the *Lactobacillus* strains (3.0×10^{10} cfu/ml) by oral gavage in a dose volume of 0.5 mL. In Group 5, ASA was administered 1-2 hrs after the *Lactobacillus* strains.

[000124] Symptoms/characteristics of DSS-induced colitis (stool consistency and faecal blood occurrence) were assessed by measuring in-life endpoints as described in Example 1, from days 1 to 11. Stool consistency scores, faecal occurrence scores and body weight loss scores were pooled to give a weighted in-life score of overall disease state, the disease activity index (DAI).

[000125] As shown in Figure 5, the combination of the *Lactobacillus* strains plus ASA (Group 5) resulted in a significant improvement in stool consistency and disease activity after 11 days, when compared to treatment with ASA alone (Group 4).

Example 3 – DSS-induced colitis model – tofacitinib and microbial biotherapeutics

[000126] Tofacitinib (sold under the brand name Xeljanz®) is a small molecule JAK inhibitor prescribed for the treatment of ulcerative colitis. In the present study the inventors used a dextran sodium sulfate (DSS)-induced model of acute colitis in mice (as described in Example 1) to compare the efficacy of tofacitinib with: (i) a combination of microbial biotherapeutic bacterial strains *Lactobacillus paracasei* (SVT 04P1), *Lactobacillus buchneri* (SVT 06B1) and *Lactobacillus zeae* (SVT 08Z1); and (ii) combination therapy comprising tofacitinib and the microbial biotherapeutics *L. paracasei* (SVT 04P1), *L. buchneri* (SVT 06B1) and *L. zeae* (SVT 08Z1).

[000127] 50 female C57BL/6NTac mice were divided into five treatment groups:

- Group 1 – non-treatment (negative control) group. n = 10.
- Group 2 – 3% DSS + vehicle (0.9% sterile saline + 2.5% sucrose). n = 10.
- Group 3 – 3% DSS + combination of SVT 04P1, SVT 06B1 and SVT 08Z1, at a concentration of 3.0×10^{10} cfu/ml. n = 10.
- Group 4 – 3% DSS + tofacitinib at dose of 30 mg/kg + vehicle (0.9% sterile saline + 2.5% sucrose). n = 10.
- Group 5 – 3% DSS + tofacitinib at dose of 30 mg/kg + combination of SVT 04P1, SVT 06B1 and SVT 08Z1, at a concentration of 3.0×10^{10} cfu/ml. n = 10.

[000128] Animals of Groups 2 to 5 received 3% DSS *ad libitum* via sterile drinking water daily from days 1 to 8, while Group 1 animals continued to receive only sterile water as drinking water. In addition to DSS, animals of Group 2 received vehicle (0.9% sterile saline + 2.5% sucrose) by oral gavage daily from days 1 to 7 in a dose volume of 1 mL. In addition to

DSS, animals of Group 3 received *Lactobacillus* strains (3.0×10^{10} cfu/ml) by oral gavage daily from days 1 to 7 in a dose volume of 0.5 mL. In addition to DSS, animals of Group 4 received tofacitinib (30 mg/kg) by oral gavage daily from days 1 to 7 in a dose volume of 10 mL/kg at a dose concentration of 3 mg/mL, 1 to 2 hours after administration of vehicle (0.9% sterile saline + 2.5% sucrose) by oral gavage in a dose volume of 1 mL. In addition to DSS, animals of Group 5 received tofacitinib (30 mg/kg) by oral gavage daily from days 1 to 7 in a dose volume of 10 mL/kg at a dose concentration of 3 mg/mL, 1 to 2 hours after administration of the *Lactobacillus* strains (3.0×10^{10} cfu/ml) by oral gavage in a dose volume of 0.5 mL. In Group 5, tofacitinib was administered 1-2 hrs after the *Lactobacillus* strains.

[000129] Symptoms/characteristics of DSS-induced colitis (stool consistency and faecal blood occurrence) were assessed by measuring in-life endpoints as described in Example 1, from days 1 to 11. Stool consistency scores, faecal occurrence scores and body weight loss scores were pooled to give a weighted in-life score of overall disease state, the disease activity index (DAI).

[000130] As shown in Figure 6, the combination of the *Lactobacillus* strains plus tofacitinib (Group 5) resulted in a significant improvement in stool consistency and disease activity after 11 days, when compared to treatment with tofacitinib alone (Group 4).

Example 4 – DSS-induced colitis model – adalimumab and microbial biotherapeutics

[000131] Adalimumab (sold under the brand name Humira®) is a monoclonal antibody TNF inhibitor prescribed for the treatment of ulcerative colitis and Crohn's disease. In the present study the inventors used a dextran sodium sulfate (DSS)-induced model of acute colitis in mice (as described in Example 1) to compare the efficacy of adalimumab with: (i) a combination of microbial biotherapeutic bacterial strains *Lactobacillus paracasei* (SVT 04P1), *Lactobacillus buchneri* (SVT 06B1) and *Lactobacillus zeae* (SVT 08Z1); and (ii) combination therapy comprising adalimumab and the microbial biotherapeutics *L. paracasei* (SVT 04P1), *L. buchneri* (SVT 06B1) and *L. zeae* (SVT 08Z1).

[000132] 50 female C57BL/6NTac mice were divided into five treatment groups:

- Group 1 – non-treatment (negative control) group. n = 10.
- Group 2 – 3% DSS + vehicle (0.9% sterile saline + 2.5% sucrose). n = 10.
- Group 3 – 3% DSS + combination of SVT 04P1, SVT 06B1 and SVT 08Z1, at a concentration of 3.0×10^{10} cfu/ml. n = 10.

- Group 4 – 3% DSS + adalimumab at dose of 3 mg/kg + vehicle (0.9% sterile saline + 2.5% sucrose). n = 10.
- Group 5 – 3% DSS + adalimumab at dose of 3 mg/kg + combination of SVT 04P1, SVT 06B1 and SVT 08Z1, at a concentration of 3.0×10^{10} cfu/ml. n = 10.

[000133] Animals of Groups 2 to 5 received 3% DSS *ad libitum* via sterile drinking water daily from days 1 to 8, while Group 1 animals continued to receive only sterile water as drinking water. In addition to DSS, animals of Group 2 received vehicle (0.9% sterile saline + 2.5% sucrose) by oral gavage daily from days 1 to 7 in a dose volume of 1 mL. In addition to DSS, animals of Group 3 received *Lactobacillus* strains (3.0×10^{10} cfu/ml) by oral gavage daily from days 1 to 7 in a dose volume of 0.5 mL. In addition to DSS, animals of Group 4 received adalimumab (3 mg/kg) by subcutaneous injection daily from days 1 to 7 in a dose volume of 10 mL/kg at a dose concentration of 0.3 mg/mL, 1 to 2 hours after administration of vehicle (0.9% sterile saline + 2.5% sucrose) by oral gavage in a dose volume of 1 mL. In addition to DSS, animals of Group 5 received adalimumab (3 mg/kg) by subcutaneous injection daily from days 1 to 7 in a dose volume of 10 mL/kg at a dose concentration of 0.3 mg/mL, 1 to 2 hours after administration of the *Lactobacillus* strains (3.0×10^{10} cfu/ml) by oral gavage in a dose volume of 0.5 mL. In Group 5, adalimumab was administered 1-2 hrs after the *Lactobacillus* strains.

[000134] Symptoms/characteristics of DSS-induced colitis (stool consistency and faecal blood occurrence) were assessed by measuring in-life endpoints as described in Example 1, from days 1 to 11. Stool consistency scores, faecal occurrence scores and body weight loss scores were pooled to give a weighted in-life score of overall disease state, the disease activity index (DAI).

[000135] As shown in Figure 7, the combination of the *Lactobacillus* strains plus adalimumab (Group 5) resulted in a significant improvement in stool consistency and disease activity after 11 days, when compared to treatment with adalimumab alone (Group 4).

Deposit Details

[000136] Details of the biological material deposited pursuant to the Budapest Treaty are provided hereinbefore in the specification. The deposited strains have been previously described in international application no. PCT/AU2019/051092. In summary:

[000137] *Lactobacillus parafarraginis* SVT 05P2 was deposited pursuant to the Budapest Treaty with the Belgian Coordinated Collections of Microorganisms (BCCM), Federal Public Planning Service Science Policy, 8, rue de la Science B-1000, Brussels, Belgium, on 27 February 2019 under Accession Number LMG P-31292.

[000138] *Lactobacillus buchneri* SVT 06B1 was deposited pursuant to the Budapest Treaty with the Belgian Coordinated Collections of Microorganisms (BCCM), Federal Public Planning Service Science Policy, 8, rue de la Science B-1000, Brussels, Belgium, on 27 February 2019 under Accession Number LMG P-31293.

[000139] *Lactobacillus zeae* SVT 08Z1 was deposited pursuant to the Budapest Treaty with the Belgian Coordinated Collections of Microorganisms (BCCM), Federal Public Planning Service Science Policy, 8, rue de la Science B-1000, Brussels, Belgium, on 27 February 2019 under Accession Number LMG P-31295.

[000140] *L. rafi* SVT 07R1 was deposited pursuant to the Budapest Treaty with the Belgian Coordinated Collections of Microorganisms (BCCM), Federal Public Planning Service Science Policy, 8, rue de la Science B-1000, Brussels, Belgium, on 27 February 2019 under Accession Number LMG P-31294.

[000141] *Lactobacillus paracasei* SVT 04P1 was deposited pursuant to the Budapest Treaty with the Belgian Coordinated Collections of Microorganisms (BCCM), Federal Public Planning Service Science Policy, 8, rue de la Science B-1000, Brussels, Belgium, on 27 February 2019 under Accession Number LMG P-31290.

[000142] *Lactobacillus diolivorans* SVT 01D1 was deposited pursuant to the Budapest Treaty with the Belgian Coordinated Collections of Microorganisms (BCCM), Federal Public Planning Service Science Policy, 8, rue de la Science B-1000, Brussels, Belgium, on 27 February 2019 under Accession Number LMG P-31287.