



(51) International Patent Classification:

C12N 1/20 (2006.01) A61K 35/741 (2015.01)

C12Q 1/689 (2018.01) A61P 1/00 (2006.01)

C12R 1/01 (2006.01) A61P 29/00 (2006.01)

A61K 35/00 (2006.01) A61P 37/00 (2006.01)

(21) International Application Number:

PCT/AU2022/050726

(22) International Filing Date:

11 July 2022 (11.07.2022)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

2021902122 10 July 2021 (10.07.2021) AU

2021902960 13 September 2021 (13.09.2021) AU

2022900261 09 February 2022 (09.02.2022) AU

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

— with sequence listing part of description (Rule 5.2(a))

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA,

(54) Title: COMPOSITIONS AND METHODS FOR TREATING DISEASE II

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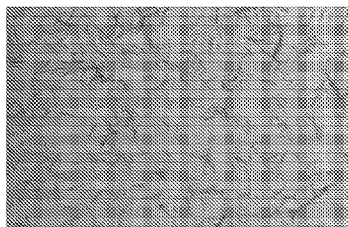


FIGURE 9

(57) Abstract: Disclosed are therapeutic compositions comprising bacterial strains and methods for the treatment or prevention of disease. More particularly, disclosed are compositions comprising bacterial strains isolated from the human digestive tract and their use in the treatment or prevention of inflammatory and autoimmune disorders. Also disclosed are novel bacterial strains and isolated populations of therapeutic importance.



WO 2023/283681 A1

TITLE OF THE INVENTION

"COMPOSITIONS AND METHODS FOR TREATING DISEASE II"

RELATED APPLICATIONS

5 **[0001]** This application claims priority to Australian Provisional Application No. 2021902122 entitled "Compositions and methods for treating disease" filed on 10 July 2021, Australian Provisional Application No. 2021902960 entitled "Compositions and methods for treating disease" filed on 13 September 2021, and Australian Provisional Application No. 2022900261 entitled "Compositions and methods for treating disease" filed on 9 February 2022, the entire contents of which are hereby incorporated herein by reference

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FIELD OF THE INVENTION

[0002] This invention relates generally to the field of therapeutic compositions comprising bacterial strains and methods for the treatment or prevention of disease. More particularly, the present invention relates to compositions comprising bacterial strains isolated from the human digestive tract and their use in the treatment or prevention of
15 inflammatory and autoimmune disorders.

BACKGROUND OF THE INVENTION

[0003] The human gut microbiota contains more than 500-1000 different phylotypes belonging to a few bacterial phylum, including Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and Verrucomicrobia. The two major phyla, the Bacteroidetes and the Firmicutes, generally represent over 90% of the gut microbiota (Arumugam et al.,
20 2011). The successful symbiotic relationships arising from bacterial colonisation of the human gut have yielded a wide variety of metabolic, structural, protective and other beneficial functions. The gut bacteria are key regulators of digestion along the gastrointestinal (GI) tract; with commensal bacterial playing an important role in the
25 extraction, synthesis, and absorption of many nutrients and metabolites, including bile acids, lipids, amino acids, vitamins, and short-chain fatty acids (SCFAs). More recently, the immunological importance of the gut microbiota and their products in regulating the development, homeostasis, and function of innate and adaptive immune cells have been recognised (Brestoff and Atris, 2013).

30 **[0004]** It is now increasingly recognised that the gut microbiome regulates host intestinal mucosal immunity and predisposition to inflammation (Geva-Zatorsky et al., 2017; Kabat et al., 2014), opening new avenues for novel therapeutic interventions.

[0005] Dramatic changes in microbiota composition have been documented in many inflammatory and autoimmune disorders, including inflammatory bowel disease (IBD).
35 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. Certain strains, including Lactobacillus and Bifidobacterium strains, have been proposed for use in treating various extraintestinal inflammatory and autoimmune disorders (see, Goldin & Gorbach, 2008; Azad et al., 2013). However, the precise effects of particular bacterial

strains locally at the GI tract and systemically throughout the body are unresolved. As a result, the relationships between different diseases and different bacterial strains in the human GI tract are yet to be clearly elucidated.

5 **[0006]** IBD (including the two major disease subtypes Crohn's disease (CD) and ulcerative colitis (UC)) is characterised by episodic and disabling inflammation of the GI tract. In 2017, it is estimated that 6.8 million people globally suffered from IBD, with the highest prevalence in the United States and Europe (GBD 2017; Inflammatory Bowel Disease Collaborators, 2019). Up to 20% of patients are diagnosed before the age of 16 and
10 paediatric-onset IBD (PIBD) is associated with a more complicated and aggressive disease with adverse impacts on growth and psychosocial development.

[0007] There is currently no cure for IBD, and long-term clinical management requires effective therapeutics with an excellent safety profile. However, existing treatments show a range of deficiencies and remission is generally short. Moreover, IBD therapeutics are ineffective where early onset coupled with more aggressive disease result in progressive
15 bowel damage and need for surgery. There is an urgent need to develop more effective and safe therapies to improve patient quality of life, maintain remission over long periods, reduce surgery and curtail individual and public health costs.

[0008] Existing treatments for IBD are sub-optimal with strong adverse effects, low compliance (50% average non-adherence rates (see, Chan et al., 2017)) and high cost.
20 Furthermore, there is no effective solution to maintaining extended periods of disease-free remission. Mesalmine, one of the most widely used first line therapies for mild to moderate flares of ulcerative colitis and for maintenance of remission, has response rates between 40%–70% and remission rates of 15%–20% (Karagozian & Burakoff, 2007).

[0009] There is a requirement in the art for new methods of treating
25 inflammatory and autoimmune disorders. 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.

SUMMARY OF THE INVENTION

[0010] The present invention is predicated in part on the inventors identifying a
30 number of bacterial strains, including *Alistipes shahii*, *Gemmiger formicilis*, and *Colonithrix sana*, enhance or improve gut barrier function. Based on this consideration, it is proposed that strains of these and closely related species are particularly suited to therapeutic applications for treating and preventing inflammatory and autoimmune disorders, as described hereinafter.

35 **[0011]** The inventors have developed new compositions comprising a viable bacterial strain of a species selected from *Alistipes shahii*, *Gemmiger formicilis*, and *Colonithrix sana*, that can be used for treating and preventing inflammatory and autoimmune disorders.

[0012] Accordingly, in one aspect the invention provides a cell of the *Alistipes shahii* strain deposited under any one of accession numbers V21/014432, V21/014433, or V21/014434, or a derivative thereof.

[0013] In some embodiments, the cell is at least partially isolated.

5 **[0014]** In another aspect, the invention provides a biologically pure culture of the *Alistipes shahii* strain deposited under any one of accession numbers V21/014432, V21/014433, or V21/014434, or a derivative thereof.

[0015] In another aspect, the present invention provides a composition comprising the cell or culture as described above and elsewhere herein.

10 **[0016]** In yet another aspect, the present invention provides a composition comprising a bacterial strain with a 16S rRNA sequence that is at least about 97.5%, 98%, 98.5% 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9%, identical to any one of SEQ ID NOs: 1-3 or 44; or with a 16S rRNA sequence represented by any one of SEQ ID NOs: 1-3 or 44. In some embodiments, the bacterial strain comprises two
15 or more copies of a 16S rRNA sequence in its genome (e.g., two copies, three copies, four copies, five copies, six copies, seven copies, eight copies).

[0017] In some embodiments, the composition further comprises a pharmaceutically acceptable excipient, diluent, or carrier.

[0018] In yet another aspect, the present invention provides a pharmaceutical
20 composition comprising a bacterial strain with a 16S rRNA sequence that is at least about 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to the 16S rRNA sequence of a bacterial strain of the *Alistipes shahii*, together with a pharmaceutically acceptable carrier, diluent, or excipient.

[0019] In a related aspect, the present invention provides a pharmaceutical
25 composition comprising an effective amount of a bacterial strain that is a phylogenetic descendant of the most recent common ancestor (MRCA) of *A. timonensis* and *A. shahii*, together with a pharmaceutically acceptable carrier, diluent, or excipient. Suitably, the MRCA is defined at node 35262 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB). In some embodiments, the phylogenetic tree is created by release 89 of
30 the GTDB, however, any subsequent release is considered to give equally as applicable results. In some preferred embodiments of this type, the bacterial strain is of the species *A. shahii*.

[0020] Typically, the bacterial strain is at least partially isolated.

[0021] In some embodiments, the bacterial strain is live. In some alternative
35 embodiments, the bacterial strain is dead.

[0022] In some embodiments, the compositions further comprise a prebiotic.

[0023] In some embodiments, the composition is formulated in a dried form. Typically, the composition is dried using techniques selected from lyophilisation, spray drying, fluidized bed drying, vacuum drying, or a combination thereof.

5 **[0024]** In some embodiments, the composition is formulated for oral administration.

[0025] In some embodiments, the bacterial strain produces an agent that attenuates or impairs signal transducer and activator of transcription 3 (STAT3) signalling in a cell.

10 **[0026]** In some embodiments of this type, the agent is a small molecule, peptide, or nucleotide. Typically, the agent is released by the bacterial strain.

[0027] In some embodiments, the agent binds specifically to any one of STAT3, JAK2, TYK2, or IL-23.

15 **[0028]** In some embodiments, the *A. shahii* metabolizes one or more agents selected from the group comprising or consisting of starch, glucose, fructose, gluconate, lactose, trehalose, and lactaldehyde as a carbon source.

[0029] In another aspect, the present invention provides a method of restoring or improving gut barrier function in a subject, the method comprising administering to the subject a bacterial strain of the species *A. shahii*, to thereby restore or improve gut barrier function.

20 **[0030]** In some preferred embodiments, restoring or improving gut barrier function is characterised by at least one of: (i) an increase in the quality and/or quantity of mucin; (ii) improvement in integrity of tight junction proteins; (iii) reduction in translocation of luminal contents into systemic circulation; or (iv) reduction of intestinal ulcers and/or intestinal wounds.

25 **[0031]** In some embodiments, the luminal contents includes lipopolysaccharide (LPS).

30 **[0032]** In some embodiments, the restoration or improvement in gut barrier function results in a reduction in systemic inflammation in the subject. In some embodiments of this type, the systemic inflammation is characterized by elevated levels of an inflammatory cytokine (e.g., IL-1 β , IL-8, IL-6, and TNF) in the subject as compared to the level of the inflammatory cytokine in a healthy subject.

35 **[0033]** In yet another aspect, the present invention provides a method of maintaining gut barrier function in a subject, the method comprising administering to the subject a bacterial strain of the species *A. shahii*, to thereby maintain gut barrier function in the subject.

[0034] In yet another aspect, the present invention provides a method of reducing inflammation in a subject, the method comprising administering to the subject a bacterial strain of the strain *A. shahii*, to thereby reduce inflammation in the subject.

[0035] In some embodiments, the inflammation is local to the gut environment, or systemic inflammation.

[0036] In another aspect, the present invention provides a method of inducing or enhancing mucosal healing in a subject, the method comprising administering to the subject
5 a bacterial strain of the species *A. shahii* in an amount sufficient to induce epithelial cell migration, proliferation and/or differentiation, to thereby induce mucosal healing in the subject.

[0037] In some embodiments, mucosal healing in the subject can be measured using one or more fecal or serum markers. By way of an illustrative example, one or more
10 fecal markers may be selected from the group comprising calprotectin, lactoferrin, metalloproteinase (MMP)-9, and lipocalin-2.

[0038] In some embodiments, the bacterial strain reduces inflammation by attenuating the NFκB pathway. In some embodiments of this type, the bacterial strain inhibits the production of one or more transcription factors, cytokines, or chemokines
15 selected from the group comprising NFκB, TNF, IFN-γ, IL-1β, IL-8, and MCP-1.

[0039] In yet another aspect, the present invention provides methods of blocking or otherwise inhibiting STAT3 signalling in a target cell, the method comprising contacting the cell with at least a soluble component of a bacterial cell preparation of the species *A. shahii*, to block or otherwise inhibit STAT3 signalling in the cell. Typically, the method of this
20 aspect is performed *in vitro*.

[0040] In some embodiments, the target cell is selected from a reporter cell (e.g., a HEK cell), an immune cell (e.g., a Th17 immune cell), an epithelial cell, or an endothelial cell. In some embodiments, the target cell is a mammalian cell, and preferably, a human cell.

[0041] In some embodiments, the bacterial cell preparation is a bacterial cell culture. The soluble component may therefore comprise, consist, or consist essentially of, the soluble fraction of the bacterial cell culture (e.g., the cell culture supernatant). The soluble component may further comprise some insoluble components of the bacterial cell culture. For example, the soluble component may include substantially all of the bacterial
25 culture. Preferably, the soluble component is substantially depleted of bacterial cells.

[0042] In some alternative embodiments, the bacterial cell preparation is a bacterial cell lysate. In exemplary embodiments of this type, the soluble component may relate to the soluble fraction of the cell lysate. A soluble fraction can suitably be achieved by any method, including by centrifugation.

[0043] In still yet another aspect, the present invention provides a method of blocking or otherwise inhibiting STAT3 signalling in a cell, the method comprising administering a bacterial strain of the species *A. shahii* to the subject, thereby blocking or otherwise inhibiting STAT3 signalling in the cell. Typically, the methods of this aspect are performed *in vivo*.
35

[0044] In some embodiments, the cell is an immune cell (e.g., a Th17 immune cell) or epithelial cell.

[0045] In some embodiments, the cell is an epithelial cell, and the bacterial strain or a metabolite produced by the bacterial strain increases the production of IL-22 in the
5 subject.

[0046] In some embodiments, the bacterial strain produces a molecule that is a direct inhibitor or an indirect inhibitor of STAT3. For example, the bacterial strain may produce a metabolite that directly inhibits at least one of an IL-23 polypeptide, a JAK2 polypeptide, a TYK2 polypeptide, or a STAT3 polypeptide.

10 **[0047]** In some embodiments, the bacterial strains used in the methods described above and elsewhere herein produce one or more metabolites selected from butyrate, acetate, ethanol and fumarate.

[0048] In some embodiments the bacterial strain has a 16S rRNA sequence that is at least about 97.5%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%,
15 99.7%, 99.8%, 99.9% identical to the 16S rRNA sequence of a bacterial strain of *A. shahii*.

[0049] In some alternative embodiments, the bacterial strain has a 16S rRNA sequence that is at least about 97.5%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% identical to any one of SEQ ID NOs: 1-3 or 44, or when the bacterial strain has the 16S rRNA gene sequence represented by any one of SEQ ID NOs: 1-3
20 or 44. In some embodiments, the bacterial strain comprises two or more copies (e.g., two copies, three copies, four copies, five copies, six copies, seven copies, eight copies) independently selected from the 16S rRNA sequences set forth in any one of SEQ ID NOs: 1-3 or 44.

[0050] In some embodiments, the bacterial strain is the *A. shahii* strain deposited
25 under any one of accession numbers V21/014432, V21/014433, or V21/014434, or a derivative thereof.

[0051] Preferably, the bacterial strain is at least partially isolated.

[0052] In some embodiments, the bacterial strain is formulated as a pharmaceutical composition, further comprising a pharmaceutically acceptable carrier,
30 diluent or excipient. In some embodiments, the pharmaceutical composition is a dry composition. In some embodiments, the dry composition is selected from the group consisting of particles, granules, and powder. By way of an illustrative example the pharmaceutical composition may be lyophilised, spray dried, fluidized bed dried, vacuum dried, or a combination thereof.

35 **[0053]** In some embodiments, the pharmaceutical composition is formulated for oral administration.

[0054] In still yet other aspects, the present invention provides a method of treating an inflammatory or autoimmune disorder in a subject, the method comprising

administering an effective amount of a bacterial strain of *A. shahii* to the subject, to thereby treat or prevent the inflammatory or autoimmune disorder.

5 **[0055]** In some embodiments, the inflammatory or autoimmune disorder is selected from the group comprising an inflammatory bowel disease (such as Crohn's disease or ulcerative colitis); asthma (such as allergic asthma or neutrophilic asthma); fatty liver disease (such as non-alcoholic fatty liver disease (NAFLD)); ankylosing spondylitis; systemic lupus erythematosus (SLE); scleroderma; Sjogren's syndrome; and vasculitis. Preferably, the inflammatory or autoimmune disorder is an inflammatory bowel disease (IBD).

10 **[0056]** In some embodiments, the bacterial strain blocks or otherwise inhibits STAT3 signalling in at least a cell of the subject. Typically, the cell is an epithelial cell, endothelial cell, or an immune cell (e.g., a Th17 immune cell).

[0057] In some embodiments, the bacterial strain has a 16S rRNA sequence that is at least about 98%, 98.5, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to the 16S rRNA sequence of a bacterial strain of *A. shahii*.

15 **[0058]** Alternatively, in some embodiments the bacterial strain has a 16S rRNA sequence that is at least about 98%, 98.5, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to any one of SEQ ID NOs: 1-4 or 44, or when the bacterial strain has the 16S rRNA gene sequence represented by any one of SEQ ID NOs: 1-3 or 44.

20 **[0059]** Preferably, the bacterial strain is at least partially isolated.

[0060] In some embodiments, the bacterial strain is formulated as a pharmaceutical composition, together with a pharmaceutically acceptable carrier, diluent, and/or excipient. In some embodiments, the composition is a dry composition selected from the group consisting of particles, granules, and powder. For example, the composition may be lyophilised. Alternatively, the composition may be spray dried, fluidized bed dried, or vacuum dried.

[0061] In some embodiments, the composition is formulated for oral administration.

30 **[0062]** In one aspect, the present invention provides a composition comprising a bacterial strain of the genus *Alistipes* for use in therapy.

[0063] In another aspect, the present invention provides a composition comprising a bacterial strain of *A. shahii*, for use in therapy. In some of the same embodiments and some other embodiments, the bacterial strain has a 16S rRNA sequence that is at least about 98%, 98.5, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to the 16S rRNA sequence of a bacterial strain of *A. shahii*.

[0064] Alternatively, the bacterial strain may have a 16S rRNA sequence that is at least about 98%, 98.5, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%,

99.8%, or 99.9% identical to any one of SEQ ID NOs: 1-3 or 44, or when the bacterial strain has the 16S rRNA gene sequence represented by any one of SEQ ID NOs: 1-3 or 44.

5 **[0065]** In yet another aspect, the present invention provides a composition comprising a bacterial strain of the genus *Alistipes*, for use in the treatment or prevention of an inflammatory or autoimmune disorder.

[0066] In still yet another aspect, the present invention provides a composition comprising a bacterial strain of *A. shahii*, for use in the treatment or prevention of an inflammatory or autoimmune disorder.

10 **[0067]** In some embodiments, the bacterial strain is the *A. shahii* strain deposited under any one of accession numbers V21/014432, V21/014433, or V21/014434, or a derivative thereof.

[0068] In some embodiments, the inflammatory or autoimmune disorder is selected from an inflammatory bowel disease (such as Crohn's disease or ulcerative colitis); asthma (such as allergic asthma or neutrophilic asthma); arthritis (such as rheumatoid
15 arthritis, osteoarthritis, psoriatic arthritis, or juvenile idiopathic arthritis); fatty liver disease (such as nonalcoholic fatty liver disease (NAFLD)); ankylosing spondylitis; psoriasis; systemic lupus erythematosus (SLE); scleroderma; Sjogren's syndrome; and type 1 diabetes mellitus. In some preferred embodiments, the inflammatory or autoimmune disorder is an inflammatory bowel disease (IBD).

20 **[0069]** In one aspect, the present invention provides a composition for use in treating an inflammatory or autoimmune disorder, the composition comprising a bacterial strain of *A. shahii*; and an ancillary treatment agent.

[0070] In some embodiments, the ancillary treatment agent is an anti-inflammatory agent. By way of an illustrative example, the anti-inflammatory agent is
25 selected from the group comprising 5-aminosalicylates, corticosteroids, azathioprine, or a combination thereof. In some other embodiments, the ancillary treatment is an antibody (e.g., a monoclonal antibody). By way of an illustrative example, the antibody may be selected from infliximab, adalimumab, golimumab, certolizumab pegol, natalizumab, and vedolizumab.

30 **[0071]** In another aspect, the present invention provides a composition for use in treating an inflammatory or autoimmune disorder, the composition comprises a bacterial strain of *A. shahii*; and a nutritional supplement. In embodiments of this type, the nutritional supplement improves engraftment of the bacterial stain.

[0072] In another aspect the invention provides a cell of the *C. sana* strain
35 deposited under the accession number V21/019213 or V21/019214, or a derivative thereof.

[0073] In some embodiments, the cell is at least partially isolated.

[0074] In another aspect, the invention provides a biologically pure culture of the *C. sana* strain deposited under accession number V21/019213 or V21/019214, or a derivative thereof.

[0075] In another aspect, the present invention provides a composition comprising the cell or culture as described above and elsewhere herein.

[0076] In yet another aspect, the present invention provides a composition comprising a bacterial strain with a 16S rRNA sequence that is at least about 97.5%, 98%, 98.5% 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9%, identical to any one of SEQ ID NOs: 11-18; or with a 16S rRNA sequence represented by any one of SEQ ID NOs: 11-18. In some embodiments, the bacterial strain comprises two or more copies of a 16S rRNA sequence in its genome (e.g., two copies, three copies, four copies, five copies, six copies, seven copies, eight copies). For example, the bacterial strain may comprise four copies of a 16S rRNA sequence in its genome (e.g., each of SEQ ID NOs: 11-14; or each of SEQ ID NOs: 15-18).

[0077] In some embodiments, the composition further comprises a pharmaceutically acceptable excipient, diluent, or carrier.

[0078] In yet another aspect, the present invention provides a pharmaceutical composition comprising a bacterial strain with a 16S rRNA sequence that is at least about 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to the 16S rRNA sequence of a bacterial strain of the *C. sana*, together with a pharmaceutically acceptable carrier, diluent, or excipient.

[0079] In a related aspect, the present invention provides a pharmaceutical composition comprising an effective amount of a bacterial strain that is a phylogenetic descendant of the most recent common ancestor (MRCA) of *C. sana* and *C. sp002437735*, together with a pharmaceutically acceptable carrier, diluent, or excipient. Suitably, the MRCA is defined at node 23879 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB). In some embodiments, the phylogenetic tree is created by release 89 of the GTDB, however, any subsequent release is considered to give equally as applicable results. In some preferred embodiments of this type, the bacterial strain is of *C. sana*.

[0080] Typically, the bacterial strain is at least partially isolated.

[0081] In some embodiments, the bacterial strain is live. In some alternative embodiments, the bacterial strain is dead.

[0082] In some embodiments, the compositions further comprise a prebiotic.

[0083] In some embodiments, the composition is formulated in a dried form. Typically, the composition is dried using techniques selected from lyophilization, spray drying, fluidized bed drying, vacuum drying, or a combination thereof.

[0084] In some embodiments, the composition is formulated for oral administration.

[0085] In some embodiments, the bacterial strain produces an agent that attenuates or impairs signal transducer and activator of transcription 3 (STAT3) signalling in a cell.

[0086] In some embodiments of this type, the agent is a small molecule, peptide, or nucleotide. Typically, the agent is released by the bacterial strain.

[0087] In some embodiments, the agent binds specifically to any one of STAT3, JAK2, TYK2, or IL-23.

5 **[0088]** In some embodiments, the *C. sana* metabolizes one or more agents selected from the group comprising or consisting of starch, glucose, fructose, gluconate, lactose, trehalose, and lactaldehyde as a carbon source.

[0089] In another aspect, the present invention provides a method of restoring or improving gut barrier function in a subject, the method comprising administering to the
10 subject a bacterial strain of the species *C. sana*, to thereby restore or improve gut barrier function.

[0090] In some preferred embodiments, restoring or improving gut barrier function is characterised by at least one of: (i) an increase in the quality and/or quantity of mucin; (ii) improvement in integrity of tight junction proteins; (iii) reduction in translocation
15 of luminal contents into systemic circulation; or (iv) reduction of intestinal ulcers and/or intestinal wounds.

[0091] In some embodiments, the luminal contents includes lipopolysaccharide (LPS).

[0092] In some embodiments, the restoration or improvement in gut barrier
20 function results in a reduction in systemic inflammation in the subject. In some embodiments of this type, the systemic inflammation is characterised by elevated levels of an inflammatory cytokine (e.g., IL-1 β IL-8, IL-6, and TNF) in the subject as compared to the level of the inflammatory cytokine in a healthy subject.

[0093] In yet another aspect, the present invention provides a method of
25 maintaining gut barrier function in a subject, the method comprising administering to the subject a bacterial strain of the species *C. sana*, to thereby maintain gut barrier function in the subject.

[0094] In yet another aspect, the present invention provides a method of
30 reducing inflammation in a subject, the method comprising administering to the subject a bacterial strain of the strain *C. sana*, to thereby reduce inflammation in the subject.

[0095] In some embodiments, the inflammation is local to the gut environment, or systemic inflammation.

[0096] In another aspect, the present invention provides a method of inducing or
35 enhancing mucosal healing in a subject, the method comprising administering to the subject a bacterial strain of the species *C. sana* in an amount sufficient to induce epithelial cell migration, proliferation and/or differentiation, to thereby induce mucosal healing in the subject.

[0097] In some embodiments, mucosal healing in the subject can be measured using one or more fecal or serum markers. By way of an illustrative example, one or more fecal markers may be selected from the group comprising calprotectin, lactoferrin, metalloproteinase (MMP)-9, and lipocalin-2.

5 **[0098]** In some embodiments, the bacterial strain reduces inflammation by attenuating the NFκB pathway. In some embodiments of this type, the bacterial strain inhibits the production of one or more transcription factors, cytokines, or chemokines selected from the group comprising NFκB, TNF, IFN-γ, IL-1β, IL-8, and MCP-1.

[0099] In yet another aspect, the present invention provides methods of blocking
10 or otherwise inhibiting STAT3 signalling in a target cell, the method comprising contacting the cell with at least a soluble component of a bacterial cell preparation of the species *C. sana*, to block or otherwise inhibit STAT3 signalling in the cell. Typically, the method of this aspect is performed *in vitro*.

[0100] In some embodiments, the target cell is selected from a reporter cell
15 (e.g., a HEK cell), an immune cell (e.g., a Th17 immune cell), an epithelial cell, or an endothelial cell. In some embodiments, the target cell is a mammalian cell, and preferably, a human cell.

[0101] In some embodiments, the bacterial cell preparation is a bacterial cell culture. The soluble component may therefore comprise, consist, or consist essentially of,
20 the soluble fraction of the bacterial cell culture (e.g., the cell culture supernatant). The soluble component may further comprise some insoluble components of the bacterial cell culture. For example, the soluble component may include substantially all of the bacterial culture. Preferably, the soluble component is substantially depleted of bacterial cells.

[0102] In some alternative embodiments, the bacterial cell preparation is a
25 bacterial cell lysate. In exemplary embodiments of this type, the soluble component may relate to the soluble fraction of the cell lysate. A soluble fraction can suitably be achieved by any method, including by centrifugation.

[0103] In still yet another aspect, the present invention provides a method of blocking or otherwise inhibiting STAT3 signalling in a cell, the method comprising
30 administering a bacterial strain of the species *C. sana* to the subject, thereby blocking or otherwise inhibiting STAT3 signalling in the cell. Typically, the methods of this aspect are performed *in vivo*.

[0104] In some embodiments, the cell is an immune cell (e.g., a Th17 immune cell) or epithelial cell.

35 **[0105]** In some embodiments, the cell is an epithelial cell, and the bacterial strain or a metabolite produced by the bacterial strain increases the production of IL-22 in the subject.

[0106] In some embodiments, the bacterial strain produces a molecule that is a direct inhibitor or an indirect inhibitor of STAT3. For example, the bacterial strain may

produce a metabolite that directly inhibits at least one of an IL-23 polypeptide, a JAK2 polypeptide, a TYK2 polypeptide, or a STAT3 polypeptide.

[0107] In some embodiments, the bacterial strains used in the methods described above and elsewhere herein produce one or more metabolites selected from butyrate, acetate, ethanol and fumarate.

[0108] In some embodiments the bacterial strain has a 16S rRNA sequence that is at least about 97.5%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% identical to the 16S rRNA sequence of a bacterial strain of *C. sana*.

[0109] In some alternative embodiments, the bacterial strain has a 16S rRNA sequence that is at least about 97.5%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% identical to any one of SEQ ID NOs: 11-18; or with a 16S rRNA sequence represented by any one of SEQ ID NOs: 11-18. In some embodiments, the bacterial strain comprises two or more copies of a 16S rRNA sequence in its genome (e.g., two copies, three copies, four copies, five copies, six copies, seven copies, eight copies). For example, the bacterial strain may comprise four copies of a 16S rRNA sequence in its genome (e.g., each of SEQ ID NOs: 11-14; or each of SEQ ID NOs: 15-18).

[0110] In some embodiments, the bacterial strain is the *C. sana* strain deposited under accession number V21/019213 or V21/019214, or a derivative thereof.

[0111] Preferably, the bacterial strain is at least partially isolated.

[0112] In some embodiments, the bacterial strain is formulated as a pharmaceutical composition, further comprising a pharmaceutically acceptable carrier, diluent or excipient. In some embodiments, the pharmaceutical composition is a dry composition. In some embodiments, the dry composition is selected from the group consisting of particles, granules, and powder. By way of an illustrative example the pharmaceutical composition may be lyophilized, spray dried, fluidized bed dried, vacuum dried, or a combination thereof.

[0113] In some embodiments, the pharmaceutical composition is formulated for oral administration.

[0114] In still yet other aspects, the present invention provides a method of treating an inflammatory or autoimmune disorder in a subject, the method comprising administering an effective amount of a bacterial strain of *C. sana* to the subject, to thereby treat or prevent the inflammatory or autoimmune disorder.

[0115] In some embodiments, the inflammatory or autoimmune disorder is selected from the group comprising an inflammatory bowel disease (such as Crohn's disease or ulcerative colitis); asthma (such as allergic asthma or neutrophilic asthma); fatty liver disease (such as non-alcoholic fatty liver disease (NAFLD)); ankylosing spondylitis; systemic lupus erythematosus (SLE); scleroderma; Sjogren's syndrome; and vasculitis. Preferably, the inflammatory or autoimmune disorder is an inflammatory bowel disease (IBD).

[0116] In some embodiments, the bacterial strain blocks or otherwise inhibits STAT3 signalling in at least a cell of the subject. Typically, the cell is an epithelial cell, endothelial cell, or an immune cell (e.g., a Th17 immune cell).

5 **[0117]** In some embodiments, the bacterial strain has a 16S rRNA sequence that is at least about 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% identical to the 16S rRNA sequence of a bacterial strain of the genus *C. sana*.

[0118] Alternatively, in some embodiments the bacterial strain has a 16S rRNA sequence that is at least about 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% identical to any one of SEQ ID NOs: 11-18; or with a 16S rRNA sequence represented by any one of SEQ ID NOs: 11-18. In some embodiments, the bacterial strain comprises two or more copies of a 16S rRNA sequence in its genome (e.g., two copies, three copies, four copies, five copies, six copies, seven copies, eight copies). For example, the bacterial strain may comprise four copies of a 16S rRNA sequence in its genome (e.g., each of SEQ ID NOs: 11-14; or each of SEQ ID NOs: 15-18).

15 **[0119]** Preferably, the bacterial strain is at least partially isolated.

[0120] In some embodiments, the bacterial strain is formulated as a pharmaceutical composition, together with a pharmaceutically acceptable carrier, diluent, and/or excipient. In some embodiments, the composition is a dry composition selected from the group consisting of particles, granules, and powder. For example, the composition may be lyophilized. Alternatively, the composition may be spray dried, fluidized bed dried, or vacuum dried.

[0121] In some embodiments, the composition is formulated for oral administration.

25 **[0122]** In one aspect, the present invention provides a composition comprising a bacterial strain of the genus *C. sana* for use in therapy.

[0123] In another aspect, the present invention provides a composition comprising a bacterial strain of *C. sana*, for use in therapy.

30 **[0124]** In yet another aspect, the present invention provides a composition comprising a bacterial strain of the genus *C. sana*, for use in the treatment or prevention of an inflammatory or autoimmune disorder.

[0125] In still yet another aspect, the present invention provides a composition comprising a bacterial strain of *C. sana*, for use in the treatment or prevention of an inflammatory or autoimmune disorder.

35 **[0126]** In some embodiments, the bacterial strain is the *C. sana* strain deposited under accession number V21/019213 or V21/019214, or a derivative thereof.

[0127] In some embodiments, the inflammatory or autoimmune disorder is selected from an inflammatory bowel disease (such as Crohn's disease or ulcerative colitis); asthma (such as allergic asthma or neutrophilic asthma); arthritis (such as rheumatoid

arthritis, osteoarthritis, psoriatic arthritis, or juvenile idiopathic arthritis); fatty liver disease (such as nonalcoholic fatty liver disease (NAFLD)); ankylosing spondylitis; psoriasis; systemic lupus erythematosus (SLE); scleroderma; Sjogren's syndrome; and type 1 diabetes mellitus. In some preferred embodiments, the inflammatory or autoimmune disorder is an inflammatory bowel disease (IBD).

[0128] In one aspect, the present invention provides a composition for use in treating an inflammatory or autoimmune disorder, the composition comprising a bacterial strain of *C. sana*; and an anti-inflammatory agent.

[0129] In some embodiments, the anti-inflammatory agent is selected from the group comprising 5-aminosalicylates, corticosteroids, azathioprine, infliximab, and adalimumab.

[0130] In another aspect, the present invention provides a composition for use in treating an inflammatory or autoimmune disorder, the composition comprises a bacterial strain of *C. sana*; and a nutritional supplement. In embodiments of this type, the nutritional supplement improves engraftment of the bacterial stain.

[0131] In still yet another aspect, Accordingly, in one aspect the invention provides a cell of the *Gemmiger formicilis* strain deposited under accession number V21/011520, or a derivative thereof.

[0132] In some embodiments, the cell is at least partially isolated.

[0133] In another aspect, the invention provides a biologically pure culture of the *Gemmiger formicilis* strain deposited under accession number V21/011520, or a derivative thereof.

[0134] In another aspect, the present invention provides a composition comprising the cell or culture as described above and elsewhere herein.

[0135] In yet another aspect, the present invention provides a composition comprising a bacterial strain with a 16S rRNA sequence that is at least about 97.5%, 98%, 98.5% 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9%, identical to SEQ ID NO: 27 or one or more of SEQ ID NOs: 45-47; or with a 16S rRNA sequence represented by SEQ ID NO: 27 or one or more of SEQ ID NOs: 45-47. In some embodiments, the bacterial strain comprises two or more copies of a 16S rRNA sequence in its genome (e.g., two copies, three copies, four copies, five copies, six copies, seven copies, eight copies).

[0136] In some embodiments, the composition further comprises a pharmaceutically acceptable excipient, diluent, or carrier.

[0137] In yet another aspect, the present invention provides a pharmaceutical composition comprising a bacterial strain with a 16S rRNA sequence that is at least about 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to the 16S rRNA sequence of a bacterial strain of the *Gemmiger formicilis*, together with a pharmaceutically acceptable carrier, diluent, or excipient.

- [0138]** In a related aspect, the present invention provides a pharmaceutical composition comprising an effective amount of a bacterial strain that is a phylogenetic descendant of the most recent common ancestor (MRCA) of *G. variabile* and *G. sp002306375*, together with a pharmaceutically acceptable carrier, diluent, or excipient.
- 5 Suitably, the MRCA is defined at node 23818 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB). In some embodiments, the phylogenetic tree is created by release 89 of the GTDB, however, any subsequent release is considered to give equally as applicable results. In some preferred embodiments of this type, the bacterial strain is of *G. formicilis*.
- 10 **[0139]** Typically, the bacterial strain is at least partially isolated.
- [0140]** In some embodiments, the bacterial strain is live. In some alternative embodiments, the bacterial strain is dead.
- [0141]** In some embodiments, the compositions further comprise a prebiotic.
- [0142]** In some embodiments, the composition is formulated in a dried form.
- 15 Typically, the composition is dried using techniques selected from lyophilization, spray drying, fluidized bed drying, vacuum drying, or a combination thereof.
- [0143]** In some embodiments, the composition is formulated for oral administration.
- [0144]** In some embodiments, the bacterial strain produces an agent that
- 20 attenuates or impairs signal transducer and activator of transcription 3 (STAT3) signalling in a cell.
- [0145]** In some embodiments of this type, the agent is a small molecule, peptide, or nucleotide. Typically, the agent is released by the bacterial strain.
- [0146]** In some embodiments, the agent binds specifically to any one of STAT3,
- 25 JAK2, TYK2, or IL-23.
- [0147]** In some embodiments, the *G. formicilis* metabolizes one or more agents selected from the group comprising or consisting of starch, glucose, fructose, gluconate, lactose, trehalose, and lactaldehyde as a carbon source.
- [0148]** In another aspect, the present invention provides a method of restoring or
- 30 improving gut barrier function in a subject, the method comprising administering to the subject a bacterial strain of the species *G. formicilis*, to thereby restore or improve gut barrier function.
- [0149]** In some preferred embodiments, restoring or improving gut barrier function is characterised by at least one of: (i) an increase in the quality and/or quantity of
- 35 mucin; (ii) improvement in integrity of tight junction proteins; (iii) reduction in translocation of luminal contents into systemic circulation; or (iv) reduction of intestinal ulcers and/or intestinal wounds.

[0150] In some embodiments, the luminal contents includes lipopolysaccharide (LPS).

[0151] In some embodiments, the restoration or improvement in gut barrier function results in a reduction in systemic inflammation in the subject. In some embodiments of this type, the systemic inflammation is characterized by elevated levels of an inflammatory cytokine (e.g., IL-1 β IL-8, IL-6, and TNF) in the subject as compared to the level of the inflammatory cytokine in a healthy subject.

[0152] In yet another aspect, the present invention provides a method of maintaining gut barrier function in a subject, the method comprising administering to the subject a bacterial strain of the species *G. formicilis*, to thereby maintain gut barrier function in the subject.

[0153] In yet another aspect, the present invention provides a method of reducing inflammation in a subject, the method comprising administering to the subject a bacterial strain of the strain *G. formicilis*, to thereby reduce inflammation in the subject.

[0154] In some embodiments, the inflammation is local to the gut environment, or systemic inflammation.

[0155] In another aspect, the present invention provides a method of inducing or enhancing mucosal healing in a subject, the method comprising administering to the subject a bacterial strain of the species *G. formicilis* in an amount sufficient to induce epithelial cell migration, proliferation and/or differentiation, to thereby induce mucosal healing in the subject.

[0156] In some embodiments, mucosal healing in the subject can be measured using one or more fecal or serum markers. By way of an illustrative example, one or more fecal markers may be selected from the group comprising calprotectin, lactoferrin, metalloproteinase (MMP)-9, and lipocalin-2.

[0157] In some embodiments, the bacterial strain reduces inflammation by attenuating the NF κ B pathway. In some embodiments of this type, the bacterial strain inhibits the production of one or more transcription factors, cytokines, or chemokines selected from the group comprising NF κ B, TNF, IFN- γ , IL-1 β , IL-8, and MCP-1.

[0158] In yet another aspect, the present invention provides methods of blocking or otherwise inhibiting STAT3 signalling in a target cell, the method comprising contacting the cell with at least a soluble component of a bacterial cell preparation of the species *G. formicilis*, to block or otherwise inhibit STAT3 signalling in the cell. Typically, the method of this aspect is performed *in vitro*.

[0159] In some embodiments, the target cell is selected from a reporter cell (e.g., a HEK cell), an immune cell (e.g., a Th17 immune cell), an epithelial cell, or an endothelial cell. In some embodiments, the target cell is a mammalian cell, and preferably, a human cell.

[0160] In some embodiments, the bacterial cell preparation is a bacterial cell culture. The soluble component may therefore comprise, consist, or consist essentially of, the soluble fraction of the bacterial cell culture (e.g., the cell culture supernatant). The soluble component may further comprise some insoluble components of the bacterial cell culture. For example, the soluble component may include substantially all of the bacterial culture. Preferably, the soluble component is substantially depleted of bacterial cells.

[0161] In some alternative embodiments, the bacterial cell preparation is a bacterial cell lysate. In exemplary embodiments of this type, the soluble component may relate to the soluble fraction of the cell lysate. A soluble fraction can suitably be achieved by any method, including by centrifugation.

[0162] In still yet another aspect, the present invention provides a method of blocking or otherwise inhibiting STAT3 signalling in a cell, the method comprising administering a bacterial strain of the species *G. formicilis* to the subject, thereby blocking or otherwise inhibiting STAT3 signalling in the cell. Typically, the methods of this aspect are performed *in vivo*.

[0163] In some embodiments, the cell is an immune cell (e.g., a Th17 immune cell) or epithelial cell.

[0164] In some embodiments, the cell is an epithelial cell, and the bacterial strain or a metabolite produced by the bacterial strain increases the production of IL-22 in the subject.

[0165] In some embodiments, the bacterial strain produces a molecule that is a direct inhibitor or an indirect inhibitor of STAT3. For example, the bacterial strain may produce a metabolite that directly inhibits at least one of an IL-23 polypeptide, a JAK2 polypeptide, a TYK2 polypeptide, or a STAT3 polypeptide.

[0166] In some embodiments, the bacterial strains used in the methods described above and elsewhere herein produce one or more metabolites selected from butyrate, acetate, ethanol and fumarate.

[0167] In some embodiments the bacterial strain has a 16S rRNA sequence that is at least about 97.5%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% identical to the 16S rRNA sequence of a bacterial strain of *G. formicilis*.

[0168] In some alternative embodiments, the bacterial strain has a 16S rRNA sequence that is at least about 97.5%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% identical to SEQ ID NO: 27 or one or more of SEQ ID NOs: 45-47 or when the bacterial strain has the 16S rRNA gene sequence represented by SEQ ID NO: 27 or one or more of SEQ ID NOs: 45-47. In some embodiments, the bacterial strain comprises two or more copies (e.g., two copies, three copies, four copies, five copies, six copies, seven copies, eight copies) independently selected from the 16S rRNA sequences set forth in SEQ ID NO: 27 or one or more of SEQ ID NOs: 45-47.

[0169] In some embodiments, the bacterial strain is the *G. formicilis* strain deposited under accession number V21/011520, or a derivative thereof.

[0170] Preferably, the bacterial strain is at least partially isolated.

[0171] In some embodiments, the bacterial strain is formulated as a pharmaceutical composition, further comprising a pharmaceutically acceptable carrier, diluent or excipient. In some embodiments, the pharmaceutical composition is a dry composition. In some embodiments, the dry composition is selected from the group consisting of particles, granules, and powder. By way of an illustrative example the pharmaceutical composition may be lyophilized, spray dried, fluidized bed dried, vacuum dried, or a combination thereof.

[0172] In some embodiments, the pharmaceutical composition is formulated for oral administration.

[0173] In still yet another aspect, the present invention provides a method of blocking or otherwise inhibiting STAT3 signalling in a cell, the method comprising administering a bacterial strain of the species *Gemmiger sp. MD158* to the subject, thereby blocking or otherwise inhibiting STAT3 signalling in the cell. Typically, the methods of this aspect are performed *in vivo*.

[0174] In still yet other aspects, the present invention provides a method of treating an inflammatory or autoimmune disorder in a subject, the method comprising administering an effective amount of a bacterial strain of *G. formicilis* to the subject, to thereby treat or prevent the inflammatory or autoimmune disorder.

[0175] In some embodiments, the inflammatory or autoimmune disorder is selected from the group comprising an inflammatory bowel disease (such as Crohn's disease or ulcerative colitis); asthma (such as allergic asthma or neutrophilic asthma); fatty liver disease (such as non-alcoholic fatty liver disease (NAFLD)); ankylosing spondylitis; systemic lupus erythematosus (SLE); scleroderma; Sjogren's syndrome; and vasculitis. Preferably, the inflammatory or autoimmune disorder is an inflammatory bowel disease (IBD).

[0176] In some embodiments, the bacterial strain blocks or otherwise inhibits STAT3 signalling in at least a cell of the subject. Typically, the cell is an epithelial cell, endothelial cell, or an immune cell (e.g., a Th17 immune cell).

[0177] In some embodiments, the bacterial strain has a 16S rRNA sequence that is at least about 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% identical to the 16S rRNA sequence of a bacterial strain of the genus *Gemmiger*.

[0178] Alternatively, in some embodiments the bacterial strain has a 16S rRNA sequence that is at least about 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% identical to SEQ ID NO: 27 or one or more of SEQ ID NOs: 45-47, or when the bacterial strain has the 16S rRNA gene sequence represented by SEQ ID NO: 27 or one or more of SEQ ID NOs: 45-47.

[0179] Preferably, the bacterial strain is at least partially isolated.

[0180] In some embodiments, the bacterial strain is formulated as a pharmaceutical composition, together with a pharmaceutically acceptable carrier, diluent, and/or excipient. In some embodiments, the composition is a dry composition selected from the group consisting of particles, granules, and powder. For example, the composition may be lyophilized. Alternatively, the composition may be spray dried, fluidized bed dried, or vacuum dried.

[0181] In some embodiments, the composition is formulated for oral administration.

[0182] In one aspect, the present invention provides a composition comprising a bacterial strain of the genus *Gemmiger* for use in therapy.

[0183] In another aspect, the present invention provides a composition comprising a bacterial strain of *G. formicilis*, for use in therapy.

[0184] In another aspect, the present invention provides a composition comprising a bacterial strain of *Gemmiger* sp. MD158, for use in therapy.

[0185] In yet another aspect, the present invention provides a composition comprising a bacterial strain of the genus *Gemmiger*, for use in the treatment or prevention of an inflammatory or autoimmune disorder.

[0186] In still yet another aspect, the present invention provides a composition comprising a bacterial strain of *G. formicilis*, for use in the treatment or prevention of an inflammatory or autoimmune disorder.

[0187] In some embodiments, the bacterial strain is the *G. formicilis* strain deposited under accession number V21/011520, or a derivative thereof.

[0188] In some embodiments, the bacterial strain is the *Gemmiger* sp. MD158 strain with a genome sequence that comprises a sequence set forth in at least one, two, three, four, or all of SEQ ID NOs: 34-38.

[0189] In some embodiments, the inflammatory or autoimmune disorder is selected from an inflammatory bowel disease (such as Crohn's disease or ulcerative colitis); asthma (such as allergic asthma or neutrophilic asthma); arthritis (such as rheumatoid arthritis, osteoarthritis, psoriatic arthritis, or juvenile idiopathic arthritis); fatty liver disease (such as nonalcoholic fatty liver disease (NAFLD)); ankylosing spondylitis; psoriasis; systemic lupus erythematosus (SLE); scleroderma; Sjogren's syndrome; and type 1 diabetes mellitus. In some preferred embodiments, the inflammatory or autoimmune disorder is an inflammatory bowel disease (IBD).

[0190] In one aspect, the present invention provides a composition for use in treating an inflammatory or autoimmune disorder, the composition comprising a bacterial strain of *G. formicilis*; and an anti-inflammatory agent.

[0191] In some embodiments, the anti-inflammatory agent is selected from the group comprising 5-aminosalicylates, corticosteroids, azathioprine, infliximab, and adalimumab.

[0192] In another aspect, the present invention provides a composition for use in treating an inflammatory or autoimmune disorder, the composition comprises a bacterial strain of *a Gemmiger species*; and a nutritional supplement. In embodiments of this type, the nutritional supplement improves engraftment of the bacterial stain. In some embodiments of this type, the bacterial strain of the the species *G. formicilis*. In some other embodiments, the bacterial strain is of the specifies *Gemmiger sp. MD158*.

[0193] In some related aspects, the technology described above and/or elsewhere herein provides bacterial species and compositions comprising them in for form of probiotics. Preferably, such probiotics are effective to improve intestinal microbial ecology, alleviate symptoms of microbial dysbiosis, promote wellness, and/or treat or prevent inflammatory and/or autoimmune disorders.

BRIEF DESCRIPTION OF THE FIGURES

[0194] The following figures form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The disclosure may be better understood by reference to one or more of these figures in combination with the detailed description of specific embodiments presented herein.

[0195] **Figure 1 shows a GTDB species representative phylogenetic tree** (r89; methods described in Parks et al. 2018), focusing on the phylogenetic neighbourhood of *A. shahii*. The most recent common ancestor node of the health-associated species *A. shahii* with a high stability (bootstrap value >90%) is highlighted.'

[0196] **Figure 2 provides graphical representations of the association between bacteria of the *Alistipes* genus and representative inflammatory and/or autoimmune disorders.** (A) Using high resolution gut metagenomic data of 6,020 subjects, we identified *A. shahii*, *A. finegoldii*, *A. senegalensis*, *A. onderdonkii*, and *A. sp000434235* as being prevalent in healthy humans (dark bar) but depleted in Crohn's disease and ulcerative colitis (striped bars); (B) These observations were validated in an independent IBD cohort previously published by Harvard (Franzosa et al., 2019); (C) *A. shahii*; (D) *A. finegoldii*; and (E) *A. senegalensis* are significantly less prevalent in a range of inflammatory and autoimmune disorders (striped bars) ($P < 0.05$, Fisher's exact test).

[0197] **Figure 3 provides graphical representation of the morphology and phylogeny of *A. shahii* strains.** (A) Gram-staining of *A. shahii* MH21-1 isolate showing cell morphology. (B) A genome tree constructed with an alignment of 120 bacteria-specific single copy marker genes from high quality reference genomes (GTDB r89). Tips are labelled with the NCBI organism name and NCBI GenBank ID. Clades are labelled with GTDB r89 taxonomy. Non-parametric bootstrap values were calculated from 1000 iterations.

[0198] **Figure 4 provides graphical representations that *A. shahii* does not affect healthy gut function in naïve C57Bl/6 mice.** (A) Overview of the model used to

assess the effect of *A. shahii* MH21-1 on naïve C57Bl/6 mice. (B) Treatment with *A. shahii* MH21-1 has little effect on body weight of naïve animals. (C)-(D) Treatment with *A. shahii* MH21-1 has no effect on colon length or colon weight/length ratio relative to vehicle treated controls in naïve animals. (E)-(G) Treatment with *A. shahii* MH21-1 has no effect on epithelial injury, inflammation, hypervascularization relative to vehicle treated controls in naïve animals. (H) Treatment with *A. shahii* MH21-1 has no effect on gut histology relative to vehicle treated controls in naïve animals. All data reported as mean and standard deviation. ns, not significant.

[0199] Figure 5 provides graphical representations that *A. shahii* restores gut barrier function. (A) Overview of the DSS model used to assess the therapeutic efficacy of *A. shahii*. (B) Effect of daily treatment of vehicle, prednisone and *A. shahii* on body weight of healthy and DSS treated mice. All treatment groups were compared to the DSS + vehicle group. Significance was determined using a two-way Anova with Tukey's test for multiple comparison. (C) Representative gut histology images of C57Bl/6 mice treated with vehicle, prednisone, or *A. shahii*. (D) DSS treatment results in an increase in the histopathological score that is ameliorated by treatment with prednisone, *F. prausnitzii* A2-165 and *A. shahii*. All data presented as mean and standard deviation. All groups were compared to the DSS + vehicle group and significance was determined using a one-way Anova with Dunnett's test for multiple comparison. (E) DSS treatment results in an increase in epithelial injury that is ameliorated by treatment with prednisone or *A. shahii*. All data presented as mean and standard deviation. All groups were compared to the DSS + vehicle group using a Kruskal-Wallis test with Dunn's correction for multiple comparisons. (F) DSS treatment results in a significant decrease in goblet cells that is ameliorated by treatment with prednisone or *A. shahii*. All data presented as mean and standard deviation. All groups were compared to the DSS + vehicle group using a Kruskal-Wallis test with Dunn's correction for multiple comparisons. (G) DSS treatment results in a significant decrease in intraepithelial lymphocytes that is ameliorated by treatment with *A. shahii*. All data presented as mean and standard deviation. All groups were compared to the DSS + vehicle group using a Kruskal-Wallis test with Dunn's correction for multiple comparisons. (H) Overview of the SKG model used to assess the therapeutic efficacy of *A. shahii*. (I) Curdlan treatment results in an increase in the histopathological score that is ameliorated by treatment with anti-IL-23 antibody and *A. shahii*. All data presented as mean and standard deviation. All groups were compared to the curdlan + vehicle group using an Anova with Dunnett's test for multiple comparisons. (J)-(M) Curdlan treatment results in an increase in IL-23, IL-12p70, IL-6 and GM-CSF that is ameliorated by treatment with *A. shahii*. All data presented as mean and standard deviation. For IL-23, IL-12p70 and GM-CSF, all groups were compared to the DSS + vehicle group using a Kruskal-Wallis test with Dunn's correction for multiple comparisons. For IL-6, all groups were compared to the curdlan + vehicle group using an Anova with Dunnett's test for multiple comparisons. For all data, ns: not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ ****, $p < 0.0001$.

[0200] Figure 6 provides a graphical representation of *A. shahii* suppressing STAT3 *in vitro*. (A)-(C) STAT3 signalling is inhibited when HEKBlue IL23

reporter cells are treated with cell-free supernatant raw or < 3KDa fractionated culture supernatant of *A. shahii* strain MH21-1 (t-test, n = 9, **** p < 0.0001; *** p = 0.0004), *A. shahii* MH21-3 and *A. shahii* MH21-6. (D) *A. shahii* culture supernatant was size fractionated and heat treated at 37°C or 97°C, then tested using the HEKBlue IL-23 reporter cell lines. After heat treatment, all fractions were able to suppress STAT3 signalling (t-test, n = 9; **** p < 0.0001; * p = 0.0362; **** p < 0.0001). (E) Cell-free supernatant from *A. shahii* MH21-1 grown in PYG exhibits potent STAT3 suppressive activity but no significant activity when grown in WCB or MCM (One-way ANOVA, n = 9; ns p = 0.4896, and 0.7489; **** p < 0.0001 and <0.0001, ns p = 0.3677 and 0.4524). (F) STAT3 signalling is inhibited when HEKBlue IL-6 reporter cells are treated with cell-free supernatant raw or < 3KDa fractionated culture supernatant of *A. shahii* strain MH21-2 and *A. shahii* strain MH21-3 (t-test, **** p < 0.0001).

[0201] Figure 7 provides a graphical representation showing that *A. shahii* promotes the migration of human gut epithelial cells. (A) Transwell migration assays were employed to study the effect of sterile culture supernatant extracts from *A. shahii* on the migration of HCT116 colon cancer cells. In serum-starved conditions (0.5% FBS), the addition of *A. shahii* 1 x extract to the bottom of the chamber significantly increased the movement of HCT116 cells to the basolateral side compared to the TYG-M medium extract control. (TYG-M, Untreated n = 6 technical replicates; *A. shahii* n = 4 technical replicates, for 3 biological replicates each; Unpaired t-test, two-tailed P = 0.0047). (B) As a second readout for cell migration, the Incucyte scratch wound assay was performed. The relative wound confluence was measured every two hours after the HCT116 cell monolayer was scratched. 90 hours post scratch, serum-starved HCT116 cells incubated in 0.6x extract from *A. shahii* MH21-1 showed significantly higher wound confluence, compared to cells treated with TYG-M medium extract. (TYG-M, Untreated n = 16 technical replicates; *A. shahii* (n = 16 technical replicates, for 3 biological replicates each); Unpaired t-test, two-tailed p = 0.0069). (C) The TEER across T84 cell monolayers was assessed during and after 48-hour basolateral treatment with barrier disruptor IFN-γ. T84 cells treated apically with *A. shahii* cell free culture supernatant show faster recovery ameliorates after IFN-γ induced changes to loss of barrier integrity following 48 hours of treatment relative to the PYG medium control as indicated by higher TEER values (Ohms) as assessed by TEER (each condition n = 4 replicates). (D) T84 cells treated apically with *A. shahii* cell free culture supernatant show significantly higher TEER ameliorates 7 days post IFN-γ induced changes to mediated loss of barrier integrity in TEER following 9 days of treatment relative to the PYG medium control as assessed by TEER (each condition n = 4 replicates). Samples were compared and statistical significance was determined using an unpaired t-test. **** p < 0.0001. (E) The TEER across T84 cell monolayers was assessed during and after 48-hour basolateral treatment with barrier disruptor IFN-γ. T84 cells treated apically with *A. shahii* cell free culture supernatant ameliorates show faster recovery from IFN-γ induced changes to loss of barrier integrity following 48 hours of treatment relative to the PYG medium control as assessed by TEER. All samples were normalized to untreated cells and presented is the percentage difference in TEER compared to the control. (F) T84 cells treated apically with

A. shahii cell free culture supernatant show significantly higher TEER ameliorates 7 days after IFN- γ induced changes to barrier integrity in TEER following 9 days of treatment relative to the PYG medium control as assessed by TEER. All samples were normalized to untreated cells. Samples were compared using an unpaired t-test. **** $p < 0.0001$. (G) Overview of the gating strategy used to isolate *A. shahii* MH21-6.

[0202] **Figure 8** provides graphical representation that *Colonithrix* species are significantly less prevalent in the gut microbiome of humans with inflammatory and autoimmune diseases compared to healthy control. Using high resolution gut metagenomic data of 6,020 subjects.

[0203] **Figure 9** provides graphical representation of the phylogeny and morphology of *C. sana* strains. (A) Gram-staining of a *C. sana* isolate showing morphology. (B) A genome tree constructed with an alignment of 120 bacteria-specific single copy marker genes from high quality reference genomes (GTDB r89; methods described in Parks et al., 2018). Tips are labelled with the NCBI organism name and NCBI GenBank ID. Clades are labelled with GTDB r89 taxonomy. Non-parametric bootstrap values were calculated from 1000 iterations. The tree focuses on the phylogenetic neighbourhood of the *C. sana* genus (i.e., around node 23879). Ancestor nodes with high stability (bootstrap value >90%) of the health-associated species *C. sana* up to the most recent common ancestor of the *Colonithrix* genus are labelled.

[0204] **Figure 10** provides graphical representations that *C. sana* restores gut barrier function. (A) Overview of the DSS model used to assess the therapeutic efficacy of *Colonithrix* species. (B) DSS treatment results in an increase in the histopathological score that is ameliorated by treatment with *C. sana* MH35-1 and *C. sana* MH35-2. All data presented as mean and standard deviation. All groups were compared to the DSS + vehicle group and significance was determined using an unpaired t-test with Welch's correction. (C) DSS treatment results in an increase in epithelial injury that is ameliorated by treatment with *C. sana* MH35-1 and *C. sana* MH35-2. All data presented as mean and standard deviation. All groups were compared to the DSS + vehicle group using a Kruskal-Wallis. For all data, ns: not significant; *, $p < 0.05$.

[0205] **Figure 11** provides a graphical representation of *C. sana* suppressing STAT3 activation *in vitro*. STAT3 signalling is inhibited when HEKBlue IL23 reporter cell lines are treated with cell-free supernatant raw or <3KDa filtered of (A) *C. sana* MH35-1 and (B) *C. sana* MH35-2 (t-test, $n = 9$, A **** $p < 0.0001$; B **** $p < 0.0001$).

[0206] **Figure 12** provides a graphical representation of *C. sana* suppressing IL-6 mediated STAT3 activation *in vitro*. (A-B) STAT3 signalling is inhibited when HEKBlue IL-6 reporter cells are treated with cell-free supernatant raw or <3KDa fractionated culture supernatant of *C. sana* MH35-1 (A) and *C. sana* MH35-2 (B) at 10% v/v (unpaired t-test, $n = 18$, **** $p < 0.0001$). (C) STAT3 signalling is inhibited when HEKBlue IL-6 reporter cells are treated with 1x extract of *C. sana* MH35-1 and *C. sana* MH35-2 (unpaired t-test, $n = 24$, **** $p < 0.0001$).

[0207] Figure 13 provides a graphical representation showing that *C. sana* promotes the migration of human gut epithelial cells. (A) Transwell migration assays were employed to study the effect of sterile culture supernatant extracts from *C. sana* MH35-1 and *C. sana* MH35-2 on the migration of HCT116 colon cancer cells. In serum-starved conditions (0.5% FBS), untreated HCT116 cells and cells treated with 0.5x bacterial medium extract showed comparable background level of migration to the basolateral side of the Transwell chamber. The addition of 0.5x *C. sana* MH35-1 and *C. sana* MH35-2 extract to the bottom of the chamber significantly increased the movement of HCT116 cells to the basolateral side compared to the medium control (Untreated n = 6 technical replicates; Medium control n = 6 technical replicates; *C. sana* MH35-1 n = 4 technical replicates, for 2 biological replicates each; *C. sana* MH35-2 n = 4 technical replicates, for 3 biological replicates each; Unpaired t-test, two tailed *** P = 0.0003 for *C. sana* MH35-1 and *** P = 0.0009 for *C. sana* MH35-2 (B) As a second readout for cell migration, the Incucyte scratch wound assay was performed. The relative wound confluence was measured every two hours after the HCT116 cell monolayer was scratched. Untreated n = 18 technical replicates; Medium control n = 18 technical replicates; *C. sana* MH35-1 n = 36 technical replicates, for 2 biological replicates; *C. sana* MH35-2 n = 36 technical replicates, for 3 biological replicates. (C) 24 hours post scratch, serum-starved HCT116 cells incubated in 0.5x extract from *C. sana* MH35-1 and *C. sana* MH35-2 showed significantly higher wound confluence, compared to cells treated with bacterial medium extract. Unpaired t-test *** P = 0.0008 for *C. sana* MH35-1 and *** P = 0.0004 for *C. sana* MH35-2.

[0208] Figure 14 provides graphical representation that *Gemmiger* species are significantly less prevalent in the gut microbiome of humans with inflammatory and autoimmune diseases compared to healthy control. Using high resolution gut metagenomic data of 6,020 subjects, we identified (A) *Gemmiger formicilis*; (B) *Gemmiger species_C*; (C) *Gemmiger species_A*; and (D) *Gemmiger sp003476825* as being prevalent in healthy humans (dark bar) but depleted in a range of inflammatory and autoimmune disorders (striped bars). (E) The strongest effect was observed for IBD, including both major subtypes ulcerative colitis and Crohn's disease. (F) These observations were validated in an independent IBD cohort previously published by Harvard. All shown association have a P < 0.05 (Fisher's exact test).

[0209] Figure 15 provides graphical representation of the phylogeny and morphology of *G. formicilis* strains. (A) Gram-staining of *G. formicilis* MH32-1 isolate showing morphology. (B) Top panel, photograph of *G. formicilis* MH32-2 on plate, showing round entire raised colonies after 48 hours on TY medium. Lower panel, gram-staining of *G. formicilis* MH32-2 isolate showing morphology. (C) A genome tree constructed with an alignment of 120 bacteria-specific single copy marker genes from high quality reference genomes (GTDB r89). Tips are labelled with the NCBI organism name and NCBI GenBank ID. Clades are labelled with GTDB r89 taxonomy. Non-parametric bootstrap values were calculated from 1000 iterations. (D) GTDB species representative tree (r89; methods described in Parks et al., 2018), focusing on the phylogenetic neighbourhood of the *Gemmiger* genus (i.e., around node 23818). Ancestor nodes with high stability (bootstrap

value >90%) of the health-associated species *G. variabile* up to the most recent common ancestor of the *Gemmiger* genus are labelled.

[0210] Figure 16 provides graphical representations that *G. formicilis* does not affect healthy gut function in naïve C57Bl/6 mice. (A) Overview of the model used to assess the effect of *G. formicilis* MH32-1 on naïve C57Bl/6 mice. (B) Treatment with MH32-1 has little effect on body weight of naïve animals. (C)-(D) Treatment with MH32-1 has no effect on colon length or colon weight/length ratio relative to vehicle treated controls in naïve animals. (E)-(F) Treatment with *G. formicilis* MH32-1 has no effect on epithelial injury or hypervascularization relative to vehicle treated controls in naïve animals. (G) Treatment with *G. formicilis* MH32-1 has no effect on gut histology relative to vehicle treated controls in naïve animals. All data reported as mean and standard deviation. ns, not significant; * $p < 0.05$.

[0211] Figure 17 provides graphical representations that *G. formicilis* restores gut barrier function. (A) Overview of the DSS model used to assess the therapeutic efficacy of *G. formicilis* MH32-1. (B) Effect of daily treatment of vehicle, prednisone and *G. formicilis* MH32-1 in healthy and DSS treated mice. All treatment groups were compared to the DSS + vehicle group. Significance was determined using a two-way Anova with Tukey's test for multiple comparison. (C) Endoscopic assessment of colitis as assessed on days 1, 2 and 6. All groups were compared to the DSS + vehicle group for each individual day using a Kruskal-Wallis test with Dunn's correction for multiple comparisons (day 1) or one-way Anova with Dunnett's correction for multiple comparison (day 2, 6) as appropriate. All data presented as mean and standard deviation. (D) DSS treatment results in a reduction in colon length that is ameliorated by *F. prausnitzii* A2-165 and *G. formicilis* MH32-1, but not prednisone. All data presented as mean and standard deviation. All groups were compared to the DSS + vehicle group and significance was determined using a one-way Anova with Dunnett's test for multiple comparison. (E) Representative gut histology images of C57Bl/6 mice treated with vehicle, prednisone, or *G. formicilis* MH32-1. (F) DSS treatment results in an increase in the histopathological score that is ameliorated by treatment with prednisone, *F. prausnitzii* A2-165 and *G. formicilis* MH32-1. All data presented as mean and standard deviation. All groups were compared to the DSS + vehicle group and significance was determined using a one-way Anova with Dunnett's test for multiple comparison. (G) DSS treatment results in an increase in epithelial injury that is ameliorated by treatment with prednisone or *G. formicilis* MH32-1. All data presented as mean and standard deviation. All groups were compared to the DSS + vehicle group using a Kruskal-Wallis test with Dunn's correction for multiple comparisons. (H) Lipocalin-2 concentration in faeces of C57Bl/6 mice treated with vehicle, prednisone, *F. prausnitzii* or *G. formicilis* MH32-1. Significance was determined using a one-way Anova with Dunnett's test for multiple comparison. (I) Overview of the SKG model used to assess the therapeutic efficacy of *G. formicilis* MH32-1. (J) Curdlan treatment results in an increase in the histopathological score that is ameliorated by treatment with anti-IL-23 antibody and *G. formicilis* MH32-1. All data presented as mean and standard deviation. All groups were compared to the curdlan + vehicle group using an

Anova with Dunnett's test for multiple comparisons. For all data, ns: not significant; *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.0001$.

[0212] Figure 18 provides a graphical representation of *G. formicilis* suppressing STAT3 activation *in vitro*. (A) STAT3 signalling is inhibited when HEKBlue IL23 reporter cell lines are treated with cell-free supernatant raw or < 3KDa filtered of *G. formicilis* MH32-1 (t-test, $n = 9$, $p < 0.0001$, $p = 0.0001$). (B) Cell-free supernatant of *G. formicilis* MH32-1 was size fractionated and heat treated at 37°C or 97°C, then tested HEKBlue IL-23 reporter cell lines. After heat treatment, all fractions are still able to inhibit STAT3 signalling (t-test, $n = 9$; $p = 0.0421$, $p < 0.0001$, $p = 0.0009$). (C) Cell-free supernatant of *G. formicilis* MH32-1 was size fractionated and treated with Proteinase K at 37°C for 30 minutes, then tested HEKBlue IL-23 reporter cell lines. After Proteinase K treatment, all fractions are still able to inhibit STAT3 signalling (t-test, $n = 9$; $p < 0.0001$, $p < 0.0001$). (D) Cell-free supernatant from MH32-1 grown in TY exhibits potent STAT3 suppressive activity but minimal activity when grown in PYG, WCB and MCM (t-test, $n = 9$; $p < 0.0001$, $p = 0.0027$, $p = 0.0203$, $p = 0.0114$). (E-F) STAT3 signalling is inhibited when HEKBlue IL23 reporter cell lines are treated with cell-free supernatant raw or < 3KDa filtered of *G. formicilis* MH32-2 and *G. formicilis* ATCC 27749 (t-test, $n = 9$, $p < 0.0001$, $p = 0.0001$). (G) STAT3 signalling is inhibited when HEKBlue IL23 reporter cell lines are treated with cell-free supernatant raw or < 3KDa filtered of *Gemmiger* sp. MD158 (Samples were compared using an unpaired t-test. ****, $p < 0.001$; *****, $p < 0.0001$). (H) PBMCs show decreased secretion of IL-6 when pre-treated with cell-free supernatant of *G. formicilis* MH32-1 and then stimulated with PIM relative to the TY medium control. Normally distributed data was analysed using an ordinary one-way Anova with Šidák's multiple comparison test ($n=7$ per treatment, ns = not significant; * = $p < 0.05$; *** = $p < 0.001$; **** = $p < 0.0001$). All data presented as mean and standard deviation. (I) *G. formicilis* MH32-1 significantly suppresses IL-6 secretion from LPS-stimulated T84 gut epithelial cells compared to the TY medium control. The suppressive activity is also retained in the <3 kDa fraction of the *G. formicilis* culture supernatant. All data presented as mean and standard deviation. T test **** $p < 0.0001$. (J) STAT3 signalling is inhibited when HEKBlue IL-6 reporter cell lines are treated with < 3KDa filtered cell-free supernatant of *G. formicilis* MH32-1 (t-test, $n = 9$; *** $p = 0.0009$).

[0213] Figure 19 provides a graphical representation showing that *G. formicilis* promotes the migration of human gut epithelial cells. (A) Transwell migration assays were employed to study the effect of sterile culture supernatant extracts from *G. formicilis* MH32-1 on the migration of HCT116 colon cancer cells. In serum-starved conditions (0.5% FBS), untreated HCT116 cells and cells treated with TY medium extract showed comparable background level of migration to the basolateral side of the Transwell chamber. The addition of *G. formicilis* MH32-1 extract to the bottom of the chamber significantly increased the movement of HCT116 cells to the basolateral side compared to the TY control (TY, $n = 6$ technical replicates; MH32-1 $n = 4$ technical replicates, for 3 biological replicates each; Dunnett's multiple comparisons test **** $P < 0.0001$). (B) Representative brightfield images at 10x magnification for Transwell® migration experiments.

Image size is 570 µm x 570 µm. (C) As a second readout for cell migration, the Incucyte scratch wound assay was performed. The relative wound confluence was measured every two hours after the HCT116 cell monolayer was scratched. TY, C n = 27 technical replicates; *G. formicilis* MH32-1 n = 18 technical replicates, for 3 biological replicates each. (D) 18 hours post scratch, serum-starved HCT116 cells incubated in 0.3x extract from *G. formicilis* MH32-1 showed significantly higher wound confluence, compared to cells treated with TY medium extract. TY, C n = 27 technical replicates; *G. formicilis* MH32-1 n = 18 technical replicates, for 3 biological replicates each; Unpaired t-test ** p < 0.0028.

[0214] Figure 20 provides a graphical representation showing that *G. formicilis* restores gut barrier integrity. Effect of treatment with *G. formicilis* MH32-1 and vehicle control on T84 cells stimulated with IFN-γ as assessed through transepithelial electrical resistance (TEER) using the Maestro Pro system. T84 cells were grown as monolayers in 96-well CytoView-Z plates and stimulated with IFNγ (100 ng/mL) once stable TEER values were established. After 72 hours, cells were washed and media replaced with fresh media alone or supplemented with 1x *G. formicilis* MH32-1 extract or vehicle control. At 45 hours post-treatment TEER measurements were recorded, and the percentage difference in TEER value compared to the control (untreated T84 cells) was visualised in histograms as mean ± SD. Statistical significance was determined by unpaired t-test; ** p < 0.01; **** p < 0.0001. (B) Overview of the gating strategy used to isolate *Gemmiger* sp. MD158.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:	Sequence Description
1	16S rRNA sequence of <i>A. shahii</i> strain MH21-1
2	16S rRNA sequence of <i>A. shahii</i> strain MH21-2
3	16S rRNA sequence of <i>A. shahii</i> strain MH21-3
4	Genome sequence of <i>A. shahii</i> strain MH21-1
5	Genome sequence of <i>A. shahii</i> strain MH21-2
6	Genome sequence of <i>A. shahii</i> strain MH21-3
7	16S rRNA sequence of <i>A. finegoldii</i> type strain AHN 2437
8	16S rRNA sequence of <i>A. onderdonkii</i> type strain WAL 8169
9	Genome sequence of <i>A. finegoldii</i> type strain AHN 2437
10	Genome sequence of <i>A. onderdonkii</i> type strain WAL 8169
11	16S rRNA sequence copy 1 of <i>C. sana</i> strain MH35-1
12	16S rRNA sequence copy 2 of <i>C. sana</i> strain MH35-1
13	16S rRNA sequence copy 3 of <i>C. sana</i> strain MH35-1
14	16S rRNA sequence copy 4 of <i>C. sana</i> strain MH35-1
15	16S rRNA sequence copy 1 of <i>C. sana</i> strain MH35-2
16	16S rRNA sequence copy 2 of <i>C. sana</i> strain MH35-2
17	16S rRNA sequence copy 3 of <i>C. sana</i> strain MH35-2
18	16S rRNA sequence copy 4 of <i>C. sana</i> strain MH35-2
19	Genome sequence of <i>C. sana</i> strain MH35-1 scaffold 1

20	Genome sequence of <i>C. sana</i> strain MH35-1 scaffold 2
21	Genome sequence of <i>C. sana</i> strain MH35-1 scaffold 3
22	Genome sequence of <i>C. sana</i> strain MH35-1 scaffold 4
23	Genome sequence of <i>C. sana</i> strain MH35-2 scaffold 1
24	Genome sequence of <i>C. sana</i> strain MH35-2 scaffold 2
25	Genome sequence of <i>C. sana</i> strain MH35-2 scaffold 3
26	Genome sequence of <i>C. sana</i> strain MH35-2 scaffold 3
27	16S rRNA sequence of <i>G. formicilis</i> strain MH32-1
28	Genome sequence of <i>G. formicilis</i> strain MH32-1
29	Genome sequence of <i>G. formicilis</i> strain MH32-2 scaffold 1
30	Genome sequence of <i>G. formicilis</i> strain MH32-2 scaffold 2
31	Genome sequence of <i>G. formicilis</i> strain MH32-2 scaffold 3
32	Genome sequence of <i>G. formicilis</i> strain MH32-2 scaffold 4
33	Genome sequence of <i>G. formicilis</i> strain MH32-2 scaffold 5
34	Genome sequence of <i>Gemmiger sp. MD158</i> scaffold 1
35	Genome sequence of <i>Gemmiger sp. MD158</i> scaffold 2
36	Genome sequence of <i>Gemmiger sp. MD158</i> scaffold 3
37	Genome sequence of <i>Gemmiger sp. MD158</i> scaffold 4
38	Genome sequence of <i>Gemmiger sp. MD158</i> scaffold 5
39	Genome sequence of <i>A. shahii</i> strain MH21-6 scaffold 1
40	Genome sequence of <i>A. shahii</i> strain MH21-6 scaffold 2
41	Genome sequence of <i>A. shahii</i> strain MH21-6 scaffold 3
42	Genome sequence of <i>A. shahii</i> strain MH21-6 scaffold 4
43	Genome sequence of <i>A. shahii</i> strain MH21-6 scaffold 5
44	16S rRNA sequence of <i>A. shahii</i> strain MH21-6
45	16S rRNA sequence of <i>G. formicilis</i> strain MH32-2
46	16S rRNA sequence of <i>Gemmiger sp. MD158</i> strain

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

[0215] 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 invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

10 [0216] 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.

[0217] The term “about” as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter *per se*.

5 **[0218]** As used herein, the term “administering,” refers to the placement of an agent (e.g., bacteria) as disclosed herein into a subject by a method or route which results in at least partial delivery of the agent at the desired site. Compositions comprising the compounds disclosed herein can be administered by any appropriate route which results in an effective biological activity or therapeutic effect in the subject. In some embodiments,
10 administration comprises physical human activity (e.g., an injection, act of ingestion, an act of application, and/or manipulation of a delivery device or machine). Such activity can be performed (e.g., by a medical professional and/or the subject being treated).

[0219] Specifically, as used herein “administer” and “administration” encompasses embodiments in which one person directs another to consume live bacteria,
15 dead bacteria, spent mediums derived from bacteria, cell pellets of bacteria, purified metabolites produced by bacteria, purified proteins produced by bacteria, prebiotics, small molecules, or combinations thereof in a certain manner and/or for a certain purpose independently of or in variance to any instructions received from a second person. Non-limiting examples of embodiments include the situation in which one person directs another
20 to consume live bacteria, dead bacteria, spent mediums derived from bacteria, cell pellets of bacteria, purified metabolites produced by bacteria, purified proteins produced by bacteria, prebiotics, small molecules, or combinations thereof in a certain manner and/or for a certain purpose include when a physician prescribes a course of conduct and/or treatment to a patient, when a parent commands a minor user (such as a child) to consume such a product,
25 when a trainer advises a user (such as an athlete) to follow a particular course of conduct and/or treatment, or when a manufacturer, distributor, or marketer recommends conditions of use to an end user, for example through advertisements or labeling on packing or on other materials provided in association with the sale or marketing of a product. In some embodiments, the disclosed compositions can be administered orally, intravenously,
30 intramuscularly, intrathecally, subcutaneously, sublingually, buccally, rectally, vaginally, by the ocular route, by the optic route, nasally, via inhalation, by nebulization, cutaneously, transdermally, or combinations thereof, and formulated for delivery with a pharmaceutically acceptable excipient, carrier or diluent. Of note, although the disclosed compositions encompass multiple formulations and modes of delivery for treatments to ameliorate
35 dysbiosis and its sequelae, it should be noted that live biotherapeutic products such as probiotics are not typically administered intravenously, intramuscularly, or intraperitoneally. These modes of delivery would likely be reserved for small-molecule products of bacterial metabolism.

[0220] The terms “administration concurrently” or “administering concurrently” or
40 “co-administering” and the like refer to the administration of a single composition containing two or more actives, or the administration of each active as separate compositions and/or

delivered by separate routes either contemporaneously or simultaneously or sequentially within a short enough period of time that the effective result is equivalent to that obtained when all such actives are administered as a single composition. By "simultaneously" is meant that the active agents are administered at substantially the same time, and desirably together in the same formulation. By "contemporaneously" it is meant that the active agents are administered closely in time, *e.g.*, one agent is administered within from about one minute to within about one day before or after another. Any contemporaneous time is useful. However, it will often be the case that when not administered simultaneously, the agents will be administered within about one minute to within about eight hours and suitably within less than about one to about four hours. When administered contemporaneously, the agents are suitably administered at the same site on the subject. The term "same site" includes the exact location, but can be within about 0.5 to about 15 centimeters, preferably from within about 0.5 to about 5 centimeters. The term "separately" as used herein means that the agents are administered at an interval, for example at an interval of about a day to several weeks or months. The active agents may be administered in either order. The term "sequentially" as used herein means that the agents are administered in sequence, for example at an interval or intervals of minutes, hours, days or weeks. If appropriate the active agents may be administered in a regular repeating cycle.

[0221] The term "agent" includes a compound that induces a desired pharmacological and/or physiological effect. The term also encompasses pharmaceutically acceptable and pharmacologically active ingredients of those compounds specifically mentioned herein including but not limited to salts, esters, amides, prodrugs, active metabolites, analogs and the like. When the above term is used, then it is to be understood that this includes the active agent *per se* as well as pharmaceutically acceptable, pharmacologically active salts, esters, amides, prodrugs, metabolites, analogs, etc. The term "agent" is not to be construed narrowly but extends to small molecules, proteinaceous molecules such as peptides, polypeptides and proteins as well as compositions comprising them and genetic molecules such as RNA, DNA and mimetics and chemical analogs thereof as well as cellular agents. The term "agent" includes a cell that is capable of producing and secreting a polypeptide referred to herein as well as a polynucleotide comprising a nucleotide sequence that encodes that polypeptide. Thus, the term "agent" extends to nucleic acid constructs including vectors such as viral or non-viral vectors, expression vectors and plasmids for expression in and secretion in a range of cells.

[0222] The "amount" or "level" of a biomarker is a detectable level in a sample. These can be measured by methods known to one skilled in the art and also disclosed herein. The expression level or amount of biomarker assessed can be used to determine the response to treatment.

[0223] As used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (or).

[0224] The term "anaerobic" means not requiring oxygen for growth. Anaerobic bacterial strains comprise bacterial strains that are obligate anaerobes (*i.e.*, those that are harmed by the presence of oxygen); aerotolerant anaerobes, (*i.e.*, those that cannot use oxygen for growth, but tolerate its presence); and facultative anaerobes (*i.e.*, those that can grow without oxygen, but can use oxygen if it is present).

[0225] "Anaerobic conditions" are defined as conditions under which the oxygen concentration in the fermentation medium is too low for the microorganism to use as a terminal electron acceptor. "Anaerobic conditions" can be further defined as conditions under which no or small amounts of oxygen are added to the medium at rates of <3 mmol/L/h, preferably <2.5 mmol/L/h, more preferably <2 mmol/L/h, and most preferably <1.5 mmol/L/h. "Anaerobic conditions" means in particular completely oxygen-free (=0 mmol/L/h oxygen) or with small amounts of oxygen added to the medium at rates of e.g., <0.5 to <1 mmol/L/h. "Anaerobic metabolism" refers to a biochemical process in which oxygen is not the final acceptor of electrons contained in NADH. Anaerobic metabolism can be divided into anaerobic respiration, in which compounds other than oxygen serve as the terminal electron acceptor, and substrate level phosphorylation, in which the electrons from NADH are utilized to generate a reduced product via a fermentative pathway.

[0226] The term "carbon source" generally refers to a substrate or compound suitable for sustaining microorganism growth. Carbon sources may be in various forms, including, but not limited to polymers, carbohydrates, alcohols, acids, aldehydes, ketones, amino acids, peptides, etc. For example, these may include monosaccharides (such as glucose, fructose, xylose), oligosaccharides (*i.e.*, sucrose, lactose), polysaccharides (*i.e.*, starch, cellulose, hemicellulose), lignocellulosic materials, fatty acids (*i.e.*, succinate, lactate, acetate), glycerol, etc. or a mixture thereof. The carbon source may be a product of photosynthesis, such as glucose or cellulose.

[0227] Monosaccharides used as carbon sources may be the product of hydrolysis of polysaccharides, such as acid or enzymatic hydrolysates of cellulose, starch and pectin. The term "energy source" may be used here interchangeably with carbon source since in chemoorganotrophic metabolism the carbon source is used both as an electron donor during catabolism and as a carbon source during cell growth.

[0228] Throughout this specification, unless the context requires otherwise, the words "comprise", "comprises" and "comprising" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements. Thus, use of the term "comprising" and the like indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting

essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

[0229] As used herein, "culturing", "culture" and the like refer to the set of procedures used *in vitro* where a population of cells (or a single cell) is incubated under conditions which have been shown to support the growth or maintenance of the cells *in vitro*. The art recognizes a wide number of formats, media, temperature ranges, gas concentrations etc. which need to be defined in a culture system. The parameters will vary based on the format selected and the specific needs of the individual who practices the methods herein disclosed. However, it is recognized that the determination of culture parameters is routine in nature.

[0230] The terms "decrease", "reduced", "reduction", "inhibit", "suppress", "attenuate" and the like are all used herein to mean a decrease by a statistically significant amount. In some embodiments, these terms typically mean a decrease by at least 10% as compared to a reference level (e.g., the absence of a given treatment or agent) and can include, for example, a decrease by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or more. As used herein "reduction", "suppression", and "inhibition" does not necessitate a complete inhibition or reduction as compared to a reference level. "Complete inhibition" and the like is a 100% inhibition as compared to a reference level. A decrease can be preferably down to a level accepted as within the range of normal (e.g., for an individual without a given disorder).

[0231] The terms "increased", "increase", "enhance", or "activate" are all used herein to mean an increase by a statistically significant amount. In some embodiments, the terms "increased", "increase", "enhance", or "activate" can mean an increase of at least 10% as compared to a reference level (e.g., the absence of a given treatment or agent) and can include, for example, of at least about 10% as compared to a reference level, for example an increase of at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or up to and including a 100% increase or any increase between 10-100% as compared to a reference level or at least about a 2-fold, or at least about a 3-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold, or at least about a 10-fold increase, or any increase between 2-fold and 10-fold or greater as compared to a reference level. In the context of a marker or symptom, an "increase" is a statistically significant increase in such level.

[0232] As used herein, the term "isolated" encompasses a bacterium or other entity or substance that has been (1) separated from at least some of the components with

which it was associated when initially produced (whether in nature, such as human stool, or in an experimental setting, such as a Petri plate consisting of artificial growth medium), and/or (2) produced, prepared, purified, and/or manufactured by the hand of man. Isolated bacterial, proteins, metabolites, or combinations thereof may be separated from at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more of the other components with which they were initially associated. In some embodiments, isolated bacteria, proteins, metabolites, or combinations thereof are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components (such as other bacterial species). The terms "purify", "purifying", and "purified" refer to a bacterium or other material that has been separated from at least some of the components with which it was associated either when initially produced or generated (e.g., when in nature or in an experimental setting), or during any time after its initial production, as recognized by those skilled in the art of bacterial cultivation or of relevant skill (e.g., chemistry). A bacterium or bacterial population can be considered purified if it is isolated at or after production, such as from a material or environment containing the bacterial or bacterial population, and a purified bacterium or bacterial population can contain other material up to about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90% or above about 90% and still be considered "isolated". In some embodiments, purified bacterial and bacterial populations are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more than about 99% pure. In the instance of bacterial compositions provided herein, the one or more bacterial types present in the composition can be independently purified from one or more other bacteria produced and/or present in the material or environment containing the bacterial type. In some embodiments, a bacterium or population of bacteria is "isolated" if it comprises a single strain of bacteria. In some embodiments, such isolated bacteria can be admixed or administered with other isolated bacteria (e.g., in a defined consortium of isolated bacteria). Bacterial compositions and the bacterial components thereof are generally purified from residual habitat products.

[0233] The term "genome" as used herein includes the DNA comprising the genes (the coding nucleic acid sequences) and the noncoding nucleic acid sequences of a microorganism, and therefore includes introduction of the nucleic acid into, for example, the coding and noncoding DNA of the microorganism.

[0234] The term "Gram-variable" means giving a positive result and/or negative result in the Gram stain test (*i.e.*, retaining the colour of the crystal violet staining reagent). Retention of crystal violet staining by a bacterium is linked to the thickness of the peptidoglycan layer in the bacterial cell wall. Gram-positive bacteria have a thicker peptidoglycan layer. Gram-staining is commonly used to help classify bacterial strains in the field of microbiology.

[0235] As used herein, the term "gut" is understood to refer to the human gastrointestinal tract, also known as the alimentary canal. The gut includes the mouth, pharynx, oesophagus, stomach, small intestine (duodenum, jejunum, ileum), large intestines (cecum and colon) and rectum. While the entire alimentary canal can be colonized by varying species of microbes, the majority of the gut microbiome, in terms of both numbers of species and biomass, resides in the intestines (small and large).

[0236] The terms "marker", "biomarker" and the like, refer to any compound that can be measured as an indicator of the physiological status of a biological system. The marker may be a biomarker that comprises an amino acid sequence, a nucleic acid sequence and fragments thereof. Exemplary biomarkers include, but are not limited to cytokines, chemokines, growth and angiogenic factors, metastasis related molecules, cancer antigens, apoptosis related proteins, enzymes, proteases, adhesion molecules, cell signalling molecules and hormones. The marker may also be a sugar that, in some embodiments, may not be significantly metabolized in the biological system. The sugar may be, for example, mannitol, lactulose, sucrose, sucralose and combinations of any of the foregoing.

[0237] "Measuring" or "measurement" means assessing the presence, absence, quantity or amount (which can be an effective amount) of a given substance within a sample, including the derivation of qualitative or quantitative concentration levels of such substances, or otherwise evaluating the values or categorization of a subject's clinical parameters. Alternatively, the term "assaying," "detecting" or "detection" may be used to refer to all measuring or measurement as described in this specification.

[0238] The term "mucosal healing" as used herein, means an improvement in one or more characteristics of that indicate an impaired mucosal layer. Such characteristics are usually determined by colonic endoscopy and include, but are not limited to, erythema, loss of vascular pattern, friability, bleeding, erosions and ulcers. In some circumstances, mucosal healing refers to a complete amelioration of detrimental effects that characterize an impaired mucosal layer. Alternatively, mucosal healing may refer to a reduction or improvement of one or more of the negative effects that characterize an impaired mucosal layer.

[0239] As used herein, the term "pharmaceutical composition" refers to the active agent in combination with a pharmaceutically acceptable carrier (e.g., a carrier commonly used in the pharmaceutical industry). The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. In some embodiments of any of the aspects, a pharmaceutically acceptable carrier can be a carrier other than water. In some embodiments, any of the aspects a pharmaceutically acceptable carrier can be a cream, emulsion, gel, liposome, nanoparticle, and/or ointment. In some embodiments of any of the aspects, a pharmaceutically acceptable carrier can be an artificial or engineered carrier (e.g., a carrier that the active ingredient would not be found to occur in or within nature).

[0240] The term "phylogenetic tree" refers to a graphical representation of the evolutionary relationships of one genetic sequence to another that is generated using defined set of phylogenetic reconstruction algorithms (e.g., parsimony, maximum likelihood, or Bayesian). Nodes in the tree represent distinct ancestral sequences and the confidence of any node is provided by a bootstrap or Bayesian posterior probability, which measures branch uncertainty.

[0241] In some embodiments, the term "strain", refers to a terminal leaf in a phylogenetic tree and is defined by a specific genetic sequence. The specific genetic sequence may be a concatenated alignment of 120 ubiquitous single-copy proteins (Parks et al., 2018) extracted from a genome assembly using GTDB-tk (Chaumeil et al., 2020) or other tools known in the art.

[0242] The term "clade" refers to the set of members of a phylogenetic tree downstream of a stable node (bootstrap value >90%) in a phylogenetic tree. A clade is a group of related organisms representing all of the phylogenetic descendants of a common ancestor. The clade comprises a set of terminal leaves in the phylogenetic tree that is a distinct monophyletic evolutionary unit.

[0243] As used herein, "prebiotic" is understood to mean an ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that may (or may not) confer benefits upon the host. Favoured prebiotics will be those which encourage growth of probiotic compositions or their beneficial functions, but not growth of pathogens nor genes associated with pathogenicity (e.g., toxins).

[0244] As used herein, "probiotic" is understood to mean "live microorganisms which when administered in adequate amounts confer a health benefit on the host", as currently defined by the World Health Organization.

[0245] The term "species" is defined as a collection of closely related organisms with greater than 97% 16S ribosomal RNA (rRNA) sequence homology and greater than 70% genomic hybridization and sufficiently different from all other organisms so as to be recognized as a distinct unit. Species and other phylogenic identifications are according to the classification known to a person skilled in the art of microbiology.

[0246] As used herein, a "subject" means a human or animal. Usually the animal is a vertebrate such as a primate, rodent, domestic animal or game animal. Primates include chimpanzees, cynomolgus monkeys, spider monkeys, and macaques (e.g., Rhesus). Rodents include mice, rats, woodchucks, ferrets, rabbits, and hamsters. Domestic and game animals include cows, horses, pigs, deer, bison, buffalo, feline species (e.g., domestic cat), canine species (e.g., dog, fox, wolf), avian species (e.g., chicken, emu, ostrich), and fish (e.g., trout, catfish, and salmon). In some embodiments the subject is a mammal (e.g., a primate (e.g., a human)). The terms "individual", "patient" and "subject" are used interchangeably herein.

[0247] Preferably the subject is a mammal. The mammal can be a human, non-human primate, mouse, rat, dog, cat, horse or cow, but is not limited to these examples.

Mammals other than humans can be advantageously used as subjects that represent animal models of inflammatory and autoimmune disorders (e.g., models of gut barrier function). A subject can be male or female.

[0248] As used herein, the terms “treat”, “treatment”, “treating” and the like, refer to therapeutic treatments, wherein the object is to reverse, alleviate, ameliorate, inhibit, slow down or stop the progression or severity of a condition associated with a disease or disorder (e.g., an inflammatory or autoimmune disorder). The term “treating” includes reducing or alleviating at least one adverse effect or symptom of a condition, disease or disorder associated with an inflammatory or autoimmune disorder. Treatment is generally “effective” if one or more symptoms or clinical markers are reduced. Alternatively, treatment is “effective” if the progression of a disease is reduced or halted. That is, “treatment” includes not just the improvement of symptoms or markers, but also a cessation of, or at least slowing of, progress or worsening of symptoms compared to what would be expected in the absence of treatment. Beneficial or desired clinical results include, but are not limited to, alleviation of one or more symptom(s), diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration, or palliation of the disease state, remission (whether partial or total), and/or decreased mortality, whether detectable or undetectable. The term “treatment” of a disease also includes providing relief from the symptoms or side-effects of the disease (including palliative treatment). A treatment need not cure a disorder (i.e., complete reversal or absence of disease) to be considered effective.

[0249] In some embodiments, sequencing comprises 16S rRNA gene sequencing, which can also be referred to as “16S ribosomal RNA sequencing”, “16S rDNA sequencing” or “16S rRNA sequencing”. Sequencing of the 16S rRNA gene can be used for genetic studies as it is highly conserved between different species of bacteria, but it is not present in eukaryotic species. In addition to highly conserved regions, the 16S rRNA gene also comprises nine hypervariable regions (V1-V9) that vary between species. 16S rRNA gene sequencing typically comprises using a plurality of universal primers that bind to conserved regions of the 16S rRNA gene, PCT amplifying the bacterial 16S rRNA gene regions (including hypervariable regions), and sequencing the amplified 16S rRNA genes with a next-generation sequencing technology as described herein (see, also e.g., U.S. Patent Nos. 5,654,418; 6,344,316; and 8,889,358; and U.S. Patent Publication Nos. 2013/157,265 and 2018/195,111, which are each incorporated by reference in their entireties).

[0250] Each embodiment described herein is to be applied *mutatis mutandis* to each and every embodiment unless specifically stated otherwise.

2. Bacterial strains – *Alistipes* species

[0251] The compositions of the invention comprise a bacterial strain of the genus *Alistipes*. The examples demonstrate that bacteria of this genus are useful for treating or preventing diseases associated with an impaired gut barrier function. The preferred bacterial strains are of the species *A. shahii*.

[0252] *Alistipes* is a genus of bacteria in the class Clostridia. The scientific classification is as follows: bacteria (kingdom); *Bacteroidetes* (phylum); *Bacteroidia* (class); *Bacteroidales* (order); *Rikenellaceae* (family); *Alistipes* (genus). Bacteria within the *Alistipes* genus are Gram-negative, non-motile bacteria with a short rod shape, and are obligate anaerobes. These criteria are important because they can inform the phylogenetic classification of bacterial strains.

[0253] *A. shahii* typically grows in circular colonies of about 0.5 mm to 1.0 mm and are pigment producing and non-fluorescing when grown on rabbit blood agar.

[0254] The *A. shahii* species was first described in Song et al., 2006. The *A. shahii* type strain WAL 8301(= ATCC BAA-1179) was isolated from human appendix tissue (Song et al., 2006). The GenBank accession number for the 16S rRNA gene sequence of the *A. shahii* type strain ATCC 27749 is NR_104846.

[0255] The breadth of the *Alistipes* genus and *A. shahii* species may be as defined by a Genome Taxonomy Database reference tree, a taxonomic classification system as described in Parks et al., 2018.

[0256] The *A. shahii* bacterium deposited under accession number V21/014432 (i.e., *A. shahii* MH21-1) was tested in the Examples and is one of the preferred strains of the invention. *A. shahii* strain MH21-1 was deposited with the international depositary authority National Measurement Institute (NMI, 1/153 Bertie Street, Port Melbourne, Victoria, 3207, Australia) by Microba IP Pty Ltd (388 Queen Street, Brisbane, Queensland 4000, Australia) on 22nd July 2021 as "*Alistipes shahii* MH21-1" and was assigned accession number V21/014432.

[0257] An exemplary 16S rRNA sequence for the *A. shahii* MH21-1 strain that was tested is set forth in SEQ ID NO: 1. Bacterial strains of the species *A. shahii* may comprise a single 16S rRNA sequence within its genome, or alternatively, may comprise two or more 16S rRNA sequences within its genome (e.g., two copies, three copies, four copies, five copies, six copies, seven copies, eight copies, or more than eight copies). In some embodiments, a bacterial strain may be identified as being of the *A. shahii* MH21-1 strain by determining whether the strain comprises a 16S rRNA sequence that corresponds to SEQ ID NO: 1, by any method known in the art. A chromosome sequence for strain *A. shahii* MH211-1 is provided in SEQ ID NO: 4. This sequence was generated using the Illumina NovSeq6000 platform.

[0258] Bacterial strains closely related to the strains MH21-1 are also shown in the examples to be effective for treating or preventing inflammatory and autoimmune disorders, through their beneficial effects on restoring gut barrier function.

[0259] For example, the *A. shahii* bacterium deposited under accession number V21/014433 (i.e., *A. shahii* MH23-2) was tested in the Examples and is another one of the preferred strains of the invention. An exemplary 16S rRNA sequence for the *A. shahii* MH212 strain that was tested is set forth in SEQ ID NO: 2. In some embodiments, a bacterial strain may be identified as being of the *A. shahii* MH21-2 strain by determining whether the strain

comprises a 16S rRNA sequence that corresponds to SEQ ID NO: 2, by any method known in the art. Strain *A. shahii* MH21-2 was deposited with the international depositary authority National Measurement Institute (NMI, 1/153 Bertie Street, Port Melbourne, Victoria, 3207, Australia) by Microba IP Pty Ltd (388 Queen Street, Brisbane, QLD 4000, Australia) on 22nd July 2021 as "*Alistipes shahii* MH21-2" and was assigned accession number V21/014433. The genome of strain *A. shahii* MH21-2 comprises a chromosome with a sequence as set forth in SEQ ID NO: 5.

[0260] For example, the *A. shahii* bacterium deposited under accession number V21/014434 (i.e., *A. shahii* MH23-3) was tested in the Examples and is another one of the preferred strains of the invention. An exemplary 16S rRNA sequence for the *A. shahii* MH21-3 strain that was tested is set forth in SEQ ID NO: 3. In some embodiments, a bacterial strain may be identified as being of the *A. shahii* MH21-3 strain by determining whether the strain comprises a 16S rRNA sequence that corresponds to SEQ ID NO: 3, by any method known in the art. Strain *A. shahii* MH21-3 was deposited with the international depositary authority National Measurement Institute (NMI, 1/153 Bertie Street, Port Melbourne, Victoria, 3207, Australia) by Microba IP Pty Ltd (388 Queen Street, Brisbane, QLD 4000, Australia) on 22nd July 2021 as "*Alistipes shahii* MH21-3" and was assigned accession number V21/014434. The genome of strain *A. shahii* MH21-3 comprises a chromosome with a sequence as set forth in SEQ ID NO: 6.

[0261] An exemplary 16S rRNA sequence for the *A. shahii* MH21-6 strain that was tested in the Examples is set forth in SEQ ID NO: 44. In some embodiments, a bacterial strain may be identified as being of the *A. shahii* MH21-6 strain by determining whether the strain comprises a 16S rRNA sequence that corresponds to SEQ ID NO: 44, by any method known in the art. The genome of strain *A. shahii* MH21-6 comprises a chromosome with a sequence as set forth in one or more of SEQ ID NOs: 38-43.

[0262] In certain embodiments, the bacterial strains of the invention have a 16S rRNA sequence that is at least 97.5%, 98%, 98.5%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to the 16S rRNA sequence of a bacterial strain of *A. shahii*. Preferably, the bacterial strain of the invention has a 16S rRNA sequence that is at least 97.5%, 98%, 98.5%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to any one of SEQ ID NOs: 1-3 and 44. In some preferred, the bacterial strain of the invention has a 16S rRNA sequence represented by any one of SEQ ID NOs: 1-3 and 44.

[0263] The genome of the bacterial strain may comprise the 16S rRNA sequence set forth in any one of SEQ ID NOs: 1-3 and 44.

[0264] In certain embodiments, the bacterial strain of the invention has a chromosome with sequence identity to SEQ ID NO: 4. In preferred embodiments, the bacterial strain of the invention has a chromosome with at least 90% sequence identity (e.g., at least 92%, 94%, 95%, 96%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, or 100% sequence identity) to SEQ ID NO: 4 across at least 60% (e.g., at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of SEQ ID NO: 4. For example, the bacterial strain of the invention may have a chromosome with at least 90% sequence identity to SEQ ID NO: 4

across 70% of SEQ ID NO: 4, or at least 90% sequence identity to SEQ ID NO: 4 across 80% of SEQ ID NO: 4, or at least 90% sequence identity to SEQ ID NO: 4 across 90% of SEQ ID NO: 4, or at least 90% sequence identity to SEQ ID NO: 4 across 100% of SEQ ID NO: 4, or at least 95% sequence identity to SEQ ID NO: 4 across 70% of SEQ ID NO: 4, or at least 95% sequence identity to SEQ ID NO: 4 across 80% of SEQ ID NOs: 4, or at least 95% sequence identity to SEQ ID NO: 4 across 90% of SEQ ID NO: 4, or at least 95% sequence identity to SEQ ID NO: 4 across 100% of SEQ ID NO: 4, or at least 98% sequence identity to SEQ ID NO: 4 across 70% of SEQ ID NO: 4, or at least 98% sequence identity to SEQ ID NO: 4 across 80% of SEQ ID NO: 4, or at least 98% sequence identity to SEQ ID NO: 4 across 90% of SEQ ID NO: 4, or at least 98% sequence identity to SEQ ID NO: 4 across 100% of SEQ ID NO: 4. A particularly preferred strain of the invention is the *A. shahii* strain deposited under accession number V21/014432. This is the exemplary MH21-1 strain tested in the DSS mouse model presented in the examples and shown to be effective for treating disease. Therefore, the invention provides a cell, such as an isolated cell, of the *A. shahii* strain deposited under accession number V21/014432, or a derivative thereof. The invention also provides a composition comprising a cell of the *A. shahii* strain deposited under accession number V21/014432, or a derivative thereof. The invention also provides a biologically pure culture of the *A. shahii* MH21-1 strain deposited under accession number V21/014432.

[0265] In certain embodiments, the bacterial strain of the invention has a chromosome with sequence identity to SEQ ID NO: 5. In preferred embodiments, the bacterial strain of the invention has a chromosome with at least 90% sequence identity (*e.g.*, at least 92%, 94%, 95%, 96%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, or 100% sequence identity) to SEQ ID NO: 5 across at least 60% (*e.g.*, at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of SEQ ID NO: 5. For example, the bacterial strain of the invention may have a chromosome with at least 90% sequence identity to SEQ ID NO: 5 across 70% of SEQ ID NO: 5, or at least 90% sequence identity to SEQ ID NO: 5 across 80% of SEQ ID NO: 5, or at least 90% sequence identity to SEQ ID NO: 5 across 90% of SEQ ID NO: 5, or at least 90% sequence identity to SEQ ID NO: 5 across 100% of SEQ ID NO: 5, or at least 95% sequence identity to SEQ ID NO: 5 across 70% of SEQ ID NO: 5, or at least 95% sequence identity to SEQ ID NO: 5 across 80% of SEQ ID NOs: 5, or at least 95% sequence identity to SEQ ID NO: 5 across 90% of SEQ ID NO: 5, or at least 95% sequence identity to SEQ ID NO: 5 across 100% of SEQ ID NO: 5, or at least 98% sequence identity to SEQ ID NO: 5 across 70% of SEQ ID NO: 5, or at least 98% sequence identity to SEQ ID NO: 5 across 80% of SEQ ID NO: 5, or at least 98% sequence identity to SEQ ID NO: 5 across 90% of SEQ ID NO: 5, or at least 98% sequence identity to SEQ ID NO: 5 across 100% of SEQ ID NO: 5. A particularly preferred strain of the invention is the *A. shahii* strain deposited under accession number V21/014433. This is the exemplary MH21-2 strain tested in the DSS mouse model presented in the examples and shown to be effective for treating disease. Therefore, the invention provides a cell, such as an isolated cell, of the *A. shahii* strain deposited under accession number V21/014433, or a derivative thereof. The invention also provides a composition comprising a cell of the *A. shahii* strain deposited under

accession number V21/014433, or a derivative thereof. The invention also provides a biologically pure culture of the *A. shahii* MH21-2 strain deposited under accession number V21/01443.

[0266] In certain embodiments, the bacterial strain of the invention has a chromosome with sequence identity to SEQ ID NO: 6. In preferred embodiments, the bacterial strain of the invention has a chromosome with at least 90% sequence identity (e.g., at least 92%, 94%, 95%, 96%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, or 100% sequence identity) to SEQ ID NO: 6 across at least 60% (e.g., at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of SEQ ID NO: 6. For example, the bacterial strain of the invention may have a chromosome with at least 90% sequence identity to SEQ ID NO: 6 across 70% of SEQ ID NO: 6, or at least 90% sequence identity to SEQ ID NO: 6 across 80% of SEQ ID NO: 6, or at least 90% sequence identity to SEQ ID NO: 6 across 90% of SEQ ID NO: 6, or at least 90% sequence identity to SEQ ID NO: 6 across 100% of SEQ ID NO: 6, or at least 95% sequence identity to SEQ ID NO: 6 across 70% of SEQ ID NO: 6, or at least 95% sequence identity to SEQ ID NO: 6 across 80% of SEQ ID NOs: 6, or at least 95% sequence identity to SEQ ID NO: 6 across 90% of SEQ ID NO: 6, or at least 95% sequence identity to SEQ ID NO: 6 across 100% of SEQ ID NO: 6, or at least 98% sequence identity to SEQ ID NO: 6 across 70% of SEQ ID NO: 6, or at least 98% sequence identity to SEQ ID NO: 6 across 80% of SEQ ID NO: 6, or at least 98% sequence identity to SEQ ID NO: 6 across 90% of SEQ ID NO: 6, or at least 98% sequence identity to SEQ ID NO: 6 across 100% of SEQ ID NO: 6. A particularly preferred strain of the invention is the *A. shahii* strain deposited under accession number V21/014434. This is the exemplary MH21-3 strain tested in the DSS mouse model presented in the examples and shown to be effective for treating disease. Therefore, the invention provides a cell, such as an isolated cell, of the *A. shahii* strain deposited under accession number V21/014434, or a derivative thereof. The invention also provides a composition comprising a cell of the *A. shahii* strain deposited under accession number V21/014434, or a derivative thereof. The invention also provides a biologically pure culture of the *A. shahii* MH21-3 strain deposited under accession number V21/01444.

[0267] In certain embodiments, the bacterial strain of the invention has a chromosome with sequence identity to one or more of SEQ ID NOs: 38-43. In preferred embodiments, the bacterial strain of the invention has a chromosome with at least 90% sequence identity (e.g., at least 92%, 94%, 95%, 96%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, or 100% sequence identity) to one or more of SEQ ID NOs: 38-43 across at least 60% (e.g., at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of one or more of SEQ ID NOs: 38-43. For example, the bacterial strain of the invention may have a chromosome with at least 90% sequence identity to one or more of SEQ ID NOs: 38-43 across 70% of one or more of SEQ ID NOs: 38-43, or at least 90% sequence identity to one or more of SEQ ID NOs: 38-43 across 80% of one or more of SEQ ID NOs: 38-43, or at least 90% sequence identity to one or more of SEQ ID NOs: 38-43 across 90% of one or more of SEQ ID NOs: 38-43, or at least 90% sequence identity to one or more of SEQ ID NOs: 38-43 across 100% of one or more of SEQ ID NOs: 38-43, or at least 95% sequence

identity to one or more of SEQ ID NOs: 38-43 across 70% of one or more of SEQ ID NOs: 38-43, or at least 95% sequence identity to one or more of SEQ ID NOs: 38-43 across 80% of one or more of SEQ ID NOs: 38-43, or at least 95% sequence identity to one or more of SEQ ID NOs: 38-43 across 90% of one or more of SEQ ID NOs: 38-43, or at least 95% sequence identity to one or more of SEQ ID NOs: 38-43 across 100% of one or more of SEQ ID NOs: 38-43, or at least 98% sequence identity to one or more of SEQ ID NOs: 38-43 across 70% of one or more of SEQ ID NOs: 38-43, or at least 98% sequence identity to one or more of SEQ ID NOs: 38-43 across 80% of one or more of SEQ ID NOs: 38-43, or at least 98% sequence identity to one or more of SEQ ID NOs: 38-43 across 90% of one or more of SEQ ID NOs: 38-43, or at least 98% sequence identity to one or more of SEQ ID NOs: 38-43 across 100% of one or more of SEQ ID NOs: 38-43. In preferred embodiments of this type, the bacterial strain has the stated sequence identity across each of SEQ ID Nos: 38-43.

[0268] A derivative of the strains deposited under any one of the accession numbers V21/014432, V21/014433, or V21/014434 may be a daughter strain (progeny) or a strain cultured (subcloned) from the original. A derivative of a strain of the invention may be modified, for example at the genetic level, without ablating the biological activity. In particular, a derivative strain of the invention is therapeutically active. A derivative strain will have comparable activity to the original V21/014432, V21/014433, or V21/014434 strains from which it is derived. In particular, a derivative strain will elicit comparable effects in at least one disease model (e.g., colitis) as shown in the Examples, which may be identified by using the culturing and administration protocols described in the Examples. A derivative of any one of the V21/014432, V21/014433, or V21/014434 strains will generally be a biotype of the respective V21/014432, V21/014433, or V21/014434 strains.

[0269] References to cells of the *A. shahii* strain deposited under accession number V21/014432 include any cells that have the same safety and therapeutic efficacy characteristics as the strains deposited under any one of accession numbers V21/014432, V21/014433, or V21/014434, and such cells are encompassed by the invention.

2.1 Bacteria biotypes

[0270] Bacterial strains that are biotypes of a bacterium deposited under any one of accession numbers V21/014432, V21/014433, or V21/014434 are also expected to be effective for treating or preventing inflammatory and autoimmune disorders. A biotype is a closely related strain that has the same or very similar physiological and biochemical characteristics.

[0271] Strains that are biotypes of a bacterium deposited under any one of the accession numbers V21/014432, V21/014433, or V21/014434 and that are suitable for use in the invention may be identified by sequencing other nucleotide sequences for a bacterium deposited under any one of accession numbers V21/014432, V21/014433, or V21/014434. For example, substantially the whole genome may be sequenced and a biotype strain of the invention may have at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity across at least 80% of its whole genome (e.g., across at least 85%, 90%, 95% or 99%, or across its whole genome). Other suitable sequences for use in identifying biotype

strains may include hsp60 or repetitive sequences such as BOX, ERIC, (GTG)₅, or REP (Masco et al., 2003; Kim et al., 2019). Biotype strains may have sequences with at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of a bacterium deposited under any one of accession numbers V21/014432, V21/014433, or V21/014434.

[0272] Alternatively, strains that are biotypes of a bacterium deposited under any one of the accession numbers V21/014432, V21/014433, or V21/014434, 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 rRNA sequencing. In some preferred embodiments, such techniques may be used to identify other suitable *A. shahii* strains.

[0273] In certain embodiments, strains that are biotypes of a bacterium deposited under any one of accession numbers V21/014432, V21/014433, or V21/014434, and that are suitable for use in the invention are strains that provide the same pattern as a bacterium deposited under any one of accession numbers V21/014432, V21/014433, or V21/014434 when analysed by amplified ribosomal DNA restriction analysis (ARDRA), for example when using Sau3AI restriction enzyme (for exemplary methods and guidance see, for example, Srůtková et al., 2011). Alternatively, biotype strains are identified as strains that have the same carbohydrate fermentation patterns as a bacterium deposited under any one of accession numbers V21/014432, V21/014433, or V21/014434.

[0274] In some embodiments, bacterial strains useful in the invention may be identified by routinely profiling the production and consumption of metabolites by a bacterial strain. It is predicted that the bacterial strains described above and elsewhere herein effect production of acetate, butyrate, ethanol and fumarate. Therefore, in some embodiments, the bacterial strains of the invention induce the production *in vivo* of one or more of the metabolites acetate, ethanol, butyrate, and fumarate. Additionally, in some embodiments the bacterial strains of the invention do not produce butyrate.

[0275] Other *Alistipes* strains that are useful in the compositions and methods of the invention, such as biotypes of a bacterium deposited under accession numbers V21/014432, V21/014433, or V21/014434, 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 TY or PYG media and/or administering the bacteria to the DSS-induced gut barrier function model and then assessing cytokine/chemokine levels, as described in the Examples. In particular, bacterial strains that have similar growth patterns, metabolic type and/or surface antigens to a bacterium deposited under accession number V21/014432, V21/014433, or V21/014434 may be useful in the invention. A useful strain will have comparable immunomodulatory activity to the V21/014432, V21/014433, or V21/014434 strain. In particular, a biotype strain will elicit comparable effects on host gut function. Furthermore, it is expected that a biotype will have a similar effect in a disease model (e.g., colitis, asthma, arthritis, multiple sclerosis and uveitis disease models) and comparable effects on cytokine/chemokine levels to the effects

shown in the Examples, and which may be identified by using the culturing and administration protocols described in the Examples.

[0276] In some embodiments, the bacterial strain may be of the species *A. finegoldii*. The *A. finegoldii* species was first described in Rautio et al., 2003. The *A. shahii* type strain CCUG 406020(T) (= AHN 2437) was isolated from human appendix tissue from children (Rautio et al., 2003). The GenBank accession number for the 16S rRNA gene sequence of the *A. finegoldii* type strain AHN 2437 is NR_043064.

[0277] An exemplary 16S rRNA sequence for an *A. finegoldii* strain that was tested is set forth in SEQ ID NO: 7. Bacterial strains of the species *A. finegoldii* may comprise a single 16S rRNA sequence within its genome, or alternatively, may comprise two or more 16S rRNA sequences within its genome (e.g., two copies, three copies, four copies, five copies, six copies, seven copies, eight copies, or more than eight copies). In some embodiments, a bacterial strain may be identified as being of the *A. finegoldii* strain by determining whether the strain comprises a 16S rRNA sequence that corresponds to SEQ ID NO: 7, by any method known in the art. A chromosome sequence for strain *A. finegoldii* is provided in SEQ ID NO: 9. This sequence was obtained from NCBI Genbank accession no. CP003274, which relates to the *A. finegoldii* strain DSM 17242.

[0278] Bacterial strains of the *A. finegoldii* species and bacterial strains closely related to *A. finegoldii* strains are considered to be effective for treating or preventing inflammatory and autoimmune disorders (e.g., IBD), through their beneficial effects on restoring gut barrier function.

[0279] The *A. onderdonkii* species was first described in Song et al., 2006. The *A. onderdonkii* type strain WAL 8169 (= ATCC BAA-1178) was isolated from human appendix tissue (Song et al., 2006). The GenBank accession number for the 16S rRNA gene sequence of the *A. onderdonkii* type strain WAL 8169 is NR_043318.1.

[0280] The breadth of the *A. shahii onderdonkii* species may be as defined by a Genome Taxonomy Database reference tree, a taxonomic classification system as described in Parks et al., 2018.

[0281] An exemplary 16S rRNA sequence for an *A. onderdonkii* strain that is set forth in SEQ ID NO: 8. Bacterial strains of the species *A. shahii* may comprise a single 16S rRNA sequence within its genome, or alternatively, may comprise two or more 16S rRNA sequences within its genome (e.g., two copies, three copies, four copies, five copies, six copies, seven copies, eight copies, or more than eight copies). In some embodiments, a bacterial strain may be identified as being of the *A. onderdonkii* species by determining whether the strain comprises a 16S rRNA sequence that highly corresponds to SEQ ID NO: 8, by any method known in the art. A chromosome sequence for an *A. onderdonkii* strain is provided in SEQ ID NO: 10. This sequence was obtained from NCBI Genbank accession no. NR_043318.1, which relates to the *A. onderdonkii* strain WAL 8169.

[0282] Bacterial strains of the *A. onderdonkii* species and bacterial strains closely related to *A. onderdonkii* strains are considered to be effective for treating or preventing

inflammatory and autoimmune disorders (e.g., IBD), through their beneficial effects on restoring gut barrier function.

2.2 Bacterial strain viability.

[0283] In preferred embodiments, the bacterial strains in the compositions of the invention are viable. In preferred embodiments, the bacterial strains in the compositions of the invention are viable and capable of partially or totally colonising the intestine. In some preferred embodiments, the bacterial strains in the compositions of the invention are live. By way of an example, the bacterial strains in the compositions of the invention have not been heat-killed. The bacteria of the invention may have immune modulatory effects that would not be exhibited by non-viable bacteria, for example because non-viable bacteria cannot produce metabolites and interact with the immune system in a different manner. The cell surface of a viable bacterium is also likely to be significantly different to a killed bacterium, in particular a heat-killed bacterium.

[0284] In some alternative embodiments, that bacteria are not viable. For example, in some embodiments the bacteria are heat-killed.

[0285] In some preferred embodiments, the bacterial strain for use in the invention is naturally-occurring. For example, the bacterial strain has been isolated from the mammalian digestive tract.

[0286] In some preferred embodiments, the bacterial strain for use in the invention has not been genetically engineered. For example, the bacterial strain has not been transformed with recombinant DNA.

3. ***Compositions – Alistipes species***

[0287] Provided herein are compositions that comprise, consist, or consist essentially of a therapeutically effective amount of a bacterial strain or strains described above and/or elsewhere herein. In some embodiments, the bacteria in the compositions may be identified by strain, species, operational taxonomic unit (OTU), whole genome sequence, 16S rRNA sequence, or other methods known in the art for defining different types of bacteria.

3.1 Most recent common ancestor (MRCA)

[0288] In some embodiments, the compositions comprise an effective amount of a bacterial strain that is a phylogenetic descendant of the MRCA of *A. timonensis* and *A. sp000434235* (see, Figure 3b). Preferably, the phylogenetic classification is as defined by the GTDB (Parks et al., 2018). In some embodiments, the phylogenetic classification is as defined in release 89 (r89) of the GTDB.

[0289] In some embodiments, determining if a bacterial strain is a descendant of a MRCA of *A. timonensis* and *A. sp000434235* may be performed using phylogenetic grouping procedures known in the art. In some embodiments, a rooted phylogenetic tree with *A. timonensis* and *A. sp000434235*, and a third taxon of interest (e.g., a taxon to be classified) may be used, with the following analysis packages being applied: Analyses of

Phylogenetics and Evolution ("ape"; <https://cran.r-project.org/web/packages/ape/index>) and Phylogenetic Tool for Comparative Biology ("phytools"; <http://cran.r-project.org/web/packages/phytools/index.html>), in order to determine whether the taxon of interest is useful for the compositions of the present invention. Both ape and phytools are
 5 packages written in the R language for use in studying molecular evolution and phylogenetics. The ape and phytools packages provide methods for phylogenetic and evolutionary analysis and their use is known to one of skill in the art. In some embodiments, the following script may be used:

```

    library("ape")
  10    library("phytools")

    input.tree = read.tree(file="tree_file")

    alistip c("s__Alistipes sp900083545", "s__Alistipes sp000434235")

    alistip.node = getMRCA(input.tree, alistip)

    alistip.tree = extract.clade(input.tree, alistip.node)
  15    print(alistip.tree$tip.label)
  
```

[0290] In some embodiments, after the script is run, if the taxon of interest is in the printed list, it is a descendant of a MRCA of the two species.

[0291] In other embodiments, different phylogenetic grouping methods known in the art may be used to determine if a bacterial strain is a descendant of a MRCA of
 20 *A. timonensis* and *A. sp000434235* (see, Figure 3B), including methods that use different analysis packages and are based on different programming languages.

[0292] In some other embodiments, the bacterial strain is a phylogenetic descendant of the MRCA of *A. timonensis* and *A. sp000434235*, together with a pharmaceutically acceptable carrier, diluent, or excipient. Suitably, the MRCA is defined at
 25 node 35260 of the bac120 phylogenetic tree from the GTDB. In some embodiments, the phylogenetic tree is created by release 89 of the GTDB, however, any suitable subsequent release is considered to give equally as applicable results.

[0293] In some other embodiments, the bacterial strain is a phylogenetic descendant of the MRCA of *A. timonensis* and *A. fingoldii*, together with a pharmaceutically
 30 acceptable carrier, diluent, or excipient. Suitably, the MRCA is defined at node 35261 of the bac120 phylogenetic tree from the GTDB. In some embodiments, the phylogenetic tree is created by release 89 of the GTDB, however, any suitable subsequent release is considered to give equally as applicable results.

[0294] In some other embodiments, the bacterial strain is a phylogenetic
 35 descendant of the MRCA of *A. timonensis* and *A. shahii*, together with a pharmaceutically acceptable carrier, diluent, or excipient. Suitably, the MRCA is defined at node 35261 of the bac120 phylogenetic tree from the GTDB. In some embodiments, the phylogenetic tree is created by release 89 of the GTDB, however, any suitable subsequent release is considered to give equally as applicable results.

[0295] In some preferred embodiments, the bacterial strain is a phylogenetic descendant of the MRCA of *A. shahii* and *A. fingoldii*, together with a pharmaceutically acceptable carrier, diluent, or excipient. Suitably, the MRCA is defined at node of the bac120 phylogenetic tree from the GTDB. In some embodiments, the phylogenetic tree is created by release 89 of the GTDB, however, any suitable subsequent release is considered to give equally as applicable results.

[0296] In some embodiments, the compositions disclosed herein are substantially free of any bacteria of the species *A. senegalensis*. In some embodiments, the bacterial strain is not of the species *A. senegalensis*.

10 3.2 16S rRNA sequence identity.

[0297] In some embodiments, the 16S rRNA sequence is obtained or determined for a bacterial species to be classified. This query 16S rRNA sequence is compared to 16S rRNA sequences from bacterial species already classified as members of the *Alistipes* genus. In some embodiments, the query 16S rRNA sequence is compared to the 16S rRNA sequences set forth in SEQ ID NO: 1. In some embodiments, the query 16S rRNA sequence is compared to all known 16S rRNA sequences for bacterial species already classified as members of the *Alistipes* genus. In other embodiments, the query 16S rRNA sequence is compared to a subset of all known 16S rRNA sequences for bacterial species already classified as members of the *Alistipes* genus. A percent identity between the query sequence and the compared sequences is determined. If the percent identity of the query sequence is determined to be above a defined threshold, then the bacterial species to be classified is classified as member of the *Alistipes* genus.

[0298] In some embodiments, the threshold sequence identity is 95%. In some other embodiments, the threshold sequence identity is 97.5%. In some other embodiments, the threshold sequence identity is 99.0%. In some embodiments, the threshold sequence identity is 94.5%, 94.6%, 94.7%, 94.8%, 94.9%, 95.0%, 95.1%, 95.2%, 95.3%, 95.4%, 95.5%, 95.6%, 95.7%, 95.8%, 95.9%, 96.0%, 96.1%, 96.2%, 96.3%, 96.4%, 96.5%, 96.6%, 96.7%, 96.8%, 96.9%, 97.0%, 97.1%, 97.2%, 97.3%, 97.4%, 97.5%, 97.6%, 97.7%, 97.8%, 97.9%, 98.0%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99.0%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100%.

[0299] In some embodiments, the 16S rRNA sequence is obtained or determined for a bacterial species to be classified. This query 16S rRNA sequence is compared to 16S rRNA sequences from bacterial species already classified as members of the family *Rikenellaceae* (including those set forth in any one of SEQ ID NO: 1-3). In some embodiments, the query 16S rRNA sequence is compared to all known 16S rRNA sequences for bacterial species already classified as members of the family *Rikenellaceae*. In other embodiments, the query 16S rRNA sequence is compared to a subset of all known 16S rRNA sequences for bacterial species already classified as members of the family *Rikenellaceae*. A percent identity between the query sequence and the compared sequences is determined. If

the percent identity of the query sequence is determined to be above a defined threshold, then the bacterial species to be classified is classified as member of the family.

[0300] In some embodiments, the threshold sequence identity is 95%. In some embodiments, the threshold sequence identity is 98.7%. In some embodiments, the threshold sequence identity is 94.8%. In some embodiments, the threshold sequence identity is 94.5%, 94.6%, 94.7%, 94.8%, 94.9%, 95.0%, 95.1%, 95.2%, 95.3%, 95.4%, 95.5%, 95.6%, 95.7%, 95.8%, 95.9%, 96.0%, 96.1%, 96.2%, 96.3%, 96.4%, 96.5%, 96.6%, 96.7%, 96.8%, 96.9%, 97.0%, 97.1%, 97.2%, 97.3%, 97.4%, 97.5%, 97.6%, 97.7%, 97.8%, 97.9%, 98.0%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99.0%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100%.

[0301] In some embodiments, the compositions comprise an at least partially isolated bacterial strain of *A. shahii* describe above and/or elsewhere herein.

[0302] In certain embodiments, the bacterial strains of the invention have a 16S rRNA sequence that is at least 97.5%, 98%, 98.5%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to the 16S rRNA sequence of a bacterial strain of *A. shahii*. Preferably, the bacterial strain of the invention has a 16S rRNA sequence that is at least 97.5%, 98%, 98.5%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to any one of SEQ ID NOs: 1-3. In some preferred, the bacterial strain of the invention has a 16S rRNA sequence represented by any one of SEQ ID NOs: 1-3.

[0303] The genome of the bacterial strain may comprise the 16S rRNA sequence set forth in any one of SEQ ID NOs: 1-3.

3.3 Genomic sequence identity.

[0304] In certain embodiments, the bacterial strain of the invention has a chromosome with sequence identity to any one of SEQ ID NOs: 4-6. In preferred embodiments, the bacterial strain of the invention has a chromosome with at least 90% sequence identity (e.g., at least 92%, 94%, 95%, 96%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, or 100% sequence identity) to any one of SEQ ID NOs: 4-6, across at least 60% (e.g., at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of SEQ ID NOs: 4-6. For example, the bacterial strain of the invention may have a chromosome with at least 90% sequence identity to any one of SEQ ID NOs: 4-6 across 70% of SEQ ID NOs: 4-6, or at least 90% sequence identity to any one of SEQ ID NOs: 4-6 across 80% of SEQ ID NOs: 4-6, or at least 90% sequence identity to any one of SEQ ID NOs: 4-6 across 90% of SEQ ID NOs: 4-6, or at least 90% sequence identity to any one of SEQ ID NOs: 4-6 across 100% of SEQ ID NOs: 4-6, or at least 95% sequence identity to any one of SEQ ID NOs: 4-6 across 70% of SEQ ID NOs: 4-6, or at least 95% sequence identity to any one of SEQ ID NOs: 4-6 across 80% of SEQ ID NOs: 4-6, or at least 95% sequence identity to any one of SEQ ID NOs: 4-6 across 90% of SEQ ID NOs: 4-6, or at least 95% sequence identity to any one of SEQ ID NOs: 4-6 across 100% of SEQ ID NOs: 4-6, or at least 98% sequence identity to any one of SEQ ID NOs: 4-6 across 70% of SEQ ID NOs: 4-6, or at least 98% sequence identity

to any one of SEQ ID NOs: 4-6 across 80% of SEQ ID NOs: 4-6, or at least 98% sequence identity to any one of SEQ ID NOs: 4-6 across 90% of SEQ ID NOs: 4-6, or at least 98% sequence identity to any one of SEQ ID NOs: 4-6 across 100% of SEQ ID NOs: 4-6. A particularly preferred strain of the invention is the *A. shahii* strain deposited under accession number V21/014432. This is the exemplary *A. shahii* MH21-1 strain tested in the DSS mouse model presented in the examples and shown to be effective for treating disease. Therefore, the invention provides a cell, such as an isolated cell, of the *A. shahii* strain deposited under accession number V21/014432, or a derivative thereof. The invention also provides a composition comprising a cell of the *A. shahii* strain deposited under accession number V21/014432, or a derivative thereof. The invention also provides a biologically pure culture of the *A. shahii* MH21-1 strain deposited under accession number V21/014432.

[0305] The invention also provides a composition comprising a cell of the *A. shahii* strain deposited under accession number V21/014433, or a derivative thereof. The invention also provides a biologically pure culture of the *A. shahii* MH21-1 strain deposited under accession number V21/014433.

[0306] The invention also provides a composition comprising a cell of the *A. shahii* strain deposited under accession number V21/014434, or a derivative thereof. The invention also provides a biologically pure culture of the *A. shahii* MH21-1 strain deposited under accession number V21/014434.

[0307] In certain embodiments, the compositions of the invention comprise an at least partially purified strain of *A. finegoldii*. In some embodiments, the strain of *A. finegoldii* has a chromosome with sequence identity to SEQ ID NO: 9. In preferred embodiments, the bacterial strain of *A. finegoldii* has a chromosome with at least 90% sequence identity (e.g., at least 92%, 94%, 95%, 96%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, or 100% sequence identity) to SEQ ID NO: 9 across at least 60% (e.g., at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of SEQ ID NO: 9. For example, the bacterial strain of *A. finegoldii* may have a chromosome with at least 90% sequence identity to SEQ ID NO: 9 across 70% of SEQ ID NO: 9, or at least 90% sequence identity to SEQ ID NO: 9 across 80% of SEQ ID NO: 9, or at least 90% sequence identity to SEQ ID NO: 9 across 90% of SEQ ID NO: 9, or at least 90% sequence identity to SEQ ID NO: 9 across 100% of SEQ ID NO: 9, or at least 95% sequence identity to SEQ ID NO: 9 across 70% of SEQ ID NO: 9, or at least 95% sequence identity to SEQ ID NO: 9 across 80% of SEQ ID NOs: 9, or at least 95% sequence identity to SEQ ID NO: 9 across 90% of SEQ ID NO: 9, or at least 95% sequence identity to SEQ ID NO: 9 across 100% of SEQ ID NO: 9, or at least 98% sequence identity to SEQ ID NO: 9 across 70% of SEQ ID NO: 9, or at least 98% sequence identity to SEQ ID NO: 9 across 80% of SEQ ID NO: 9, or at least 98% sequence identity to SEQ ID NO: 9 across 90% of SEQ ID NO: 9, or at least 98% sequence identity to SEQ ID NO: 9 across 100% of SEQ ID NO: 9.

[0308] In certain embodiments, the compositions of the invention comprise an at least partially purified strain of *A. onderdonkii*. In some embodiments, the strain of *A. onderdonkii* has a chromosome with sequence identity to SEQ ID NO: 10. In preferred

embodiments, the bacterial strain of *A. onderdonkii* has a chromosome with at least 90% sequence identity (e.g., at least 92%, 94%, 95%, 96%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, or 100% sequence identity) to SEQ ID NO: 10 across at least 60% (e.g., at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of SEQ ID NO: 10. For example, the bacterial strain of *A. onderdonkii* may have a chromosome with at least 90% sequence identity to SEQ ID NO: 10 across 70% of SEQ ID NO: 10, or at least 90% sequence identity to SEQ ID NO: 10 across 80% of SEQ ID NO: 10, or at least 90% sequence identity to SEQ ID NO: 10 across 90% of SEQ ID NO: 10, or at least 90% sequence identity to SEQ ID NO: 10 across 100% of SEQ ID NO: 10, or at least 95% sequence identity to SEQ ID NO: 10 across 70% of SEQ ID NO: 10, or at least 95% sequence identity to SEQ ID NO: 10 across 80% of SEQ ID NO: 10, or at least 95% sequence identity to SEQ ID NO: 10 across 90% of SEQ ID NO: 10, or at least 95% sequence identity to SEQ ID NO: 10 across 100% of SEQ ID NO: 10, or at least 98% sequence identity to SEQ ID NO: 10 across 70% of SEQ ID NO: 10, or at least 98% sequence identity to SEQ ID NO: 10 across 80% of SEQ ID NO: 10, or at least 98% sequence identity to SEQ ID NO: 10 across 90% of SEQ ID NO: 10, or at least 98% sequence identity to SEQ ID NO: 10 across 100% of SEQ ID NO: 10.

4. Bacterial strains – *Colonithrix sana*

[0309] The compositions of the invention comprise a bacterial strain of the genus *Colonithrix*. The examples demonstrate that bacteria of this genus are useful for treating or preventing diseases associated with an impaired gut barrier function. The preferred bacterial strains are of the specie *Colonithrix sana*.

[0310] *Colonithrix* is a genus of bacteria in the class Clostridia. The scientific classification is as follows: bacteria (kingdom); *Firmicutes_A* (phylum); *Clostridia* (class); *Oscillospirales* (order); *Oscillospiraceae* (family); *Colonithrix* (genus). Bacteria within the *Colonithrix* genus are Gram-negative to Gram-variable, non-motile bacteria with a long, thin rod-like shape, and are obligate anaerobes. These criteria are important because they can inform the phylogenetic classification of bacterial strains. For instance, the bacterial species *C. sana* has previously been classified as belonging to the genus *ER* and/or *Ruminococcaceae*, based on these criteria in particular.

[0311] The breadth of the *Colonithrix* genus and *C. sana* species are as defined by a Genome Taxonomy Database reference tree, a taxonomic classification system as described in Parks et al., 2018.

[0312] The *C. sana* bacterium deposited under accession numbers V21/019213 (i.e., *C. sana* MH35-1) was tested in the Examples and is one of the preferred strains of the invention. *C. sana* strain MH35-1 and MH35-2 were deposited with the international depositary authority National Measurement Institute (NMI, 1/153 Bertie Street, Port Melbourne, Victoria, 3207, Australia) by Microba IP Pty Ltd (388 Queen Street, Brisbane, QLD 4000, Australia) on 28 September 2021 as "*Colonithrix sana* MH35-1" and "*Colonithrix sana* MH35-2" and was assigned accession numbers V21/019213 and V21/019214, respectively.

[0313] Exemplary 16S rRNA sequences for the *C. sana* MH35-1 strain that was tested is set forth in any one of SEQ ID NOs: 11-14. Bacterial strains of the species may comprise a single 16S rRNA sequence within its genome, or alternatively, may comprise two or more 16S rRNA sequences within its genome (e.g., two copies, three copies, four copies, 5 five copies, six copies, seven copies, eight copies, or more than eight copies). In some preferred embodiments, the bacterial strain of the species *C. sana* comprises four copies of a 16S rRNA sequence within its genome. In some embodiments, a bacterial strain may be identified as being of the *C. sana* MH35-1 strain by determining whether the strain comprises a 16S rRNA sequence that corresponds to any one of SEQ ID NOs: 11-14, by any method 10 known in the art. A chromosome sequence for strain *C. sana* MH35-1 is provided in one or more of SEQ ID NOs: 19-22. In some embodiments, a chromosome sequence for strain *C. sana* MH35-1 comprises each of the sequences set forth in SEQ ID NOs: 19-22. These sequences were generated using the Illumina NovSeq6000 platform.

[0314] Exemplary 16S rRNA sequences for the *C. sana* MH35-2 strain that was 15 tested is set forth in any one of SEQ ID NOs: 15-18. Bacterial strains of the species may comprise a single 16S rRNA sequence within its genome, or alternatively, may comprise two or more 16S rRNA sequences within its genome (e.g., two copies, three copies, four copies, five copies, six copies, seven copies, eight copies, or more than eight copies). In some preferred embodiments, the bacterial strain of the species *C. sana* comprises four copies of a 20 16S rRNA sequence within its genome. In some embodiments, a bacterial strain may be identified as being of the *C. sana* MH35-2 strain by determining whether the strain comprises a 16S rRNA sequence that corresponds to any one of SEQ ID NOs: 15-18, by any method known in the art. A chromosome sequence for strain *C. sana* MH35-1 is provided in one or more of SEQ ID NOs: 23-26. In some embodiments, a chromosome sequence for strain 25 *sana* MH35-2 comprises each of the sequences set forth in SEQ ID NOs: 23-26. These sequences were generated using the Illumina NovSeq6000 platform.

[0315] Bacterial strains closely related to the *C. sana* strains MH35-1 and MH35-2 are also considered to be effective for treating or preventing inflammatory and *autoimmune* disorders, through their beneficial effects on restoring gut barrier function.

[0316] In certain embodiments, the bacterial strains of the invention have a 16S 30 rRNA sequence that is at least 97.5%, 98%, 98.5%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to the 16S rRNA sequence of a bacterial strain of *C. sana*. Preferably, the bacterial strain of the invention has a 16S rRNA sequence that is at least 97.5%, 98%, 98.5%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to any one of SEQ ID NOs: 35 11-18. In some preferred embodiments, the bacterial strain of the invention has a 16S rRNA sequence represented by any one of SEQ ID NOs: 11-18. In some of the same preferred embodiments, the bacterial strain of the invention has four copies of a 16S rRNA sequence represented by at least one, two, three, or all of SEQ ID NOs: 11-14. In some other preferred embodiments, the bacterial strain of the invention has four copies of a 16S rRNA 40 sequence represented by at least one, two, three, or all of SEQ ID NOs: 15-18.

[0317] The genome of the bacterial strain may comprise each of the 16S rRNA sequences set forth in SEQ ID NO: 11-14. Alternatively, the genome of the bacterial strain may comprise each of the 16S rRNA sequences set forth in SEQ ID NO: 15-18.

[0318] In certain embodiments, the bacterial strain of the invention has a chromosome with sequence identity to any one of SEQ ID NOs: 19-22 or 23-26. In preferred embodiments, the bacterial strain of the invention has a chromosome with at least 90% sequence identity (e.g., at least 92%, 94%, 95%, 96%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, or 100% sequence identity) to any one of SEQ ID NOs: 19-22 or 23-26 across at least 60% (e.g., at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of any one of SEQ ID NOs: 19-22 or 23-26. For example, the bacterial strain of the invention may have a chromosome with at least 90% sequence identity to any one of SEQ ID NOs: 19-22 or 23-26 across 70% of any one of SEQ ID NOs: 19-22 or 23-26, or at least 90% sequence identity to any one of SEQ ID NOs: 19-22 or 23-26 across 80% of any one of SEQ ID NOs: 19-22 or 23-26, or at least 90% sequence identity to any one of SEQ ID NOs: 19-22 or 23-26 across 90% of any one of SEQ ID NOs: 19-22 or 23-26, or at least 90% sequence identity to any one of SEQ ID NOs: 19-22 or 23-26 across 100% of any one of SEQ ID NOs: 19-22 or 23-26, or at least 95% sequence identity to any one of SEQ ID NOs: 19-22 or 23-26 across 70% of any one of SEQ ID NOs: 19-22 or 23-26, or at least 95% sequence identity to any one of SEQ ID NOs: 19-22 or 23-26 across 80% of any one of SEQ ID NOs: 19-22 or 23-26, or at least 95% sequence identity to any one of SEQ ID NOs: 15-18 across 90% of any one of SEQ ID NOs: 19-22 or 23-26, or at least 95% sequence identity to any one of SEQ ID NOs: 19-22 or 23-26 across 100% of any one of SEQ ID NOs: 19-22 or 23-26, or at least 98% sequence identity to any one of SEQ ID NOs: 19-22 or 23-26 across 70% of any one of SEQ ID NOs: 19-22 or 23-26, or at least 98% sequence identity to any one of SEQ ID NOs: 19-22 or 23-26 across 80% of any one of SEQ ID NOs: 19-22 or 23-26, or at least 98% sequence identity to any one of SEQ ID NOs: 19-22 or 23-26 across 90% of any one of SEQ ID NOs: 19-22 or 23-26, or at least 98% sequence identity to any one of SEQ ID NOs: 19-22 or 23-26 across 100% of any one of SEQ ID NOs: 19-22 or 23-26. A particularly preferred strain of the invention is the *C. sana* strain deposited under accession numbers V21/019213 and/or V21/019214. This is the exemplary *C. sana* MH35-1 and *C. sana* MH35-2 strains tested in the DSS mouse model presented in the examples and shown to be effective for treating disease. Therefore, the invention provides a cell, such as an isolated cell, of the *C. sana* strains deposited under accession numbers V21/019213 and/or V21/019214, or a derivative thereof. The invention also provides a composition comprising a cell of the *C. sana* strains deposited under accession numbers V21/019213 and/or V21/019214, or a derivative thereof. The invention also provides a biologically pure culture of the *C. sana* MH35-1 and MH35-2 strains deposited under accession numbers V21/019213 and V21/019214, respectively.

[0319] A derivative of the strains deposited under the accession number V21/019213 or V21/019214, may be daughter strains (progeny) or strains cultured (subcloned) from the original. A derivative of a strain of the invention may be modified, for example at the genetic level, without ablating the biological activity. In particular, a

derivative strain of the invention is therapeutically active. A derivative strain will have comparable activity to the original V21/019213 or V21/019214 strains from which it is derived. In particular, a derivative strain will elicit comparable effects in at least one disease model (e.g., colitis) as shown in the Examples, which may be identified by using the culturing and administration protocols described in the Examples. A derivative of any one of the V21/019213 or V21/019214 strains will generally be a biotype of the respective V21/019213 and V21/019214 strains.

[0320] References to cells of the *C. sana* strain deposited under accession numbers V21/019213 or V21/019214 include any cells that have the same safety and therapeutic efficacy characteristics as the strains deposited under accession numbers V21/019213 or V21/019214, and such cells are encompassed by the invention.

4.1 Bacteria biotypes

[0321] Bacterial strains that are biotypes of a bacterium deposited under accession numbers V21/019213 or V21/019214 are also expected to be effective for treating or preventing inflammatory and autoimmune disorders. A biotype is a closely related strain that has the same or very similar physiological and biochemical characteristics.

[0322] Strains that are biotypes of a bacterium deposited under the accession numbers V21/019213 or V21/019214 and that are suitable for use in the invention may be identified by sequencing other nucleotide sequences for a bacterium deposited under accession numbers V21/019213 or V21/019214. For example, substantially the whole genome may be sequenced and a biotype strain of the invention may have at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity across at least 80% of its whole genome (e.g., across at least 85%, 90%, 95% or 99%, or across its whole genome). Other suitable sequences for use in identifying biotype strains may include hsp60 or repetitive sequences such as BOX, ERIC, (GTG)₅, or REP (Masco et al., 2003; Kim et al., 2019). Biotype strains may have sequences with at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of a bacterium deposited under accession numbers V21/019213 or V21/019214.

[0323] Alternatively, strains that are biotypes of a bacterium deposited under the accession numbers V21/019213 or V21/019214, 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 rRNA sequencing. In some preferred embodiments, such techniques may be used to identify other suitable *C. sana* strains.

[0324] In certain embodiments, strains that are biotypes of a bacterium deposited under accession numbers V21/019213 or V21/019214, and that are suitable for use in the invention are strains that provide the same pattern as a bacterium deposited under accession numbers V21/019213 or V21/019214 when analysed by amplified ribosomal DNA restriction analysis (ARDRA), for example when using Sau3AI restriction enzyme (for exemplary methods and guidance see, for example, Srůtková et al., 2011). Alternatively,

biotype strains are identified as strains that have the same carbohydrate fermentation patterns as a bacterium deposited under accession numbers V21/019213 or V21/019214.

[0325] In some embodiments, bacterial strains useful in the invention may be identified by routinely profiling the production and consumption of metabolites by a bacterial strain. It is predicted that the bacterial strains described above and elsewhere herein effect production of acetate, butyrate, ethanol and fumarate. Therefore, in some embodiments, the bacterial strains of the invention induce the production *in vivo* of one or more of the metabolites acetate, ethanol, butyrate, and fumarate. Additionally, in some embodiments the bacterial strains of the invention do not produce butyrate.

[0326] Other *Colonithrix* strains that are useful in the compositions and methods of the invention, such as biotypes of a bacterium deposited under accession numbers V21/019213 and/or V21/019214, 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 TY or PYG media and/or administering the bacteria to the DSS-induced gut barrier function model and then assessing cytokine/chemokine levels, as described in the Examples. In particular, bacterial strains that have similar growth patterns, metabolic type and/or surface antigens to a bacterium deposited under accession numbers V21/019213 and/or V21/019214 may be useful in the invention. A useful strain will have comparable immunomodulatory activity to the V21/019213 and/or V21/019214 strains. In particular, a biotype strain will elicit comparable effects on host gut function. Furthermore, it is expected that a biotype will have a similar effect in a disease model (e.g., colitis, asthma, arthritis, multiple sclerosis and uveitis disease models) and comparable effects on cytokine/chemokine levels to the effects shown in the Examples, and which may be identified by using the culturing and administration protocols described in the Examples.

4.2 Bacterial strain viability.

[0327] In preferred embodiments, the bacterial strains in the compositions of the invention are viable. In preferred embodiments, the bacterial strains in the compositions of the invention are viable and capable of partially or totally colonising the intestine. In some preferred embodiments, the bacterial strains in the compositions of the invention are live. By way of an example, the bacterial strains in the compositions of the invention have not been heat-killed. The bacteria of the invention may have immune modulatory effects that would not be exhibited by non-viable bacteria, for example because non-viable bacteria cannot produce metabolites and interact with the immune system in a different manner. The cell surface of a viable bacterium is also likely to be significantly different to a killed bacterium, in particular a heat-killed bacterium.

[0328] In some alternative embodiments, that bacteria are not viable. For example, in some embodiments the bacteria are heat-killed.

[0329] In some preferred embodiments, the bacterial strain for use in the invention is naturally-occurring. For example, the bacterial strain has been isolated from the mammalian digestive tract.

[0330] In some preferred embodiments, the bacterial strain for use in the invention has not been genetically engineered. For example, the bacterial strain has not been transformed with recombinant DNA.

5. Compositions – *Colonithrix* species

[0331] Provided herein are compositions that comprise, consist, or consist essentially of a therapeutically effective amount of a bacterial strain or strains described above and/or elsewhere herein. In some embodiments, the bacteria in the compositions may be identified by strain, species, operational taxonomic unit (OTU), whole genome sequence, 16S rRNA sequence, or other methods known in the art for defining different types of bacteria.

5.1 Most recent common ancestor (MRCA)

[0332] In some embodiments, the compositions comprise an effective amount of a bacterial strain that is a phylogenetic descendant of the MRCA of *C. sp003522105* and *C. sp002437735* (see, Figure 8). Preferably, the phylogenetic classification is as defined by the GTDB (Parks et al., 2018). In some embodiments, the phylogenetic classification is as defined in release 89 (r89) of the GTDB.

[0333] In some embodiments, determining if a bacterial strain is a descendant of a MRCA of *C. sp003522105* and *C. sp002437735* may be performed using phylogenetic grouping procedures known in the art. In some embodiments, a rooted phylogenetic tree with *C. sp003522105*, *C. sp002437735*, and a third taxon of interest (e.g., a taxon to be classified) may be used, with the following the analysis packages being applied: Analyses of Phylogenetics and Evolution (ape: <https://cran.r-project.org/web/packages/ape/index.html>) and Phylogenetic Tool for Comparative Biology ("phytools"; <http://cran.r-project.org/web/packages/phytools/index.html>), in order to determine whether the taxon of interest is useful for the compositions of the present invention. Both ape and phytools are packages written in the R language for use in studying molecular evolution and phylogenetics. The ape and phytools packages provide methods for phylogenetic and evolutionary analysis and their use is known to one of skill in the art. In some embodiments, the following script may be used:

```
library("ape")

library("phytools")

input.tree = read.tree(file="tree_file")

colonithrix = c("s__C. sp003522105", "s__C. sp002437735"))

colonithrix.node = getMRCA(input.tree, colonithrix)

colonithrix.tree = extract.clade(input.tree, colonithrix.node)

print(colonithrix.tree$tip.label)
```

[0334] In some embodiments, after the script is run, if the taxon of interest is in the printed list, it is a descendant of a MRCA of the two species.

[0335] In other embodiments, different phylogenetic grouping methods known in the art may be used to determine if a bacterial strain is a descendant of a MRCA of *C. sp003522105* and *C. sp002437735* (see, Figure 8), including methods that use different analysis packages and are based on different programming languages.

[0336] In some other embodiments, the bacterial strain is a phylogenetic descendant of the MRCA of *C. sp003522105* and *C. sp002437735*, together with a pharmaceutically acceptable carrier, diluent, or excipient. Suitably, the MRCA is defined at node 23879 of the bac120 phylogenetic tree from the GTDB. In some embodiments, the phylogenetic tree is created by release 89 of the GTDB, however, any suitable subsequent release is considered to give equally as applicable results.

[0337] In some other embodiments, the bacterial strain is a phylogenetic descendant of the MRCA of *C. sp003522105* and *C. sp002437735*, together with a pharmaceutically acceptable carrier, diluent, or excipient. Suitably, the MRCA is defined at node 23820 of the bac120 phylogenetic tree from the GTDB. In some embodiments, the phylogenetic tree is created by release 89 of the GTDB, however, any suitable subsequent release is considered to give equally as applicable results.

[0338] In other embodiments, a bacterial species is a member of the family *Oscillospiraceae* if the species has a 16S rRNA sequence with sequence identity to 16S rRNA sequences from species already identified as a member of the family *Oscillospiraceae*. In some embodiments, identification of whether a bacterial species is a member of the family *Oscillospiraceae* is performed using the methods described in Yarza et al., 2014, *Nature Reviews Microbiology* 12:635-645, and Stackebrandt, E. & Ebers, J., 2006, *Microbiol. Today* 8:6-9, which are hereby incorporated by reference herein.

5.2 16S rRNA Sequence Identity

[0339] In some embodiments, the 16S rRNA sequence is obtained or determined for a bacterial species to be classified. This query 16S rRNA sequence is compared to 16S rRNA sequences from bacterial species already classified as members of the *Colonithrix* genus. In some embodiments, the query 16S rRNA sequence is compared to the 16S rRNA sequences set forth in any one of SEQ ID NOs: 11-18. In some embodiments, the query 16S rRNA sequence is compared to all known 16S rRNA sequences for bacterial species already classified as members of the *Colonithrix* genus. In other embodiments, the query 16S rRNA sequence is compared to a subset of all known 16S rRNA sequences for bacterial species already classified as members of the *Colonithrix* genus. A percent identity between the query sequence and the compared sequences is determined. If the percent identity of the query sequence is determined to be above a defined threshold, then the bacterial species to be classified is classified as member of the *Colonithrix* genus.

[0340] In some embodiments, the threshold sequence identity is 95%. In some other embodiments, the threshold sequence identity is 97.5%. In some other embodiments,

the threshold sequence identity is 99.0%. In some embodiments, the threshold sequence identity is 94.5%, 94.6%, 94.7%, 94.8%, 94.9%, 95.0%, 95.1%, 95.2%, 95.3%, 95.4%, 95.5%, 95.6%, 95.7%, 95.8%, 95.9%, 96.0%, 96.1%, 96.2%, 96.3%, 96.4%, 96.5%, 96.6%, 96.7%, 96.8%, 96.9%, 97.0%, 97.1%, 97.2%, 97.3%, 97.4%, 97.5%, 97.6%, 97.7%, 97.8%, 97.9%, 98.0%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99.0%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100%.

[0341] In some embodiments, the 16S rRNA sequence is obtained or determined for a bacterial species to be classified. This query 16S rRNA sequence is compared to 16S rRNA sequences from bacterial species already classified as members of the family *Oscillospiraceae*. In some embodiments, the query 16S rRNA sequence is compared to all known 16S rRNA sequences for bacterial species already classified as members of the family *Oscillospiraceae*. In other embodiments, the query 16S rRNA sequence is compared to a subset of all known 16S rRNA sequences for bacterial species already classified as members of the family *Oscillospiraceae*. A percent identity between the query sequence and the compared sequences is determined. If the percent identity of the query sequence is determined to be above a defined threshold, then the bacterial species to be classified is classified as member of the family *Oscillospiraceae*.

[0342] In some embodiments, the threshold sequence identity is 95%. In some embodiments, the threshold sequence identity is 98.7%. In some embodiments, the threshold sequence identity is 94.8%. In some embodiments, the threshold sequence identity is 94.5%, 94.6%, 94.7%, 94.8%, 94.9%, 95.0%, 95.1%, 95.2%, 95.3%, 95.4%, 95.5%, 95.6%, 95.7%, 95.8%, 95.9%, 96.0%, 96.1%, 96.2%, 96.3%, 96.4%, 96.5%, 96.6%, 96.7%, 96.8%, 96.9%, 97.0%, 97.1%, 97.2%, 97.3%, 97.4%, 97.5%, 97.6%, 97.7%, 97.8%, 97.9%, 98.0%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99.0%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100%.

6. Bacterial strains – *Gemmiger* species

[0343] The compositions of the invention comprise a bacterial strain of the genus *Gemmiger*. The examples demonstrate that bacteria of this genus are useful for treating or preventing diseases associated with an impaired gut barrier function. Some of the preferred bacterial strains are of the species *G. formicilis*.

[0344] *Gemmiger* is a genus of bacteria in the class Clostridia. The scientific classification is as follows: bacteria (kingdom); *Firmicutes* (phylum); *Clostridia* (class); *Clostridiales* (order); *Ruminococcaceae* (family); *Gemmiger* (genus). Bacteria within the *Gemmiger* genus are Gram-negative to Gram-variable, non-motile bacteria with a “budding” shape, and are obligate anaerobes. These criteria are important because they can inform the phylogenetic classification of bacterial strains. For instance, the bacterial species *G. formicilis* has previously been classified as belonging to the genus *Subdoligranulum*, based on these criteria in particular.

[0345] *G. formicilis* strains (previously characterized as *Subdoligranulum formicilis*) are described in Gossling and Moore, 1975. The type strain *G. formicilis* Virginia Polytechnic Institute strain X2-56 (= ATCC 27749) was isolated from human feces (Gossling and Moore, 1975). The GenBank accession number for the 16S rRNA gene sequence of the
5 *G. formicilis* type strain ATCC 27749 is NR_104846.

[0346] The breadth of the *Gemmiger* genus and *G. formicilis* species are as defined by a Genome Taxonomy Database reference tree, a taxonomic classification system as described in Parks et al., 2018.

[0347] The *G. formicilis* bacterium deposited under accession number
10 V21/011520 (i.e., *G. formicilis* MH32-1) was tested in the Examples and is one of the preferred strains of the invention. *G. formicilis* strain MH32-1 was deposited with the international depositary authority National Measurement Institute (NMI, 1/153 Bertie Street, Port Melbourne, Victoria, 3207, Australia) by Microba IP Pty Ltd (388 Queen Street, Brisbane, QLD 4000, Australia) on 11th June 2021 as "*Gemmiger formicilis* MH32-1" and was assigned
15 accession number V21/011520.

[0348] An exemplary 16S rRNA sequence for the MH321 strain that was tested is set forth in SEQ ID NO: 27. Bacterial strains of the species *G. formicilis* may comprise a single 16S rRNA sequence within its genome, or alternatively, may comprise two or more 16S rRNA sequences within its genome (e.g., two copies, three copies, four copies, five
20 copies, six copies, seven copies, eight copies, or more than eight copies). In some embodiments, a bacterial strain may be identified as being of the *G. formicilis* MH32-1 strain by determining whether the strain comprises a 16S rRNA sequence that corresponds to SEQ ID NO: 27, by any method known in the art. A chromosome sequence for strain MH32-1 is provided in SEQ ID NO: 28. This sequence was generated using the Illumina NovSeq6000
25 platform.

[0349] Bacterial strains closely related to the strains *G. formicilis* MH32-1 and/or *G. formicilis* MH32-2 are also considered to be effective for treating or preventing inflammatory and autoimmune disorders, through their beneficial effects on restoring gut barrier function.

[0350] In certain embodiments, the bacterial strains of the invention have a 16S rRNA sequence that is at least 97.5%, 98%, 98.5%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to the 16S rRNA sequence of a bacterial strain of *G. formicilis*. Preferably, the bacterial strain of the invention has a 16S rRNA sequence that is at least 97.5%, 98%, 98.5%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to SEQ ID NO: 27 or one or
30 more of SEQ ID NOs: 45-47. In some preferred, the bacterial strain of the invention has a 16S rRNA sequence represented by SEQ ID NO: 27 or one or more of SEQ ID NOs: 45-47.
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[0351] The genome of the bacterial strain may comprise the 16S rRNA sequence set forth in SEQ ID NO: 27. Alternatively, the genome of the bacterial strain may comprise the 16S rRNA sequence set forth in one or more of SEQ ID NO: 45-47.

[0352] In certain embodiments, the bacterial strain of the invention has a chromosome with sequence identity to SEQ ID NO: 28. In preferred embodiments, the bacterial strain of the invention has a chromosome with at least 90% sequence identity (e.g., at least 92%, 94%, 95%, 96%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, or 100% sequence identity) to SEQ ID NO: 28 across at least 60% (e.g., at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of SEQ ID NO: 28. For example, the bacterial strain of the invention may have a chromosome with at least 90% sequence identity to SEQ ID NO: 28 across 70% of SEQ ID NO: 28, or at least 90% sequence identity to SEQ ID NO: 28 across 80% of SEQ ID NO: 28, or at least 90% sequence identity to SEQ ID NO: 28 across 90% of SEQ ID NO: 28, or at least 90% sequence identity to SEQ ID NO: 28 across 100% of SEQ ID NO: 28, or at least 95% sequence identity to SEQ ID NO: 28 across 70% of SEQ ID NO: 28, or at least 95% sequence identity to SEQ ID NO: 28 across 80% of SEQ ID NOs: 28, or at least 95% sequence identity to SEQ ID NO: 28 across 90% of SEQ ID NO: 28, or at least 95% sequence identity to SEQ ID NO: 28 across 100% of SEQ ID NO: 28, or at least 98% sequence identity to SEQ ID NO: 28 across 70% of SEQ ID NO: 28, or at least 98% sequence identity to SEQ ID NO: 28 across 80% of SEQ ID NO: 28, or at least 98% sequence identity to SEQ ID NO: 28 across 90% of SEQ ID NO: 28, or at least 98% sequence identity to SEQ ID NO: 28 across 100% of SEQ ID NO: 28. A particularly preferred strain of the invention is the *G. formicilis* strain deposited under accession number V21/011520. This is the exemplary *G. formicilis* MH32-1 strain tested in the DSS mouse model presented in the examples and shown to be effective for treating disease. Therefore, the invention provides a cell, such as an isolated cell, of the *G. formicilis* strain deposited under accession number V21/011520, or a derivative thereof. The invention also provides a composition comprising a cell of the *G. formicilis* strain deposited under accession number V21/011520, or a derivative thereof. The invention also provides a biologically pure culture of the *G. formicilis* MH32-1 strain deposited under accession number V21/011520.

[0353] In other embodiments, the bacterial strain of the invention has a chromosome with sequence identity to any one of SEQ ID NOs: 29-33. In preferred embodiments, the bacterial strain of the invention has a chromosome with at least 90% sequence identity (e.g., at least 92%, 94%, 95%, 96%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, or 100% sequence identity) any one of SEQ ID NOs: 29-33 across at least 60% (e.g., at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of any one of SEQ ID NOs: 29-33. For example, the bacterial strain of the invention may have a chromosome with at least 90% sequence identity to any one of SEQ ID NOs: 29-33 across 70% of any one of SEQ ID NOs: 29-33, or at least 90% sequence identity to any one of SEQ ID NOs: 29-33 across 80% of any one of SEQ ID NOs: 29-33, or at least 90% sequence identity to any one of SEQ ID NOs: 29-33 across 90% of any one of SEQ ID NOs: 29-33, or at least 90% sequence identity to any one of SEQ ID NOs: 29-33 across 100% of any one of SEQ ID NOs: 29-33, or at least 95% sequence identity to any one of SEQ ID NOs: 29-33 across 70% of any one of SEQ ID NOs: 29-33, or at least 95% sequence identity to any one of SEQ ID NOs: 29-33 across 80% of any one of SEQ ID NOs: 29-33, or at least 95% sequence identity to any one of SEQ ID NOs: 29-33 across 90% of any one of SEQ ID NOs:

29-33, or at least 95% sequence identity to any one of SEQ ID NOs: 29-33 across 100% of any one of SEQ ID NOs: 29-33, or at least 98% sequence identity to any one of SEQ ID NOs: 29-33 across 70% of any one of SEQ ID NOs: 29-33, or at least 98% sequence identity to any one of SEQ ID NOs: 29-33 across 80% of any one of SEQ ID NOs: 29-33, or at least 98% sequence identity to any one of SEQ ID NOs: 29-33 across 90% of any one of SEQ ID NOs: 29-33, or at least 98% sequence identity to any one of SEQ ID NOs: 29-33 across 100% of any one of SEQ ID NOs: 29-33.

[0354] In certain embodiments, the bacterial strains of the invention have a 16S rRNA sequence that is at least 97.5%, 98%, 98.5%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to the 16S rRNA sequence of a bacterial strain of *Gemmiger sp.* MD158. Preferably, the bacterial strain of the invention has a 16S rRNA sequence that is at least 97.5%, 98%, 98.5%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to SEQ ID NO: 48. In some preferred, the bacterial strain of the invention has a 16S rRNA sequence represented by SEQ ID NO: 48.

[0355] The genome of the bacterial strain may comprise the 16S rRNA sequence set forth in SEQ ID NO: 48.

[0356] In some other embodiments, the bacterial strain of the invention is a bacterial strain of the species *Gemmiger sp.* MD158. By way of an example, the bacterial strain may have a chromosome with sequence identity to any one of SEQ ID NOs: 34-38. In preferred embodiments, the bacterial strain of the invention has a chromosome with at least 90% sequence identity (e.g., at least 92%, 94%, 95%, 96%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, or 100% sequence identity) any one of SEQ ID NOs: 29-33 across at least 60% (e.g., at least 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99% or 100%) of any one of SEQ ID NOs: 34-38. For example, the bacterial strain of the invention may have a chromosome with at least 90% sequence identity to any one of SEQ ID NOs: 34-38 across 70% of any one of SEQ ID NOs: 34-38, or at least 90% sequence identity to any one of SEQ ID NOs: 34-38 across 80% of any one of SEQ ID NOs: 34-38, or at least 90% sequence identity to any one of SEQ ID NOs: 34-38 across 90% of any one of SEQ ID NOs: 34-38, or at least 90% sequence identity to any one of SEQ ID NOs: 34-38 across 100% of any one of SEQ ID NOs: 29-33, or at least 95% sequence identity to any one of SEQ ID NOs: 34-38 across 70% of any one of SEQ ID NOs: 34-38, or at least 95% sequence identity to any one of SEQ ID NOs: 34-38 across 80% of any one of SEQ ID NOs: 34-38, or at least 95% sequence identity to any one of SEQ ID NOs: 34-38 across 90% of any one of SEQ ID NOs: 34-38, or at least 95% sequence identity to any one of SEQ ID NOs: 34-38 across 100% of any one of SEQ ID NOs: 34-38, or at least 98% sequence identity to any one of SEQ ID NOs: 34-38 across 70% of any one of SEQ ID NOs: 34-38, or at least 98% sequence identity to any one of SEQ ID NOs: 34-38 across 80% of any one of SEQ ID NOs: 34-38, or at least 98% sequence identity to any one of SEQ ID NOs: 34-38 across 90% of any one of SEQ ID NOs: 34-38, or at least 98% sequence identity to any one of SEQ ID NOs: 34-38 across 100% of any one of SEQ ID NOs: 34-38.

[0357] A derivative of the strains deposited under the accession number V21/011520, may be a daughter strain (progeny) or a strain cultured (subcloned) from the original. A derivative of a strain of the invention may be modified, for example at the genetic level, without ablating the biological activity. In particular, a derivative strain of the invention is therapeutically active. A derivative strain will have comparable activity to the original V21/011520 strain from which it is derived. In particular, a derivative strain will elicit comparable effects in at least one disease model (e.g., colitis) as shown in the Examples, which may be identified by using the culturing and administration protocols described in the Examples. A derivative of any one of the V21/011520 strains will generally be a biotype of the respective V21/011520 strain.

[0358] References to cells of the *G. formicilis* strain deposited under accession number V21/011520 include any cells that have the same safety and therapeutic efficacy characteristics as the strains deposited under accession number V21/011520, and such cells are encompassed by the invention.

6.1 Bacteria biotypes

[0359] Bacterial strains that are biotypes of a bacterium deposited under accession number V21/011520 are also expected to be effective for treating or preventing inflammatory and autoimmune disorders. A biotype is a closely related strain that has the same or very similar physiological and biochemical characteristics.

[0360] Strains that are biotypes of a bacterium deposited under the accession number V21/011520 and that are suitable for use in the invention may be identified by sequencing other nucleotide sequences for a bacterium deposited under accession number V21/011520. For example, substantially the whole genome may be sequenced and a biotype strain of the invention may have at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity across at least 80% of its whole genome (e.g., across at least 85%, 90%, 95% or 99%, or across its whole genome). Other suitable sequences for use in identifying biotype strains may include hsp60 or repetitive sequences such as BOX, ERIC, (GTG)₅, or REP (Masco et al., 2003; Kim et al., 2019). Biotype strains may have sequences with at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of a bacterium deposited under accession number V21/011520.

[0361] Alternatively, strains that are biotypes of a bacterium deposited under the accession number V21/011520, 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 rRNA sequencing. In some preferred embodiments, such techniques may be used to identify other suitable *G. formicilis* strains.

[0362] In certain embodiments, strains that are biotypes of a bacterium deposited under accession number V21/011520, and that are suitable for use in the invention are strains that provide the same pattern as a bacterium deposited under accession number V21/011520 when analysed by amplified ribosomal DNA restriction

analysis (ARDRA), for example when using Sau3AI restriction enzyme (for exemplary methods and guidance see, for example, Srůtková et al., 2011). Alternatively, biotype strains are identified as strains that have the same carbohydrate fermentation patterns as a bacterium deposited under accession number V21/011520.

5 **[0363]** In some embodiments, bacterial strains useful in the invention may be identified by routinely profiling the production and consumption of metabolites by a bacterial strain. It is predicted that the bacterial strains described above and elsewhere herein effect production of acetate, butyrate, ethanol and fumarate. Therefore, in some embodiments, the bacterial strains of the invention induce the production *in vivo* of one or more of the
10 metabolites acetate, ethanol, butyrate, and fumarate. Additionally, in some embodiments the bacterial strains of the invention do not produce butyrate.

[0364] Other *Gemmiger* strains that are useful in the compositions and methods of the invention, such as biotypes of a bacterium deposited under accession number V21/011520, may be identified using any appropriate method or strategy, including the
15 assays described in the examples. For instance, strains for use in the invention may be identified by culturing in anaerobic TY or PYG media and/or administering the bacteria to the DSS-induced gut barrier function model and then assessing cytokine/chemokine levels, as described in the Examples. In particular, bacterial strains that have similar growth patterns, metabolic type and/or surface antigens to a bacterium deposited under accession number
20 V21/011520 may be useful in the invention. A useful strain will have comparable immunomodulatory activity to the V21/011520 strain. In particular, a biotype strain will elicit comparable effects on host gut function. Furthermore, it is expected that a biotype will have a similar effect in a disease model (e.g., colitis, asthma, arthritis, multiple sclerosis and uveitis disease models) and comparable effects on cytokine/chemokine levels to the effects
25 shown in the Examples, and which may be identified by using the culturing and administration protocols described in the Examples.

Bacterial strain viability.

[0365] In preferred embodiments, the bacterial strains in the compositions of the invention are viable. In preferred embodiments, the bacterial strains in the compositions of
30 the invention are viable and capable of partially or totally colonising the intestine. In some preferred embodiments, the bacterial strains in the compositions of the invention are live. By way of an example, the bacterial strains in the compositions of the invention have not been heat-killed. The bacteria of the invention may have immune modulatory effects that would not be exhibited by non-viable bacteria, for example because non-viable bacteria cannot
35 produce metabolites and interact with the immune system in a different manner. The cell surface of a viable bacterium is also likely to be significantly different to a killed bacterium, in particular a heat-killed bacterium.

[0366] In some alternative embodiments, that bacteria are not viable. For example, in some embodiments the bacteria are heat-killed.

[0367] In some preferred embodiments, the bacterial strain for use in the invention is naturally-occurring. For example, the bacterial strain has been isolated from the mammalian digestive tract.

[0368] In some preferred embodiments, the bacterial strain for use in the invention has not been genetically engineered. For example, the bacterial strain has not been transformed with recombinant DNA.

7. Compositions – *Gemmiger species*

[0369] Provided herein are compositions that comprise, consist, or consist essentially of a therapeutically effective amount of a bacterial strain or strains described above and/or elsewhere herein. In some embodiments, the bacteria in the compositions may be identified by strain, species, operational taxonomic unit (OTU), whole genome sequence, 16S rRNA sequence, or other methods known in the art for defining different types of bacteria.

7.1 Most recent common ancestor (MRCA)

[0370] In some embodiments, the compositions comprise an effective amount of a bacterial strain that is a phylogenetic descendant of the MRCA of *G. variable* and *G. sp002306375* (see, Figure 15). Preferably, the phylogenetic classification is as defined by the GTDB (Parks et al., 2018). In some embodiments, the phylogenetic classification is as defined in release 89 (r89) of the GTDB.

[0371] In some embodiments, determining if a bacterial strain is a descendant of a MRCA of *G. variable* and *G. sp002306375* may be performed using phylogenetic grouping procedures known in the art. In some embodiments, a rooted phylogenetic tree with *G. variable*, *G. sp002306375*, and a third taxon of interest (e.g., a taxon to be classified) may be used, with the following the analysis packages being applied: Analyses of Phylogenetics and Evolution ("ape"; <https://cran.r-project.org/web/packages/ape/index.html>) and Phylogenetic Tool for Comparative Biology ("phytools";), in order to determine whether the taxon of interest is useful for the compositions of the present invention. Both ape and phytools are packages written in the R language for use in studying molecular evolution and phylogenetics. The ape and phytools packages provide methods for phylogenetic and evolutionary analysis and their use is known to one of skill in the art. In some embodiments, the following script may be used:

```
library("ape")

library("phytools")

input.tree = read.tree(file="tree_file")

gemmi c("s__Gemmiger sp900554145", "s__Gemmiger sp004555405")

gemmi.node = getMRCA(input.tree, gemmi)

gemmi.tree = extract.clade(input.tree, gemmi.node)

print(gemmi.tree$tip.label)
```

[0372] In some embodiments, after the script is run, if the taxon of interest is in the printed list, it is a descendant of a MRCA of the two species.

[0373] In other embodiments, different phylogenetic grouping methods known in the art may be used to determine if a bacterial strain is a descendant of a MRCA of *G. variabile* and *G. sp002306375* (see, Figure 15B,C), including methods that use different analysis packages and are based on different programming languages.

[0374] In some other embodiments, the bacterial strain is a phylogenetic descendant of the MRCA of *G. variabile* and *G. sp003476825*, together with a pharmaceutically acceptable carrier, diluent, or excipient. Suitably, the MRCA is defined at node 23819 of the bac120 phylogenetic tree from the GTDB. In some embodiments, the phylogenetic tree is created by release 89 of the GTDB, however, any suitable subsequent release is considered to give equally as applicable results.

[0375] In some other embodiments, the bacterial strain is a phylogenetic descendant of the MRCA of *G. variabile* and *G. formicilis*, together with a pharmaceutically acceptable carrier, diluent, or excipient. Suitably, the MRCA is defined at node 23820 of the bac120 phylogenetic tree from the GTDB. In some embodiments, the phylogenetic tree is created by release 89 of the GTDB, however, any suitable subsequent release is considered to give equally as applicable results.

[0376] In other embodiments, a bacterial species is a member of the family *Ruminococcaceae* if the species has a 16S rRNA sequence with sequence identity to 16S rRNA sequences from species already identified as a member of the family *Ruminococcaceae*. In some embodiments, identification of whether a bacterial species is a member of the family *Ruminococcaceae* is performed using the methods described in Yarza et al., 2014, Nature Reviews Microbiology 12:635-645, and Stackebrandt, E. & Ebers, J., 2006, Microbiol. Today 8:6–9, which are hereby incorporated by reference herein.

7.2 16S rRNA Sequence Identity

[0377] In some embodiments, the 16S rRNA sequence is obtained or determined for a bacterial species to be classified. This query 16S rRNA sequence is compared to 16S rRNA sequences from bacterial species already classified as members of the *Gemmiger* genus. In some embodiments, the query 16S rRNA sequence is compared to the 16S rRNA sequences set forth in SEQ ID NO: 27. In some embodiments, the query 16S rRNA sequence is compared to all known 16S rRNA sequences for bacterial species already classified as members of the *Gemmiger* genus. In other embodiments, the query 16S rRNA sequence is compared to a subset of all known 16S rRNA sequences for bacterial species already classified as members of the *Gemmiger* genus. A percent identity between the query sequence and the compared sequences is determined. If the percent identity of the query sequence is determined to be above a defined threshold, then the bacterial species to be classified is classified as member of the *Gemmiger* genus.

[0378] In some embodiments, the threshold sequence identity is 95%. In some other embodiments, the threshold sequence identity is 97.5%. In some other embodiments,

the threshold sequence identity is 99.0%. In some embodiments, the threshold sequence identity is 94.5%, 94.6%, 94.7%, 94.8%, 94.9%, 95.0%, 95.1%, 95.2%, 95.3%, 95.4%, 95.5%, 95.6%, 95.7%, 95.8%, 95.9%, 96.0%, 96.1%, 96.2%, 96.3%, 96.4%, 96.5%, 96.6%, 96.7%, 96.8%, 96.9%, 97.0%, 97.1%, 97.2%, 97.3%, 97.4%, 97.5%, 97.6%, 97.7%, 97.8%, 97.9%, 98.0%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99.0%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100%.

[0379] In some embodiments, the 16S rRNA sequence is obtained or determined for a bacterial species to be classified. This query 16S rRNA sequence is compared to 16S rRNA sequences from bacterial species already classified as members of the family *Ruminococcaceae*. In some embodiments, the query 16S rRNA sequence is compared to all known 16S rRNA sequences for bacterial species already classified as members of the family *Ruminococcaceae*. In other embodiments, the query 16S rRNA sequence is compared to a subset of all known 16S rRNA sequences for bacterial species already classified as members of the family *Ruminococcaceae*. A percent identity between the query sequence and the compared sequences is determined. If the percent identity of the query sequence is determined to be above a defined threshold, then the bacterial species to be classified is classified as member of the family *Ruminococcaceae*.

[0380] In some embodiments, the threshold sequence identity is 95%. In some embodiments, the threshold sequence identity is 98.7%. In some embodiments, the threshold sequence identity is 94.8%. In some embodiments, the threshold sequence identity is 94.5%, 94.6%, 94.7%, 94.8%, 94.9%, 95.0%, 95.1%, 95.2%, 95.3%, 95.4%, 95.5%, 95.6%, 95.7%, 95.8%, 95.9%, 96.0%, 96.1%, 96.2%, 96.3%, 96.4%, 96.5%, 96.6%, 96.7%, 96.8%, 96.9%, 97.0%, 97.1%, 97.2%, 97.3%, 97.4%, 97.5%, 97.6%, 97.7%, 97.8%, 97.9%, 98.0%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99.0%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100%.

8. Functional features of bacterial strains

[0381] Gut barrier dysregulation is a key pathway leading to systemic inflammation. As demonstrated in the examples, the bacterial strains of the invention, and compositions comprising said strains, are effective at enhancing gut barrier function.

[0382] All inflammatory or autoimmune disorders mediated by gut barrier dysregulation causing systemic inflammation in the subject are applicable for treatment with the bacterial strains described above and/or elsewhere herein.

8.1 Gut barrier function.

[0383] Gut barrier (also known as intestinal barrier) function regulates transport and host defense mechanisms at the mucosal interface with the outside world. Transcellular and paracellular fluxes are tightly controlled by membrane pumps, ion channels and tight junctions, adapting permeability to physiological needs.

[0384] The translocation of foreign (i.e., non-host) substances such as lipopolysaccharide (LPS) and other inflammatory compounds from the luminal side of the intestine into the circulating system is inhibited by the epithelial barrier. One of the functions of this epithelial barrier is performed by the tight junctions. Tight junctions, or *zonula occludens*, are the closely associated areas of two epithelial cells whose membranes join together forming a virtually impermeable barrier to fluid, thereby separating the vascular system from the lumen of the digestive tract. Disturbance at any level, but particularly bacterial translocation due to increased permeability and breakdown of oral tolerance due to compromised epithelial and T cell interaction, can result in inflammation and tissue damage. Thus, a reduction of the tight junction barrier function has been demonstrated to result in an increased translocation of undesirable substances such as LPS from intestinal lumen into the circulating system.

[0385] The present invention provides methods of restoring or improving gut barrier function in a subject, the method comprising administering to the subject a composition that comprises a bacterial strain as described above and/or elsewhere herein, to thereby restore or improve the gut barrier function in the subject. In some specific embodiments, the present invention provides methods of restoring or improving gut barrier function in a subject, the method comprising administering to the subject a composition that comprises a bacterial strain of one or more of *A. shahii*, *C. sana*, and *G. formicilis*, to thereby restore or improve the gut barrier function in the subject. As used in this specification, gut barrier integrity refers to a measure of gut barrier function. High gut barrier integrity can be associated with a lack of gut or intestinal permeability, wherein a high level of gut permeability is indicative of low gut barrier integrity. In a related embodiment, the invention also provides methods of maintaining healthy or normal gut barrier function. Such methods may be used to prevent gut barrier dysregulation in subjects considered to be at high risk of gut barrier dysregulation (e.g., subjects in remission of IBD). In some embodiments the composition comprises a bacterial strain of *A. shahii*. In some other embodiments the composition comprises a bacterial strain of *C. sana*. In yet some other embodiments, the composition comprises a bacterial strain of *G. formicilis*.

[0386] In some embodiments, at least one biomarker measured in a sample (and, in particular, a biological sample) is used to assess the change, in particular, an improvement, in the gut barrier integrity of a subject.

[0387] In some embodiments of the methods and uses provided in this specification, the composition comprising a bacterial strain of *A. shahii* may increase or decrease the levels of one or more biomarkers of gut barrier integrity in a sample from a subject.

[0388] In some embodiments of the methods and uses provided in this specification, the composition comprising a bacterial strain of *C. sana* may increase or decrease the levels of one or more biomarkers of gut barrier integrity in a sample from a subject.

[0389] Furthermore, in some embodiments of the methods and uses provided in this specification, the composition comprising a bacterial strain of *G. formicilis* may increase or decrease the levels of one or more biomarkers of gut barrier integrity in a sample from a subject.

5 **[0390]** In some embodiments, depending on the particular biomarker, either an increase or a decrease in the level of the marker is indicative of an increase in gut barrier integrity and/or a decrease in gut permeability. In some embodiments, the biomarker is selected from a cytokine, chemokine, growth factor, angiogenic factor, enzyme, protease, adhesion molecule, cell signalling molecule, hormone or sugar. In some embodiments, the
10 biomarker comprises a cytokine. In some embodiments, the marker comprises a chemokine. In some embodiments, the marker comprises a growth factor. In some embodiments, the marker comprises an angiogenic factor. In some embodiments, the marker comprises an enzyme. In some embodiments, the marker comprises a protease. In some embodiments, the marker comprises an adhesion molecule. In some embodiments, the marker comprises a
15 cell signalling molecule. In some embodiments, the marker comprises a hormone. In some embodiments, the marker comprises a sugar.

[0391] This specification provides assays for biomarkers of intestinal permeability. Biological samples from the subject such as blood (plasma, or serum) or tissue may be used to measure levels of any suitable biomarker including one or more of LPS,
20 lipopolysaccharide binding protein (LPSBP), intestinal fatty acid binding protein (IFABP), Zonulin, bacterial and/or 16S rRNA, but is not limited to these markers. LPS, I-FABP and Zonulin may be measured by enzyme-linked immunosorbent assay ("ELISA"). Techniques and kits for ELISA are well known to those in the art. In some embodiments, elevated LPS, I-FABP and/or Zonulin, when compared to a control in blood, serum, saliva, urine and/or
25 plasma, is used as an indicator of increased intestinal permeability, and, thus, lower gut barrier integrity.

[0392] LPSBP may also be measured by ELISA. In some embodiments, significant changes in LPSBP either higher or lower, when compared to a control, may be used as an indicator of increased intestinal permeability and can confirm a reduced gut barrier integrity.

30 **[0393]** In some embodiments, increases in bacterial 16S rRNA is used as an indicator of increased intestinal permeability, and, therefore, a reduction in gut barrier integrity. Bacterial 16S rRNA may be purified from blood, serum, organ tissue or urine using standard nucleic acid isolation protocols. These are, for example, commercially available. The isolated nucleic acids may be detected by qPCR amplification using primers specific for
35 bacterial 16S rRNA sequences or amplification using primers specific for bacterial 16S rRNA and sequencing the resultant amplicons.

[0394] Tight junction proteins that are expressed by the intestinal epithelial cells and regulate intestinal permeability may also be used as biomarkers of intestinal permeability. In some embodiments, tight junction proteins are assayed to determine
40 alterations in intestinal permeability and gut barrier integrity. In some embodiments, the proteins measured may include, but are not limited to, claudins, occludin, ZO-1, and E-

cadherin (adherens junction) proteins. Other tight junction proteins may also be assayed. In some embodiments, the tight junction proteins are measured using an immunohistochemical stain. In some embodiments, the tight junction proteins are measured using ELISA.

5 **[0395]** In some embodiments, plasma citrulline is assayed to determine alterations in intestinal permeability and gut barrier integrity. A reduction in plasma citrulline levels corresponds to a loss in epithelial cell mass indicating an increase in gut barrier permeability.

10 **[0396]** In some embodiments, the method includes oral administration of an insoluble sugar such as sucralose, collection of a bodily fluid such as urine or blood after one or more defined periods of time, and measurement of the insoluble sugar contained in the bodily fluid through standard clinical analytical techniques. The insoluble sugars may include, but are not limited to, mannitol, lactulose, sucrose, sucralose and combinations of any of the foregoing.

15 **[0397]** In some embodiments, gut barrier integrity is measured using an *in vitro* assay. A particularly preferred *in vitro* assay suitable for measuring gut barrier function is by trans-epithelial electrical resistance (TEER). Such assays are well known in the field (e.g., Srinivasan, 2015; and Lea, 2015).

8.2 Mucosal healing

20 **[0398]** Mucosal healing has become an important endpoint to assess the therapeutic effect in inflammatory and autoimmune disorders. The definition of full mucosal healing currently used in IBD (e.g., CD and UC) clinical trials is the “complete absence of all inflammatory and ulcerative lesions”, but this definition lacks validation and does not include mucosal improvement and grading of mucosal healing.

25 **[0399]** Mucosal healing is predominantly defined by endoscopic assessment of intestinal inflammation. In order to evaluate the presence or absence of mucosal healing on endoscopy, various endoscopic scoring systems have been developed. These indices allow for the determination of improvements of endoscopic lesions, even when the rather rigid endpoint of mucosal healing and thereby the total disappearance of all mucosal ulcerations is not met. The endoscopic component of the clinical Mayo score, introduced in 1987, is currently the most used score of the mucosal layer in clinical practice (see, Schroeder et al., 1987). It includes the variables erythema, loss of vascular pattern, friability, bleeding, erosions and ulcers, and ranges from 0 to 3. Mucosal healing is classically considered to be a score of 0 (normal mucosa) or 1 (mucosal erythema, decreased vascular pattern, mild friability) (D’Haens, 2007).

35 **[0400]** In some other embodiments, mucosal healing is determined to have occurred when the patient is determined to have an endoscopy sub-score of 0 or 1 as assessed by flexible sigmoidoscopy. In certain such embodiments, patients who experience mucosal healing are determined to have an endoscopy sub-score of 0.

40 **[0401]** Both corticosteroids and aminosalicylates have been used for decades and are among the most commonly prescribed drugs for repairing the mucous layer (e.g., in

patients with UC) (Carvalho and Cotter, 2017). The mechanisms through which they reduce mucosal inflammation include controlling nuclear factor (NF)-kB expression and inflammatory cytokines (directly modulating cell migration and proliferation of epithelial cell lines). Anti-TNF drugs (e.g., infliximab, adalimumab, and golimumab) act at several steps of mucosal injury, restricting the inflammatory infiltrate and T cell proliferation within the lamina propria (Baert, 1999), and downregulating the expression of metalloproteinases and proinflammatory molecules (Baert, 1999). They also act on the regenerative process, restoring the protective capabilities of the mucosa by reinforcing intestinal permeability and mucosal secretion, activating fibroblasts, and maintaining epithelial regeneration (Suenart, 2002).

[0402] Other measures of assessing mucosal healing are well known in the art, including the measurement of biomarkers C-reactive protein and calprotectin. An advantage of using *in vitro* biomarker assays for the assessment of mucosal healing is that such assays are typically far less invasive for the subject. Histopathology is another measure of inflammation, which has been cited as being particularly informative for mucosal healing.

8.3 *STAT3 signalling pathway*

[0403] Cytokine pathways mediate a broad range of biological functions, including many aspects of inflammation and immunity. The Janus kinases (JAK), including JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2), are cytoplasmic tyrosine kinases that associate with type I and type II cytokine receptors and regulate cytokine signal transduction. Cytokine engagement with cognate receptors triggers activation of receptor associated JAKs and this leads to JAK-mediated tyrosine phosphorylation of signal transducer and activator of transcription (STAT) proteins and ultimately transcriptional activation of specific gene sets (Schindler et al., 2007, *J. Biol. Chem.* 282: 20059-63). Cytokine receptors are typically functional as heterodimers, and as a result, more than one type of JAK kinase is usually associated with a cytokine receptor complex. The specific JAKs associated with different cytokine receptor complexes have been determined in many cases through genetic studies and corroborated by other experimental evidence.

[0404] STAT3 plays an important role in the activation of several autoimmune and inflammatory disorders, including IBD. The bacterial strains of the present invention significantly suppress IL-23-mediated STAT3 activation. Thus, the present invention provides methods of suppressing or otherwise inhibiting STAT3 signalling in a subject (i.e., IL-23-mediated STAT3 signalling), the method comprising administering to the subject a composition that comprises bacterial strain as described above and/or elsewhere herein. Accordingly, in some embodiments the bacterial strains described herein directly or indirectly suppress STAT3 activity. In some embodiments, the strain of *A. shahii* produces a bioactive molecule that binds directly to a STAT3 polypeptide. In some alternative embodiments, the bacterial strain is an indirect inhibitor of STAT3 activation, for example, by binding to a molecule upstream of STAT3 in the IL-23-mediated STAT3 signalling pathway, or by binding to a molecule that regulates STAT3 activity (e.g., ubiquitination). By way of an illustrative

example, the bioactive agent may directly bind or antagonize any one of IL23, JAK2, or TYK2 in order to suppress the IL-23-mediated STAT3 signalling pathway.

8.4 Th17 inflammatory response

[0405] Some bacterial compositions of the invention are effective for reducing the Th17 inflammatory response. In particular, treatment with the compositions described above and elsewhere herein may modulate Th17 pathway cytokines (including TNF, IL-22, IL-21, and IL-17), and result in clinical improvements in animal models of conditions mediated by the Th17 pathway. Therefore, the compositions of the invention may be useful for treating or preventing inflammatory and autoimmune disorders, and in some embodiments, diseases or conditions mediated by Th17. In particular, the compositions of the invention may be useful for reducing or preventing elevation of the Th17 inflammatory response.

[0406] Th17 cells are a subset of T helper cells that produce, among other cytokines, IL17A, IL17F, IL-21 and IL-22. Th17 cell differentiation 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 disorders (as described in, for example, Ye, 2015; Fabro, 2015; Yin, 2014; Cheluvappa, 2014; Schieck, 2014; Balato, 2014). Some diseases that are mediated by Th17 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 Monteleone, 2011). Diseases mediated by the Th17 pathway may be characterised by increased expression of Th17-related genes, such as STAT3 or IL-23 receptor. Diseases mediated by the Th17 pathway may be associated with increased levels of Th17 cells.

[0407] IL-17 is a key cytokine that links T cells activation to neutrophil activation and mobilization, hence IL-17 plays a pivotal role in innate immunity. However, due to its role in neutrophil activation, can contribute to inflammatory autoimmune diseases such as inflammatory bowel disease, psoriasis, and rheumatoid arthritis. 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.

[0408] Examples of diseases and conditions that may be mediated by the Th17 pathway include (but are not limited to) inflammatory bowel disease (such as Crohn's disease and ulcerative colitis); multiple sclerosis; arthritis (such as rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and juvenile idiopathic arthritis); neuromyelitis optica (Devic's disease); ankylosing spondylitis; spondyloarthritis; psoriasis; systemic lupus

erythematosus; celiac disease; asthma (such as allergic asthma or neutrophilic asthma); chronic obstructive pulmonary disease (COPD); cancer (such as breast cancer, colon cancer, lung cancer or ovarian cancer); uveitis; scleritis; vasculitis; Behcet's disease; atherosclerosis; atopic dermatitis; emphysema; periodontitis; allergic rhinitis; and allograft rejection. Accordingly, in some aspects the present invention provides methods for treating or preventing one or more of these conditions or diseases, by administering a composition as described above and/or elsewhere herein. In further preferred embodiments, these conditions or diseases are mediated by the STAT3 signalling pathway. In further preferred embodiments, these conditions or diseases are mediated through the Th17 pathway.

10 **[0409]** In certain embodiments, the present invention provides methods compositions of the invention are for use in a method of reducing Th17 cell differentiation in the treatment or prevention of a disease or condition mediated by the Th17 pathway. In certain embodiments, the compositions of the invention are for use in treating or preventing an inflammatory or autoimmune disorder, wherein said treatment or prevention is achieved
15 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 disorder, 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 disorder
20 or condition, or the composition of the invention may be for use in preventing an inflammatory or autoimmune disorder or condition developing into a chronic inflammatory or autoimmune disorder or condition. In certain embodiments, the disease or condition may not be responsive to treatment with TNF inhibitors. These uses of the invention may be applied to any of the specific disease or conditions listed in the preceding paragraph.

25 **[0410]** The Th17 pathway are often associated with chronic inflammatory and autoimmune disorders, 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
30 disease.

[0411] The compositions of the invention may be useful for treating diseases and conditions mediated by 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
35 responded to other therapies (such as treatment with TNF inhibitors), and/or treating or preventing the tissue damage and symptoms associated with 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.

40 **[0412]** 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. Such reduction or prevention of elevated levels of these cytokines may be useful for treating or preventing inflammatory and autoimmune disorders and conditions, in particular those mediated by the Th17 pathway.

5 8.5 Th1 inflammatory response

[0413] CD4⁺ T cells play an important role in inflammatory disease/disorder pathogenesis, with many subsets of CD4⁺ T cells having been identified as drivers in perpetuating chronic intestinal inflammation (see, Imam et al., 2018). For example, T helper type 1 (Th1) cells accumulate in the intestinal tract of individuals with IBD, and are directly
10 associated with disease. Interferon- γ (IFN- γ) is the defining cytokine produced by Th1 cells. During intestinal inflammation IFN- γ in combination with TNF is proposed to drive intestinal epithelial cell β -catenin signalling and limit their differentiation and proliferation (Imam et al., 2018).

9. Methods of Treatment

[0414] In some embodiments, the present invention provides methods of treating or preventing an inflammatory or autoimmune disorder in a subject, the methods comprising administering to the subject a bacterial strain as described above and/or elsewhere herein.

[0415] Suitably, the inflammatory or autoimmune disorder is selected from the group comprising: an inflammatory bowel disease (such as Crohn's disease or ulcerative
20 colitis); asthma (such as allergic asthma or neutrophilic asthma); arthritis (such as rheumatoid arthritis, osteoarthritis, psoriatic arthritis, or juvenile idiopathic arthritis); fatty liver disease (such as nonalcoholic fatty liver disease (NAFLD)); ankylosing spondylitis; psoriasis; systemic lupus erythematosus (SLE); scleroderma; Sjogren's syndrome; vasculitis; and type 1 diabetes mellitus.

25 9.1 Inflammatory Bowel Disease (IBD)

[0416] The examples demonstrate that the compositions of the invention have a beneficial restorative effect on gut barrier function and that they also have anti-inflammatory properties, and so they may be useful in the treatment of IBD.

[0417] Accordingly, in some embodiments the invention provides a composition
30 comprising a bacterial strain of the genus *Alistipes* for use in a method of treating or preventing an inflammatory bowel disease. The inventors have identified that treatment with *Alistipes* strains reduces severity of colitis in a mouse model of disease. Thus, the compositions of the invention may be useful in the treatment of inflammatory diseases. In some embodiments, the compositions of the invention are for use in the treatment or
35 prevention of an IBD. In some embodiments, the invention provides methods of treating or preventing ulcerative colitis. In some embodiments, the invention provides methods of treating or preventing of Crohn's disease. In certain embodiments, the invention provides methods of treating or preventing ulcerations and/or bleeding in the treatment of an IBD, in particular in the treatment of colitis and ulcerative colitis. In preferred embodiments, the
40 invention provides a method of treating or preventing IBD in a subject, the method

comprising administering to the subject a composition comprising a bacterial strain of the species *A. shahii*. In further preferred embodiments, the invention provides a method of treating or preventing colitis, (particularly ulcerative colitis) in a subject, the method comprising administering to the subject a composition comprising a bacterial strain of the species *A. shahii*. In further preferred embodiments, the invention provided methods of reducing at least one side effect of colitis (particularly ulcerative colitis), including ulcerations and/or bleeding.

[0418] In addition, in some embodiments the invention provides a composition comprising a bacterial strain of the genus *Colonithrix* for use in a method of treating or preventing an inflammatory bowel disease. The inventors have identified that treatment with *Colonithrix* strains reduces severity of colitis in a mouse model of disease. Thus, the compositions of the invention may be useful in the treatment of inflammatory diseases. In some embodiments, the compositions of the invention are for use in the treatment or prevention of an IBD. In some embodiments, the invention provides methods of treating or preventing ulcerative colitis. In some embodiments, the invention provides methods of treating or preventing of Crohn's disease. In certain embodiments, the invention provides methods of treating or preventing ulcerations and/or bleeding in the treatment of an IBD, in particular in the treatment of colitis and ulcerative colitis. In preferred embodiments, the invention provides a method of treating or preventing IBD in a subject, the method comprising administering to the subject a composition comprising a bacterial strain of the species *C. sana*. In further preferred embodiments, the invention provides a method of treating or preventing colitis, (particularly ulcerative colitis) in a subject, the method comprising administering to the subject a composition comprising a bacterial strain of the species *C. sana*. In further preferred embodiments, the invention provided methods of reducing at least one side effect of colitis (particularly ulcerative colitis), including ulcerations and/or bleeding.

[0419] Furthermore, in some embodiments the invention provides a composition comprising a bacterial strain of the genus *Gemmiger* for use in a method of treating or preventing an inflammatory bowel disease. The inventors have identified that treatment with *Gemmiger* strains reduces severity of colitis in a mouse model of disease. Thus, the compositions of the invention may be useful in the treatment of inflammatory diseases. In some embodiments, the compositions of the invention are for use in the treatment or prevention of an IBD. In some embodiments, the invention provides methods of treating or preventing ulcerative colitis. In some embodiments, the invention provides methods of treating or preventing of Crohn's disease. In certain embodiments, the invention provides methods of treating or preventing ulcerations and/or bleeding in the treatment of an IBD, in particular in the treatment of colitis and ulcerative colitis. In preferred embodiments, the invention provides a method of treating or preventing IBD in a subject, the method comprising administering to the subject a composition comprising a bacterial strain of the species *G. formicilis*. In further preferred embodiments, the invention provides a method of treating or preventing colitis, (particularly ulcerative colitis) in a subject, the method comprising administering to the subject a composition comprising a bacterial strain of the

species *G. formicilis*. In further preferred embodiments, the invention provided methods of reducing at least one side effect of colitis (particularly ulcerative colitis), including ulcerations and/or bleeding.

[0420] IBD is a complex disease that can be caused by multiple environmental and genetic factors. Factors contributing to the onset of IBD include diet, microbiota, intestinal permeability, and genetic susceptibility to increased inflammatory response to gut infection. Symptoms of inflammatory bowel disease include abdominal pain, vomiting, diarrhea, rectal bleeding, severe internal cramps/muscle spasms in the pelvic region, weight loss and anaemia. In certain embodiments, the compositions are for use in reducing one or more symptoms associated with IBD. In certain embodiments, the compositions of the invention are for use in preventing one or more symptoms of IBD.

[0421] IBD may accompany other diseases or conditions, such as cardiovascular disease, neuropsychological disorders, and metabolic syndrome. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of one or more diseases or conditions that accompany IBD.

[0422] IBD is generally diagnosed by biopsy or colonoscopy. Measurements of faecal calprotectin is useful for the preliminary diagnosis of IBD. Other laboratory test for the diagnosis of IBD include, complete blood count, erythrocyte sedimentation rate, comprehensive metabolic panel, faecal occult blood test or C-reactive protein test. Typically, a combination of laboratory testing and biopsy/colonoscopy will be used to confirm diagnosis of IBD. In certain embodiments, the compositions of the invention are for use in a subject diagnosed with IBD.

[0423] In certain embodiments the IBD is Crohn's disease and/or ulcerative colitis. As broadly described above, studies have shown that several inflammatory cytokines are upregulated in the inflammatory mucosa of patients with Crohn's disease and ulcerative colitis, including but not limited to STAT3 signalling and NFκB signalling pathway-mediated cytokines (e.g., IL-17, TNF, IL-21, IL-22). Therefore, inhibition of STAT3 signalling pathway-mediated cytokine activity and/or NFκB signalling pathway-mediated cytokines may be useful in the treatment of Crohn's disease and ulcerative colitis. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of Crohn's disease and/or ulcerative colitis.

[0424] Crohn's disease and ulcerative colitis are complex diseases with an array of probable causes, including genetic risk factors, diet, other lifestyle factors, such as smoking and alcohol consumption, and microbiome composition. Crohn's disease can manifest anywhere along the GI tract, whereas ulcerative colitis is typically prevalent in the large intestine and colon.

[0425] Gastrointestinal symptoms of IBD range from mild to severe and include abdominal pain, diarrhea, faecal blood, ileitis, increased bowel movements, increased flatulence, intestinal stenosis, vomiting, and perianal discomfort. The compositions of the

invention may be for use in the treatment of prevention of one or more gastrointestinal symptoms of Crohn's disease and/or ulcerative colitis.

[0426] Systemic symptoms of Crohn's disease and ulcerative colitis include growth defects, such as the inability to maintain growth during puberty, decreased appetite, fever and weight loss. Extra-intestinal features of Crohn's disease include uveitis, photobia, episcleritis, gall stones, seronegative spondyloarthropathy, arthritis, enthesitis, erythema nodosum, pyoderma gangrenosum, deep venous thrombosis, pulmonary embolism, autoimmune haemolytic anaemia, clubbing and osteoporosis. Extra-intestinal features are additional conditions associated with Crohn's disease and/or ulcerative colitis that manifest outside the GI tract. Subjects with Crohn's disease also exhibit increased susceptibility to neurological complications such as seizures, strokes, myopathy, peripheral neuropathy, headache and depression. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of one or more systemic symptoms of Crohn's disease and/or ulcerative colitis. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of one or more extra-intestinal features of Crohn's disease and/or ulcerative colitis.

[0427] The diagnosis of Crohn's disease and ulcerative colitis usually involves carrying out multiple tests and surgical procedures, such as gastroscopy and/or colonoscopy and biopsy, typically of the ileum, radiologic tests, complete blood counts, C-reactive protein tests and erythrocyte sedimentation rates. In certain embodiments, the compositions of the invention are for use in subjects diagnosed with Crohn's disease or ulcerative colitis. In some embodiments, compositions of the invention are for use in treating a subject who has been diagnosed with Crohn's disease or ulcerative colitis.

[0428] Crohn's disease and ulcerative colitis are classified depending on the extent of the region of the GI tract affected (Gasche et al., 2000). A Crohn's disease of both the ileum and colon is classified as Ileocolic Crohn's. In some embodiments, the compositions are for use in the treatment or prevention of Ileocolic Crohn's. In some embodiments, the compositions are for use in a subject diagnosed with Ileocolic Crohn's/Crohn's ileitis is classified if only the ileum is affected. Crohn's colitis is classified if only the colon is affected. In certain embodiments, the compositions are for use in the treatment or prevention of Crohn's ileitis. In some embodiments, the compositions are for use in a subject diagnosed with Crohn's ileitis. In certain embodiments, the compositions are for use in the treatment or prevention of Crohn's colitis. In some embodiments, the compositions are for use in a subject diagnosed with Crohn's colitis.

[0429] Crohn's disease and ulcerative colitis may be treated with a number of therapeutic agents, such as corticosteroids, such as prednisone, immunosuppressive agents, such as azathioprine, or biologics, such as infliximab, adalimumab, and golimumab, vedolizumab and etrolizumab. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of Crohn's disease or ulcerative colitis in combination with an additional therapeutic agent, including but not limited to those listed above. In

certain embodiments, the additional therapeutic agent is for use in the treatment or prevention of Crohn's disease and/or ulcerative colitis.

9.2 Autoimmune disorders

[0430] In humans, signs of intestinal inflammation are detectable before the clinical onset of many autoimmune disorders, such as type 1 diabetes (T1D) (Bosi, 2006). Similarly, augmented gut permeability appears before the development of insulinitis in diabetes-prone rats in comparison with diabetes-resistant rats (Meddings, 1999; Neu, 2005). Those findings indicate that the breakage of gut barrier integrity with subsequent increased antigen trafficking and occurrence of low-grade intestinal inflammation precede the onset of T1D and are directly related to its pathogenesis, rather than secondary to diabetes-induced metabolic alterations (*i.e.*, hyperglycemia). The gastrointestinal barrier is a fundamental gatekeeper to avoid the contact between luminal content and the human body. The barrier is composed of a mucus layer and an intestinal epithelial barrier (IEB), and both are crucial to prevent the passage of commensal bacteria, pathogens, and food antigens from the lumen into the gut tissue and systemic circulation. The IEB is a single layer of epithelial cells held together by a complex junctional system composed of tight junctional adhesion molecules (JAMs), tricellulin, and angulins whose interaction between themselves and with intracellular scaffolding proteins, *i.e.*, zonula occludens proteins (ZO), is fundamental to maintain tight junction integrity and control paracellular trafficking. In patients and rat models of T1D alterations of the IEB have been reported in association with gut inflammation (Meddings, 1999; Sapone, 2006). Furthermore, the importance of the gut mucus layer, an important gut barrier containing immunoregulatory molecules such as antimicrobial peptides and mucins, has recently been reported (see, Sorini et al., 2019).

[0431] In some embodiments, bacterial strains from the species *A. shahii* may provide therapeutic benefits in the treatment or prevention of asthma, such as allergic asthma or neutrophilic asthma. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of asthma in a subject. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *A. shahii* for use in the treatment or prevention of asthma.

[0432] In some embodiments, bacterial strains from the species *A. shahii* may provide therapeutic benefits in the treatment or prevention of GVHD. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of GVHD in a subject. In preferred embodiments, the invention provides a composition comprising a bacterial strain of the species *A. shahii* for use in the treatment or prevention of GVHD.

[0433] In some embodiments, bacterial strains from the species *A. shahii* may provide therapeutic benefits in the treatment or prevention of arthritis, such as rheumatoid arthritis, osteoarthritis, psoriatic arthritis, or juvenile idiopathic arthritis. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of arthritis in a subject. In certain embodiments, the invention provides a composition

comprising a bacterial strain of the species *A. shahii* for use in the treatment or prevention of arthritis.

5 **[0434]** In some embodiments, bacterial strains from the species *A. shahii* may provide therapeutic benefits in the treatment or prevention of multiple sclerosis. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of multiple sclerosis in a subject. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *A. shahii* for use in the treatment or prevention of multiple sclerosis.

10 **[0435]** In some embodiments, bacterial strains from the species *A. shahii* may provide therapeutic benefits in the treatment or prevention of psoriasis. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of psoriasis in a subject. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *A. shahii* or use in the treatment or prevention of psoriasis.

15 **[0436]** In some embodiments, bacterial strains from the species *A. shahii* may provide therapeutic benefits in the treatment or prevention of systemic lupus erythematosus (SLE). In certain embodiments, the compositions of the invention are for use in the treatment or prevention of SLE in a subject. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *A. shahii* for use in the treatment or prevention of SLE.

20 **[0437]** In some embodiments, bacterial strains from the species *A. shahii* may provide therapeutic benefits in the treatment or prevention of allograft rejection. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of allograft rejection in a subject. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *A. shahii* for use in the treatment or prevention of allograft rejection.

30 **[0438]** In some embodiments, bacterial strains from the species *C. sana* may provide therapeutic benefits in the treatment or prevention of asthma, such as allergic asthma or neutrophilic asthma. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of asthma in a subject. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *C. sana* for use in the treatment or prevention of asthma.

35 **[0439]** In some embodiments, bacterial strains from the species *C. sana* may provide therapeutic benefits in the treatment or prevention of GVHD. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of GVHD in a subject. In preferred embodiments, the invention provides a composition comprising a bacterial strain of the species *C. sana* for use in the treatment or prevention of GVHD.

40 **[0440]** In some embodiments, bacterial strains from the species *C. sana* may provide therapeutic benefits in the treatment or prevention of arthritis, such as rheumatoid

arthritis, osteoarthritis, psoriatic arthritis, or juvenile idiopathic arthritis. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of arthritis in a subject. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *C. sana* for use in the treatment or prevention of
5 arthritis.

[0441] In some embodiments, bacterial strains from the species *C. sana* may provide therapeutic benefits in the treatment or prevention of multiple sclerosis. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of multiple sclerosis in a subject. In certain embodiments, the invention provides a composition
10 comprising a bacterial strain of the species *C. sana* for use in the treatment or prevention of multiple sclerosis.

[0442] In some embodiments, bacterial strains from the species *C. sana* may provide therapeutic benefits in the treatment or prevention of psoriasis. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of psoriasis in a subject. In certain embodiments, the invention provides a composition
15 comprising a bacterial strain of the species *C. sana* or use in the treatment or prevention of psoriasis.

[0443] In some embodiments, bacterial strains from the species *C. sana* may provide therapeutic benefits in the treatment or prevention of systemic lupus erythematosus (SLE). In certain embodiments, the compositions of the invention are for use in the treatment or prevention of SLE in a subject. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *C. sana* for use in the treatment or prevention of SLE.
20

[0444] In some other embodiments, bacterial strains from the species *C. sana* may provide therapeutic benefits in the treatment or prevention of allograft rejection. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of allograft rejection in a subject. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *C. sana* for use in the treatment or prevention of allograft rejection.
25

[0445] In some embodiments, bacterial strains from the species *G. formicilis* may provide therapeutic benefits in the treatment or prevention of asthma, such as allergic asthma or neutrophilic asthma. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of asthma in a subject. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *G. formicilis*
30 for use in the treatment or prevention of asthma.
35

[0446] In some embodiments, bacterial strains from the species *G. formicilis* may provide therapeutic benefits in the treatment or prevention of GVHD. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of GVHD in a subject. In preferred embodiments, the invention provides a composition

comprising a bacterial strain of the species *G. formicilis* for use in the treatment or prevention of GVHD.

5 **[0447]** In some embodiments, bacterial strains from the species *G. formicilis* may provide therapeutic benefits in the treatment or prevention of arthritis, such as rheumatoid arthritis, osteoarthritis, psoriatic arthritis, or juvenile idiopathic arthritis. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of arthritis in a subject. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *G. formicilis* for use in the treatment or prevention of arthritis.

10 **[0448]** In some embodiments, bacterial strains from the species *G. formicilis* may provide therapeutic benefits in the treatment or prevention of multiple sclerosis. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of multiple sclerosis in a subject. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *G. formicilis* for use in the treatment or
15 prevention of multiple sclerosis.

[0449] In some embodiments, bacterial strains from the species *G. formicilis* may provide therapeutic benefits in the treatment or prevention of psoriasis. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of psoriasis in a subject. In certain embodiments, the invention provides a composition
20 comprising a bacterial strain of the species *G. formicilis* or use in the treatment or prevention of psoriasis.

[0450] In some embodiments, bacterial strains from the species *G. formicilis* may provide therapeutic benefits in the treatment or prevention of systemic lupus erythematosus (SLE). In certain embodiments, the compositions of the invention are for use in the
25 treatment or prevention of SLE in a subject. In certain embodiments, the invention provides a composition comprising a bacterial strain of the species *G. formicilis* for use in the treatment or prevention of SLE.

[0451] In some other embodiments, bacterial strains from the species
30 *G. formicilis* may provide therapeutic benefits in the treatment or prevention of allograft rejection. In certain embodiments, the compositions of the invention are for use in the treatment or prevention of allograft rejection in a subject. In certain other embodiments, the invention provides a composition comprising a bacterial strain of the species *G. formicilis* for use in the treatment or prevention of allograft rejection.

10. Formulations

35 **[0452]** In some embodiments, the compositions of the invention comprises fewer than 40 different bacterial strains. In some embodiments, the composition comprises fewer than 30 different bacterial strains. In some embodiments, the composition comprises fewer than 20 different bacterial strains. In some embodiments, the composition comprises fewer than 10 different bacterial strains. In some embodiments, the composition comprises fewer
40 than 5 different bacterial strains. In some preferred embodiments, the composition

comprises fewer than 3 different bacterial strains. In some preferred embodiments, the composition comprises a single bacterial strain. In some embodiments, the composition does not comprise bacteria of the genus *Clostridium*. In some of the same embodiments and some other embodiments, the composition does not comprise bacteria of the species *Alistipes*
5 *senegalensis*.

[0453] The compositions of the invention comprise bacteria (i.e., live bacteria and/or killed bacteria). In preferred embodiments of the invention, the composition is formulated in freeze-dried form. The composition of the invention may comprise granules or gelatin capsules, for example hard gelatin capsules, comprising a bacterial strain of the
10 invention. Preferably, the composition of the invention comprises lyophilised bacteria. Lyophilisation of bacteria is a well-established procedure and relevant guidance is available in, for example, references (Miyamoto-Shinohara, 2008; and Day & Stacey, 2007).

[0454] The composition of the invention may comprise a live, active bacterial culture. The examples demonstrate that cultures of the bacteria of the invention are
15 therapeutically effective.

[0455] 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
20 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
25 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.

[0456] In some embodiments, the composition comprises a mixture of live
30 bacterial strains and bacterial strains that have been killed. In preferred embodiments, the composition of the invention is encapsulated to enable delivery of the bacterial strain to the intestine. Encapsulation protects the composition from degradation until delivery at the target location through, for example, rupturing with chemical or physical stimuli such as pressure, enzymatic activity, or physical disintegration, which may be triggered by changes
35 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 widely available in the art
40 (for example, in Mitropoulou, 2013; and Kailasapathy, 2002).

[0457] The composition may be administered orally and may be in the form of a tablet, capsule or powder. Encapsulated products are preferred because bacteria of the genus *Alistipes* are obligate anaerobes.

5 **[0458]** 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.

10 **[0459]** A suitable daily dose of the bacteria, for example for an adult human, may be from about 1×10^3 to about 1×10^{11} colony forming units (CFU); for example, from about 1×10^7 to about 1×10^{10} CFU; in another example from about 1×10^6 to about 1×10^{10} CFU; in another example from about 1×10^7 to about 1×10^{11} CFU; in another example from about 1×10^8 to about 1×10^{10} CFU; in another example from about 1×10^8 to about 1×10^{11} CFU.

15 **[0460]** In certain embodiments, the dose of the bacteria is at least 10^9 cells per day, such as at least 10^{10} , at least 10^{11} , or at least 10^{12} cells per day.

[0461] In certain embodiments, a dose of the composition may comprise the bacterial strain in an amount of from about 1×10^6 to about 1×10^{11} colony forming units (CFU)/g, respect to the weight of the composition. The dose may be suitable for an adult
20 human. For example, the composition may comprise the bacterial strain from about 1×10^3 to about 1×10^{11} CFU/g; for example, from about 1×10^7 to about 1×10^{10} CFU/g; in another example from about 1×10^6 to about 1×10^{10} CFU/g; in another example from about 1×10^7 to about 1×10^{11} CFU/g; in another example from about 1×10^8 to about 1×10^{10} CFU/g; in another example from about 1×10^8 to about 1×10^{11} CFU/g, from about 1×10^8 to about 1×10^{10} CFU/g. For example, from about 1×10^8 to about 1×10^{10} CFU/g. The
25 dose may be, for example, up to or over 1 g, 3 g, 5 g, and 10 g.

[0462] In some embodiments, the compositions described above and/or elsewhere herein comprise, consist, or consist essentially of an amount of bacterial strain from about 1×10^3 to about 1×10^{11} colony forming units per gram with respect to a weight
30 of the composition.

[0463] In some embodiments, the compositions described above and/or elsewhere herein comprise the bacterial strain at a dose of between 500 mg and 1000 mg, between 600 mg and 900 mg, between 700 mg and 800 mg, between 500 mg and 750 mg or between 750 mg and 1000 mg. In certain embodiments, the invention provides the above
35 pharmaceutical composition, wherein the lyophilised bacteria in the pharmaceutical composition is administered at a dose of between 500 mg and 1000 mg, between 600 mg and 900 mg, between 700 mg and 800 mg, between 500 mg and 750 mg, or between 750 mg and 1000 mg.

[0464] The composition may be formulated as a probiotic. A probiotic is defined by the FAO/WHO as a live microorganism that, when administered in adequate amounts, confers a health benefit on the host.

[0465] Typically, a probiotic, such as the composition of the invention, is
5 optionally combined with at least one suitable prebiotic compound. A prebiotic compound is usually a non-digestible carbohydrate such as an oligosaccharide 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 transgalactoligosaccharides.

[0466] Other prebiotic compounds (such as vitamin C, for example), may be
10 included as oxygen scavengers and 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.

[0467] In certain embodiments, the probiotic composition of the present
15 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). Known prebiotics include commercial products such as inulin and transgalactoligosaccharides.

[0468] In some embodiments, the prebiotic is a carbohydrate selected from the group comprising or consisting of fructooligosaccharides (or FOS), short-chain
20 fructooligosaccharides, inulin, isomaltoligosaccharides, pectins, xylooligosaccharides (or XOS), chitosan oligosaccharides (or COS), beta-glucans, arable gum modified and resistant starches, polydextrose, tagatose, acacia fibers, carob, oats, and citrus fibers. In one aspect, the prebiotics are the short-chain fructooligosaccharides. Short-chain FOS are not digestible carbohydrates, generally obtained by the conversion of the beet sugar and including a
25 saccharose molecule to which three glucose molecules are bonded.

[0469] The compositions of the invention may comprise pharmaceutically acceptable excipients or carriers, such as those described in Handbook of Pharmaceutical Excipients. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art and are described, for example, in Remington's Pharmaceutical Sciences.
30 Examples of suitable carriers include lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol and the like. Examples of suitable diluents include ethanol, glycerol and water. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as, or in addition to, the carrier, excipient or
35 diluent one or more suitable binders, lubricants, suspending agents, coating agents, and/or solubilising agents. Examples of suitable binders include starch, gelatin, natural sugars such as glucose, anhydrous lactose, free-flow lactose, β -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,
40 magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Preservatives, stabilizers, dyes and even flavouring agents may be provided in the

pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid, cysteine and esters of 4-hydroxybenzoic acid, for example, in some embodiments the preservative is selected from sodium benzoate, sorbic acid and esters of 4-hydroxybenzoic acid. Antioxidants and suspending agents may be also used. A further example of a suitable carrier is saccharose. A further example of a suitable preservative is cysteine.

[0470] 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-based 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. Alternatively, the milk could be a plant-based milk, including for example, soy milk, oat milk, almond milk, coconut milk, or macadamia milk. 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.

[0471] In some embodiments, the compositions disclosed herein comprise one or more bacterial strains of the genus *Alistipes* and do not contain bacteria from any other species, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another species. Thus, in some embodiments, the invention provides a composition comprising one or more bacterial strains of the genus *Alistipes* (e.g., *Alistipes shahii*), which does not contain bacteria from any other species or which comprises only *de minimis* or biologically irrelevant amounts of bacteria from another species, for use in therapy.

[0472] In some embodiments, the compositions comprise one or more bacterial strains of the genus *Alistipes* and do not contain bacteria from any other genus or comprise only *de minimis* or biologically irrelevant amounts of bacteria from another. In some embodiments, the compositions comprise one or more bacterial strains of the genus *Alistipes* (e.g., *Alistipes shahii*) and do not contain bacteria from any other genus or comprise only *de minimis* or biologically irrelevant amounts of bacteria from another.

[0473] In certain embodiments, the compositions disclosed herein contain a single bacterial species and do not contain any other bacterial species. In certain embodiments, the compositions disclosed herein contain a single bacterial strain and do not contain any other bacterial strains. For example, the compositions of the invention may comprise bacteria only of a strain of *A. shahii*. Such compositions may comprise only *de minimis* or biologically irrelevant amounts of other bacterial strains or species. Such compositions may be a culture that is substantially free from other species of organism. In

some embodiments, such compositions may be a in a dried form and be substantially free from other species of organism.

[0474] In some embodiments, the invention provides a composition comprising a single bacterial strain of the genus *Alistipes* 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. In some embodiments, the invention provides a composition comprising a single bacterial strain of the species *Alistipes shahii* (e.g., *Alistipes shahii* MH21-1, *Alistipes shahii* MH21-2, or *Alistipes shahii* MH21-3) and 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.

[0475] In some embodiments, the compositions disclosed herein comprise one or more bacterial strains of the genus *Colonithrix* and do not contain bacteria from any other species, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another species. Thus, in some embodiments, the invention provides a composition comprising one or more bacterial strains of the genus *Colonithrix* (e.g., *C. sana*), which does not contain bacteria from any other species or which comprises only *de minimis* or biologically irrelevant amounts of bacteria from another species, for use in therapy.

[0476] In some embodiments, the compositions comprise one or more bacterial strains of the genus *Colonithrix* and do not contain bacteria from any other genus or comprise only *de minimis* or biologically irrelevant amounts of bacteria from another. In some embodiments, the compositions comprise one or more bacterial strains of the genus *Colonithrix* and do not contain bacteria from any other genus or comprise only *de minimis* or biologically irrelevant amounts of bacteria from another.

[0477] In certain embodiments, the compositions disclosed herein contain a single bacterial species and do not contain any other bacterial species. In certain embodiments, the compositions disclosed herein contain a single bacterial strain and do not contain any other bacterial strains. For example, the compositions of the invention may comprise bacteria only of a strain of *C. sana*. Such compositions may comprise only *de minimis* or biologically irrelevant amounts of other bacterial strains or species. Such compositions may be a culture that is substantially free from other species of organism. In some embodiments, such compositions may be a in a dried form and be substantially free from other species of organism.

[0478] In some embodiments, the invention provides a composition comprising a single bacterial strain of the genus *Colonithrix* 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.

[0479] In some embodiments, the invention provides a composition comprising a single bacterial strain of the species *Colonithrix sana* (e.g., *C. sana* MH35-1, or *C. sana* MH35-2) and 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.

5 **[0480]** In some embodiments, the compositions disclosed herein comprise one or more bacterial strains of the genus *Gemmiger* and do not contain bacteria from any other species, or which comprise only de *minimis* or biologically irrelevant amounts of bacteria from another species. Thus, in some embodiments, the invention provides a composition comprising one or more bacterial strains of the genus *Gemmiger* (e.g., *Gemmiger formicilis*), which does not contain bacteria from any other species or which comprises only de *minimis* or biologically irrelevant amounts of bacteria from another species, for use in therapy.

10 **[0481]** In some embodiments, the compositions comprise one or more bacterial strains of the genus *Gemmiger* and do not contain bacteria from any other genus or comprise only de *minimis* or biologically irrelevant amounts of bacteria from another. In some embodiments, the compositions comprise one or more bacterial strains of the genus *Gemmiger* (e.g., *Gemmiger formicilis*) and do not contain bacteria from any other genus or
15 comprise only de *minimis* or biologically irrelevant amounts of bacteria from another.

[0482] In certain embodiments, the compositions disclosed herein contain a single bacterial species and do not contain any other bacterial species. In certain embodiments, the compositions disclosed herein contain a single bacterial strain and do not contain any other bacterial strains. For example, the compositions of the invention may
20 comprise bacteria only of a strain of *G. formicilis*. Such compositions may comprise only de *minimis* or biologically irrelevant amounts of other bacterial strains or species. Such compositions may be a culture that is substantially free from other species of organism. In some embodiments, such compositions may be a in a dried form and be substantially free from other species of organism.

25 **[0483]** In some embodiments, the invention provides a composition comprising a single bacterial strain of the genus *Gemmiger* 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. In some embodiments, the invention provides a composition comprising a single bacterial strain of the species *Gemmiger* (e.g., *Gemmiger*
30 formicilis MH32-1) and 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.

[0484] 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
35 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.

[0485] In certain embodiments, the compositions of the invention consist of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 bacterial strains or species. In certain
40 embodiments, the compositions consist of from 1 to 10, preferably from 1 to 5 bacterial

strains or species. In some embodiments, the compositions disclosed herein 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 disclosed herein 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 disclosed herein comprise 1-40, 1-30, 1-20, 1-19, 1-18, 1-15, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-50, 2-40, 2-30, 2-20, 2-15, 2-10, 2-5, 6-30, 6-15, 16-25, or 31-50 strains from within the same species and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions disclosed herein comprise more than one species from within the same genus (e.g., more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, 23, 25, 30, 35 or 40 species), and, optionally, do not contain bacteria from any other genus. In some embodiments, the compositions disclosed herein comprise less than 50 species from within the same genus (e.g., less than 50, 45, 40, 35, 30, 25, 20, 15, 12, 10, 8, 7, 6, 5, 4 or 3 species), and, optionally, do not contain bacteria from any other genus. In some embodiments, the compositions disclosed herein comprise 1-50, 1-40, 1-30, 1-20, 1-15, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-50, 2-40, 2-30, 2-20, 2-15, 2-10, 2-5, 6-30, 6-15, 16-25, or 31-50 species from within the same genus and, optionally, do not contain bacteria from any other genus. The invention comprises any combination of the foregoing.

[0486] In some embodiments, the compositions of the invention comprise more than one bacterial strain or species. For example, in some embodiments, the compositions of the invention comprise more than one strain from within the same species (e.g., more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40 or 45 strains), and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise less than 50 strains from within the same species (e.g., less than 45, 40, 35, 30, 25, 20, 15, 12, 10, 9, 8, 7, 6, 5, 4 or 3 strains), and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise 1-40, 1-30, 1-20, 1-19, 1-18, 1-15, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-50, 2-40, 2-30, 2-20, 2-15, 2-10, 2-5, 6-30, 6-15, 16-25, or 31-50 strains from within the same species and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise more than one species from within the same genus (e.g., more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, 23, 25, 30, 35 or 40 species), and, optionally, do not contain bacteria from any other genus. In some embodiments, the compositions of the invention comprise less than 50 species from within the same genus (e.g., less than 50, 45, 40, 35, 30, 25, 20, 15, 12, 10, 8, 7, 6, 5, 4 or 3 species), and, optionally, do not contain bacteria from any other genus. In some embodiments, the compositions of the invention comprise 1-50, 1-40, 1-30, 1-20, 1-15, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-50, 2-40, 2-30, 2-20, 2-15, 2-10, 2-5, 6-30, 6-15, 16-25, or 31-50 strains from within the same genus and, optionally, do not contain bacteria from any other genus. The invention comprises any combination of the foregoing.

[0487] In certain embodiments, the pharmaceutical composition of the invention comprises between 1-50 distinct bacterial strains, such as between 1-50, 1-40, 1-30, 1-20, 1-19, 1-18, 1-17, 1-16, 1-15, 1-14, 1-13, 1-12, 1-11, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3 or 2 distinct bacterial strains. In certain embodiments, the pharmaceutical composition of the invention comprises between 1-50 distinct bacterial strains, such as between 1-50, 1-40, 1-30, 1-20, 1-19, 1-18, 1-17, 1-16, 1-15, 1-14, 1-13, 1-12, 1-11, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3 or 2 distinct bacterial strains.

[0488] In some embodiments, the composition of the invention additionally comprises a bacterial strain that has the same safety and therapeutic efficacy characteristics as a strain deposited at the NMI (Australia) under accession no. V21/014432, V21/014433, and/or V21/014434.

[0489] In some embodiments, the composition of the invention additionally comprises a bacterial strain that has the same safety and therapeutic efficacy characteristics as a strain deposited at the NMI (Australia) under accession no. V21/019213 and/or V21/019214.

[0490] In some other embodiments, the composition of the invention additionally comprises a bacterial strain that has the same safety and therapeutic efficacy characteristics as a strain deposited at the NMI (Australia) under accession no. V21/011520.

[0491] 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.

[0492] Preferably, the compositions disclosed herein are to be administered to the gastrointestinal (GI) tract in order to enable delivery to, and/or partial or total colonisation of, the intestine with the bacterial strain of the invention. In other words, the bacteria may colonise some or all of the GI tract and such colonisation may be transient or permanent. More specifically, the phrase "*total colonisation of the intestine*" means that bacteria have colonised all parts of the intestine (*i.e.*, the small intestine, large intestine and rectum). Additionally or alternatively, the term "*total colonisation*" means that the bacteria engraft permanently in some or all parts of the intestine.

[0493] Similarly, the phrase "*partial colonisation of the intestine*" means that bacteria have colonised some but not all parts of the intestine. Additionally or alternatively, the term "*partial colonisation*" means that the bacteria engraft transiently in some or all parts of the intestine.

[0494] The transience of engraftment of bacteria can be determined by assessing (*e.g.*, in a fecal sample) the abundance of the bacterial strain of the invention periodically

(e.g., daily or weekly) following the end of a dosing interval to determine the washout period, i.e., the period between conclusion of the dosing interval and there being no detectable levels of the bacterial strain of the invention present. In some embodiments, the washout period is 14 days or less, 12 days or less, 10 days or less, 7 days or less, 4 days or less, 3 days or less, 2 days or less, or 1 day or less.

[0495] In some embodiments, the bacteria described above or elsewhere herein engraft transiently in the large intestine.

[0496] In some embodiments, the bacterial strains of the invention are 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 these human adult faeces and being used in a composition of the invention.

[0497] In some embodiments, the one or more *Alistipes* bacterial strain is/are the only therapeutically active agents in a composition of the invention. In some embodiments, the bacterial strains in the composition is/are the only therapeutically active agents in a composition of the invention.

[0498] The compositions for use in accordance with the invention may or may not require marketing approval.

[0499] In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is in a dried form. In some cases, the bacterial strain is reconstituted prior to administration. In some cases, the reconstitution is by use of a diluent described herein. 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 dried (e.g., lyophilised or spray dried) and wherein it is viable and capable of partially or totally colonising the intestine. In some of the same embodiments and some alternative embodiments, the bacterial strain transiently colonises the intestine.

[0500] 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.

[0501] The compositions of the invention can comprise pharmaceutically acceptable excipients, diluents or carriers.

[0502] In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain of the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat or prevent an inflammatory or autoimmune disorder when administered to a subject in need thereof. In some preferred embodiments, the inflammatory or autoimmune disorder is selected from the group comprising: an inflammatory bowel disease (such as Crohn's disease or ulcerative colitis); asthma (such as allergic asthma or neutrophilic asthma); arthritis (such as rheumatoid arthritis, osteoarthritis, psoriatic arthritis, or juvenile idiopathic arthritis); fatty liver disease (such as nonalcoholic fatty liver disease (NAFLD)); ankylosing spondylitis; psoriasis; systemic lupus erythematosus (SLE); scleroderma; Sjogren's syndrome; vasculitis; and type 1 diabetes mellitus.

[0503] In certain embodiments, the invention provides pharmaceutical composition comprising: a bacterial strain of the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat or prevent an inflammatory or autoimmune disorder mediated by the STAT3 signalling pathway. In preferred embodiments, said disorder is selected from the group consisting of an inflammatory bowel disease (such as Crohn's disease or ulcerative colitis); asthma (such as allergic asthma or neutrophilic asthma); arthritis (such as rheumatoid arthritis, osteoarthritis, psoriatic arthritis, or juvenile idiopathic arthritis); fatty liver disease (such as nonalcoholic fatty liver disease (NAFLD)); ankylosing spondylitis; psoriasis; systemic lupus erythematosus (SLE); scleroderma; Sjogren's syndrome; vasculitis; and type 1 diabetes mellitus.

[0504] In certain embodiments, the invention provides the above pharmaceutical composition, wherein the amount of the bacterial strain is from about 1×10^3 to about 1×10^{11} colony forming units (CFU) per gram with respect to a weight of the composition.

[0505] In certain embodiments, the invention provides the above pharmaceutical composition, wherein the composition is administered at a dose of up to or over 1 g, 3 g, 5 g or 10 g.

[0506] 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.

[0507] 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.

[0508] In certain embodiments, the invention provides the above pharmaceutical composition, comprising a diluent selected from the group consisting of ethanol, glycerol and water.

[0509] 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.

5 **[0510]** In certain embodiments, the invention provides the above pharmaceutical composition, further comprising at least one of a preservative, an antioxidant and a stabilizer.

[0511] 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 4-hydroxybenzoic acid.

10 **[0512]** In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is in a dried form (e.g., lyophilised, spray dried, fluidized bed dried, etc.).

[0513] 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
15 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.

[0514] In some embodiments, the composition of the invention is provided in a
20 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.

[0515] The composition of the present invention may, in some embodiments, be provided as a pharmaceutical formulation. For example, the composition may be provided as
25 a tablet or capsule. In some embodiments, the capsule is a gelatine capsule ("gel-cap"). The capsule can be a hard or a soft capsule. 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
30 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
35 process.

[0516] In some embodiments, the compositions disclosed herein are administered orally. Oral administration may involve swallowing, so that the compound enters the GI tract.

[0517] Pharmaceutical formulations suitable for oral administration include solid plugs, solid microparticulates, semi-solid and liquid (including multiple phases or dispersed
40 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.

5 **[0518]** 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).

10 **[0519]** 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-
15 insoluble polymer).

20 **[0520]** 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
25 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 (as described in United States Patent Publication No. 2016/0067188). In some embodiments, the
30 formulation is an intrinsically enteric capsule (for example, VCAPS® from Capsugel).

35 **[0521]** In some embodiments, the composition is a probiotic or a medical food comprising a bacterial strain of *A. shahii*. The bacteria can be administered, for instance, as a probiotic, as a capsule, tablet, caplet, pill, troche, lozenge, power, and/or granule. This strain can also be formulated as a nutraceutical, conventional food, medical food, or drug. The bacteria can also be administered as part of a fecal transplant or via suppository. In some
40 embodiments, the composition is formulated for delivery to the gut, as described further herein, in some embodiments had the composition further comprise a prebiotic.

Co-administering with additional agents

35 **[0522]** In some embodiments, the methods described herein can further comprise co-administering a second agent and/or treatment to the subject (e.g., as part of a therapy). The combination therapy, where employed, can be tailored to the particular indication. For example, where a strain of the species *A. shahii* is administered to treat an inflammatory disorder (e.g., an inflammatory bowel disease), it can be administered in combination with
40 an anti-inflammatory agent or therapy as known in the art of approved for clinical treatment

of an inflammatory disorder. Other indications can be similarly treated with, for example, strains of the species *A. shahii* as described herein in combination with agents known in the art or approved for the clinical treatment of those indications.

5 **[0523]** Similarly, where a strain of the species *C. sana* is administered to treat an inflammatory disorder (e.g., an inflammatory bowel disease), it can be administered in combination with an anti-inflammatory agent or therapy as known in the art of approved for clinical treatment of an inflammatory disorder. Other indications can be similarly treated with, for example, strains of the species *C. sana* as described herein in combination with agents known in the art or approved for the clinical treatment of those indications.

10 **[0524]** Similarly, where a strain of the species *G. formicilis* is administered to treat an inflammatory disorder (e.g., an inflammatory bowel disease), it can be administered in combination with an anti-inflammatory agent or therapy as known in the art of approved for clinical treatment of an inflammatory disorder. Other indications can be similarly treated with, for example, strains of the species *G. formicilis* as described herein in combination with
15 agents known in the art or approved for the clinical treatment of those indications.

[0525] Suitable anti-inflammatory agents that could be used in the treatment of an inflammatory bowel disease include, but not necessarily limited are, the group comprising 5-aminosalicylates, corticosteroids, azathioprine, infliximab, and adalimumab.

[0526] The present invention also includes the compositions as described above,
20 further comprising an anti-inflammatory agent. Such compositions can optionally be in the form of a single composition, or alternatively, two of more separate compositions.

11. Screening methods

[0527] The invention also includes methods of identifying bacterial strains that are suitable for use in the methods of the present invention. Such methods typically include
25 screening for a bacterial strain with a particular functional activity. Suitable assays include those described in the below examples, but any assay for measuring gut barrier function, mucosal healing, modulation of NF- κ B activation, or modulation of STAT3 signalling are equally as applicable.

[0528] In some embodiments, the screening method identifies the ability of a
30 bacterial strain of *Alistipes* to modulate STAT3 signalling pathway. By way of an illustrative example, the invention provides a method of blocking or otherwise inhibiting the activation of STAT3 signalling in a target cell, the method comprising contacting the target cell with at least a soluble component of a bacterial cell preparation of the species *Alistipes shahii*, to block or otherwise inhibit the activation of STAT3 signalling in the target cell.

[0529] In some embodiments, the screening method identifies the ability of a
35 bacterial strain of *Colonithrix* to modulate STAT3 signalling pathway. By way of an illustrative example, the invention provides a method of blocking or otherwise inhibiting the activation of STAT3 signalling in a target cell, the method comprising contacting the target cell with at least a soluble component of a bacterial cell preparation of the specie *C. sana*, to block or
40 otherwise inhibit the activation of STAT3 signalling in the target cell.

[0530] In some embodiments, the screening method identifies the ability of a bacterial strain of *Gemmiger* to modulate STAT3 signalling pathway. By way of an illustrative example, the invention provides a method of blocking or otherwise inhibiting the activation of STAT3 signalling in a target cell, the method comprising contacting the target cell with at least a soluble component of a bacterial cell preparation of the species *G. formicilis*, to block or otherwise inhibit the activation of STAT3 signalling in the target cell.

[0531] In some embodiments of this type, the target cell is selected from the group comprising screening a bacterial strain for a functional reporter cell (e.g., a HEK cell), an immune cell (e.g., a Th17 immune cell), an epithelial cell, and an endothelial cell.

10 [0532] In some embodiments, the bacterial cell preparation comprises a bacterial cell culture. Suitably, the soluble component may comprise the supernatant of the bacterial cell culture. In some embodiments of this type, the soluble component is substantially depleted of bacterial cells.

15 [0533] In some alternative embodiments, the bacterial cell preparation comprises a bacterial cell pellet. Preferably, the bacterial cells of the cell pellet are lysed by any means known in the art. After cell lysis, it is typical for the cell lysate soluble fraction to be separated from the insoluble fraction. The cell lysate may be subject to further processing before being during the screening assay. (e.g., diluted in a buffer), or exposed to a processing reagent.

20 **12. Modes of administration**

[0534] Preferably, the compositions of the invention are to be administered to the GI tract in order to enable delivery to the intestine with the bacterial strain of the invention. Preferably, the compositions of the invention are formulated to be administered to the GI tract in order to enable delivery to the intestine with the bacterial strain of the invention. In some embodiments the compositions of the invention are formulated to be administered to the GI tract in order to enable delivery to, and partial or total colonization of, the intestine with the bacterial strain of the invention.

[0535] In certain embodiments, the compositions of the invention may be administered as a foam, as a spray or a gel.

30 [0536] In certain embodiments, the compositions of the invention may be administered as a suppository, such as a rectal suppository, for example in the form of a theobroma oil (cocoa butter), synthetic hard fat (e.g., suppocire®, WITEPSOL), glycerogelatin, polyethylene glycol, or soap glycerin composition.

[0537] In certain embodiments, the compositions of the invention are administered to the GI 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.

[0538] 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 (either once or several times). In certain embodiments, the compositions disclosed herein are administered regularly, such as daily, every two days, or weekly, for an extended period of time, such as for at least one week, two weeks, one month, two months, six months, or one year.

[0539] In some embodiments, the compositions disclosed herein are administered for 7 days, 14 days, 16 days, 21 days or 28 days or no more than 7 days, 14 days, 16 days, 21 days, or 28 days. For example, in some embodiments the compositions disclosed herein are administered for 16 days.

[0540] 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.

[0541] 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 disorder (such as those disclosed herein) developing in her child *in utero* and/or after it is born.

[0542] The compositions of the invention may be administered to a patient that has been diagnosed with: a disease or condition mediated by gut barrier function dysregulation; or that has been identified as being at risk of a disease or condition mediated by gut barrier dysfunction; a disease or condition mediated by the STAT3 signalling pathway, or that has been identified as being at risk of a disease or condition mediated by the STAT3 signalling pathway; or an inflammatory or autoimmune disorder (such as those disclosed herein). The compositions may also be administered as a prophylactic measure to prevent the development of diseases or conditions mediated by the STAT3 signalling pathway in a healthy patient.

[0543] The compositions disclosed herein may be administered to a patient that has been diagnosed with an inflammatory or autoimmune disorder, in particular an inflammatory or autoimmune disorder mediated by the microbiota-gut axis, or that has been identified as being at risk of an inflammatory or autoimmune disorder, in particular an inflammatory or autoimmune disorder mediated by the microbiota-gut axis. The compositions may also be administered as a prophylactic measure to prevent the development of inflammatory or autoimmune disorders, in particular inflammatory or autoimmune disorders mediated by the microbiota-gut axis in a healthy patient.

[0544] 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 bacteria of the genus *Alistipes*, in particular *A. shahii*. Alternatively, or in addition, the patient may have reduced or absent colonisation by bacteria

of the genus *Colonithrix*, in particular *C. sana*. Alternatively or in addition, the patient may have reduced or absent colonisation by bacteria of the genus *Gemmiger*, in particular *G. formicilis*.

[0545] The compositions of the invention may be administered as a food product,
5 such as a nutritional supplement.

[0546] Generally, the compositions of the invention are for the prevention or treatment of human diseases, 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
10 administered to animals, oral gavage may be used.

[0547] In some embodiments, the subject to whom the composition is to be administered is an adult human. In some embodiments, the subject to whom the composition is to be administered is an infant human.

13. Culturing methods

[0548] The bacterial strains for use in the present invention can be cultured using standard microbiology techniques as detailed in, for example, reference as taught in
15 McSweeney, 2005.

[0549] The solid or liquid medium used for culture may, for example, be selected from TY or PYG medium.

[0550] Illustrative media formulations that are suitable for use with the present
20 invention include those provided in Table 1.

TABLE 1
Culture Media Formulations

	As_v1	TY	PYG	PYGM	YG/V
Tryptone	15 g	10 g	20 g	20 g	
Soy Peptone	5 g				
Vegetable tryptone					20g
Yeast extract	5 g	2.5 g	10 g	10 g	10 g
Glucose	2.5g	4 g	10 g	10 g	10 g
Raffinose	2.5 g				
Cellobiose		1 g	---		
Maltose		1 g	---		
Sodium chloride	5 g				
Hemin solution	10 mL	1 ml	1 mL		
Menadione (10 mg/ml)	0.1 mL			1 mL	
Salts 2*	38 mL	38 mL	38 mL	38 mL	38 mL
Salts 3*	38 mL	38 mL	38 mL	38 mL	38 mL

Sodium bicarbonate	8 g	8 g	8 g	8 g	8 g
Resazurin	1 mL	1 mL	1 mL	1 mL	1 mL
Acetic acid		1.9 mL			
Cysteine	1 g	1 g	1 g	1 g	1 g
Water	to 1000 mL	to 1000 mL	to 1000 mL	to 1000 mL	to 1000 mL
* Salts 2: K ₂ HPO ₄ 6 g/L					
* Salts 3: KH ₂ PO ₄ 6 g/L, (NH ₄) ₂ SO ₄ 6 g/L, NaCl 12 g/L, MgSO ₄ ·7H ₂ O 2.5 g/L, CaCl ₂ ·2H ₂ O 1.6 g/L					

[0551] In order that the invention may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting experimental examples.

5

EXAMPLES

EXAMPLE 1

Alistipes association with health, IBD and other diseases.

[0552] Inflammatory bowel disease is characterised by structure-function changes to the microbiome with a significant reduction in both the prevalence and abundance of select gut bacteria in the IBD gut when compared to the healthy gut. Several studies have shown that these bacteria may modulate IBD pathogenesis (Mallone et al., 2011; and Sokol et al., 2008) however a key obstacle to using these bacteria to develop new therapeutics has been that low resolution 16S rRNA based profiling do not provide sufficient resolution to accurately discriminate against health and IBD associated strains at a low taxonomic level (i.e., genus, species, strain).

[0553] The inventors used the Microba Disease Database (MDD), which contains high resolution faecal gut metagenomic data and associated host metadata for 6,020 adults, to study the prevalence of species in the *Alistipes* "Clade A" (as shown in Figure 1) in inflammatory and autoimmune diseases. Metagenomic sequence reads were analysed using the Microba Community Profiler (MCP) (Parks et al., 2021).

[0554] At least five *Alistipes* Clade A species were detected in more than 10% of MDD samples: *A. shahii*, *A. finegoldii*, *A. senegalensis*, *A. onderdonkii* and the uncultured species *A. sp000434235*.

[0555] The inventors found that four detected *Alistipes* Clade A species are prevalent in healthy humans but rarely detected in inflammatory and autoimmune diseases (Figure 2A-E). The strongest effect was observed for IBD, including both major subtypes ulcerative colitis and Crohn's disease (Figure 2, and Table 2, below). All five *Alistipes* species (*A. shahii*, *A. finegoldii*, *A. onderdonkii*, *A. senegalensis*, and *A. sp000434235*) were significantly less prevalent in IBD as compared to healthy (Figure 2A, Table 2). The strongest reduction in IBD was observed for *A. shahii* (Figure 2A, Table 2). These observations were

validated in an independent IBD cohort previously published by Franzosa et al., (2018) (Figure 2B, Table 2).

- 5 **[0556]** *A. shahii*, *A. finegoldii* and *A. senegalensis* were significantly less prevalent in a range of inflammatory and autoimmune conditions compared to healthy controls (Figure 2C-E and Table 3).

TABLE 2**ASSOCIATION STATISTICS BETWEEN *ALISTIPES* SPECIES AND IBD**

Taxon	Prevalence (%)						P Cochran-Mantel-Haenszel test
	Control MDD	CD MDD	UC MDD	Control Harvard	CD Harvard	UC Harvard	
<i>A. shahii</i>	79.8	48.8	51.2	63.0	14.9	26.2	1.90×10^{-15}
<i>A. finegoldii</i>	66.9	34.9	46.3	45.7	17.9	23.0	8.00×10^{-09}
<i>A. senegalensis</i>	37.8	14.0	17.1	13.0	3.0	8.2	2.40×10^{-06}
<i>A. onderdonkii</i>	73.4	62.8	56.1	52.2	20.9	45.9	0.00017
<i>A. sp000434235</i>	14.6	11.6	4.9	4.3	0.0	3.3	0.019

TABLE 3**ASSOCIATION BETWEEN *ALISTIPES* SPECIES AND INFLAMMATORY DISEASES**

Condition	P Fisher's Exact Test				
	<i>Alistipes shahii</i>	<i>Alistipes finegoldii</i>	<i>Alistipes senegalensis</i>	<i>Alistipes onderdonkii</i>	<i>Alistipes sp000434245</i>
Asthma	0.0016	0.007	2.0×10^{-5}	--	--
Other autoimmune conditions affecting the joints	0.027	0.039	0.012	--	--
Psoriatic arthritis	--	0.048	--	--	--
Rheumatoid arthritis	0.026	--	0.00085	--	--
Fatty liver	0.026	--	0.0033	--	--
IBD	2.3×10^{-9}	1.9×10^{-6}	3.03×10^{-6}	0.0021	0.026
Crohn's disease	3.0×10^{-5}	7.6×10^{-5}	0.002	--	--
Ulcerative colitis	4.3×10^{-7}	0.00088	0.00027	0.0031	0.016
Non-alcohol fatty liver disease	3.5×10^{-5}	--	0.014	--	--
Systemic autoimmune disease	6.6×10^{-5}	0.0049	0.011	--	--

Type 1 diabetes mellitus	--	0.011	--	--	--
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Isolation and Genome-Scale Analysis of *A. shahii*

[0557] To better understand the role of *A. shahii* in health and the pathogenesis of IBD *A. shahii* MH21-1, MH21-2 and MH21-3 strains were isolated from healthy human
5 donors. *A. shahii* grew well on As_v1 and PYG media and was typically observed as a Gram-negative staining short rod (Figure 3A).

[0558] Phylogenetic reconstruction of the *Alistipes* genus using high-quality GenBank and RefSeq genomes from the NCBI and *A. shahii* MH21-1, MH21-2, and MH21-3 revealed the isolates placed with high confidence within the *Alistipes shahii* clade, close to
10 several uncultured species (Figure 3B). *A. shahii* MH21-1, MH21-2, and MH21-3 are sugar fermenters, and are predicted to utilize starch, glucose, gluconate, lactose, melibiose, and galactose as a carbon source. Complete, or near complete biosynthetic pathways were identified for most amino acids except tryptophan, aspartate, cysteine, and tyrosine. Several
15 pathways for the uptake and fermentation of amino acids (Ala, Try, Asp, Cys, Glu, Gln, His, and Met) were also identified, but are likely not primary sources of energy. Metabolic modelling of the *A. shahii* isolate revealed that when fed with glucose and other hexose sugars, primary by-products of fermentation are acetate and propionate. Acetate is predicted to be the major by-product of fermentation by *A. shahii* MH21-1, MH21-2, and MH21-3 isolates. The biosynthetic potential of MH21-1 was assessed by searching for well
20 characterised, and putative biosynthetic gene clusters (BGCs) using AntiSmash v5.0 and deepBGC, respectively. AntiSmash did not identify any BGCs, but DeepBGC identified the 3 unclassified BGC with >1 coding region and >0.75 deepBGC score, with two predicted to produce antibacterial products.

A. shahii Enhances Gut Barrier Function.

[0559] To assess the role of *A. shahii* in the healthy gut, naive C57Bl/6 SPF mice
25 were treated for 8 days with *A. shahii* MH21-1 (Figure 4A). During this treatment period, there was no morbidity, and no changes in general appearance, behaviour, posture, mobility and neurological behaviour as compared to vehicle control, were observed. Similarly, there was no significant change in body weight in *A. shahii* MH21-1 treated animals relative to the
30 vehicle control and colon length and weight/length ratio were also unaffected (Figures 4B-D). *A. shahii* MH21-1 did not result in any significant histological changes in the colon when compared to the vehicle as determined by assessing epithelial injury, inflammation and hypervascularization alone, or as a combined histopathological score (Figure 4E-H).

[0560] It was hypothesised that the big-data approach described above could be
35 used to identify novel candidate live biotherapeutics for inflammatory and autoimmune diseases (e.g., IBD). The inventors therefore tested the therapeutic efficacy of *A. shahii* MH21-1 in an acute model of DSS induced murine colitis (Figure 5A) as this is a well-documented model of epithelial injury and repair. DSS treatment resulted in significant disease activity relative to the vehicle control. There was a significant reduction in body

weight (Figure 5B) which has been shown to be an accurate and reliable indicator of colitis (Britto et al., 2019). As expected, prednisone exacerbated the DSS induced weight loss (Yamamoto et al., 2013), however DSS induced weight loss was ameliorated by treatment with *A. shahii* MH21-1 or *F. prausnitzii* A2-165 (Figure 5B).

5 **[0561]** Histological analysis of DSS treated mice revealed significant gut damage characterised by crypt loss, epithelial erosion and ulceration. Notably, treatment with *A. shahii* MH21-1 resulted in a significant improvement in pathology characterised by crypt re-formation and re-epithelisation (Figure 5C) as evidenced by improvements in histopathological healing (Figure 5D) and epithelial injury (Figure 5E). Treatment with
10 *A. shahii* MH21-1 also resulted in a significant increase in goblet cells and intraepithelial lymphocytes relative to the vehicle treated control (Figure 5F-G). As expected, prednisone also resulted in a significant improvement in disease pathology.

[0562] The IL-23-Th17 cell immune axis is central to the pathogenesis of IBD and is a validated therapeutic target (Martin, et al., 2017). As the DSS model of gut barrier
15 function is largely underpinned by Th1 polarised immunity (Marks et al., 2015) the therapeutic efficacy of *A. shahii* MH21-1 in the murine SKG model was also tested (Figure 5H). SKG mice carry a mutation in the ZAP-70 gene and develop IL-23 driven Crohn's like ileitis following disease initiation with curdlan treatment (Figure 4I, (Liu et al., 2019)). Histological analysis of vehicle treated mice revealed significant gut damage characterised by
20 infiltration of inflammatory cells and granuloma formation 7 days after curdlan treatment. As expected, treatment with the anti-IL-23 monoclonal antibody resulted in a significant reduction in histological damage. Treatment with *A. shahii* MH21-1 also resulted in significant improvement in pathology as evidenced by improvements in the histopathological score (Figure 5I). Similarly, treatment with *A. shahii* MH21-1 also resulted in significant reduction
25 in the production of cytokines central to disease pathogenesis including IL-23, IL-12 (IL-12p70), IL-6 and GM-CSF (Figure 5J-M).

[0563] Taken together, these data revealed that *A. shahii* MH21-1 did not cause any adverse effects in naïve, DSS treated or SKG mice, and that *A. shahii* MH21-1 enhanced gut barrier function and promoted mucosal healing in DSS treated and SKG mice.

30 *A. shahii* suppresses STAT3 activation in vitro.

[0564] Given the dramatic effects on histological inflammation and re-epithelialisation observed in the DSS treated animals, we next examined the ability of *A. shahii* to modulate IBD associated immune pathways. IL-23 driven immune responses are central to the pathogenesis of IBD and are a clinically recognised target (Friedrich et al.,
35 2019; Yang et al., 2017). Therefore, the ability of *A. shahii* MH21-1 to suppress IL-23-mediated activation of STAT3 was examined, using the HEK-BLUE™ IL-23 reporter cell line. The HEK-BLUE™ IL-23 reporter cell line carries a STAT3 inducible SEAP reporter that is responsive to IL-23 stimulation. As expected, IL-23 mediated activation of STAT3 could be prevented by tofacitinib (Figure 5A). Strikingly, cell free culture supernatant and the <3 kDa
40 filtered fraction prepared from *A. shahii* MH21-1, *A. shahii* MH21-3 and *A. shahii* MH21-6 (grown in As_v1, PYG and TY medium respectively) suppressed SEAP reporter activity

(Figure 6A-C). No cytotoxic effects were observed following treatment with the *A. shahii* culture supernatants. Furthermore, the biochemical characteristics of the CS by size fractionation and heat treatment were analysed. Through this approach, we determined that the STAT3 suppressive activity of the *A. shahii* MH21-1 <3 kDa fraction was unaffected by heat treatment (Figure 6D).

[0565] Finally, the impact of growth medium dependent effects on IL-23 mediated STAT3 activation was analysed. CS prepared from strains grown in PYG medium exhibited potent STAT3 suppressive activities. Interestingly, there was no STAT3 suppressive activity when the strains were grown in Wilkins-Chalgren broth or MCM (Figure 6E). This is consistent with previous reports (e.g., Giri et al., 2019; Schreiner et al., 2019) and suggests that the immunomodulatory activity *A. shahii* MH21-1 is affected by nutritional factors.

A. shahii suppresses IL-6 mediated activation of STAT3.

[0566] Increased levels of the pro-inflammatory cytokine IL-6 and signalling via its receptor IL-6R is associated with IBD pathogenesis. Notably, IL-6 contributes to chronic inflammation in the gut due to its pro-inflammatory and anti-apoptotic effects on immune cells. These effects are mediated by IL-6 receptor binding which causes JAK kinase activation and STAT3 dimerization in combination with activation of MAPK/ERK and other downstream kinases. Here, the inventors examined whether *A. shahii* MH21-2 and *A. shahii* MH21-3 are able to suppress IL-6-mediated activation of STAT3 using the HEK-Blue™ IL-6 reporter cell line. The HEK-Blue™ IL-6 reporter cell line constitutively expresses the human IL-6 receptor and binding of IL-6 to the receptor triggers expression of a STAT3-responsive SEAP reporter. Tofacitinib was used as a control, and as expected, IL-6 mediated activation of STAT3 was fully prevented by tofacitinib (Figure 6F). These data clearly show that *A. shahii* MH21-2 and *A. shahii* MH21-3 raw culture supernatant and its <3 kDa filtered fraction significantly suppress SEAP reporter activity. There was a modest effect on HEK cell viability (approximately 17% reduction relative to the medium control (range 14-21% reduction)) as assessed using an ATP assay.

A. shahii promotes human intestinal epithelial cell migration.

[0567] Damage of the intestinal barrier commonly occurs in IBD. The rapid migration of intestinal epithelial cells is a crucial component of the wound healing process to re-establish homeostasis. To investigate whether bioactives derived from *A. shahii* can affect the motility of intestinal epithelial cells, a TRANSWELL® migration assay was employed. HCT116 cells were seeded apically in a TRANSWELL® chamber and the ability of *A. shahii* extract to promote migration to the basolateral side of the chamber was assessed. *A. shahii* MH21-1 significantly promoted the migration of HCT116 cells to the basolateral side relative to the TYG-M medium extract control (Figure 7A). The pro-migratory effects of *A. shahii* were further confirmed using the IncuCyte scratch wound assay. At 90 hours post induction of a scratch wound, HCT116 cells showed an accelerated rate of wound closure in the presence of extract from *A. shahii* MH21-1, compared to the control cells treated with TYG-M medium extract control (Figure 7B).

A. shahii supports effective gut barrier integrity.

[0568] Intestinal epithelial cells form a physical and biochemical barrier that separates host tissue from gut microbes and luminal contents (Peterson et al., 2014). Impaired gut barrier function has been implicated in the pathogenesis of several diseases including IBD (Vanuytsel et al., 2021) and has been proposed as a therapeutic target to improve disease outcomes (Sommer et al., 2021). The integrity of the gut epithelial cell barrier can be assessed using a simple, non-invasive method termed trans-epithelial electrical resistance (TEER). In the TEER assay, an electrical current is applied across an epithelial cell layer and the resistance is measured. A reduction in the TEER value is indicative of a compromised barrier. To assess the ability of *A. shahii* to modulate barrier function, the TEER was monitored in T84 gut epithelial cell monolayers cultured with or without *A. shahii* MH21-1 culture supernatant, before and after barrier integrity was disrupted with IFN- γ . Following a 48-hour incubation with IFN- γ , a 90% drop in TEER was observed in T84 cells indicating an increased barrier permeability (Figure 7 C, E). The treatment with *A. shahii* MH21-1 culture supernatant was not able to prevent this drop in TEER. However, after removal of the disruptor, a quicker increase in TEER indicating a faster recovery in barrier integrity could be observed in T84 cells in the presence of *A. shahii* MH21-1 culture supernatant compared to cells treated with bacterial medium PYG alone (Figure 7 C, E). One week after IFN- γ was removed, the TEER of *A. shahii* MH21-1 treated cells was comparable to those cells that never received IFN γ treatment, whereas the cells treated with bacterial medium only still showed an almost 60% reduced TEER (Figure 7 D, F).

[0569] The present inventors came to the realization that *A. shahii* produces metabolites that contribute to therapeutic efficacy. 34 metabolites (classified as Level 1 or 2a) were identified in the cell free culture supernatant that were ≥ 2 -fold increase relative to the PYG medium control (Table 4). These included metabolites previously shown to modulate inflammation, immune cell infiltration, oxidative stress and barrier function (e.g., urocanic acid, allopurinol, N-acetyl-cysteine, ornithine, and propionylcarnitine, amongst others (see, Albert et al., 2010; Schlesinger et al., 2020; Shirazi et al., 2021; You et al., 2009; Qi et al., 2019; and Scioli et al., 2014).

TABLE 4
METABOLITES PRODUCED BY *A. SHAHII* FOLLOWING GROWTH IN PYG MEDIUM

Name	Fold change
Urocanic acid	894.6551508
Phenylacetic acid	179.4364595
4-Methyl-2-oxovaleric acid	163.5753332
Glutamine	121.6146497
5-Methylcytosine	103.1114623
Deoxyinosine	103.0815557
Thymine	101.5947171
5-(Hydroxymethyl)uracil	94.63791225
2-Oxo-3-phenylpropanoic acid	76.02671611

NAD+	61.30470622
Succinic acid	60.4820872
Argininosuccinic acid	38.01824958
Uracil	11.51841613
Choline	6.52417322
Glycine	6.180949626
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	5.921702634
1-Methyl Adenosine	5.402707811
2'-Deoxyuridine	4.335844251
Alanine	3.684617389
Homocysteine	3.310067513
O-Acetyl-serine	3.305068149
Allopurinol	3.154638493
DOPA	3.132608227
Riboflavin	3.09084237
Propionylcarnitine	2.906766968
4-Hydroxybenzaldehyde	2.678856081
Proline	2.647814705
Carbamoylaspartate	2.492066105
Ornithine	2.282564761
Inosine	2.277347411
Guanosine	2.272928277
Pyridoxal	2.093546775
N-Acetyl-cysteine	2.041929474
Crotonic acid	2.02419823

Materials & Methods

Bacterial strains, culture conditions and analyses.

[0570] Stool samples were collected from healthy human adults with no history of gastrointestinal disorders and mixed with an equal weight per volume of sterile oxygen free glycerol solution (McSweeney, 2005). Donors had not consumed antibiotics in the three months prior to collection of the faecal samples. *A. shahii* and *Faecalibacterium prausnitzii* were routinely processed in a Coy vinyl anaerobic chamber with an oxygen free atmosphere (85% N₂:10% CO₂:5% H₂) atmosphere. *A. shahii* was routinely cultured in As_v1 or PYG medium while *F. prausnitzii* was cultured in TY medium. All isolates were stocked by mixing 3 mL of actively growing culture with an equal volume of glycerol solution (McSweeney, 2005) and storing at -80 °C.

Isolation of *A. shahii*.

[0571] *A. shahii* strain MH21-1 was produced by inoculating a donor faecal sample with *A. shahii* present at a relative abundance of 0.24% into M4 broth. The enrichment culture was subsequently plated on Bacteroides Bile Esculin Agar and following growth colonies were picked into Schaedler broth. By this approach, an isolate was identified and termed *A. shahii* MH21-1 following whole genome sequencing. *A. shahii* strains MH21-2 and MH21-3 were produced from a donor faecal sample with *A. shahii* at 1.16% relative

abundance. Briefly, 100 μ l of a 7×10^6 cell/ml microbial cell suspension prepared from the donor faecal sample was washed and then diluted 10-fold in an anaerobic buffered diluent solution (38 ml/L each of salt solutions 2 & 3 (McSweeney et al., 2005), 1 ml.L⁻¹ of 0.1% (w/v) resazurin solution, 1 g/L L-cysteine). The cells were stained with Syto-9 and propidium iodide according to the manufacturer's instructions (Thermo Fisher Scientific) and then serially diluted to 100 cells/ml. Microbial cells were dispensed using a Namocell Hana single cell dispenser (gating parameters were set to: FSCL=20, SSCL left open, FITCL=5) into a 96 well plate containing M2GSC medium supplemented with 10% v/v faecal water. An enrichment with *A. shahii* at 90% was identified and subsequently streaked on As_v1 medium. Distinct colonies with differing morphologies were subsequently purified by repeated streak on As_v1 medium. By this approach, two isolates termed *A. shahii* MH21-2 and MH21-3 were identified by whole genome sequencing.

[0572] To isolate *A. shahii* MH21-6, 1 mL of a faecal stock sample was mixed with a further 3 mL of 30% glycerol solution and vortexed for 2 minutes with ~3.5 mm glass beads (Daintree Scientific) to dissociate bacteria from faecal debris. Aliquots of the dissociated bacteria were stored at -80°C for later processing as necessary. Bacterial cells were sorted using a BD FACSAria™ Fusion Flow Cytometer with a 100 μ m nozzle and small particle detector according to the manufacturer's instruction. The system was pressurised at 20 psi and events were triggered on scatter with thresholds set using 0.2 μ m filtered PBS which allowed a few events from the noise floor. To sort bacterial cells, an aliquot was filtered through a 40 μ m filter and a 10-3 dilution of the filtrate was subsequently prepared using a 0.2 μ m filtered anaerobic buffered diluent solution (i.e., 38 ml/L each of salt solutions 2 & 3 (McSweeney et al., 2005). The diluted filtrate was run to determine the dilution resulting in 500-1200 events per second. Targets were gated on scatter and doublets were excluded (gating strategy shown in Figure 7G). Single cells were deposited onto a TY agar plate and incubated at 37°C for up to 3 weeks. Isolates were identified by whole genome sequencing following growth in TY broth. By this approach, an isolate affiliated with *A. shahii* was tentatively identified. The isolate was purified by repeated streaking of single colonies on TY agar. The final purified isolate was glycerol stocked and submitted for whole genome sequencing and by this approach an isolate termed *A. shahii* MH21-6 was produced.

Metabolic reconstruction.

[0573] Protein coding sequences were predicted and annotated using the annotate function in enrichM (version 0.5.2). Briefly, enrichM identifies protein coding sequences using prodigal (version 2.6.3) in -p meta mode. The amino acid sequences are then searched against the UniRef100 database (downloaded November 2020) using DIAMOND (version 2.0.4), and E.C., TCDB and eggnoG classifications are inherited from the idmapping file distributed with UniRef. Hmmer hmmsearches (version 3.1b2) against Pfam (release 33.0), tigrfam (release 15.0) and dbcan2 (downloaded September 2019) were used to annotate functional domains, key metabolic markers and carbohydrate activate (CAZy) enzymes, respectively. Metabolic pathways were identified using the classify function in enrichM, which assesses annotations and their genomic position against manually defined metabolic pathway definitions. A pathway is considered present in a genome if it encoded

>80% of the required proteins, and passes all required synteny checks. These automatically predicted pathways were then manually assessed. In addition, gutSMASH (version 1.0.0) was applied to identify common biosynthetic pathways encoded by gut microorganisms.

Phylogenetic tree

5 **[0574]** A genome tree was constructed from high quality genomes, defined as $\geq 90\%$ complete and $\leq 5\%$ contamination from checkM analysis, within the *Alistipes* genus (NCBI release 89) and the four MH21 isolates. For each genome, a set of 122 bacteria-specific conserved marker genes were extracted from each genome using gtdbtk *identify*. These genes were then aligned to profile HMMs and concatenated to a single alignment with gtdbtk *align*, and a Maximum likelihood phylogenetic tree was constructed from the
10 alignments using FastTree (version 2.1.10) with gtdbtk *infer*. Non-parametric bootstrap values were inferred using GenomeTreeK (v0.1.6) from 1000 repetitions.

Preparation of bacterial strains for animal experimentation.

15 **[0575]** *A. shahii* and *F. prausnitzii* strains were grown to early stationary phase in As_v1 and TY medium respectively. The cell density of the individual cultures was calculated using a Helber Counting Chamber. To prepare the bacterial gavage solutions, individual cultures were centrifuged under a layer of sterile heavy mineral oil at 5,000 g for 10 minutes and the cell-free supernatant was then discarded. The cell pellets were washed in 1.5 mL of sterile anaerobic buffered diluent (38 mL/L each of salt solutions 2 & 3 (McSweeney et al.,
20 2005), 1 mL/L of 0.1% (w/v) resazurin solution, 1 g/L L-cysteine) and then centrifuged again. Finally, the washed cell pellet was resuspended in half strength glycerol solution (15% v/v glycerol solution in anoxic buffered diluent) to a final concentration of 1×10^9 cells/mL, aliquoted and frozen at -80°C until required. The viability of the cell preparations was confirmed by thawing a single aliquot and streaking on an agar plate. The identity and purity
25 of the individual strain preparations was confirmed by whole genome sequencing.

Acute model of DSS-induced gut barrier dysfunction.

30 **[0576]** Six-week-old C57BL/6 female mice purchased from Animal Resources Centres (Western Australia, Australia) were randomised and then co-housed for seven days prior to experimentation. To induce gut barrier dysfunction, mice were treated with 3% DSS *ad libitum* in the drinking water for six days. Naïve age matched control mice were processed and received DSS free drinking water. All treatments started one day prior to provision of DSS and all mice were sacrificed two days after the final DSS treatment. For the treatments, mice were anaesthetised with isoflurane and orally gavaged with 200 μL of bacterial preparations or vehicle control. Prednisone (2 mg/kg) was administered following
35 anaesthesia by intraperitoneal injection. Body weights and stool consistency were recorded daily. Stool samples were collected daily. Following sacrifice, the colon, liver and spleen were collected for analysis. Blood was collected by cardiac puncture.

Endoscopic and Histological scoring.

40 **[0577]** Animals were examined with a small animal gastrointestinal endoscope (Karl Storz Endoskope, Tuttlingen, Germany) on days 1, 2 and 6 to assess the extent of

colon mucosal inflammation (Marks et al., 2015; and Liu et al., 2019). Briefly, mice were anesthetised with isoflurane and a colonoscope was inserted through the rectum. Images captured by high-definition videos were examined in a blinded manner to assess the presence and extent of disease pathologies (Table 5). Histological scoring was performed essentially as previously described in the art (Marks et al., 2015). Briefly, samples were fixed in 4% formalin, paraffin embedded and sectioned. Tissue sections were hematoxylin and eosin stained to assess disease pathology and with Alcian blue to assess mucin production. Slides were imaged using the Aperio digital imaging system (Leica Biosystems, Nußloch, Germany). To grade colitis severity, the extent of inflammation and epithelial injury in the tissue sections were graded semi-quantitatively using an established scoring system (Table 6). The samples were then randomised and subsequently scored in a blinded by a trained gastrointestinal pathologist.

TABLE 5**Scoring of Gut Barrier Dysfunction by Colonoscopy**

Feature	Scoring
Thickening of the colon wall	
Transparent	0
Moderate	1
Marked	2
Intransparent	3
Changes in vascular pattern	
Normal	0
Moderate	1
Marked	2
Bleeding	3
Granularity of the mucosal surface	
None	0
Moderate	1
Marked	2
Extreme	3
Stool consistency/mucus secretion	
Normal and solid	0
Still shaped, mild mucus	1
Unshaped, mucus	2
Spread	3
Extent of involved area	
0-5% (none)	0
5-20% (patchy)	1
20-50% (moderate)	2
>50% (predominant)	3

TABLE 6**SCORING OF HISTOLOGICAL COLITIS**

Inflammation score (Scored 0-4)	
0	No evidence of inflammation
1	Low level of inflammation with scattered infiltrating mononuclear cells (1-2 foci only)
2	Moderate inflammation with multiple foci
3	High level of inflammation with increased vascular density and marked wall thickening

4 Maximal severity of inflammation with transmural leukocyte infiltration and loss of goblet cells

Injury score (Scored 0-3)

0	No epithelial injury
1	Occasional epithelial lesion
2	1-2 foci of ulceration
3	Extensive ulceration

Colitis activity (Composite score (/17) based on measures listed below)

Hypervascularisation	0-3 based on severity
Presence of mononuclear cells	0-3 based on severity
Epithelial hyperplasia	0-3 based on severity
Epithelial injury	0-3 based on severity
Presence of neutrophils	0-3 based on severity
Lymphoid aggregates	Scored 0-2, where 0 = 0, 1 = ≤ 2 , and 2 = ≥ 2

SKG model of murine ileitis.

[0578] Five-week-old SKG female mice (n = 3 male; n = 3 female) were randomised and then co-housed for 3 weeks prior to experimentation. To induce disease, mice were administered curdlan (1,3- β -glucan, 3 mg per mouse) intraperitoneal (i.p.). Naïve age matched control mice were similarly processed except that they were administered saline i.p. All treatments started prior to administration of curdlan, and all mice were sacrificed 7 days after the curdlan treatment. For the bacterial treatments, mice were orally gavaged with 200 μ l of bacterial preparations or vehicle control starting 2 days prior to the curdlan administration. Anti-mouse IL-23 p19 monoclonal antibody (Thermo Fisher, 30 μ g per mouse) was administered by i.p. injection one day prior to curdlan administration. Stool samples were collected daily. Body weights were measured at Day -2, 0 and 7. Following sacrifice, the colon, distal small intestine, mesenteric lymph nodes and spleen were collected for analysis. Blood was collected by cardiac puncture.

Clinical and histological scoring.

[0579] Histological scoring was performed essentially as described by Benham et al., (2014). Briefly, samples were fixed in 4% formalin, paraffin embedded and sectioned. Tissue sections were hematoxylin and eosin stained to assess disease pathology and with Alcian blue for goblet cell assessment. Slides were imaged using an Olympus VS120 slide scanner (Olympus Corporation, Tokyo, Japan). To grade ileitis severity, the extent of inflammation and epithelial injury in the tissue sections were graded semi-quantitatively using an established scoring system (see, Table 7, Benham et al., (2014)). The samples were then randomised and subsequently scored in a blinded manner by an independent trained pathologist.

[0580] To characterise the cytokines present in serum, the mice were euthanised by CO₂ asphyxiation after which blood was collected by cardiac bleed and serum isolated by centrifugation. Serum samples were stored at -80°C. These serum samples were subsequently thawed and used in the LEGENDplex Mouse Inflammation Panel (13-plex) (BioLegend, Cat. 740446) to determine effects on cytokine production (IL-23, IL-1 α , IFN- γ , TNF, MCP-1, IL-12p70, IL-1 β , IL-10, IL-6, IL-27, IL-17A, IFN- β , GM-CSF). Serum samples were diluted 2-fold and then incubated with capture beads conjugated to an antibody against

specific analyte. After washing, a biotinylated detection antibody mixture is added to create a "capture bead-analyte-detection antibody" sandwich. Streptavidin-phycoerythrin (SA-PE) is added to bind to the biotinylated detection antibodies. After a final wash, the mixtures are analyzed by flow cytometry where the beads are differentiated by size and internal
 5 fluorescence intensities. Cytokine concentration is determined by PE fluorescence and comparison to a standard curve of known cytokine concentrations using BioLegend's LEGENDplex data analysis software. The unique size and fluorescence characteristics of the beads allows for the simultaneous measurement of 13 cytokines.

TABLE 7**Histological scoring system for ileum of SKG mice**

Feature	Grade	Description
Inflammatory infiltrate	0	Absent
	1	Scattered PMNs or MNs in lamina propria (LP) involving <5 intercryptal spaces contiguously involved in an area
	2	Increased PMNs or MNs in LP and/or submucosa involving >5 intercryptal spaces contiguously involved in an area and $\pm$ crypt abscesses and areas of crypt/villous loss < 5 crypt widths
	3	Extensive transmural inflammatory infiltrate and areas of crypt/villous loss > 5 crypt widths and/or confluent areas of crypt abscesses and/or mucosal erosion/ulceration
Granulomata	0	Absent
	1	Aggregation of cells not definitely a granuloma
	2	A definite granuloma formation present
	3	Multiple granulomas present
Villous Distortion	0	Absent
	1	Villus height increased or decreased by <1/3 of normal
	2	Villus height increased or decreased by 1/3-2/3 of normal
	3	Villus height increased or decreased by >2/3 of normal
Cross sectional area	Score	
<1%	0.5	
1-25%	1	
26-50%	2	
51-75%	3	
>75%	4	

For total histology score add scores from sections 1, 2 and 3 and multiply by cross sectional area involved.

Characterisation of STAT3 suppressive activity.

15 **[0581]** To assess STAT3 suppressive activity of *A. shahii* MH21-1 and MH21-3, three independent colonies were inoculated and grown until early stationary phase. Then, each seed culture broth was used to inoculate two technical replicates each generating six technical replicates from three biological replicates. The technical replicates were grown until
 20 early stationary phase and then cell free culture supernatant was harvested as previously described (Giri et al., 2019). Culture supernatants were size fractionated by passing through a 3 kDa CENTRICON® column according to the manufacturer's (Merck Millipore) instructions.

To prepare *A. shahii* MH21-6 supernatant samples for assay, a seed culture was established from a single colony and grown until early stationary phase. Then, the seed culture broth was used to inoculate two technical replicates. The technical replicates were grown until early stationary phase and then cell free culture supernatant was harvested as previously
5 described (Giri et al., 2019). Culture supernatants were size fractionated by passing through a 3 kDa CENTRICON® column according to the manufacturer's (Merck Millipore) instructions.

[0582] STAT3 activity was assessed using the HEK Blue IL-23 cell line (Invivogen). Briefly, 50,000 cells per well were seeded in triplicate in a 96-well plate 24 hours prior to the start of the assay. Bacteria supernatant or sterile bacterial medium
10 were mixed at a final concentration of 10%, 25%, or 50% v/v with recombinant human IL-23 (rhIL-23, R&D systems) at a final concentration of 5 ng/mL. This mixture was then added directly to the cells and incubated at 37°C for 6 hours. The ability of the supernatants to suppress STAT3 activation was compared to the Janus kinase inhibitor tofacitinib (10 µM). STAT3 regulated SEAP reporter activity was assessed using Quanti Blue solution as
15 recommended by the manufacturer (Invivogen). Results are the average of at least three independent experiments. Cytotoxicity was assessed using an MTT assay. Briefly, MTT reagent was added to the cells at a final concentration of 1.2 mM. The cells were incubated at 37°C for 4 hours and then fixed with DMSO. Cytotoxicity was assessed by measuring absorbance at 540 nm as recommended by the manufacturer (Invitrogen, Thermo Fisher,
20 Australia).

[0583] STAT3 activity was assessed using the HEK Blue IL-6 cell line (Invivogen). Briefly, 50,000 cells per well were seeded in triplicate in a 96-well plate 24 hours prior to the start of the assay. Bacteria supernatant or sterile bacterial medium were added to the cells at a final concentration of 10% v/v for a 60-minute pretreatment. Then recombinant human
25 IL 6 (R&D systems) was added at a final concentration of 2 ng/mL and the cells were incubated at 37°C for 24 hours. The ability of the supernatants to suppress IL-6 mediated STAT3 activation was compared to the Janus kinase inhibitor tofacitinib (10 µM). After 24 hours, the STAT3 regulated SEAP reporter activity was assessed using Quanti Blue solution as recommended by the manufacturer (Invivogen). Results are the average of three
30 independent experiments. Cytotoxicity was assessed using the CellTiter-Glo® 2.0 Cell Viability Assay (Promega, Australia) as recommended by the manufacturer.

Cell migration assay.

[0584] The IncuCyte® Live-Cell Imaging System (Essen BioScience) and Transwell migration assay were used to assess the migration of HCT116 cells during exposure to sterile
35 culture supernatant extracts from *A. shahii*. These bacterial extracts were prepared using an Amberlite XAD-7 resin essentially as previously described by Colosimo et al., (2019). Briefly, a single colony was inoculated and grown until early stationary phase. This "seed culture" broth was used to inoculate 600 ml of broth and the culture was incubated until early stationary phase. Culture supernatants were prepared by centrifuging the culture at 4000 g
40 for 30 minutes and then passing the cell free supernatant through a 3 kDa filter according to the manufacturer's instructions (Sartorius Vivaflow® 50 Ultrafiltration Unit 3kDa MWCO

PES). Activated Amberlite XAD-7 resin was added to 400 mL of 3 kDa filtered cell-free supernatant (10% w/v), and the slurry was gently shaken overnight at 4°C. The resin was collected, washed with 400 mL of deionized water and then mixed with 120 mL of 100% methanol. Following 2 h incubation with gentle shaking, the methanol elution was collected. A second elution in 120 mL of 100% methanol was performed as previously described and the two elutions were ultimately combined and dried under vacuum using a rotary evaporator. The extract was fully resuspended at in 100% DMSO (thereafter referred to as 1000X) and stored at -20 °C.

[0585] Human HCT116 gut epithelial cells were maintained in McCoy's 5a medium supplemented with 10% FBS and 1% Pen/Strep. For the IncuCyte® scratch wound assay, 3.5×10^4 HCT116 cells were plated on poly-L-ornithine-coated IncuCyte® ImageLock 96-well plates (Essen BioScience). After 24 hours, the IncuCyte® WoundMaker tool was used to induce a homogeneous scratch wound in the nearly confluent cell monolayer. The cells were washed twice with DPBS and then 0.6x extract from *A. shahii* in 200 µL 0.5% FBS McCoy's 5a medium was added. Uninoculated bacterial media (1%) served as negative control. Immediately after adding the stimulants, the plate was transferred to the IncuCyte® system and cell migration was monitored by imaging each well every 2 hours over the course of 90 hours. Data analysis was performed using the integrated analysis software.

[0586] To assess cell migration via the TRANSWELL® assay, 3.5×10^4 HCT116 cells were seeded in 100 µL 10% FBS culture medium in the top compartment of a 6.5 mm insert with TC-treated polycarbonate membrane in 24-well plates (8 µm pore size, Corning Costar). 600 µL 10% FBS culture medium was added to the lower compartment. The cells were allowed to settle for 24 hours. After a DPBS wash, 100 µL and 600 µL of 0.5% FBS medium was added to the top and lower compartment, respectively. Then, 1x concentrated extract from *A. shahii* was added to the lower compartment. After 16 hours, the cells were washed with DPBS and the cells attached to the top of the membrane were carefully removed with a cotton tip. The migrated cells on the bottom of the membrane were then fixed in 70% ethanol for 10 minutes followed by staining in 0.25% crystal violet for 5 minutes. The TRANSWELL® inserts were washed with water, dried and the membrane mounted with 50% glycerol in water on glass slides and imaged immediately. TRANSWELL® experiments were performed in biological and technical triplicates and for each replicate two representative images of the membrane were taken at 10x magnification. The number of migrated cells was automatically counted using ImageJ and the average cell number displayed. The extent of cell migration was expressed as the average number of migrated cells in two microscopic fields per well from three biological and three technical replicates.

Epithelial barrier integrity assay using Trans-Epithelial Electrical Resistance (TEER).

[0587] The ability of *A. shahii* to modulate barrier integrity was assessed by measuring the transepithelial electrical resistance across confluent monolayers of T84 gut epithelial cells. T84 cells were purchased from CellBank Australia and cultured in Dulbecco's Modified Eagle Medium Nutrient Mixture 12 (DMEM/F12; ThermoFisher Scientific, Waltham, MA, USA) supplemented with 5% foetal bovine serum and 1% Penicillin-Streptomycin. T84

cells were seeded into 24-well Millicell polycarbonate cell culture inserts with 0.4 μm pore size (Merck Millipore, PSHT010R5) at a density of 60,000 cells per well in 400 μL warm T84 medium, and 24 mL of medium was added into the single-well feeder tray. Medium changes of both compartments were performed every second day. After seven days of culture, the upper part of the plate assembly with cell culture inserts were transferred from the feeder tray to a 24-well receiver tray (Merck Millipore, PSMW010R5), with each well containing 800 μL media. TEER values of each well were then measured daily using the Millicell ERS-2 Voltohmmeter. Experiments were started once the cells in all wells reached stable TEER readings above 1500 Ω . Tofacitinib (75 μM) was used as control known to accelerate restoration of barrier function (Sayoc-Becerra et al., 2020). In a prophylactic model, T84 cells were pretreated with 3kDa-filtered MH21 bacterial supernatant as well as 3kDa-filtered bacterial medium (PYG) as negative control. Both were diluted to 10% in T84 medium and added to the apical compartment. After one hour of pretreatment, IFN- γ (50 ng/mL) was added basolateral to disrupt barrier integrity, in addition to the apical treatment with *A. shahii* MH21-1 supernatant. After 48 hours, all wells were washed thoroughly with warm PBS to remove the disruptor IFN- γ . From then on, the *A. shahii* MH21-1 supernatant and control treatments in the apical chamber, and the T84 medium in the basolateral chamber, were refreshed every 24 hours, and the TEER values were measured daily in duplicate. The data from two biological replicates with duplicate measurements each were presented either as raw TEER value in Ω or as the percentage difference in TEER value compared to the untreated T84 cells. Statistical significance was determined by unpaired t-test.

Metabolomic analysis of *A. shahii*

[0588] To assess the metabolites produced by *A. shahii*, six independent colonies were inoculated and grown until early stationary phase. Then, each seed culture broth was used to inoculate two technical replicates of YG/V generating 12 technical replicates from six biological replicates. The technical replicates were grown until early stationary phase and then cell free culture supernatant was harvested following centrifugation at 12,550 g for 3 minutes in an anaerobic chamber. Culture supernatants were snap frozen on dry ice and then stored at -80°C until testing.

[0589] Sample analysis was carried out by MS-Omics (Denmark) as follows. The analysis was carried out using a Thermo Scientific Vanquish LC coupled to Thermo Q Exactive HF MS. An electrospray ionization interface was used as ionization source. Analysis was performed in negative and positive ionization mode. The UPLC was performed using a slightly modified version of the protocol described by Catalin et al. (UPLC/MS Monitoring of Water-Soluble Vitamin Bs in Cell Culture Media in Minutes, Water Application note 2011, 720004042en). Peak areas were extracted using Compound Discoverer 3.1 (Thermo Scientific). Identification of compounds were performed at four levels; Level 1: identification by retention times (compared against in-house authentic standards), accurate mass (with an accepted deviation of 3ppm), and MS/MS spectra, Level 2a: identification by retention times (compared against in-house authentic standards), accurate mass (with an accepted deviation of 3ppm). Level 2b: identification by accurate mass (with an accepted deviation of 3ppm),

and MS/MS spectra, Level 3: identification by accurate mass alone (with an accepted deviation of 3ppm).

EXAMPLE 2

Colonithrix association with health, IBD and other diseases.

5 **[0590]** Inflammatory bowel disease is characterised by structure-function changes to the microbiome with a significant reduction in both the prevalence and abundance of select gut bacteria in the IBD gut when compared to the healthy gut. Several studies have shown that these bacteria may modulate IBD pathogenesis (Mallone et al., 2011; and Sokol et al., 2008) however a key obstacle to using these bacteria to develop new
10 therapeutics has been that low resolution 16S rRNA based profiling do not provide sufficient resolution to accurately discriminate against health and IBD associated strains at a low taxonomic level (i.e., genus, species, strain).

[0591] The inventors used the Microba Disease Database (MDD), which contains high resolution faecal gut metagenomic data and associated host metadata for 6,020 adults,
15 to study the prevalence of *Colonithrix* species in inflammatory and autoimmune diseases. Metagenomic sequence reads were analysed using the Microba Community Profiler (MCP) (Parks et al., 2021).

[0592] Three *Colonithrix* species were detected in more than two individuals in the MDD: *C. sana*, and the uncultured species *C. sp900317525* and *C. sp003522105*.

20 **[0593]** The inventors found the *Colonithrix* species as being prevalent in healthy humans but rarely detected in inflammatory and autoimmune diseases (Figure 7A-D). The strongest effect was observed for IBD, including both major subtypes ulcerative colitis and Crohn's disease (Figure 8, and Table 8, below). The genus *Colonithrix* was significantly less prevalent in IBD compared to healthy individuals ($P=7.3E-17$, $FDR=8.77E-15$). Accordingly,
25 all three *Colonithrix* species (*C. sana*, *C. sp900317525*, *C. sp003522105*) were significantly less prevalent in IBD as compared to healthy (Figure 8, Table 8). The strongest reduction in IBD was observed for *C. sana* (Figure 8, Table 8). These observations were validated in an independent IBD cohort previously published by Harvard ((Franzosa et al., 2018), Figure 8, Table 8).

30

TABLE 8

ASSOCIATION STATISTICS BETWEEN COLONITHRIX SPECIES AND IBD

Taxon	PREVALENCE (%)						P Cochran-Mantel-Haenszel test association with IBD
	Healthy MDD	CD MDD	UC MDD	Healthy Harvard	CD Harvard	UC Harvard	
<i>Colonithrix sana</i>	82.4	32.6	48.8	69.6	6	37.7	8.7E-24
<i>Colonithrix sp900317525</i>	73.9	27.9	39	39.1	1.5	21.3	5.70E-18

<i>Colonithrix</i> <i>sp003522105</i>	24.4	7	2.4	13	0	11.5	4.80E-07
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Isolation and genome-scale analysis of *C. sana*

[0594] To better understand the role of *C. sana* in health and the pathogenesis of IBD two new isolates, termed *C. sana* MH35-1 and *C. sana* MH35-2, were isolated from a healthy human donor. The isolation was performed by generating a dilution-to-extinction enrichment and then plating for single colonies. *C. sana* MH35-1 and *C. sana* MH35-2 grew on TY and PYG based media and were typically observed as a Gram-variable staining long thin rod like cells (Figure 9A) although cell morphology significantly more heterogenous in older cultures. Phylogenetic reconstruction of the *ER4* genus using high-quality GenBank and RefSeq genomes from the NCBI and *C. sana* MH35-1 and *C. sana* MH35-2 revealed the isolates placed with high confidence within the *C. sana* clade, close to several uncultured species (Figure 9B), and close to the CAG-83 and *Oscillibacter* genera. *C. sana* MH35-1 and *C. sana* MH35-2 are likely amino acid fermenters, but are predicted to utilise glucose. Complete, or near complete biosynthetic pathways were identified for alanine, arginine, asparagine, aspartic acid, glutamine, glutamic acid, glycine, leucine, proline and serine. Several pathways for the uptake and fermentation of amino acids (asparagine, aspartic acid, glutamic acid, histidine, isoleucine, cysteine and leucine) were identified. Metabolic modelling of the *C. sana* MH35-1 and *C. sana* MH35-2 isolates revealed that when fed with glucose and amino acids, primary byproducts of fermentation are acetate and ammonia. AntiSmash identified a RiPP ranthipeptide BGC in both strains with no close homology to BGCs in public databases.

C. sana Enhances Gut Barrier Function

[0595] It was hypothesised that the big-data approach described above could be used to identify novel candidate live biotherapeutics for inflammatory and autoimmune diseases (e.g., IBD). The inventors therefore tested the therapeutic efficacy of *C. sana* MH35-1 and *C. sana* MH35-2 in an acute model of DSS induced murine colitis (Figure 10A) as this is a well-documented model of epithelial injury and repair. DSS treatment resulted in significant disease activity relative to the vehicle control. Histological analysis of DSS treated mice revealed significant gut damage characterized by crypt loss, epithelial erosion and ulceration. Notably, treatment with *C. sana* MH35-1 and *C. sana* MH352 resulted in significant improvement in pathology as evidenced by improvements in histopathological healing (Figure 10B) and a reduction in epithelial injury (Figure 10C). Neither prednisone nor *F. prausnitzii* A2-165 had a significant impact on histopathological healing or epithelial injury (Figure 10B-C). Taken together, these data revealed that *C. sana* MH35-1 and *C. sana* MH35-2 promoted mucosal healing in DSS treated C57Bl/6 mice.

C. sana suppresses IL-23 mediated activation of STAT3 in vitro

[0100] Given the dramatic effects on histological inflammation and re-epithelialisation observed in the DSS treated animals, the inventors next examined the ability of *C. sana* MH35-1 and *C. sana* MH35-2 to modulate IBD associated immune pathways. IL-23 driven

immune responses are central to the pathogenesis of IBD and are a clinically recognised target (Friedrich et al., 2019; Yang et al., 2017). The inventors examined the ability of *C. sana* MH35-1 and *C. sana* MH35-2 to suppress IL-23-mediated activation of STAT3 using the HEK-Blue™ IL-23 reporter cell line. The HEK-Blue™ IL-23 reporter cell line carries a STAT3 inducible SEAP reporter that is responsive to IL-23 stimulation. As expected, IL-23 mediated activation of STAT3 could be prevented by tofacitinib (Figure 11A-B). Interestingly, cell free culture supernatant prepared from *C. sana* MH35-1 and *C. sana* MH35-2 grown in PYGM medium suppressed SEAP reporter activity (Figure 11A-B). Notably, no changes were observed in cell morphology following treatment with the *C. sana* MH35-1 and *C. sana* MH35-2 culture supernatants suggesting the supernatants were not toxic.

C. sana suppresses IL-6 mediated activation of STAT3.

[0101] Increased levels of the pro-inflammatory cytokine IL-6 and signalling via its receptor IL-6R is associated with IBD pathogenesis. Notably, IL-6 contributes to chronic inflammation in the gut due to its pro-inflammatory and anti-apoptotic effects on immune cells. These effects are mediated by IL-6 receptor binding which causes JAK kinase activation and STAT3 dimerization in combination with activation of MAPK/ERK and other downstream kinases. Here, the inventors examined whether *C. sana* MH35-1 and *C. sana* MH35-2 are able to suppress IL-6-mediated activation of STAT3 using the HEK-Blue™ IL-6 reporter cell line. The HEK-Blue™ IL-6 reporter cell line constitutively expresses the human IL-6 receptor and binding of IL-6 to the receptor triggers expression of a STAT3-responsive SEAP reporter. Tofacitinib was used as a control, and as expected, IL-6 mediated activation of STAT3 was fully prevented by tofacitinib (Figure 12A, B). It was shown that *C. sana* MH35-1 and *C. sana* MH35-2 raw culture supernatant and its <3 kDa filtered fraction significantly suppressed SEAP reporter activity (Figure 12A, B). Moreover, <3 kDa filtered culture supernatant Amberlite extract from both *C. sana* MH35-1 and *C. sana* MH35-2 suppressed SEAP reporter activity by nearly 65% (Figure 12C). In both experiments, there was a minimal effect on HEK cell viability ($\leq 9\%$ reduction) as assessed using an ATP assay.

C. sana promotes human intestinal epithelial cell migration.

[0596] Damage of the intestinal barrier commonly occurs in IBD. The rapid migration of intestinal epithelial cells is a crucial component of the wound healing process to re-establish homeostasis. To investigate whether bioactives derived from *C. sana* can affect the motility of intestinal epithelial cells, a TRANSWELL® migration assay was employed. HCT116 cells were seeded apically in a TRANSWELL® chamber and the ability of *C. sana* MH35-1 and *C. sana* MH35-2 extract to promote migration to the basolateral side of the chamber was assessed. Untreated cells had a basal level of migration to the basolateral side of the membrane, and this was unaffected by treatment with PYGM medium (Figure 12A). *C. sana* MH35-1 and *C. sana* MH35-2 significantly promoted the migration of HCT116 cells to the basolateral side (Figure 13A). The pro-migratory effects of *C. sana* were further confirmed using the IncuCyte scratch wound assay. Within 72 hours post induction of a scratch wound, HCT116 cells showed an accelerated rate of wound closure in the presence of

extract from *C. sana* MH35-1 and *C. sana* MH35-2, compared to the control cells treated with PYGM medium (Figure 13B, C).

Materials & Methods

Bacterial strains, culture conditions and analyses.

5 **[0597]** Stool samples were collected from healthy human adults with no history of gastrointestinal disorders and mixed with an equal weight per volume of sterile oxygen free glycerol solution (McSweeney, 2005). Donors had not consumed antibiotics in the three months prior to collection of the faecal samples. *C. sana* and *Faecalibacterium prausnitzii* were routinely processed in a Coy vinyl anaerobic chamber with an oxygen free atmosphere
10 (85% N₂:10% CO₂:5% H₂) atmosphere. *C. sana* was routinely cultured in a modified PYG medium (PYGM, Table 1) while *F. prausnitzii* was cultured in TY medium (Table 1). All isolates were stocked by mixing 3 mL of actively growing culture with an equal volume of glycerol solution (McSweeney, 2005) and storing at –80 °C.

Isolation of *C. sana*.

15 **[0598]** An isolate of *C. sana* was produced by inoculating a donor faecal sample with *C. sana* present at a relative abundance of 1.2% into custom broth (Table 1) and then serially diluting to extinction. The dilution-to-extinction culture series was sequenced and a low diversity enrichment culture with *C. sana* at a relative abundance of 6.6% was identified, Colonies were recovered and subsequently arrayed on TY medium. Arrayed isolates were
20 screened by PCR using species specific primers and two putative *C. sana* isolates were identified and subsequently purified. By this approach, Gram-viable staining isolates with a long thin rod cell morphology were identified and termed *C. sana* MH35-1 and *C. sana* MH35-2 following whole genome sequencing.

Metabolic reconstruction.

25 **[0599]** Protein coding sequences were predicted and annotated using the annotate function in enrichM (version 0.6.2). Briefly, enrichM identifies protein coding sequences using prodigal (version 2.6.3) in -p meta mode. The amino acid sequences are then searched against the UniRef100 database (downloaded November 2020) using DIAMOND (version 2.0.4), and E.C., TCDB and eggnoG classifications are inherited from the
30 idmapping file distributed with UniRef. Hmmer hmmsearches (version 3.1b2) against Pfam (release 33.0), tigrfam (release 15.0) and dbcan2 (downloaded September 2019) were used to annotate functional domains, key metabolic markers and carbohydrate activate (CAZy) enzymes, respectively. Metabolic pathways were identified using the classify function in enrichM, which assesses annotations and their genomic position against manually defined
35 metabolic pathway definitions. A pathway is considered present in a genome if it encoded >80% of the required proteins, and passes all required synteny checks. These automatically predicted pathways were then manually assessed. In addition, gutSMASH (version 1.0.0) was applied to identify common biosynthetic pathways encoded by gut microorganisms.

Phylogenetic tree.

[0600] A genome tree was constructed from high quality genomes, defined as $\geq 90\%$ complete and $\leq 5\%$ contamination from checkM analysis, within the *Colonithrix* genus (NCBI release 89) and the two *C. sana* MH35 isolates. For each genome, a set of 122 bacteria-specific conserved marker genes were extracted from each genome using gtdbtk *identify*. These genes were then aligned to profile HMMs and concatenated to a single alignment with gtdbtk *align*, and a Maximum likelihood phylogenetic tree was constructed from the alignments using FastTree (version 2.1.10) with gtdbtk *infer*. Non-parametric bootstrap values were inferred using GenomeTreeK (v0.1.6) from 1000 repetitions.

Preparation of bacterial strains for animal experimentation.

[0601] *C. sana* and *F. prausnitzii* strains were grown to early stationary phase PYGM and TY medium, respectively. The cell density of the individual cultures was calculated using a Helber Counting Chamber. To prepare the bacterial gavage solutions, individual cultures were centrifuged under a layer of sterile heavy mineral oil at 5,000 g for 10 minutes and the cell-free supernatant was then discarded. The cell pellets were washed in 1.5 mL of sterile anaerobic buffered diluent (38 mL/L each of salt solutions 2 & 3 (McSweeney et al., 2005), 1 mL/L of 0.1% (w/v) resazurin solution, 1 g/L L-cysteine) and then centrifuged again. Finally, the washed cell pellet was resuspended in half strength glycerol solution (15% v/v glycerol solution in anoxic buffered diluent) to a final concentration of 1×10^9 cells/mL, aliquoted and frozen at -80°C until required. The viability of the cell preparations was confirmed by thawing a single aliquot and streaking on an agar plate. The identity and purity of the individual strain preparations was confirmed by whole genome sequencing.

Acute model of DSS-induced gut barrier dysfunction.

[0602] Six-week-old C57BL/6 female mice purchased from Animal Resources Centres (Western Australia, Australia) were randomised and then co-housed for seven days prior to experimentation. To induce gut barrier dysfunction, mice were treated with 3% DSS *ad libitum* in the drinking water for six days. Naïve age matched control mice were processed and received DSS free drinking water. All treatments started one day prior to provision of DSS and all mice were sacrificed two days after the final DSS treatment. For the treatments, mice were anaesthetised with isoflurane and orally gavaged with 200 μL of bacterial preparations or vehicle control. Prednisone (2 mg/kg) was administered following anaesthetisation by intraperitoneal injection. Body weights and stool consistency were recorded daily. Stool samples were collected daily. Following sacrifice, the colon, liver and spleen were collected for analysis. Blood was collected by cardiac puncture.

Endoscopic and Histological scoring.

[0603] Animals were examined with a small animal gastrointestinal endoscope (Karl Storz Endoskope, Tuttlingen, Germany) on days 1, 2 and 6 to assess the extent of colon mucosal inflammation (Marks et al., 2015; and Liu et al., 2019). Briefly, mice were anaesthetised with isoflurane and a colonoscope was inserted through the rectum. Images captured by high-definition videos were examined in a blinded manner to assess the presence and extent of disease pathologies (Table 5). Histological scoring was performed

essentially as previously described in the art (Marks et al., 2015). Briefly, samples were fixed in 4% formalin, paraffin embedded and sectioned. Tissue sections were hematoxylin and eosin stained to assess disease pathology and with Alcian blue to assess mucin production. Slides were imaged using the Aperio digital imaging system (Leica Biosystems, Nußloch, Germany). To grade colitis severity, the extent of inflammation and epithelial injury in the tissue sections were graded semi-quantitatively using an established scoring system (Table 6). The samples were then randomised and subsequently scored in a blinded by a trained gastrointestinal pathologist.

Characterisation of STAT3 suppressive activity.

[0604] To assess STAT3 suppressive activity, three independent colonies of *C. sana* MH35-1 and *C. sana* MH35-2 were inoculated and grown until early stationary phase. Then, each seed culture broth was used to inoculate two technical replicates each generating six technical replicates from three biological replicates. The technical replicates were grown until early stationary phase and then cell free culture supernatant was harvested as previously described (Giri et al., 2019). Culture supernatants were size fractionated by passing through a 3 kDa CENTRICON® column according to the manufacturer's (Merck Millipore) instructions.

[0605] STAT3 activity was assessed using the HEK Blue IL-23 cell line (Invivogen). Briefly, 50,000 cells per well were seeded in triplicate in a 96-well plate 24 hours prior to the start of the assay. Bacteria supernatant or sterile bacterial medium were mixed at a final concentration of 25% v/v with recombinant human IL-23 (rhIL-23, R&D systems) at a final concentration of 5 ng/mL. This mixture was then added directly to the cells and incubated at 37°C for 6 hours. The ability of the supernatants to suppress STAT3 activation was compared to the Janus kinase inhibitor tofacitinib (10 µM). STAT3 regulated SEAP reporter activity was assessed using Quanti Blue solution as recommended by the manufacturer (Invivogen). Results are the average of at least three independent experiments. Cytotoxicity was assessed using MTT assay. Briefly, MTT was added to the cells at a final concentration of 1.2 mM. The cells were incubated at 37°C for 4 hours and then fixed with DMSO. Cytotoxicity was assessed by measuring absorbance at 540 nm as recommended by the manufacturer (Invitrogen, Thermo Fisher, Australia).

Cytokine production assay.

[0606] 5×10^4 of T84 cells were seeded in triplicate in 96 well plates and left to settle overnight. Raw or <3 kDa-filtered bacterial culture supernatant (25% v/v) or media control were added to T84 cells. After 90-minute treatment with the bacterial culture supernatants, the cells were stimulated with LPS (100 µg/ml). After 6 hours, T84 culture supernatant was collected for IL-6 assessment. IL-6 was measured using the HEK-blue cell reporter system from InvivoGen according to standard protocol. For the ELISA assays, 1×10^4 cells/well of HCT116 cells were seeded in duplicate 96 well plates. HCT116 cells were treated with IL1β (10 ng) and indole-3-carbinol (5 µM) or culture supernatant (10% v/v) as appropriate and incubated at 37°C for 16 hours. Then, cell supernatants were collected and

analysed using an IL-8 human uncoated ELISA Kit according to the manufacturer's instructions.

Cell migration assay.

[0607] The Transwell migration assay was used to assess the migration of HCT116 cells during exposure to *C. sana* MH35-1 and *C. sana* MH35-2 culture supernatant extracts. These bacterial extracts were prepared using an Amberlite XAD-7 resin essentially as previously described by Colosimo *et al.* Briefly, a single colony was inoculated and grown until early stationary phase. This "seed culture" broth was used to inoculate 600 mL of PYGM broth and the culture was incubated until early stationary phase. Culture supernatants were prepared by centrifuging the culture at 4000 g for 30 minutes and then passing the cell free supernatant through a 3 kDa filter according to the manufacturer's instructions (Sartorius Vivaflow® 50 Ultrafiltration Unit 3kDa MWCO PES). Activated Amberlite XAD-7 resin was added to 400 mL of 3 kDa filtered cell-free supernatant (10% w/v), and the slurry was gently shaken overnight at 4°C. The resin was collected, washed with 400 mL of deionized water and then mixed with 120 mL of 100% methanol. Following 2 h incubation with gentle shaking, the methanol elution was collected. A second elution in 120 mL of 100% methanol was performed as previously described and the two elutions were ultimately combined and dried under vacuum using a rotary evaporator. The extract was fully resuspended at in 100% DMSO (thereafter referred to as 1000X) and stored at -20 °C.

[0608] Human HCT116 gut epithelial cells were maintained in McCoy's 5a medium supplemented with 10% FBS and 1% Pen/Strep. For the IncuCyte® scratch wound assay, 3.5×10^4 HCT116 cells were seeded in 100 µL 10% FBS culture medium in the top compartment of a 6.5 mm insert with TC-treated polycarbonate membrane in 24-well plates (8 µm pore size, Corning Costar). 600 µL 10% FBS culture medium was added to the lower compartment. The cells were allowed to settle for 24 hours. After a DPBS wash, 100 µL and 600 µL of 0.5% FBS medium was added to the top and lower compartment, respectively. Then, 0.5x concentrated extract from *C. sana* MH35-1 and *C. sana* MH35-2 was added to the lower compartment. After 16 hours, the cells were washed with DPBS and the cells attached to the top of the membrane were carefully removed with a cotton tip. The migrated cells on the bottom of the membrane were then fixed in 70% ethanol for 10 minutes followed by staining in 0.25% crystal violet for 5 minutes. The Transwell inserts were washed with water, dried and the membrane mounted with 50% glycerol in water on glass slides and imaged immediately. Transwell experiments were performed in biological and technical triplicates and for each replicate two representative images of the membrane were taken at 10x magnification. The number of migrated cells was automatically counted using ImageJ and the average cell number displayed. The extent of cell migration was expressed as the average number of migrated cells in two microscopic fields per well from three biological and three technical replicates.

[0609] To assess migration with via the IncuCyte scratch wound assay, 3.5×10^4 HCT116 cells were plated on poly-L-ornithine-coated IncuCyte® ImageLock 96-well plates (Essen BioScience). After 24 hours, the IncuCyte® WoundMaker tool was used to induce a

homogeneous scratch wound in the nearly confluent cell monolayer. The cells were washed twice with DPBS and then 0.5x concentrated extract from *C. sana* MH35-1 and *C. sana* MH35-2 in 200 µL 0.5% FBS McCoy's 5a medium was added. The cells were also treated with 0.5x concentrated extract from uninoculated bacterial media as a negative control.

- 5 Immediately after adding the stimulants, the plate was transferred to the IncuCyte system and cell migration was monitored by imaging each well every 2 hours over the course of 72 hours. Data analysis was performed using the integrated analysis software.

Example 3

Gemmiger association with health, IBD and other diseases.

- 10 **[0610]** Inflammatory bowel disease is characterised by structure-function changes to the microbiome with a significant reduction in both the prevalence and abundance of select gut bacteria in the IBD gut when compared to the healthy gut. Several studies have shown that these bacteria may modulate IBD pathogenesis (Mallone et al., 2011; and Sokol et al., 2008) however a key obstacle to using these bacteria to develop new
15 therapeutics has been that low resolution 16S rRNA based profiling do not provide sufficient resolution to accurately discriminate against health and IBD associated strains at a low taxonomic level (i.e., genus, species, strain).

- [0611]** The inventors used the Microba Disease Database (MDD), which contains high resolution faecal gut metagenomic data and associated host metadata for 6,020 adults, to study the prevalence of *Gemmiger* species in inflammatory and autoimmune diseases. Metagenomic sequence reads were analysed using the Microba Community Profiler (MCP) (Parks et al., 2021).

- [0612]** Five *Gemmiger* species were detected in more than two individuals in the MDD: *G. formicilis*, the uncultured species *G. sp003476825* and three uncultivated species that were discovered by Microba through the assembly of metagenomic reads into Metagenome Assembled Genomes (MAGs) (Parks et al., 2021) (*G. species_A*, *G. species_B*, and *G. species_C*).

- [0613]** The inventors found the *Gemmiger* species as being prevalent in healthy humans but rarely detected in inflammatory and autoimmune diseases (Figure 14A-D). The strongest effect was observed for IBD, including both major subtypes ulcerative colitis and Crohn's disease (Figure 14E, and Table 9, below). The genus *Gemmiger* was significantly less prevalent in IBD compared to healthy individuals ($P=1.3e-10$, $FDR=1.9e-09$). Accordingly, all
30 five *Gemmiger* species (*G. formicilis*, *G. species_A*, *G. species_B*, *G. species_C* and *G. sp003476825*) were significantly less prevalent in IBD as compared to healthy (Figure
35 14E, Table 9). The strongest reduction in IBD was observed for *G. formicilis* (Figure 14E, Table 9). These observations were validated in an independent IBD cohort previously published by Harvard ((Franzosa et al., 2018), Figure 14F, Table 9).

TABLE 9

ASSOCIATION STATISTICS BETWEEN GEMMIGER SPECIES AND IBD

Taxon	PREVALENCE (%)				P Cochran-Mantel-Haenszel test
	Healthy MDD	IBD MDD	Healthy Harvard	IBD Harvard	
<i>Gemmiger formicilis</i>	83.8	38.5	71.7	18.0	6.2×10^{-31}
<i>Gemmiger species_C</i>	44.0	12.3	39.1	2.3	2.3×10^{-16}
<i>Gemmiger species_B</i>	10.9	5.7	10.9	3.9	0.036
<i>Gemmiger species_A</i>	73.1	45.1	41.3	10.9	4.1×10^{-12}
<i>Gemmiger sp003476825</i>	90.8	68.9	80.4	39.1	1.2×10^{-13}

Isolation and genome-scale analysis of *G. formicilis*

[0614] To better understand the role of *G. formicilis* in health and the pathogenesis of IBD a new isolate, termed *G. formicilis* MH32-1 was isolated from a healthy human donor. The isolation was performed by generating a dilution-to-extinction enrichment and then plating for single colonies. *G. formicilis* MH32-1 was identified as an extremely oxygen sensitive strain that grew well on TY and PYG medium. It was typically observed as Gram-negative staining coccus-like cell with a characteristic "budding" morphology (Gossling & Moore 1975), Figure 15A). A second isolate, *G. formicilis* MH32-2 was isolated from a faecal sample archived in the Microba Faecal Biobank. A faecal donor sample with *G. formicilis* at a relative abundance of 6.48% was serially diluted and plated on TY medium. Analysis by psMGS revealed a single plate with *G. formicilis* at a relative abundance of 1.35%. Individual colonies were subsequently picked and screened using custom *G. formicilis* primers. By this process an axenic isolate was subsequently produced. *G. formicilis* MH32-2 formed raised creamy white/milky colonies with an entire margin after 48 h on TY (Figure 15B, top panel). Further analysis revealed that *G. formicilis* MH32-2 cells formed Gram-variable coccoid/ovoid cells that were often present as pairs or occasionally as short chains (Figure 15B, bottom panel).

[0615] Phylogenetic reconstruction of the *Gemmiger* genus using high-quality GenBank and RefSeq genomes from the NCBI and MH32-1 revealed the isolate placed next to several uncultured members of *G. formicilis*, close to *G. variabile*, and several uncultured species (Figure 15C, D). *G. formicilis* MH32-1 is a sugar fermenter, and is predicted to utilize starch, glucose, fructose, gluconate, lactose, trehalose, and lactaldehyde as a carbon source. Complete, or near complete biosynthetic pathways were identified for all amino acids except tryptophan, phenylalanine, serine, and tyrosine. Several pathways for the uptake and fermentation of amino acids (alanine, arginine, asparagine, cysteine, glutamic acid, glutamine, methionine, serine, and threonine) were also identified, but are likely not primary sources of energy. Metabolic modelling of the *G. formicilis* MH32-1 isolate revealed that when fed with glucose and other hexose sugars, the primary byproducts of fermentation are butyrate, acetate, ethanol, and fumarate. Pathways to produce lactate are also encoded by the *G. formicilis* MH32-1 isolate, but metabolic modelling did not predict these as major fermentation byproducts. The biosynthetic potential of *G. formicilis* MH32-1 was assessed by

searching for well characterised, and putative biosynthetic gene clusters (BGCs) using AntiSmash v5.0 and deepBGC, respectively. AntiSmash identified a single RiPP sactipeptide BGC with no close homology to BGCs in public databases. DeepBGC identified the same sactipeptide RiPP and a further eight putative BGCs with >1 coding region and >0.75 deepBGC score, all annotated as antibacterial.

G. formicilis MH32-1 Enhances Gut Barrier Function

[0616] To assess the role of *G. formicilis* in the healthy gut, naive C57Bl/6 SPF mice were treated for 8 days with *G. formicilis* MH32-1 (Figure 16A). During this treatment period, there was no morbidity, and no changes in general appearance, behaviour, posture, mobility and neurological behaviour were observed. Similarly, there was no significant change in body weight in *G. formicilis* MH32-1 treated animals relative to the vehicle control and colon length and weight/length ratio were also unaffected (Figures 16B-D). *G. formicilis* MH32-1 did not result in any significant histological changes in the colon when compared to the vehicle as determined by assessing epithelial injury and hypervascularization alone, or as a combined histopathological score (Figure 16E-G).

[0617] It was hypothesised that the big-data approach described above could be used to identify novel candidate live biotherapeutics for inflammatory and autoimmune diseases (e.g., IBD). The inventors therefore tested the therapeutic efficacy of *G. formicilis* MH32-1 in an acute model of DSS induced murine colitis (Figure 17A) as this is a well-documented model of epithelial injury and repair. DSS treatment resulted in significant disease activity relative to the vehicle control. There was a significant reduction in body weight (Figure 17B) which has been shown to be an accurate and reliable indicator of colitis (Britto et al., 2019). As expected, prednisone exacerbated the DSS induced weight loss (Yamamoto et al., 2013), however DSS induced weight loss was ameliorated by treatment with *G. formicilis* MH32-1 or *F. prausnitzii* A2-165 (Figure 17B). Endoscopic analysis revealed a progressive increase in disease activity in all treatment groups with *F. prausnitzii* A2-165 and *G. formicilis* but not prednisone resulting in lower disease activity at day 6 relative to the vehicle treatment group (Figure 17C).

[0618] Analysis of the colon revealed significant shortening (Figure 17D) following DSS treatment. Treatment with *F. prausnitzii* A2-165 and *G. formicilis* MH32-1 but not prednisone resulted in a significant reduction in colon shortening (Figure 17D). Histological analysis of DSS treated mice revealed significant gut damage characterised by crypt loss and epithelial erosion. Notably, treatment with *G. formicilis* MH32-1 resulted in significant improvement in pathology characterised by crypt re-formation and re-epithelisation (Figure 17E) as evidenced by improvements in histopathological healing (Figure 17F) and epithelial injury (Figure 17G). Consistent with this, treatment with *G. formicilis* MH32-1 resulted in decreased gut inflammation as determined by lipocalin-2 in faeces (Figure 17H (McSweeney et al., 2005)). As expected, prednisone and *F. prausnitzii* A2-165 also resulted in a significant improvement in disease pathology.

[0619] The IL-23-Th17 cell immune axis is central to the pathogenesis of IBD and is a validated therapeutic target (Martin, et al., 2017). As the DSS model of gut barrier

function is largely underpinned by Th1 polarised immunity (Marks et al., 2015) the therapeutic efficacy of *G. formicilis* MH32-1 in the murine SKG model was also tested. SKG mice carry a mutation in the ZAP-70 gene and develop IL-23 driven Crohn's-like ileitis and autoimmune inflammatory arthritis following disease initiation with curdlan treatment (Figure 17I, (Liu et al., 2019)). Histological analysis of vehicle treated mice revealed significant gut damage characterised by infiltration of inflammatory cells and granuloma formation 7 days after curdlan treatment. As expected, treatment with the anti-IL-23 monoclonal antibody resulted in a significant reduction in histological damage. Treatment with *G. formicilis* MH321 also resulted in significant improvement in pathology as evidenced by improvements in the histopathological score (Figure 17J).

[0620] Taken together, these data revealed that *G. formicilis* MH32-1 did not cause any adverse effects in DSS treated or naïve mice, and that *G. formicilis* MH32-1 enhanced gut barrier function and promoted mucosal healing in DSS treated and SKG mice.

G. formicilis suppresses STAT3 activation in vitro

[0621] Given the dramatic effects on histological inflammation and re-epithelialisation observed in the DSS treated animals, the inventors next examined the ability of *G. formicilis* to modulate IBD associated immune pathways. IL-23 driven immune responses are central to the pathogenesis of IBD and are a clinically recognised target (Friedrich et al., 2019; Yang et al., 2017). The inventors examined the ability of *G. formicilis* MH32-1 to suppress IL-23-mediated activation of STAT3 using the HEK-Blue™ IL-23 reporter cell line. The HEK-Blue™ IL-23 reporter cell line carries a STAT3 inducible SEAP reporter that is responsive to IL-23 stimulation. As expected, IL-23 mediated activation of STAT3 could be prevented by tofacitinib (Figure 18A). Interestingly, cell free culture supernatant prepared from *G. formicilis* MH32-1 grown in TY medium suppressed SEAP reporter activity (Figure 18A). Further analysis revealed the STAT3 suppressive activity of *G. formicilis* was associated with the <3 kDa fraction (Figure 18A). Notably, we did not observe any cytotoxic effects following treatment with *G. formicilis* culture supernatants. The biochemical characteristics of the CS were assessed by heat and proteinase K treatment. By this approach, it was determined that the STAT3 suppressive activity of *G. formicilis* was unaffected by heat or proteinase K (Figure 18B-C).

[0622] Finally, the inventors examined the impact of growth medium dependent effects on IL-23 mediated STAT3 activation. CS prepared from strains grown in TY or PYG medium exhibited potent STAT3 suppressive activities. Interestingly, there was minimal STAT3 suppressive activity when the strains were grown in Wilkins-Chalgren medium and MCM medium (Figure 18D). This is consistent with previous reports (e.g., Giri et al., 2019; Schreiner et al., 2019) and suggests that the immunomodulatory activity *G. formicilis* MH321 can be enhanced by nutritional factors.

[0623] In order to demonstrate that the IL-23 suppressive activity was characteristic of *G. formicilis* as a species, two additional *G. formicilis* strains were analysed. The ability of *G. formicilis* MH32-2 culture supernatant to suppress IL-23 mediated activation of STAT3 was assessed using the HEK Blue IL-23 reporter cell line. The HEK Blue IL-23 cell

line contains a STAT3 responsive SEAP reporter whose expression is induced by IL-23. Treatment with tofacitinib, a pan JAK inhibitor, inhibits IL-23 mediated SEAP expression. Treatment with TY medium resulted in modest suppression of IL-23 mediated activation of STAT3 (Figure 18E). The present inventors also interrogated the ability of culture supernatant and <3 kDa supernatant fraction to modulate STAT3 activity. The <3 kDa fraction as was analysed to determine whether the STAT3 activity was due to a low molecular weight bioactive, which would be more amenable to drug development. *G. formicilis* MH32-2 culture supernatant suppressed IL-23 mediated STAT3 activation (Figure 18E). Notably, the *G. formicilis* MH32-2 <3 kDa fractions suppressed IL-23 mediated STAT3 activation to a similar extent as the cell free culture supernatant (Figure 18E). The <3 kDa culture supernatant fraction of MH32-1 was also able to significantly suppress IL-6-induced STAT3 activation in HEK reporter cells ($p = 0.0009$) (Figure 18J). During both experiments, no cytotoxicity effects of the supernatants were detected.

[0624] In addition, the *G. formicilis* type strain (ATCC 27749) suppressed IL-23 mediated STAT3 activation, providing further evidence that this activity was representative of the *G. formicilis* species. The suppressive activity was detected in the supernatant fraction suggesting that it was mediated by a secreted bioactive (Figure 18F). Furthermore, the bioactive(s) are likely low molecular weight as the suppressive activity was detected in the <3 kDa fraction.

[0625] Next, the present inventors examined the ability of *Gemmiger* sp. MD158 culture supernatant to suppress IL-23 mediated activation of STAT3 using the HEK Blue IL-23 reporter cell line. Treatment with tofacitinib inhibited IL-23 mediated SEAP expression. Treatment with TY medium resulted in modest suppression of IL-23 mediated activation of STAT3 (Figure 18G). We examine the ability of culture supernatant and <3 kDa supernatant fraction to modulate STAT3 activity. The present inventors examined the <3 kDa fraction as we believed low molecular weight bioactives would be more amenable to drug development. *Gemmiger* sp. MD158 culture supernatant suppressed IL-23 mediated STAT3 activation (Figure 18G). Notably, the *G. Gemmiger* sp. MD158 <3 kDa fractions suppressed IL-23 mediated STAT3 activation to a similar extent as the cell free culture supernatant (Figure 18G). During the experiment, no cytotoxicity effects of the supernatants were detected.

[0626] Given the immunomodulatory capacity of the culture supernatant, the inventors realised that the bioactives produced by *G. formicilis* may have direct effects on peripheral immune cells (PBMCs). We therefore examined the effect of *G. formicilis* MH32-1 culture supernatant on IL-6 production as increased levels of the pro-inflammatory cytokine IL-6 and signalling via its receptor IL-6R is associated with IBD pathogenesis. Notably, IL-6 contributes to chronic inflammation in the gut due to its pro-inflammatory and anti-apoptotic effects on immune cells. These effects are mediated by IL-6 receptor binding which causes JAK kinase activation and STAT3 dimerization in combination with activation of MAPK/ERK and other downstream kinases. Treatment of PBMCs with PIM induced IL-6 production and this induction was unaffected by pre-treatment with TY medium (Figure 18H). Pre-treatment with *G. formicilis* MH32-1 culture supernatant suppressed PIM stimulated IL-6 secretion

relative to the TY pre-treated control (Figure 18H). Pre-treatment of T84 gut epithelial cells with *G. formicilis* MH32-1 culture supernatant or the <3 kDa culture supernatant fraction suppressed LPS mediated IL-6 secretion (Figure 18I). Taken together, these data shows that *G. formicilis* can modulate an important cytokine with a key role in innate and adaptive immune cell driven responses.

G. formicilis promotes human intestinal epithelial cell migration.

[0627] Damage of the intestinal barrier commonly occurs in IBD. The rapid migration of intestinal epithelial cells is a crucial component of the wound healing process to re-establish homeostasis. To investigate whether bioactives derived from *G. formicilis* can affect the motility of intestinal epithelial cells, a TRANSWELL® migration assay was employed. HCT116 cells were seeded apically in a TRANSWELL® chamber and the ability of *G. formicilis* extract to promote migration to the basolateral side of the chamber was assessed. Untreated cells had a basal level of migration to the basolateral side of the membrane, and this was unaffected by treatment with TY medium (Figure 19A-B). *G. formicilis* MH32-1 significantly promoted the migration of HCT116 cells to the basolateral side (Figure 19A-B). The pro-migratory effects of *G. formicilis* were further confirmed using the IncuCyte scratch wound assay. At 18 hours post induction of a scratch wound, HCT116 cells showed an accelerated rate of wound closure in the presence of extract from *G. formicilis* MH32-1, compared to the control cells treated with TY medium (Figure 19C-D).

G. formicilis supports effective gut barrier function

[0628] Intestinal epithelial cells form a physical and biochemical barrier that separates host tissue from gut microbes and luminal contents (Peterson et al., 2014). Impaired gut barrier function has been implicated in the pathogenesis of inflammatory and autoimmune diseases, including IBD (Vanuytsel et al., 2021) and has been proposed as a therapeutic target to improve disease outcome (Sommer et al., 2021).

[0629] The present inventors assessed the integrity of the gut epithelial cell barrier using a non-invasive method termed trans-epithelial electrical resistance (TEER). In this assay, an electrical current is applied across an epithelial cell layer and the resistance is recorded. A reduction in the TEER value is indicative of a compromised barrier. TEER measurements constitute the "gold standard" for non-invasive measurements of barrier integrity in mono-culture cell layers.

[0630] The ability of *G. formicilis* to modulate barrier function using T84 epithelial cells was determined. In this model, IFN- γ is known to negatively affect barrier function and was therefore utilised to simulate a diseased state. IFN- γ exposure resulted in a significant drop in recorded TEER values, indicative of a reduced barrier integrity. Treatment with <3 kDa fractionated culture supernatant from *G. formicilis* MH32-1 significantly mitigated the IFN- γ -mediated reduction in TEER relative to the YG/V medium control (Figure 20A) and thus, *G. formicilis* may promote the restoration of barrier integrity.

Materials & Methods

Bacterial strains, culture conditions and analyses.

[0631] Stool samples were collected from healthy human adults with no history of gastrointestinal disorders and mixed with an equal weight per volume of sterile oxygen free glycerol solution (McSweeney, 2005). Donors had not consumed antibiotics in the three months prior to collection of the faecal samples. *G. formicilis* and *Faecalibacterium prausnitzii* were routinely processed in a Coy vinyl anaerobic chamber with an oxygen free atmosphere (85% N₂: 10% CO₂: 5% H₂) atmosphere. *G. formicilis* was routinely cultured in TY or PYG medium while *F. prausnitzii* was cultured in TY medium. All isolates were stocked by mixing 3 mL of actively growing culture with an equal volume of glycerol solution (McSweeney, 2005) and storing at -80 °C.

Isolation of *G. formicilis* MH32-1.

[0632] An isolate of *G. formicilis* was produced by inoculating a donor faecal sample with *G. formicilis* present at a relative abundance of 2.48% into TY medium and then serially diluting to extinction. Colonies were recovered on TY medium and then screened essentially as previously described to identify extremely oxygen sensitive isolates. Here, individual colonies were replica plate over 2 TY agar plates in an anaerobic chamber. One plate was subsequently removed from the anaerobic chamber and exposed to atmospheric conditions for 1 hour. The plate was then returned to the anaerobic chamber and both plates were incubated at 37°C. Extremely oxygen sensitive isolates were identified by their failure to grow following 1 hour exposure to atmospheric conditions. A single isolate was purified by streaking on TY agar and *G. formicilis* MH32-1 was identified by whole genome sequencing.

Isolation of *G. formicilis* MH32-2.

[0633] Donor faecal sample BBD6936 with *G. formicilis* present at a relative abundance of 6.48% (see Experimental Data/Faecal Biobank data) was serially diluted and plated on TY medium. Following 5 days growth, the plates were examined and plates with >50 colonies were divided into 6 equal parts. The culturable microbial diversity on each plate was assessed by plate scrape metagenomic sequencing (psMGS). Briefly, the colonies in at least two parts were independently scraped off the plate into sterile anaerobic diluent and DNA was extracted and submitted for metagenomic sequencing. The sequence data was analysed using the Microba Analysis Pipeline and the diversity in each section was assessed. Single colonies were subsequently arrayed onto TY agar and incubated at 37°C until grown. DNA was extracted from individual patches and analysed by qPCR using custom *G. formicilis* targeting primers (forward primer P_f 5' -CGCGCAGTCGAACGCAATGTTCGTGAC C-3' ; reverse primer P_r 5' -GGCACAGCGCACTGGAGATGAAGCGC-3'). By this approach, a single candidate *G. formicilis* isolate was identified and streaked onto TY agar. A single colony was picked and streaked on TY agar and this process was repeated once more. The identity and purity of the final isolate was assessed by whole genome sequencing and microscopy. The axenic isolate was determined to be Gram-variable staining coccoid isolate with a characteristic dumbbell morphology and was termed *G. formicilis* MH32-2 following whole genome sequencing.

[0634] *G. formicilis* MH32-2 was examined by microscopy using standard protocols in the art. Briefly, a broth culture (usually 0.2 mL) was centrifuged, and the cell pellet was resuspended in 3-6 μ L of PBS. The cell pellet was transferred onto a glass slide and smeared with a 1 μ L loop, whereafter it was air-dried, or heat fixed. The dried/heat-fixed smear was flooded with crystal violet staining reagent for 30 seconds and the slide was then washed in an indirect stream of tap water to remove unbound stain. The slide was next flooded with Gram's iodine for 30 seconds and the slide was again washed in an indirect stream of tap water to remove unbound stain. The slide was next flooded with decolorizing agent and then immediately washed with tap water. Finally, the slide was flooded with safranin for 30 seconds and the slide was then washed in an indirect stream of tap water until the water ran clean. The slide was allowed to air dry, and cells were then visualised under an oil immersion using a Brightfield microscope.

Isolation of Gemmiger sp. MD158

[0635] To isolate *Gemmiger* sp. MD158, 1 mL of a faecal stock sample was mixed with a further 3 mL of 30% glycerol solution and vortexed for 2 minutes with $\sim$ 3.5mm glass beads (Daintree Scientific) to dissociate bacteria from faecal debris. Aliquots of the dissociated bacteria were stored at -80°C for later processing as necessary. Bacterial cells were sorted using a BD FACSAria™ Fusion Flow Cytometer with a 100 μ m nozzle and small particle detector according to the manufacturer's instruction. The system was pressurised at 20 psi and events were triggered on scatter with thresholds set using 0.2 μ m filtered PBS which allowed a few events from the noise floor. To sort bacterial cells, an aliquot was filtered through a 40 μ m filter and a 10^{-3} dilution of the filtrate was subsequently prepared using a 0.2 μ m filtered anaerobic buffered diluent solution (i.e., 38 ml/L each of salt solutions 2 & 3 (McSweeney et al., 2005)). The diluted filtrate was run to determine the dilution resulting in 500-1200 events per second. Targets were gated on scatter and doublets were excluded (gating strategy shown in Figure 20B). Single cells were deposited onto a TY agar plate and incubated at 37°C for up to 3 weeks. Isolates were identified by whole genome sequencing following growth in TY broth. By this approach, an isolate affiliated with *Gemmiger* sp. was tentatively identified. The final purified isolate was glycerol stocked and submitted for whole genome sequencing and by this approach an isolate termed *Gemmiger* sp. MD158 was produced.

Metabolic reconstruction.

[0636] Protein coding sequences were predicted and annotated using the annotate function in enrichM (version 0.5.2). Briefly, enrichM identifies protein coding sequences using prodigal (version 2.6.3) in -p meta mode. The amino acid sequences are then searched against the UniRef100 database (downloaded November 2020) using DIAMOND (version 2.0.4), and E.C., TCDB and eggnoG classifications are inherited from the idmapping file distributed with UniRef. Hmmer hmmsearches (version 3.1b2) against Pfam (release 33.0), tigrfam (release 15.0) and dbcan2 (downloaded September 2019) were used to annotate functional domains, key metabolic markers and carbohydrate activate (CAZy) enzymes, respectively. Metabolic pathways were identified using the classify function in

enrichM, which assesses annotations and their genomic position against manually defined metabolic pathway definitions. A pathway is considered present in a genome if it encoded >80% of the required proteins, and passes all required synteny checks. These automatically predicted pathways were then manually assessed. In addition, gutSMASH (version 1.0.0)
 5 was applied to identify common biosynthetic pathways encoded by gut microorganisms.

Phylogenetic tree

[0637] A genome tree was constructed from high quality genomes, defined as $\geq 90\%$ complete and $\leq 5\%$ contamination from checkM analysis, within the *Gemmiger* genus (NCBI release 89) and the four MH32 isolates. For each genome, a set of 122 bacteria-specific conserved marker genes were extracted from each genome using gtdbtk *identify*.
 10 These genes were then aligned to profile HMMs and concatenated to a single alignment with gtdbtk *align*, and a Maximum likelihood phylogenetic tree was constructed from the alignments using FastTree (version 2.1.10) with gtdbtk *infer*. Non-parametric bootstrap values were inferred using GenomeTreeK (v0.1.6) from 1000 repetitions.

Preparation of bacterial strains for animal experimentation.

[0638] *G. formicilis* and *F. prausnitzii* strains were grown to early stationary phase in TY medium. The cell density of the individual cultures was calculated using a Helber Counting Chamber. To prepare the bacterial gavage solutions, individual cultures were centrifuged under a layer of sterile heavy mineral oil at 5,000 g for 10 minutes and the cell-free supernatant was then discarded. The cell pellets were washed in 1.5 mL of sterile
 20 anaerobic buffered diluent (38 mL/L each of salt solutions 2 & 3 (McSweeney et al., 2005), 1 mL/L of 0.1% (w/v) resazurin solution, 1 g/L L-cysteine) and then centrifuged again. Finally, the washed cell pellet was resuspended in half strength glycerol solution (15% v/v glycerol solution in anoxic buffered diluent) to a final concentration of 1×10^9 cells/mL, aliquoted and
 25 frozen at -80°C until required. The viability of the cell preparations was confirmed by thawing a single aliquot and streaking on an agar plate. The identity and purity of the individual strain preparations was confirmed by whole genome sequencing.

Acute model of DSS-induced gut barrier dysfunction.

[0639] Six-week-old C57BL/6 female mice purchased from Animal Resources
 30 Centres (Western Australia, Australia) were randomised and then co-housed for seven days prior to experimentation. To induce gut barrier dysfunction, mice were treated with 3% DSS *ad libitum* in the drinking water for six days. Naïve age matched control mice were processed and received DSS free drinking water. All treatments started one day prior to provision of DSS and all mice were sacrificed two days after the final DSS treatment. For the
 35 treatments, mice were anaesthetised with isofluorane and orally gavaged with 200 μL of bacterial preparations or vehicle control. Prednisone (2 mg/kg) was administered following anaesthetisation by intraperitoneal injection. Body weights and stool consistency were recorded daily. Stool samples were collected daily. Following sacrifice, the colon, liver and spleen were collected for analysis. Blood was collected by cardiac puncture.

Endoscopic and Histological scoring.

[0640] Animals were examined with a small animal gastrointestinal endoscope (Karl Storz Endoskope, Tuttlingen, Germany) on days 1, 2 and 6 to assess the extent of colon mucosal inflammation (Marks et al., 2015; and Liu et al., 2019). Briefly, mice were anesthetised with isoflurane and a colonoscope was inserted through the rectum. Images captured by high-definition videos were examined in a blinded manner to assess the presence and extent of disease pathologies (Table 5). Histological scoring was performed essentially as previously described in the art (Marks et al., 2015). Briefly, samples were fixed in 4% formalin, paraffin embedded and sectioned. Tissue sections were hematoxylin and eosin stained to assess disease pathology and with Alcian blue to assess mucin production. Slides were imaged using the Aperio digital imaging system (Leica Biosystems, Nußloch, Germany). To grade colitis severity, the extent of inflammation and epithelial injury in the tissue sections were graded semi-quantitatively using an established scoring system (Table 6). The samples were then randomised and subsequently scored in a blinded by a trained gastrointestinal pathologist.

Characterisation of STAT3 suppressive activity.

[0641] To assess STAT3 suppressive activity of the *G. formicilis* strains, three independent colonies were inoculated and grown until early stationary phase. Then, each seed culture broth was used to inoculate two technical replicates each generating six technical replicates from three biological replicates. The technical replicates were grown until early stationary phase and then cell free culture supernatant was harvested as previously described (Giri et al., 2019). Culture supernatants were size fractionated by passing through a 3 kDa CENTRICON® column according to the manufacturer's (Merck Millipore) instructions. To prepare *Gemmiger* sp. MD158 supernatant samples for assay, a seed culture was established and grown until early stationary phase. Then, the seed culture broth was used to inoculate two technical replicates. The technical replicates were grown until early stationary phase and then cell free culture supernatant was harvested as previously described (Giri et al., 2019). Culture supernatants were size fractionated by passing through a 3 kDa CENTRICON® column according to the manufacturer's (Merck Millipore) instructions.

[0642] STAT3 activity was assessed using the HEK Blue IL-23 cell line (Invivogen). Briefly, 50,000 cells per well were seeded in triplicate in a 96-well plate 24 hours prior to the start of the assay. Bacteria supernatant or sterile bacterial medium were mixed at a final concentration of 10%, 25%, or 50% v/v with recombinant human IL23 (rhIL-23, R&D systems) at a final concentration of 5 ng/mL. This mixture was then added directly to the cells and incubated at 37°C for 6 hours. The ability of the supernatants to suppress STAT3 activation was compared to the Janus kinase inhibitor tofacitinib (10 µM). STAT3 regulated SEAP reporter activity was assessed using Quanti Blue solution as recommended by the manufacturer (Invivogen). Results are the average of at least three independent experiments. Cytotoxicity was assessed using MTT assay. Briefly, MTT was added to the cells at a final concentration of 1.2 mM. The cells were incubated at 37°C for

4 hours and then fixed with DMSO. Cytotoxicity was assessed by measuring absorbance at 540 nm as recommended by the manufacturer (Invitrogen, Thermo Fisher, Australia).

Cytokine production assay.

5 **[0643]** 5 x 10⁴ of T84 cells were seeded in triplicate in 96 well plates and left to settle overnight. Raw or <3 kDa-filtered bacterial culture supernatant (25% v/v) or media control were added to T84 cells. After 90-minute treatment with the bacterial culture supernatants, the cells were stimulated with LPS (100 ug/ml). After 6 hours, T84 culture supernatant was collected for IL-6 assessment. IL-6 was measured using the HEK-blue cell reporter system from InvivoGen according to standard protocol..

10 **[0644]** PBMCs were extracted as previously described (Mallone et al., 2011). Briefly, PBMCs were isolated from blood samples using a Ficoll/Lymphoprep gradient (Stemcell), frozen at 80°C, and stored in liquid nitrogen. For the experiments, 250,000 PBMCs/well were seeded in 24-well plates. PBMCs were stimulated with PMA/Ionomycin/Monensin (40 ng/mL, 1 mg/mL, and 2 mg/mL respectively) for 4 hours at 37°C. An
15 untreated control was used as negative control. Where indicated, PBMCs were pre-treated for 30 minutes at 37°C with bacterial supernatant or bacterial medium control at final concentration of 10% v/v. To characterise the IL-6 present in PBMC culture supernatants, cell culture media supernatants from PBMCs that were stimulated as described above were collected and stored at -80°C. These supernatants were subsequently thawed and used to
20 determine effects on cytokine production.

Cell migration assay.

[0645] The IncuCyte® Live-Cell Imaging System (Essen BioScience) and Transwell migration assay were used to assess the migration of HCT116 cells during exposure to sterile culture supernatant from *G. formicilis*. Human HCT116 gut epithelial cells were maintained in
25 McCoy's 5a medium supplemented with 10% FBS and 1% Pen/Strep. For the IncuCyte® scratch wound assay, 3.5 x 10⁴ HCT116 cells were plated on poly-L-ornithine-coated IncuCyte® ImageLock 96-well plates (Essen BioScience). After 24 hours, the IncuCyte® WoundMaker tool was used to induce a homogeneous scratch wound in the nearly confluent cell monolayer. The cells were washed twice with DPBS and then stationary phase culture
30 supernatant from *G. formicilis* at 1% (v/v) in 200 µL 0.5% FBS McCoy's 5a medium was added. Uninoculated bacterial media (1%) served as negative control. Immediately after adding the stimulants, the plate was transferred to the IncuCyte® system and cell migration was monitored by imaging each well every 2 hours over the course of 72 hours. Data analysis was performed using the integrated analysis software.

35 **[0646]** To assess cell migration via the TRANSWELL® assay, 3.5 x 10⁴ HCT116 cells were seeded in 100 µL 10% FBS culture medium in the top compartment of a 6.5 mm insert with TC-treated polycarbonate membrane in 24-well plates (8 µm pore size, Corning Costar). 600 µL 10% FBS culture medium was added to the lower compartment. The cells were allowed to settle for 24 hours. After a DPBS wash, 100 µL and 600 µL of 0.5% FBS
40 medium was added to the top and lower compartment, respectively. Then, 0.4x concentrated extract from *G. formicilis* was added to the lower compartment. These bacterial extracts

were prepared using an Amberlite XAD-7 resin as previously described. After 16 hours, the cells were washed with DPBS and the cells attached to the top of the membrane were carefully removed with a cotton tip. The migrated cells on the bottom of the membrane were then fixed in 70% ethanol for 10 minutes followed by staining in 0.25% crystal violet for 5 minutes. The TRANSWELL® inserts were washed with water, dried and the membrane mounted with 50% glycerol in water on glass slides and imaged immediately. TRANSWELL® experiments were performed in biological and technical triplicates and for each replicate two representative images of the membrane were taken at 10x magnification. The number of migrated cells was automatically counted using ImageJ and the average cell number displayed. The extent of cell migration was expressed as the average number of migrated cells in two microscopic fields per well from three biological and three technical replicates.

Epithelial barrier integrity assay using Trans-Epithelial Electrical Resistance (TEER).

[0647] The ability of *G. formicilis* to prevent loss of barrier integrity was assessed by measuring the transepithelial electrical resistance across confluent monolayers of T84 gut epithelial cells using the Maestro Pro system. T84 cells were purchased from CellBank Australia and cultured in Dulbecco's Modified Eagle Medium Nutrient Mixture 12 (DMEM/F12; ThermoFisher Scientific, Waltham, MA, USA) supplemented with 5% foetal bovine serum and 1% Penicillin-Streptomycin. T84 cells were seeded at a density of 0.1×10^6 cells in a total of 0.2 mL/well into 96-well CytoView-Z plates and incubated at 37°C, 5% CO₂ in the Maestro Pro system (Axion BioSystems, Atlanta, GA, USA). To maintain consistent humidity, sterile water was added to compartments surrounding the wells. Once stable TEER measurements were established (approximately 100 h post-seeding), cells were stimulated with recombinant human IFN- γ (100 ng/mL; R&D systems, Minneapolis, MN, USA). At 72 h post-stimulation, cells were washed with dPBS (ThermoFisher Scientific, Waltham, MA, USA) and media replaced with DMEM/F12 only, or DMEM/F12 supplemented with 1 x YG/V media control or 1 x *G. formicilis* bacterial extract prepared using Amberlite XAD-7 resin as previously outlined. At 45 h post-treatment, TEER measurements were recorded, and the percentage difference in TEER value compared to the control (untreated T84 cells) was visualised in histograms as mean $\pm$ SD. Statistical significance was determined by unpaired t-test.

[0648] The disclosure of every patent, patent application, and publication cited herein is hereby incorporated herein by reference in its entirety.

[0649] The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

[0650] Throughout the specification the aim has been to describe the preferred embodiments of the invention without limiting the invention to any one embodiment or specific collection of features. Those of skill in the art will therefore appreciate that, in light of the instant disclosure, various modifications and changes can be made in the particular embodiments exemplified without departing from the scope of the present invention. All such modifications and changes are intended to be included within the scope of the appended claims.

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WHAT IS CLAIMED IS:

1. A cell of the *Alistipes shahii* strain deposited under any one of accession numbers V21/014432, V21/014433, or V21/014434, or a derivative thereof.
- 5 2. The cell of claim 1, wherein the cell is at least partially isolated.
3. A biologically pure culture of the *Alistipes shahii* strain deposited under any one of accession numbers V21/014432, V21/014433, or V21/014434, or a derivative thereof.
- 10 4. A composition comprising the cell of claim 1 or claim 2, or the culture of claim 3.
5. A composition comprising a bacterial strain with a 16S rRNA sequence that is at least about 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9%,
15 identical to any one of SEQ ID NOs: 1-3 or 44, or which has a 16S rRNA gene sequence represented by any one of any one of SEQ ID NOs: 1-3 or 44.
6. The composition of claim 4 or claim 5, further comprising a pharmaceutically acceptable excipient, diluent, or carrier.
- 20 7. A pharmaceutical composition comprising a bacterial strain with a 16S rRNA sequence that is at least about 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to the 16S rRNA sequence of a bacterial strain of the species *Alistipes shahii*, together with a pharmaceutically acceptable carrier, diluent, or excipient.
- 25 8. A pharmaceutical composition comprising a bacterial strain that is a phylogenetic descendant of the MRCA of *A. timonensis* and *A. sp000434235*, together with a pharmaceutically acceptable carrier, diluent, or excipient.
- 30 9. The pharmaceutical composition of claim 8, wherein the MRCA is defined at node 35260 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.
10. The composition of any one of claims 5 to 9, wherein the bacterial strain is at least
35 partially isolated.
11. The composition of any one of claims 4 to 10, wherein the bacterial strain is viable or non-viable.
- 40 12. The composition of any one of claims 4 to 11, wherein the composition is in a dried form.

13. The composition of claim 12, wherein the composition is formulated in a capsule, a tablet, a pill, a troche, a lozenge, a powder, or a granule.
14. The composition of claim 12 or claim 13, wherein the composition is dried by lyophilisation, spray drying, fluidized bed drying, vacuum drying, or a combination thereof.
15. The composition of any one of claims 4 to 14, wherein the composition is formulated for delivery to the gut.
16. The composition of any one of claims 4 to 15, further comprising a prebiotic.
17. The composition of any one of claims 4 to 16, further comprising one or more additional bacterial strains.
18. The composition of claim 17, wherein the one or more additional bacterial strains are at least partially isolated.
19. The composition of any one of claims 4 to 18, wherein the composition does not comprise bacteria of the genus *Clostridium*.
20. The cell, culture, or composition according to any one of claims 1 to 19, wherein the bacterial strain produces an agent that attenuates or impairs signal transducer and activator of transcription 3 (STAT3) signalling in a cell.
21. The cell, culture or composition according to claim 20, wherein the agent is a small molecule, peptide, or nucleotide.
22. The cell, culture or composition according to claim 20 or claim 21, wherein the agent is released by the bacteria.
23. The cell, culture or composition according to any one of claims 20 to 22, wherein the agent binds specifically to any one of STAT3, JAK2, TYK, or IL-23.
24. The cell, culture, or composition according to any one of claims 1 to 23, wherein the bacterial strain produces one or more metabolite selected from butyrate, acetate, ethanol, and fumarate.
25. The cell, culture or composition according to any one of claims 1 to 24, wherein the bacterial strain is of the species *A. shahii*.
26. A food or drink product comprising the composition of any one of claims 4 to 25.

27. A method of restoring or improving gut barrier function in a subject, the method comprising administering to the subject a bacterial strain of the *Alistipes* genus, to thereby restore or improve gut barrier function.
- 5 28. The method of claim 27, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *A. timonensis* and *A. sp000434235*.
29. The product of claim 28, wherein the MRCA is defined at node 35260 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.
- 10 30. The method of any one of claims 27 to 29, wherein the bacterial strain is of the species *A. shahii*.
31. The method of any one of claims 27 to 30, wherein restoring or improving gut barrier
15 function is characterised by at least one of: (i) an increase in the quality and/or quantity of mucin; (ii) improvement in integrity of tight junction proteins; (iii) reduction in translocation of luminal contents into systemic circulation; or (iv) a reduction of intestinal ulcers and/or intestinal wounds.
- 20 32. The method of claim 31, wherein the luminal contents includes lipopolysaccharide (LPS).
33. The method of any one of claims 27 to 32, wherein the restoration or improvement in gut barrier dysfunction results in a reduction in systemic inflammation in the subject.
- 25 34. The method of claim 33, wherein systemic inflammation is identified in the subject when the level of an inflammatory cytokine (e.g., IL-1 β , IL-8, IL-6, and TNF) in a sample from the subject is above a predetermined threshold.
- 30 35. A method of inducing or enhancing mucosal healing in a subject, the method comprising administering to the subject a bacterial strain of the *Alistipes* genus in an amount sufficient to induce epithelial cell migration and/or proliferation; to thereby induce mucosal healing in the subject.
- 35 36. The method of claim 35, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *A. timonensis* and *A. sp000434235*.
37. The product of claim 36, wherein the MRCA is defined at node 35260 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.
- 40 38. The method of any one of claims 35 to 37, wherein the bacterial strain is of the species *A. shahii*.

39. The method of any one of claims 35 to 38, wherein mucosal healing is measured using one or more fecal or serum markers.
40. The method of claim 39, wherein one or more fecal markers are selected from the group comprising calprotectin, lactoferrin, metalloproteinase (MMP)-9, and lipocalin-2.
41. The method of any one of claims 35 to 40, wherein mucosal healing is measured using endoscopic score.
42. A method of reducing inflammation in a subject, the method comprising administering to the subject a therapeutically effective amount of a bacterial strain of the *Alistipes* genus, to thereby reduce inflammation in the subject.
43. The method of claim 42, wherein the bacterial species is a phylogenetic descendant of the MRCA of *A. timonensis* and *A. sp000434235*.
44. The product of claim 43, wherein the MRCA is defined at node 35260 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.
45. The method of any one of claims 42 to 44, wherein the bacterial strain is of the species *A. shahii*.
46. The method of any one of claims 42 to 45, wherein the inflammation is local to the gut environment, or systemic inflammation.
47. The method of any one of claims 42 to 46, wherein the bacterial strain attenuates the NFκB pathway (e.g., by reducing or inhibiting NFκB).
48. A method of blocking or otherwise inhibiting the activation of STAT3 signalling in a target cell, the method comprising contacting the target cell with at least a soluble component of a bacterial cell preparation of the *Alistipes* genus, to block or otherwise inhibit the activation of STAT3 signalling in the target cell.
49. The method of claim 48, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *A. timonensis* and *A. sp000434235*.
50. The product of claim 49, wherein the MRCA is defined at node 35260 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.
- The method of any one of claims 48 to 50, wherein the bacterial strain is of the species *A. shahii*.

51. The method of any one of claims 48 to 51, wherein the target cell is selected from the group comprising a reporter cell (e.g., a HEK cell), an immune cell (e.g., a Th17 immune cell), an epithelial cell, and an endothelial cell.
- 5 52. The method of any one of claims 48 to 51, wherein the bacterial cell preparation comprises a bacterial cell culture.
53. The method of claim 52, wherein the soluble component comprises the supernatant of the bacterial cell culture.
- 10 54. The method of any one of claims 48 to 53, wherein the soluble component is substantially depleted of bacterial cells.
55. The method of any one of claims 48 to 54, wherein the bacterial cell preparation
15 comprises a bacterial cell pellet.
56. The method of claim 55, wherein the soluble component comprises soluble fraction of the lysed cells.
- 20 57. The method of claim 56, wherein the soluble fraction is substantially separated from the insoluble cell fraction by centrifugation.
58. The method of any one of claims 48 to 57, wherein the method is performed *in vitro*.
- 25 59. A method of blocking or otherwise inhibiting STAT3 signalling in the gut environment of a subject, the method comprising administering to the subject a bacterial strain of the *Alistipes* genus, to block or otherwise inhibit STAT3 signalling in the gut environment of the subject.
- 30 60. The method of claim 59, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *A. timonensis* and *A. sp000434235*.
61. The product of claim 60, wherein the MRCA is defined at node 35260 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.
- 35 62. The method of any one of claims 59 to 61, wherein the bacterial strain is of the species *A. shahii*.
63. The method of any one of claims 59 to 62, wherein the cell is an immune cell (e.g., a
40 Th17 immune cell), epithelial cell, or endothelial cell.
64. The method of any one of claims 59 to 63, wherein the cell is an epithelial cell, wherein the bacterial strain or molecule increases the production of IL-22 in the subject.

65. The method of any one of claims 59 to 64, wherein the bacterial strain produces a molecule that is a direct inhibitor or an indirect inhibitor of STAT3.
- 5 66. The method of any one of claims 59 to 65, wherein the bacterial strain produces a molecule that directly inhibits at least one of an IL-23 polypeptide, a JAK2 polypeptide, a TYK2 polypeptide, or a STAT3 polypeptide.
67. The method of any one of claims 27 to 66, wherein the bacterial strain produces one
10 or more of the following metabolites: butyrate, acetate, ethanol and fumarate.
68. The method of any one of claims 27 to 67, wherein the bacterial strain has a 16S rRNA sequence that is at least about 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% identical to the 16S rRNA sequence of a bacterial
15 strain of the species *A. shahii*; or when the bacterial strain has a 16S rRNA gene sequence of a bacterial strain of *A. shahii*.
69. The method of any one of claims 27 to 68, wherein the bacterial strain has a 16S rRNA sequence that is at least about 97.5%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%,
20 99.6%, 99.7%, 99.8%, 99.9% identical to any one of SEQ ID NOs: 1-3 or 44, or when the bacterial strain has the 16S rRNA gene sequence represented by any one of SEQ ID NOs: 1-3 or 44.
70. The method of any one of claims 27 to 69, wherein the bacterial strain is the *A. shahii*
25 strain deposited under any one of accession numbers V21/014432, V21/014433, or V21/014434, or a derivative thereof
71. The method of any one of claims 27 to 70, wherein the bacterial strain is at least partially isolated.
30
72. The method of any one of claims 27 to 71, wherein the bacterial strain is formulated as a pharmaceutical composition, further comprising a pharmaceutically acceptable carrier, diluent or excipient.
- 35 73. The method of claim 72, wherein the composition is in a dried form.
74. The method of claim 73, wherein the dried form is selected from the group comprising particles, granules, and powder.
- 40 75. The method of claim 73 or claim 74, wherein the composition is dried by lyophilisation, spray drying, fluidized bed drying, vacuum drying, or a combination thereof.

76. The method of any one of claims 72 to 75, wherein the pharmaceutical composition is formulated for oral administration.

77. A method of treating or preventing an inflammatory or autoimmune disorder in a
5 subject, the method comprising administering an effective amount of a bacterial strain of the *Alistipes* genus to the subject, to thereby treat or prevent the inflammatory or autoimmune disorder.

78. The method of claim 77, wherein the bacterial strain is a phylogenetic descendant of
10 the MRCA of *A. timonensis* and *A. sp000434235*.

79. The product of claim 78, wherein the MRCA is defined at node 35260 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

80. The method of any one of claims 77 to 79, wherein the bacterial strain is of the species
15 *A. shahii*.

81. The method of any one of claims 77 to 80, wherein the inflammatory or autoimmune
20 disorder is selected from the group comprising an inflammatory bowel disease (such as Crohn's disease or ulcerative colitis); asthma (such as allergic asthma or neutrophilic asthma); fatty liver disease (such as non-alcoholic fatty liver disease (NAFLD)); ankylosing spondylitis; systemic lupus erythematosus (SLE); scleroderma; Sjogren's syndrome; and vasculitis.

82. The method of claim 77 to 81, wherein the inflammatory or autoimmune disorder is an
25 inflammatory bowel disease (IBD).

83. The method of any one of claims 77 to 82, wherein when administered to a subject,
30 the bacterial strain blocks or otherwise inhibits STAT3 signalling in at least a cell of the subject.

84. The method of claim 83 wherein the cell is an epithelial cell, immune cell (e.g., a Th17 immune cell), or an endothelial cell.

85. The method of any one of claims 77 to 84, wherein the bacterial strain produces one
35 or more metabolites selected from the group comprising butyrate, acetate, ethanol and fumarate.

86. The method according to any one of claims 77 to 85, wherein the bacterial strain has a
40 16S rRNA sequence that is at least about 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% identical to the 16S rRNA sequence of a bacterial strain of the genus *Alistipes*.

87. The method according to any one of claims 77 to 86, wherein the bacterial strain has a 16S rRNA sequence that is at least about 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% identical to any one of SEQ ID NOs: 1-3, 7, 8, or 44, or when the bacterial strain has the 16S rRNA gene sequence represented by any one of SEQ ID NOs: 1-3, 7, 8, or 44.

5

88. The method according to any one of claims 77 to 87, wherein the bacterial strain is at least partially isolated.

89. The method according to any one of claims 77 to 88, wherein the bacterial strain is formulated as a pharmaceutical composition, together with a pharmaceutically acceptable carrier, diluent, and/or excipient.

10

90. The method of claim 89, wherein the composition is in a dried form.

91. The method of claim 90, wherein the dried form is selected from the group comprising particles, granules, and powder.

15

92. The method of claim 90 or claim 91, wherein the composition is dried by lyophilisation, spray drying, fluidized bed drying, vacuum drying, or a combination thereof.

20

93. The method of any one of claims 77 to 92, wherein an anti-inflammatory agent is co-administered to the subject.

94. The method of claim 93, wherein the anti-inflammatory agent is selected from the group comprising a 5-aminosalicylate, corticosteroid, azathioprine, infliximab, and adalimumab.

25

95. The method of any one of claims 27 to 94, wherein treating comprises, prior to administering the composition to the subject, identifying that the subject has a deficiency in *A. shahii* gut bacteria.

30

96. The method of claim 95, wherein identifying the deficiency in the subject comprises measurement of *A. shahii* bacteria in the subject's stool by 16S rRNA sequencing and/or whole genome sequencing.

35

97. The composition of any one of claims 27 to 96, wherein the bacterial strain is live or dead.

98. The composition of any one of claims 27 to 97, further comprising a prebiotic.

40

99. The composition of any one of claims 27 to 98, further comprising one or more additional bacterial strains.

100. The method of any one of claims 27 to 99, wherein the subject is a mammalian subject.
101. The method of any one of claims 27 to 101, wherein the subject is a human subject.
- 5 102. A composition comprising a bacterial strain of the genus *Alistipes*, for use in therapy.
103. A composition comprising a bacterial strain of *Alistipes shahii*, for use in therapy.
- 10 104. A composition comprising a bacterial strain of the genus *Alistipes*, for use in the treatment or prevention of an inflammatory or autoimmune disorder.
105. A composition comprising a bacterial strain of *Alistipes shahii*, for use in the treatment or prevention of an inflammatory or autoimmune disorder.
- 15 106. The composition of any one of claims 102 to 105, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *A. timonensis* and *A. sp000434235*.
107. The product of claim 106, wherein the MRCA is defined at node 35260 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.
- 20 108. Use of a bacterial strain of the genus *Alistipes* in the manufacture of a medicament for the treatment of an inflammatory or autoimmune disorder.
- 25 109. The use of claim 108, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *A. timonensis* and *A. sp000434235*.
110. The product of claim 109, wherein the MRCA is defined at node 35260 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.
- 30 111. Use of a bacterial strain of *A. shahii* in the manufacture of a medicament for the treatment of an inflammatory or autoimmune disorder.
112. The composition or use of any one of claims 102 to 111, wherein the inflammatory or autoimmune disorder is selected from an inflammatory bowel disease (such as Crohn's disease or ulcerative colitis); asthma (such as allergic asthma or neutrophilic asthma); fatty liver disease (such as non-alcoholic fatty liver disease (NAFLD)); ankylosing spondylitis; systemic lupus erythematosus (SLE); scleroderma; Sjogren's syndrome; and vasculitis.
- 35 113. The composition or use of any one of claims 102 to 112, wherein the inflammatory or autoimmune disorder is an inflammatory bowel disease (e.g., Crohn's disease or ulcerative colitis).
- 40

114. The composition or use of any one of claims 102 to 115, wherein the bacterial strain is the *A. shahii* strain deposited under any one of accession numbers V21/014432, V21/014433, or V21/014434 or a derivative thereof.
- 5 115. A composition for use in treating an inflammatory or autoimmune disorder, the composition comprises a bacterial strain of the *Alistipes* genus; and an anti-inflammatory agent.
116. The composition of claim 115, wherein the bacterial strain is a phylogenetic
10 descendant of the MRCA of *A. timonensis* and *A. sp000434235*.
117. The product of claim 116, wherein the MRCA is defined at node 35260 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.
- 15 118. The composition of any one of claims 115 to 117, wherein the bacterial strain is of the species *A. shahii*.
119. The composition of claim any one of claims 115 to 118, wherein the anti-inflammatory agent is selected from the group comprising 5-aminosalicylates, corticosteroids,
20 azathioprine, infliximab, and adalimumab.
120. A composition for use in treating an inflammatory or autoimmune disorder, the composition comprises a bacterial strain of the *Alistipes* genus; and a nutritional supplement.
- 25 121. The composition of claim 120, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *A. timonensis* and *A. sp000434235*.
122. The product of claim 121, wherein the MRCA is defined at node 35260 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.
30
123. The composition of any one of claims 121 to 122, wherein the bacterial strain is of the species *A. shahii*.
124. A method of improving or maintaining health in a subject, the method comprising
35 administering to the subject a composition comprising a bacterial strain of *A. shahii*; to thereby maintain or improve health in the subject.
125. The method of claim 124, further comprising administering to the subject a nutritional supplement.
40
126. A comestible or potable product comprising a bacterial strain of the *Alistipes* genus; and a nutritional supplement.

127. The product of claim 126, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *A. timonensis* and *A. sp000434235*.

128. The product of claim 127, wherein the MRCA is defined at node 35260 of the bac120
5 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

129. The product of any one of claims 126 to 128, wherein the bacterial strain is of the species *A. shahii*.

10 130. The product of any one of claims 126 to 129, wherein the nutritional supplement is a prebiotic.

131. A cell of the *C. sana* strain deposited under accession number V21/019213 or V21/019214, or a derivative thereof.
15

132. The cell of claim 131, wherein the cell is at least partially isolated.

133. A biologically pure culture of the *C. sana* strain deposited under accession number V21/019213 or V21/019214, or a derivative thereof.
20

134. A composition comprising the cell of claim 131 or claim 132, or the culture of claim 133.

135. A composition comprising a bacterial strain with a 16S rRNA sequence that is at least
25 about 97.5%, 98%, 98.5% 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9%, any one of SEQ ID NOs: 11-18, or which has one or more 16S rRNA gene sequences represented by SEQ ID NOs: 11-18.

136. The composition of claim 134 or claim 135, further comprising a pharmaceutically
30 acceptable excipient, diluent, or carrier.

137. A pharmaceutical composition comprising a bacterial strain with a 16S rRNA sequence that is at least about 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to the 16S rRNA sequence of a bacterial strain of the specie *C. sana*, together with a
35 pharmaceutically acceptable carrier, diluent, or excipient.

138. A pharmaceutical composition comprising a bacterial strain that is a phylogenetic descendant of the MRCA of *C. sana* and *C. sp002437735*, together with a pharmaceutically acceptable carrier, diluent, or excipient.
40

139. The pharmaceutical composition of claim 138, wherein the MRCA is defined at node 23867 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.
- 5 140. The composition of any one of claims 135 to 139, wherein the bacterial strain is at least partially isolated.
141. The composition of any one of claims 134 to 140, wherein the bacterial strain is live or dead.
- 10 142. The composition of any one of claims 134 to 141, wherein the composition is in a dried form.
143. The composition of claim 142, wherein the composition is formulated in a capsule, a tablet, a pill, a troche, a lozenge, a powder, or a granule.
- 15 144. The composition of claim 142 or claim 143, wherein the composition is dried by lyophilization, spray drying, fluidized bed drying, vacuum drying, or a combination thereof.
- 20 145. The composition of any one of claims 134 to 144, wherein the composition is formulated for delivery to the gut.
146. The composition of any one of claims 134 to 145, further comprising a prebiotic.
- 25 147. The composition of any one of claims 134 to 146, further comprising one or more additional bacterial strains.
148. The composition of claim 147, wherein the one or more additional bacterial strains are at least partially isolated.
- 30 149. The composition of any one of claims 134 to 148, wherein the composition does not comprise bacteria of the genus *Clostridium*.
150. The cell, culture, or composition according to any one of claims 131 to 149, wherein the bacterial strain produces an agent that attenuates or impairs signal transducer and activator of transcription 3 (STAT3) signalling in a cell.
- 35 151. The cell, culture or composition according to claim 150, wherein the agent is a small molecule, peptide, or nucleotide.
- 40 152. The cell, culture or composition according to claim 150 or claim 151, wherein the agent is released by the bacteria.

153. The cell, culture or composition according to any one of claims 150 to 152, wherein the agent binds specifically to any one of STAT3, JAK2, TYK, or IL-23.

154. The cell, culture, or composition according to any one of claims 131 to 153, wherein
5 the bacterial strain produces one or more metabolite selected from butyrate, acetate, ethanol, and fumarate.

155. The cell, culture or composition according to any one of claims 131 to 154, wherein the
10 bacterial strain is of the species *C. sana*.

156. A food or drink product comprising the composition of any one of claims 134 to 155.

157. A method of restoring or improving gut barrier function in a subject, the method
comprising administering to the subject a bacterial strain of the *Colonithrix* genus, to thereby
15 restore or improve gut barrier function.

158. The method of claim 157, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *C. sana* and *C sp002437735*.

20 159. The method of claim 158, wherein the MRCA is defined at node 23679 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

160. The method of any one of claims 157 to 159, wherein the bacterial strain is of the species *C. sana*.

25 161. The method of any one of claims 157 to 160, wherein restoring or improving gut barrier function is characterised by at least one of: (i) an increase in the quality and/or quantity of mucin; (ii) improvement in integrity of tight junction proteins; (iii) reduction in translocation of luminal contents into systemic circulation; or (iv) a reduction of intestinal
30 ulcers and/or intestinal wounds.

162. The method of claim 161, wherein the luminal contents includes lipopolysaccharide (LPS).

35 163. The method of any one of claims 157 to 162, wherein the restoration or improvement in gut barrier dysfunction results in a reduction in systemic inflammation in the subject.

164. The method of claim 163, wherein systemic inflammation is identified in the subject when the level of an inflammatory cytokine (e.g., IL-1 β , IL-8, IL-6, and TNF) in a sample
40 from the subject is above a predetermined threshold.

165. A method of inducing or enhancing mucosal healing in a subject, the method comprising administering to the subject a bacterial strain of the *Colonithrix* genus in an amount sufficient to induce epithelial cell migration and/or proliferation; to thereby induce mucosal healing in the subject.

5

166. The method of claim 165, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *C. sana* and *C. sp002437735*.

167. The method of claim 166, wherein the MRCA is defined at node 23879 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

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168. The method of any one of claims 165 to 167, wherein the bacterial strain is of the species *C. sana*.

169. The method of any one of claims 165 to 168, wherein mucosal healing is measured using one or more fecal or serum markers.

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170. The method of claim 169, wherein one or more fecal markers are selected from the group comprising calprotectin, lactoferrin, metalloproteinase (MMP)-9, and lipocalin-2.

20

171. The method of any one of claims 165 to 170, wherein mucosal healing is measured using endoscopic score.

172. A method of reducing inflammation in a subject, the method comprising administering to the subject a therapeutically effective amount of a bacterial strain of the *Colonithrix* genus, to thereby reduce inflammation in the subject.

25

173. The method of claim 172, wherein the bacterial species is a phylogenetic descendant of the MRCA of *C. sana* and *C. sp002437735*.

30

174. The method of claim 173, wherein the MRCA is defined at node 23879 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

175. The method of any one of claims 172 to 174, wherein the bacterial strain is of the species *C. sana*.

35

176. The method of any one of claims 172 to 175, wherein the inflammation is local to the gut environment, or systemic inflammation.

177. The method of any one of claims 172 to 176, wherein the bacterial strain attenuates the NFκB pathway (e.g., by reducing or inhibiting NFκB).

40

178. A method of blocking or otherwise inhibiting the activation of STAT3 signalling in a target cell, the method comprising contacting the target cell with at least a soluble component of a bacterial cell preparation of the *Colonithrix* genus, to block or otherwise inhibit the activation of STAT3 signalling in the target cell.

5

179. The method of claim 178, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *C. sana* and *C. sp002437735*.

180. The method of claim 179, wherein the MRCA is defined at node 23879 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

10

The method of any one of claims 178 to 180, wherein the bacterial strain is of the species *C. sana*.

181. The method of any one of claims 178 to 180, wherein the target cell is selected from the group comprising a reporter cell (e.g., a HEK cell), an immune cell (e.g., a Th17 immune cell), an epithelial cell, and an endothelial cell.

15

182. The method of any one of claims 178 to 181, wherein the bacterial cell preparation comprises a bacterial cell culture.

20

183. The method of claim 182, wherein the soluble component comprises the supernatant of the bacterial cell culture.

184. The method of any one of claims 178 to 183, wherein the soluble component is substantially depleted of bacterial cells.

25

185. The method of any one of claims 178 to 184, wherein the bacterial cell preparation comprises a bacterial cell pellet.

30

186. The method of claim 185, wherein the soluble component comprises soluble fraction of the lysed cells.

187. The method of claim 186, wherein the soluble fraction is substantially separated from the insoluble cell fraction by centrifugation.

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188. The method of any one of claims 178 to 187, wherein the method is performed *in vitro*.

189. A method of blocking or otherwise inhibiting STAT3 signalling in the gut environment of a subject, the method comprising administering to the subject a bacterial strain of the

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Colonithrix genus, to block or otherwise inhibit STAT3 signalling in the gut environment of the subject.

190. The method of claim 189, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *C. sana* and *C. sp002437735*.

191. The method of claim 190, wherein the MRCA is defined at node 23879 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

192. The method of any one of claims 189 to 191, wherein the bacterial strain is of the species *C. sana*.

193. The method of any one of claims 189 to 192, wherein the cell is an immune cell (e.g., a Th17 immune cell), epithelial cell, or endothelial cell.

194. The method of any one of claims 189 to 193, wherein the cell is an epithelial cell, wherein the bacterial strain or molecule increases the production of IL-22 in the subject.

195. The method of any one of claims 189 to 194, wherein the bacterial strain produces a molecule that is a direct inhibitor or an indirect inhibitor of STAT3.

196. The method of any one of claims 189 to 195, wherein the bacterial strain produces a molecule that directly inhibits at least one of an IL-23 polypeptide, a JAK2 polypeptide, a TYK2 polypeptide, or a STAT3 polypeptide.

197. The method of any one of claims 157 to 196, wherein the bacterial strain produces one or more of the following metabolites: butyrate, acetate, ethanol and fumarate.

198. The method of any one of claims 157 to 197, wherein the bacterial strain has a 16S rRNA sequence that is at least about 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% identical to the 16S rRNA sequence of a bacterial strain of the species *C. sana*; or when the bacterial strain has a 16S rRNA gene sequence of a bacterial strain of *C. sana*.

199. The method of any one of claims 157 to 198, wherein the bacterial strain has a 16S rRNA sequence that is at least about 97.5%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% identical to any one of SEQ ID NOs: 11-18, or when the bacterial strain has the 16S rRNA gene sequence represented by one or more of SEQ ID NOs: 11-18.

200. The method of any one of claims 157 to 199, wherein the bacterial strain is the *C. sana* strain deposited under accession number V21/019213 or V21/019214, or a derivative thereof
- 5 201. The method of any one of claims 157 to 200, wherein the bacterial strain is at least partially isolated.
202. The method of any one of claims 157 to 201, wherein the bacterial strain is formulated as a pharmaceutical composition, further comprising a pharmaceutically acceptable carrier,
10 diluent or excipient.
203. The method of claim 202, wherein the composition is in a dried form.
204. The method of claim 203, wherein the dried form is selected from the group
15 comprising particles, granules, and powder.
205. The method of claim 203 or claim 204, wherein the composition is dried by lyophilization, spray drying, fluidized bed drying, vacuum drying, or a combination thereof.
- 20 206. The method of any one of claims 202 to 205, wherein the pharmaceutical composition is formulated for oral administration.
207. A method of treating or preventing an inflammatory or autoimmune disorder in a subject, the method comprising administering an effective amount of a bacterial strain of the
25 *Colonithrix* genus to the subject, to thereby treat or prevent the inflammatory or autoimmune disorder.
208. The method of claim 207, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *C. sana* and *C. sp002437735*.
30
209. The method of claim 208, wherein the MRCA is defined at node 23879 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.
210. The method of any one of claims 207 to 209, wherein the bacterial strain is of the
35 species *C. sana*.
211. The method of any one of claims 207 to 210, wherein the inflammatory or autoimmune disorder is selected from the group comprising an inflammatory bowel disease (such as Crohn's disease or ulcerative colitis); asthma (such as allergic asthma or
40 neutrophilic asthma); fatty liver disease (such as non-alcoholic fatty liver disease (NAFLD)); ankylosing spondylitis; systemic lupus erythematosus (SLE); scleroderma; Sjogren's syndrome; and vasculitis.

212. The method of claim 207 to 211, wherein the inflammatory or autoimmune disorder is an inflammatory bowel disease (IBD).

5 213. The method of any one of claims 207 to 212, wherein when administered to a subject, the bacterial strain blocks or otherwise inhibits STAT3 signalling in at least a cell of the subject.

214. The method of claim 213 wherein the cell is an epithelial cell, immune cell (e.g., a
10 Th17 immune cell), or an endothelial cell.

215. The method of any one of claims 207 to 214, wherein the bacterial strain produces one or more metabolites selected from the group comprising butyrate, acetate, ethanol and fumarate.

15

216. The method according to any one of claims 207 to 215, wherein the bacterial strain has a 16S rRNA sequence that is at least about 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% identical to the 16S rRNA sequence of a bacterial strain of the genus *Colonithrix*.

20 217. The method according to any one of claims 207 to 216, wherein the bacterial strain has a 16S rRNA sequence that is at least about 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% identical to any one of SEQ ID NO: 11-18, or when the bacterial strain has one or more 16S rRNA gene sequences represented by SEQ ID NO: 11-18.

25 218. The method according to any one of claims 207 to 217, wherein the bacterial strain is at least partially isolated.

219. The method according to any one of claims 207 to 218, wherein the bacterial strain is formulated as a pharmaceutical composition, together with a pharmaceutically acceptable
30 carrier, diluent, and/or excipient.

220. The method of claim 219, wherein the composition is in a dried form.

221. The method of claim 220, wherein the dried form is selected from the group
35 comprising particles, granules, and powder.

222. The method of claim 220 or claim 221, wherein the composition is dried by lyophilisation, spray drying, fluidized bed drying, vacuum drying, or a combination thereof.

40 223. The method of any one of claims 207 to 222, wherein an anti-inflammatory agent is co-administered to the subject.

224. The method of claim 223, wherein the anti-inflammatory agent is selected from the group comprising a 5-aminosalicylate, corticosteroid, azathioprine, infliximab, and adalimumab.
- 5 225. The method of any one of claims 157 to 224, wherein treating comprises, prior to administering the composition to the subject, identifying that the subject has a deficiency in *C. sana* gut bacteria.
- 10 226. The method of claim 225, wherein identifying the deficiency in the subject comprises measurement of *C. sana* bacteria in the subject's stool by 16S rRNA sequencing and/or whole genome sequencing.
- 15 227. The composition of any one of claims 157 to 226, wherein the bacterial strain is live or dead.
228. The composition of any one of claims 157 to 227, further comprising a prebiotic.
229. The composition of any one of claims 157 to 228, further comprising one or more additional bacterial strains.
- 20 230. The method of any one of claims 157 to 229, wherein the subject is a mammalian subject.
231. The method of any one of claims 157 to 1230, wherein the subject is a human subject.
- 25 232. A composition comprising a bacterial strain of the genus *Colonithrix*, for use in therapy.
233. A composition comprising a bacterial strain of *C. sana* for use in therapy.
- 30 234. A composition comprising a bacterial strain of the genus *Colonithrix*, for use in the treatment or prevention of an inflammatory or autoimmune disorder.
235. A composition comprising a bacterial strain of *C. sana* for use in the treatment or prevention of an inflammatory or autoimmune disorder.
- 35 236. The composition of any one of claims 232 to 235, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *C. sana* and *C. sp002437735*.
- 40 237. The composition of claim 236, wherein the MRCA is defined at node 23879 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

238. Use of a bacterial strain of the genus *Colonithrix* in the manufacture of a medicament for the treatment of an inflammatory or autoimmune disorder.
239. The use of claim 238, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *C. sana* and *C. sp0022437735*.
240. The use of claim 239, wherein the MRCA is defined at node 23879 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.
241. Use of a bacterial strain of *C. sana* in the manufacture of a medicament for the treatment of an inflammatory or autoimmune disorder.
242. The composition or use of any one of claims 232 to 241, wherein the inflammatory or autoimmune disorder is selected from an inflammatory bowel disease (such as Crohn's disease or ulcerative colitis); asthma (such as allergic asthma or neutrophilic asthma); fatty liver disease (such as non-alcoholic fatty liver disease (NAFLD)); ankylosing spondylitis; systemic lupus erythematosus (SLE); scleroderma; Sjogren's syndrome; and vasculitis.
243. The composition or use of any one of claims 232 to 242, wherein the inflammatory or autoimmune disorder is an inflammatory bowel disease (e.g., Crohn's disease or ulcerative colitis).
244. The composition or use of any one of claims 232 to 245, wherein the bacterial strain is the *C. sana* strain deposited under accession numbers V21/019213 or V21/019214, or a derivative thereof.
245. A composition for use in treating an inflammatory or autoimmune disorder, the composition comprises a bacterial strain of the *Colonithrix* genus; and an anti-inflammatory agent.
246. The composition of claim 245, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *C. sana* and *C. sp002437735*.
247. The composition of claim 246, wherein the MRCA is defined at node 23879 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.
248. The composition of any one of claims 245 to 247, wherein the bacterial strain is of the species *C. sana*.
249. The composition of claim any one of claims 245 to 248, wherein the anti-inflammatory agent is selected from the group comprising 5-aminosalicylates, corticosteroids, azathioprine, infliximab, and adalimumab.

250. A composition for use in treating an inflammatory or autoimmune disorder, the composition comprises a bacterial strain of the *Colonithrix* genus; and a nutritional supplement.

5

251. The composition of claim 250, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *C. sana* and *C. sp002437735*.

252. The composition of claim 251, wherein the MRCA is defined at node 23879 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

10

253. The composition of claim 251 or claim 252, wherein the bacterial strain is of the species *C. sana*.

254. A method of improving or maintaining health in a subject, the method comprising administering to the subject a composition comprising a bacterial strain of *C. sana*; to thereby maintain or improve health in the subject.

15

255. The method of claim 254, further comprising administering to the subject a nutritional supplement.

20

256. A comestible or potable product comprising a bacterial strain of the *Colonithrix* genus; and a nutritional supplement.

257. The product of claim 256, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *C. sana* and *C. sp002437735*.

25

258. The product of claim 257, wherein the MRCA is defined at node 23879 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

30

259. The product of any one of claims 256 to 258, wherein the bacterial strain is of the species *C. sana*.

30

260. The product of any one of claims 256 to 259, wherein the nutritional supplement is a prebiotic.

35

261. A cell of the *Gemmiger formicilis* strain deposited under accession number V21/011520, or a derivative thereof.

262. The cell of claim 261, wherein the cell is at least partially isolated.

40

263. A biologically pure culture of the *Gemmiger formicilis* strain deposited under accession number V21/011520, or a derivative thereof.

264. A composition comprising the cell of claim 261 or claim 262, or the culture of claim 263.

5 265. A composition comprising a bacterial strain with a 16S rRNA sequence that is at least about 97.5%, 98%, 98.5% 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9%, identical to SEQ ID NO: 27 or one or more of SEQ ID NOs: 45-47; or which has a 16S rRNA gene sequence represented by SEQ ID NO: 27 or one or more of SEQ ID NOs: 45-47.

10

266. The composition of claim 264 or claim 265, further comprising a pharmaceutically acceptable excipient, diluent, or carrier.

15 267. A pharmaceutical composition comprising a bacterial strain with a 16S rRNA sequence that is at least about 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to the 16S rRNA sequence of a bacterial strain of the species *Gemmiger formicilis*, together with a pharmaceutically acceptable carrier, diluent, or excipient.

20 268. A pharmaceutical composition comprising a bacterial strain that is a phylogenetic descendant of the MRCA of *G. variabile* and *G. sp002306375*, together with a pharmaceutically acceptable carrier, diluent, or excipient.

25 269. The pharmaceutical composition of claim 268, wherein the MRCA is defined at node 23818 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

270. The composition of any one of claims 265 to 269, wherein the bacterial strain is at least partially isolated.

30 271. The composition of any one of claims 264 to 270, wherein the bacterial strain is live or dead.

272. The composition of any one of claims 264 to 271, wherein the composition is in a dried form.

35

273. The composition of claim 272, wherein the composition is formulated in a capsule, a tablet, a pill, a troche, a lozenge, a powder, or a granule.

40 274. The composition of claim 272 or claim 273, wherein the composition is dried by lyophilization, spray drying, fluidized bed drying, vacuum drying, or a combination thereof.

275. The composition of any one of claims 274 to 274, wherein the composition is formulated for delivery to the gut.

276. The composition of any one of claims 274 to 275, further comprising a prebiotic.

5

277. The composition of any one of claims 274 to 276, further comprising one or more additional bacterial strains.

278. The composition of claim 277, wherein the one or more additional bacterial strains are at least partially isolated.

10

279. The composition of any one of claims 264 to 278, wherein the composition does not comprise bacteria of the genus *Clostridium*.

280. The cell, culture, or composition according to any one of claims 261 to 279, wherein the bacterial strain produces an agent that attenuates or impairs signal transducer and activator of transcription 3 (STAT3) signalling in a cell.

15

281. The cell, culture or composition according to claim 280, wherein the agent is a small molecule, peptide, or nucleotide.

20

282. The cell, culture or composition according to claim 280 or claim 281, wherein the agent is released by the bacteria.

283. The cell, culture or composition according to any one of claims 280 to 282, wherein the agent binds specifically to any one of STAT3, JAK2, TYK, or IL-23.

25

284. The cell, culture, or composition according to any one of claims 261 to 283, wherein the bacterial strain produces one or more metabolite selected from butyrate, acetate, ethanol, and fumarate.

30

285. The cell, culture or composition according to any one of claims 261 to 284, wherein the bacterial strain is of the species *G. formicilis*.

286. A food or drink product comprising the composition of any one of claims 264 to 285.

35

287. A method of restoring or improving gut barrier function in a subject, the method comprising administering to the subject a bacterial strain of the *Gemmiger* genus, to thereby restore or improve gut barrier function.

40

288. The method of claim 287, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *G. variabile* and *G. sp002306375*.

289. The method of claim 28, wherein the MRCA is defined at node 23818 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

290. The method of any one of claims 287 to 289, wherein the bacterial strain is of the
5 species *G. formicilis*.

291. The method of any one of claims 287 to 290, wherein restoring or improving gut barrier function is characterised by at least one of: (i) an increase in the quality and/or quantity of mucin; (ii) improvement in integrity of tight junction proteins; (iii) reduction in
10 translocation of luminal contents into systemic circulation; or (iv) a reduction of intestinal ulcers and/or intestinal wounds.

292. The method of claim 291, wherein the luminal contents includes lipopolysaccharide (LPS).
15

293. The method of any one of claims 287 to 292, wherein the restoration or improvement in gut barrier dysfunction results in a reduction in systemic inflammation in the subject.

294. The method of claim 293, wherein systemic inflammation is identified in the subject
20 when the level of an inflammatory cytokine (e.g., IL-1 β , IL-8, IL-6, and TNF) in a sample from the subject is above a predetermined threshold.

295. A method of inducing or enhancing mucosal healing in a subject, the method comprising administering to the subject a bacterial strain of the *Gemmiger* genus in an
25 amount sufficient to induce epithelial cell migration and/or proliferation; to thereby induce mucosal healing in the subject.

296. The method of claim 295, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *G. variabile* and *G. sp002306375*.
30

297. The method of claim 296, wherein the MRCA is defined at node 23818 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

298. The method of any one of claims 295 to 297, wherein the bacterial strain is of the
35 species *G. formicilis*.

299. The method of any one of claims 295 to 298, wherein mucosal healing is measured using one or more fecal or serum markers.

40 300. The method of claim 299, wherein one or more fecal markers are selected from the group comprising calprotectin, lactoferrin, metalloproteinase (MMP)-9, and lipocalin-2.

301. The method of any one of claims 295 to 300, wherein mucosal healing is measured using endoscopic score.

5 302. A method of reducing inflammation in a subject, the method comprising administering to the subject a therapeutically effective amount of a bacterial strain of the *Gemmiger* genus, to thereby reduce inflammation in the subject.

303. The method of claim 302, wherein the bacterial species is a phylogenetic descendant of the MRCA of *G. variabile* and *G. sp002306375*.
10

304. The method of claim 303, wherein the MRCA is defined at node 23818 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

305. The method of any one of claims 302 to 304, wherein the bacterial strain is of the species *G. formicilis*.
15

306. The method of any one of claims 302 to 305, wherein the inflammation is local to the gut environment, or systemic inflammation.

20 307. The method of any one of claims 302 to 306, wherein the bacterial strain attenuates the NFκB pathway (e.g., by reducing or inhibiting NFκB).

308. A method of blocking or otherwise inhibiting the activation of STAT3 signalling in a target cell, the method comprising contacting the target cell with at least a soluble
25 component of a bacterial cell preparation of the *Gemmiger* genus, to block or otherwise inhibit the activation of STAT3 signalling in the target cell.

309. The method of claim 308, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *G. variabile* and *G. sp002306375*.
30

310. The method of claim 309, wherein the MRCA is defined at node 23818 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

The method of any one of claims 308 to 310, wherein the bacterial strain is of the species *G. formicilis*.
35

311. The method of any one of claims 308 to 310, wherein the target cell is selected from the group comprising a reporter cell (e.g., a HEK cell), an immune cell (e.g., a Th17 immune cell), an epithelial cell, and an endothelial cell.
40

312. The method of any one of claims 308 to 311, wherein the bacterial cell preparation comprises a bacterial cell culture.

313. The method of claim 312, wherein the soluble component comprises the supernatant of the bacterial cell culture.
- 5 314. The method of any one of claims 308 to 313, wherein the soluble component is substantially depleted of bacterial cells.
315. The method of any one of claims 308 to 314, wherein the bacterial cell preparation comprises a bacterial cell pellet.
- 10 316. The method of claim 315, wherein the soluble component comprises soluble fraction of the lysed cells.
317. The method of claim 316, wherein the soluble fraction is substantially separated from the insoluble cell fraction by centrifugation.
- 15 318. The method of any one of claims 308 to 317, wherein the method is performed *in vitro*.
- 20 319. A method of blocking or otherwise inhibiting STAT3 signalling in the gut environment of a subject, the method comprising administering to the subject a bacterial strain of the *Gemmiger* genus, to block or otherwise inhibit STAT3 signalling in the gut environment of the subject.
- 25 320. The method of claim 319, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *G. variabile* and *G. sp002306375*.
321. The method of claim 320, wherein the MRCA is defined at node 23818 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.
- 30 322. The method of any one of claims 319 to 321, wherein the bacterial strain is of the species *G. formicillis*.
323. The method of any one of claims 319 to 322, wherein the cell is an immune cell (e.g., a Th17 immune cell), epithelial cell, or endothelial cell.
- 35 324. The method of any one of claims 319 to 323, wherein the cell is an epithelial cell, wherein the bacterial strain or molecule increases the production of IL-22 in the subject.
- 40 325. The method of any one of claims 319 to 324, wherein the bacterial strain produces a molecule that is a direct inhibitor or an indirect inhibitor of STAT3.

326. The method of any one of claims 319 to 325, wherein the bacterial strain produces a molecule that directly inhibits at least one of an IL-23 polypeptide, a JAK2 polypeptide, a TYK2 polypeptide, or a STAT3 polypeptide.

5 327. The method of any one of claims 287 to 326, wherein the bacterial strain produces one or more of the following metabolites: butyrate, acetate, ethanol and fumarate.

328. The method of any one of claims 287 to 327, wherein the bacterial strain has a 16S rRNA sequence that is at least about 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%,
10 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% identical to the 16S rRNA sequence of a bacterial strain of the species *G. formicilis*; or when the bacterial strain has a 16S rRNA gene sequence of a bacterial strain of *G. formicilis*.

329. The method of any one of claims 287 to 328, wherein the bacterial strain has a 16S rRNA sequence that is at least about 97.5%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%,
15 99.5%, 99.6%, 99.7%, 99.8%, 99.9% identical to SEQ ID NO: 27 or one or more of SEQ ID NOs: 45-47, or when the bacterial strain has the 16S rRNA gene sequence represented by one or more of SEQ ID NOs: 45-47.

20 330. The method of any one of claims 287 to 329, wherein the bacterial strain is the *G. formicilis* strain deposited under accession number V21/011520, or a derivative thereof

331. The method of any one of claims 287 to 330, wherein the bacterial strain is at least partially isolated.

25 332. The method of any one of claims 287 to 331, wherein the bacterial strain is formulated as a pharmaceutical composition, further comprising a pharmaceutically acceptable carrier, diluent or excipient.

30 333. The method of claim 332, wherein the composition is in a dried form.

334. The method of claim 333, wherein the dried form is selected from the group comprising particles, granules, and powder.

35 335. The method of claim 333 or claim 334, wherein the composition is dried by lyophilization, spray drying, fluidized bed drying, vacuum drying, or a combination thereof.

336. The method of any one of claims 332 to 335, wherein the pharmaceutical composition is formulated for oral administration.

40 337. A method of treating or preventing an inflammatory or autoimmune disorder in a subject, the method comprising administering an effective amount of a bacterial strain of the

Gemmiger genus to the subject, to thereby treat or prevent the inflammatory or autoimmune disorder.

338. The method of claim 337, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *G. variabile* and *G. sp002306375*.

339. The method of claim 338, wherein the MRCA is defined at node 23818 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

340. The method of any one of claims 337 to 339, wherein the bacterial strain is of the species *G. formicilis*.

341. The method of any one of claims 337 to 340, wherein the inflammatory or autoimmune disorder is selected from the group comprising an inflammatory bowel disease (such as Crohn's disease or ulcerative colitis); asthma (such as allergic asthma or neutrophilic asthma); fatty liver disease (such as non-alcoholic fatty liver disease (NAFLD)); ankylosing spondylitis; systemic lupus erythematosus (SLE); scleroderma; Sjogren's syndrome; and vasculitis.

342. The method of claim 337 to 341, wherein the inflammatory or autoimmune disorder is an inflammatory bowel disease (IBD).

343. The method of any one of claims 337 to 342, wherein when administered to a subject, the bacterial strain blocks or otherwise inhibits STAT3 signalling in at least a cell of the subject.

344. The method of claim 343 wherein the cell is an epithelial cell, immune cell (e.g., a Th17 immune cell), or an endothelial cell.

345. The method of any one of claims 337 to 344, wherein the bacterial strain produces one or more metabolites selected from the group comprising butyrate, acetate, ethanol and fumarate.

346. The method according to any one of claims 337 to 345, wherein the bacterial strain has a 16S rRNA sequence that is at least about 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% identical to the 16S rRNA sequence of a bacterial strain of the genus *Gemmiger*.

347. The method according to any one of claims 337 to 346, wherein the bacterial strain has a 16S rRNA sequence that is at least about 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% identical to SEQ ID NO: 27 or one or more of SEQ ID NOs: 45-47, or when the bacterial strain has the 16S rRNA gene sequence represented by one or more of SEQ ID NOs: 27 or 45-47.

348. The method according to any one of claims 337 to 347, wherein the bacterial strain is at least partially isolated.
- 5 349. The method according to any one of claims 337 to 348, wherein the bacterial strain is formulated as a pharmaceutical composition, together with a pharmaceutically acceptable carrier, diluent, and/or excipient.
350. The method of claim 349, wherein the composition is in a dried form.
- 10 351. The method of claim 350, wherein the dried form is selected from the group comprising particles, granules, and powder.
352. The method of claim 350 or claim 351, wherein the composition is dried by
- 15 lyophilisation, spray drying, fluidized bed drying, vacuum drying, or a combination thereof.
353. The method of any one of claims 337 to 352, wherein an anti-inflammatory agent is co-administered to the subject.
- 20 354. The method of claim 353, wherein the anti-inflammatory agent is selected from the group comprising a 5-aminosalicylate, corticosteroid, azathioprine, infliximab, and adalimumab.
355. The method of any one of claims 287 to 354, wherein treating comprises, prior to
- 25 administering the composition to the subject, identifying that the subject has a deficiency in *G. formicilis* gut bacteria.
356. The method of claim 355, wherein identifying the deficiency in the subject comprises measurement of *G. formicilis* bacteria in the subject's stool by 16S rRNA sequencing and/or
- 30 whole genome sequencing.
357. The composition of any one of claims 287 to 356, wherein the bacterial strain is live or dead.
- 35 358. The composition of any one of claims 287 to 357, further comprising a prebiotic.
359. The composition of any one of claims 287 to 358, further comprising one or more additional bacterial strains.
- 40 360. The method of any one of claims 287 to 359, wherein the subject is a mammalian subject.
361. The method of any one of claims 287 to 360, wherein the subject is a human subject.

362. A composition comprising a bacterial strain of the genus *Gemmiger*, for use in therapy.
363. A composition comprising a bacterial strain of *Gemmiger formicilis*, for use in therapy.
- 5 364. A composition comprising a bacterial strain of the genus *Gemmiger*, for use in the treatment or prevention of an inflammatory or autoimmune disorder.
365. A composition comprising a bacterial strain of *Gemmiger formicilis*, for use in the
- 10 treatment or prevention of an inflammatory or autoimmune disorder.
366. The composition of any one of claims 362 to 365, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *G. variabile* and *G. sp002306375*.
- 15 367. The composition of claim 366, wherein the MRCA is defined at node 23818 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.
368. Use of a bacterial strain of the genus *Gemmiger* in the manufacture of a medicament for the treatment of an inflammatory or autoimmune disorder.
- 20 369. The use of claim 368, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *G. variabile* and *G. sp002306375*.
370. The use of claim 369, wherein the MRCA is defined at node 23818 of the bac120
- 25 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.
371. Use of a bacterial strain of *G. formicilis* in the manufacture of a medicament for the treatment of an inflammatory or autoimmune disorder.
- 30 372. The composition or use of any one of claims 362 to 371, wherein the inflammatory or autoimmune disorder is selected from an inflammatory bowel disease (such as Crohn's disease or ulcerative colitis); asthma (such as allergic asthma or neutrophilic asthma); fatty liver disease (such as non-alcoholic fatty liver disease (NAFLD)); ankylosing spondylitis; systemic lupus erythematosus (SLE); scleroderma; Sjogren's syndrome; and vasculitis.
- 35 373. The composition or use of any one of claims 362 to 372, wherein the inflammatory or autoimmune disorder is an inflammatory bowel disease (e.g., Crohn's disease or ulcerative colitis).
- 40 374. The composition or use of any one of claims 362 to 375, wherein the bacterial strain is the *G. formicilis* strain deposited under accession number V21/011520 or a derivative thereof.

375. A composition for use in treating an inflammatory or autoimmune disorder, the composition comprises a bacterial strain of the *Gemmiger* genus; and an anti-inflammatory agent.

5

376. The composition of claim 375, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *G. variable* and *G. sp002306375*.

377. The composition of claim 376, wherein the MRCA is defined at node 23818 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

10

378. The composition of any one of claims 375 to 377, wherein the bacterial strain is of the species *G. formicilis*.

379. The composition of claim any one of claims 375 to 378, wherein the anti-inflammatory agent is selected from the group comprising 5-aminosalicylates, corticosteroids, azathioprine, infliximab, and adalimumab.

15

380. A composition for use in treating an inflammatory or autoimmune disorder, the composition comprises a bacterial strain of the *Gemmiger* genus; and a nutritional supplement.

20

381. The composition of claim 380, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *G. variable* and *G. sp002306375*.

25

382. The composition of claim 381, wherein the MRCA is defined at node 23818 of the bac120 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

383. The composition of any one of claims 380 to 382, wherein the bacterial strain is of the species *G. formicilis*.

30

384. A method of improving or maintaining health in a subject, the method comprising administering to the subject a composition comprising a bacterial strain of *G. formicilis*; to thereby maintain or improve health in the subject.

35

385. The method of claim 384, further comprising administering to the subject a nutritional supplement.

386. A comestible or potable product comprising a bacterial strain of the *Gemmiger* genus; and a nutritional supplement.

40

387. The product of claim 386, wherein the bacterial strain is a phylogenetic descendant of the MRCA of *G. variabile* and *G. sp002306375*.

388. The product of claim 387, wherein the MRCA is defined at node 23818 of the bac120
5 phylogenetic tree from the Genome Taxonomy Database (GTDB) release 89.

389. The product of any one of claims 386 to 388, wherein the bacterial strain is of the species *G. formicilis*.

10 390. The product of any one of claims 386 to 389, wherein the nutritional supplement is a prebiotic.

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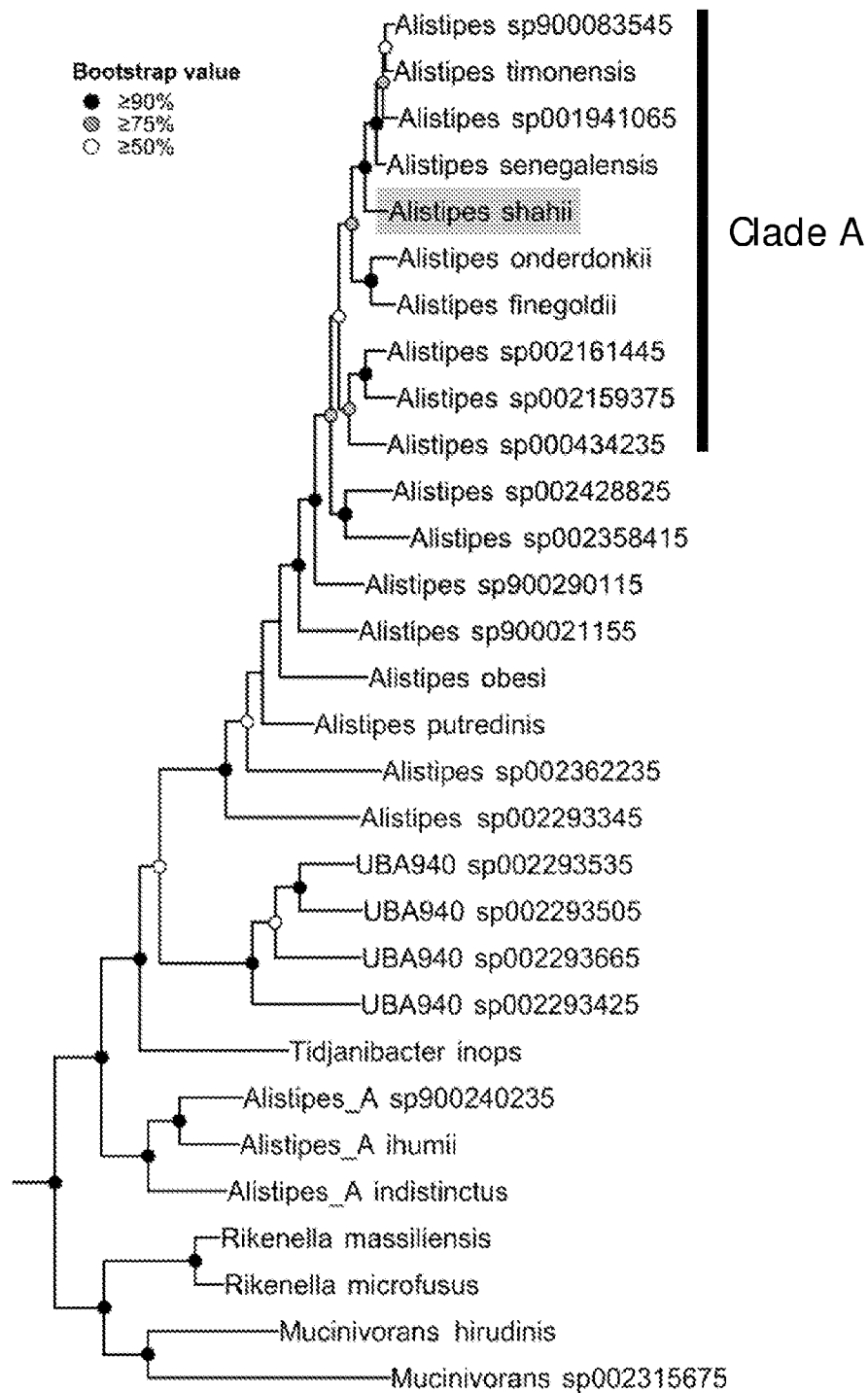
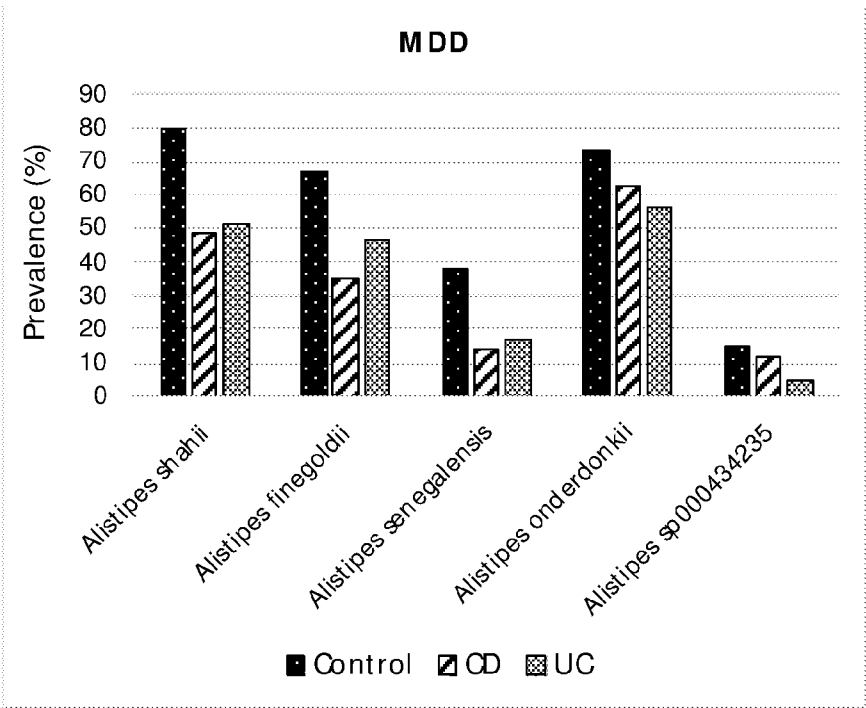


FIGURE 1

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A



B

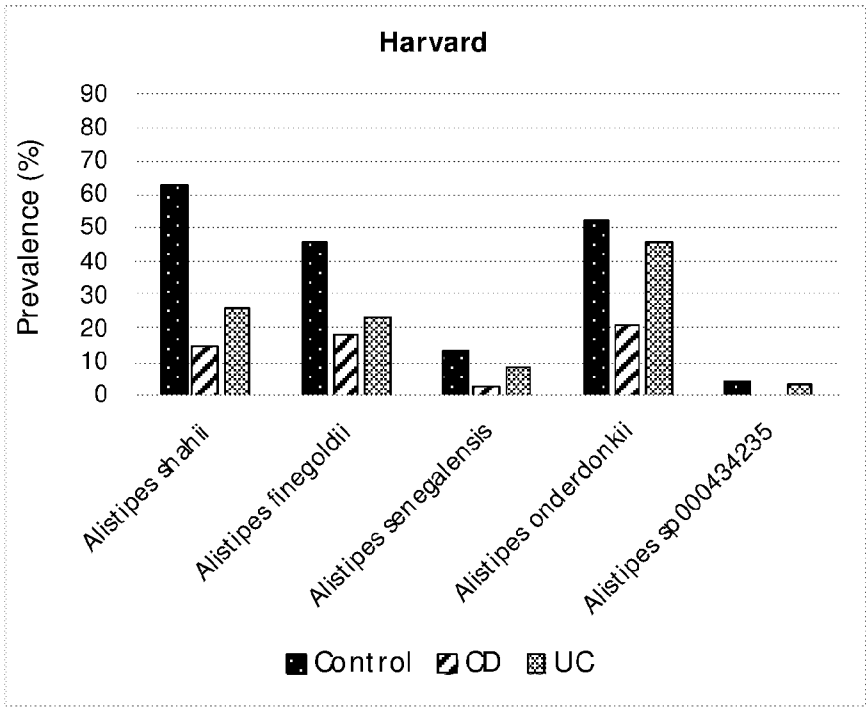


FIGURE 2

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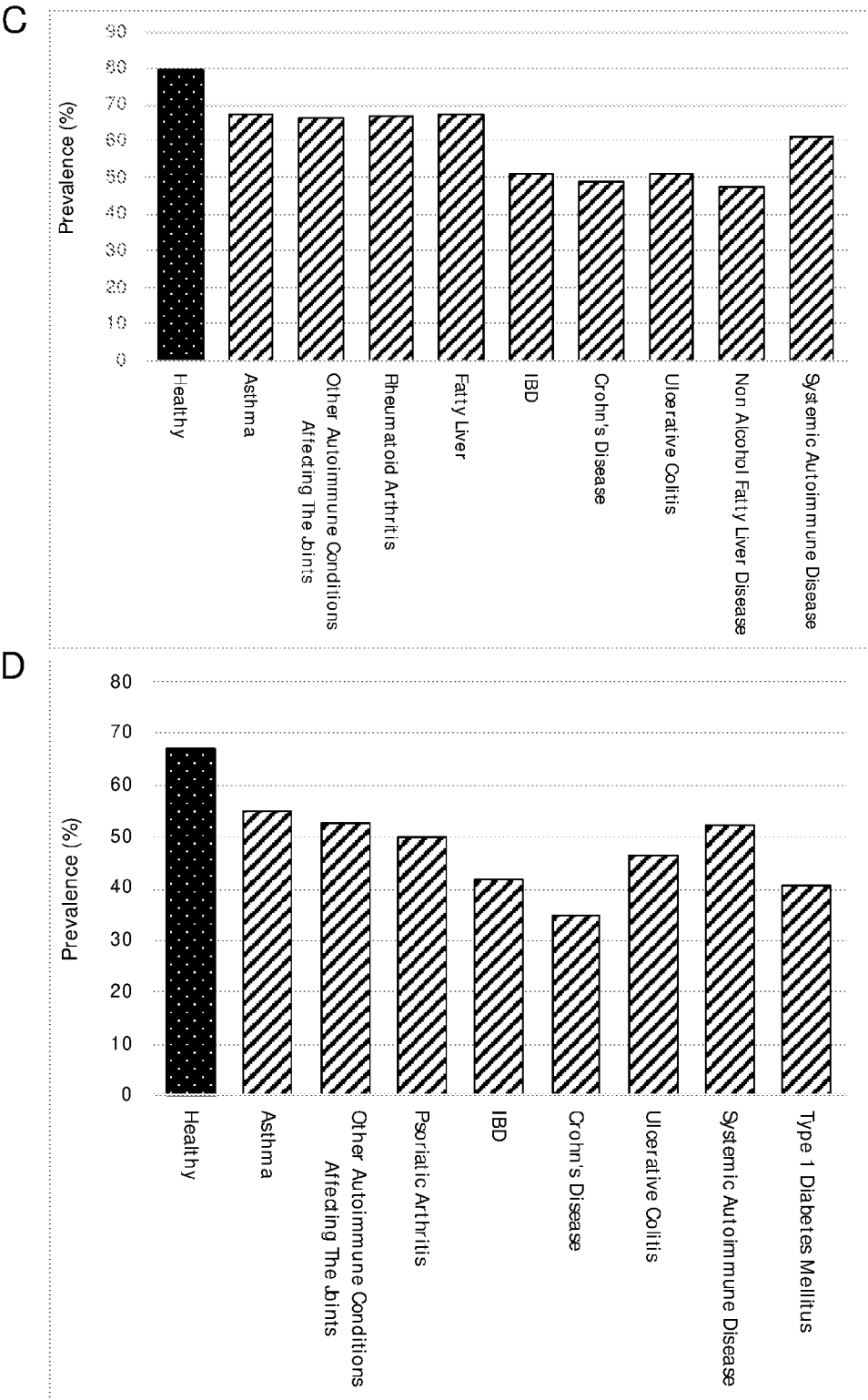


FIGURE 2 CON'T

4/25

E

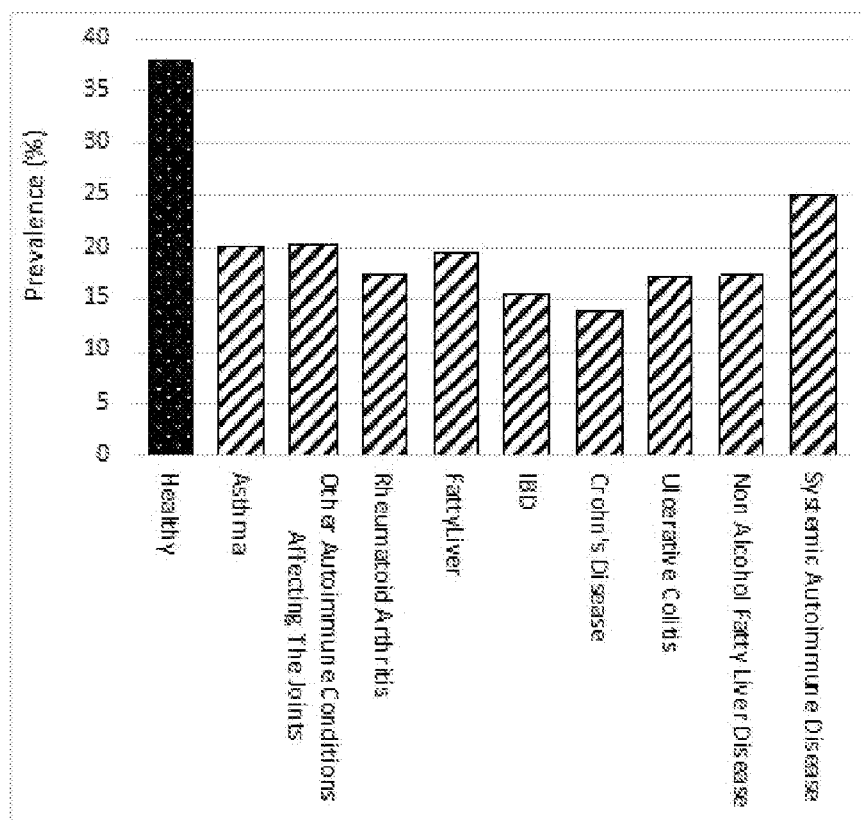
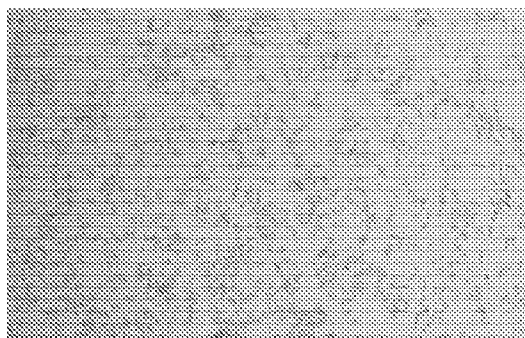


FIGURE 2 CON'T

5/25

A



B

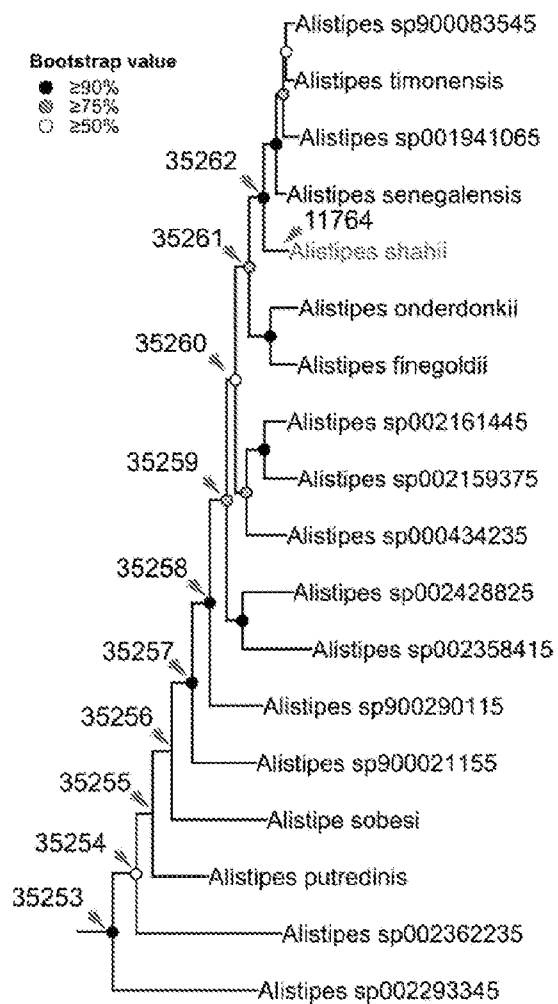


FIGURE 3

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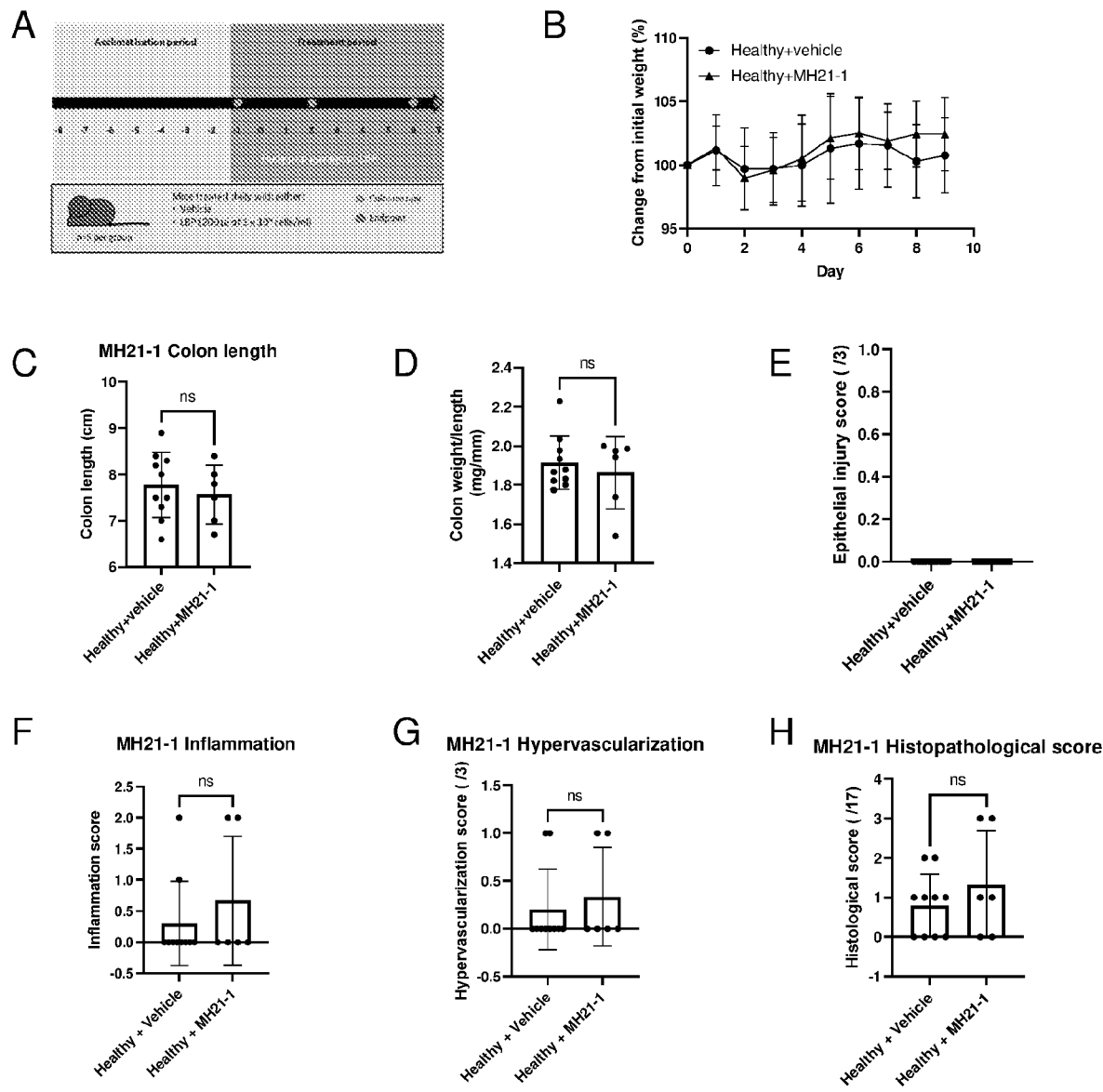


FIGURE 4

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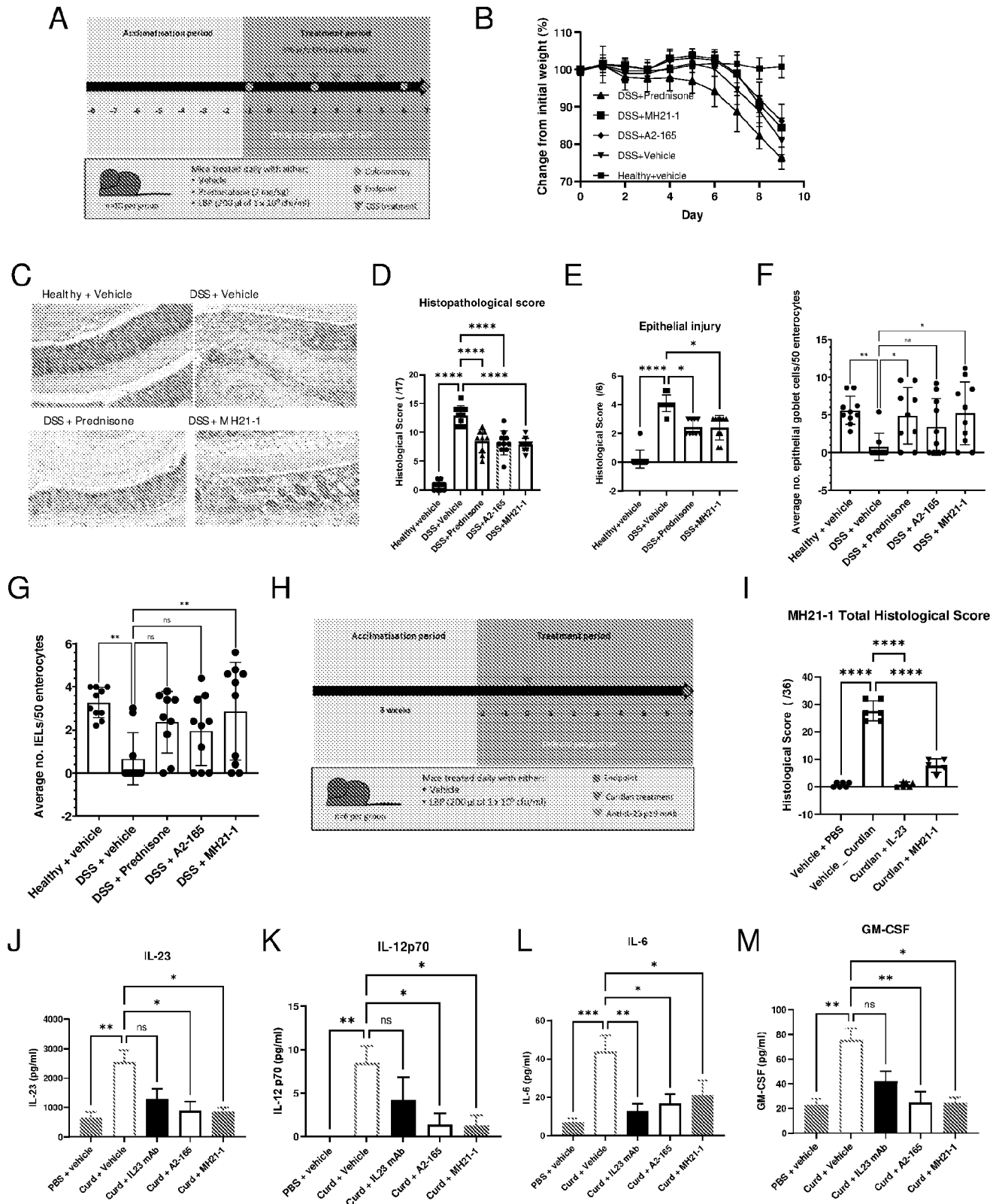


FIGURE 5

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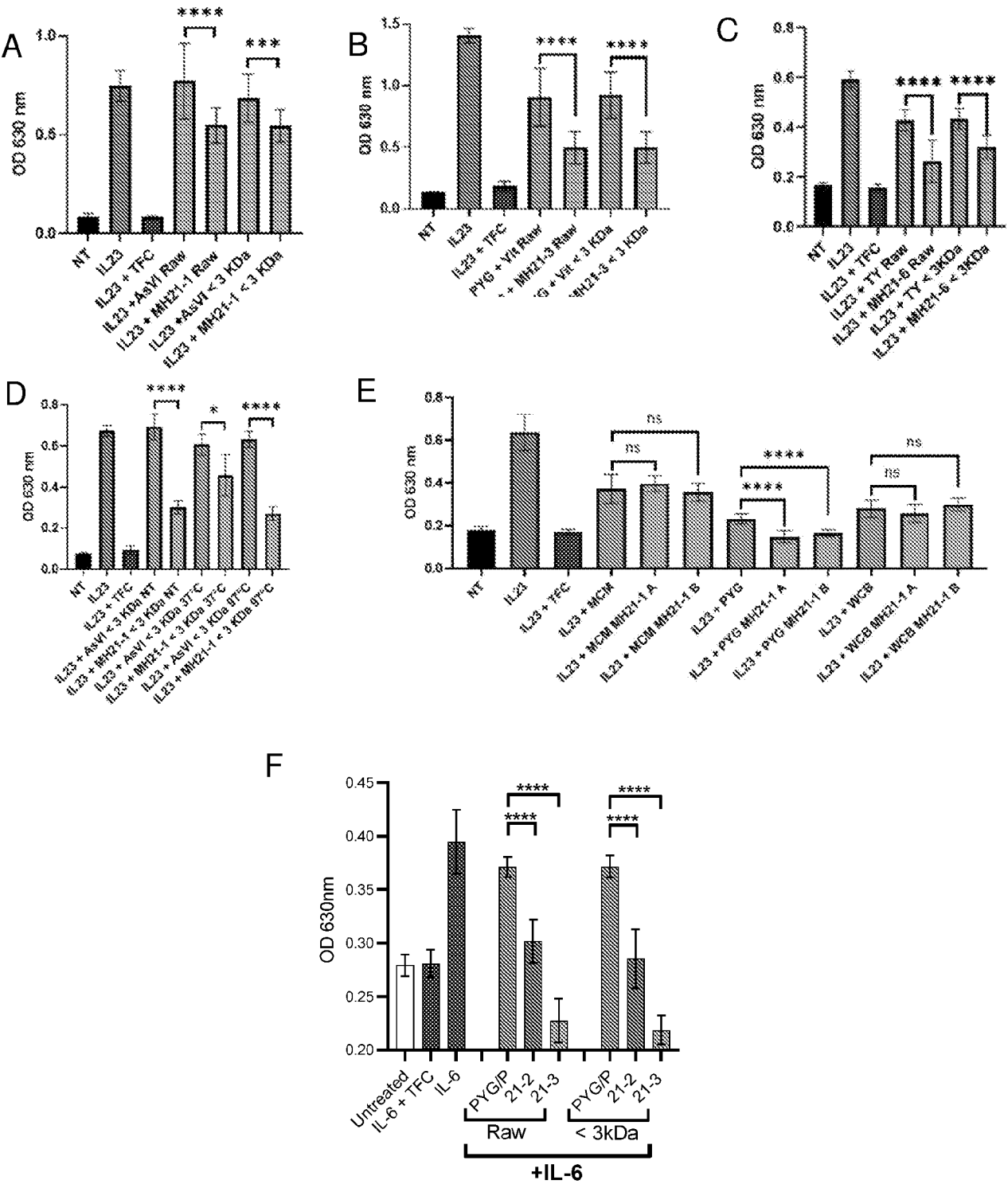


FIGURE 6

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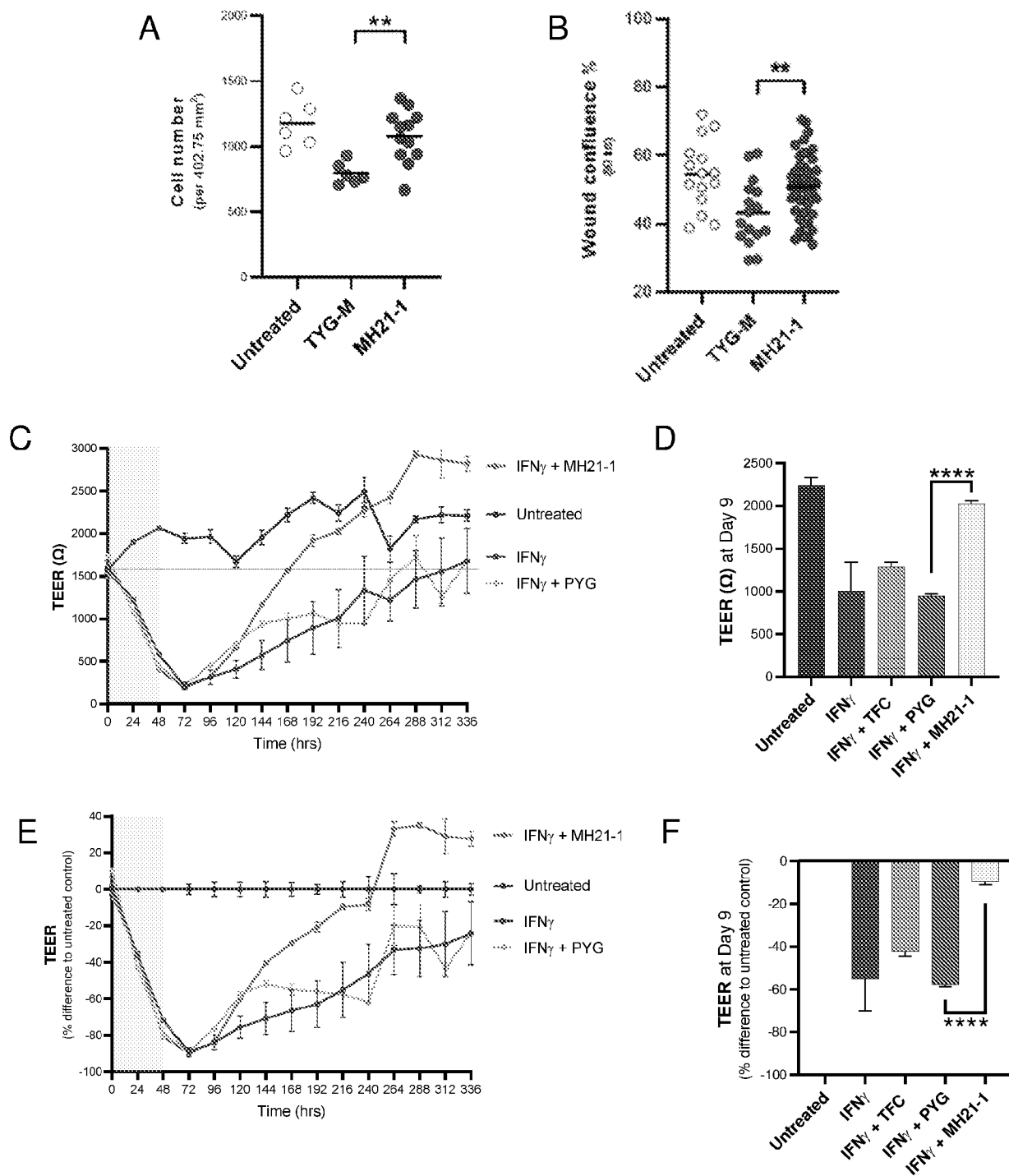
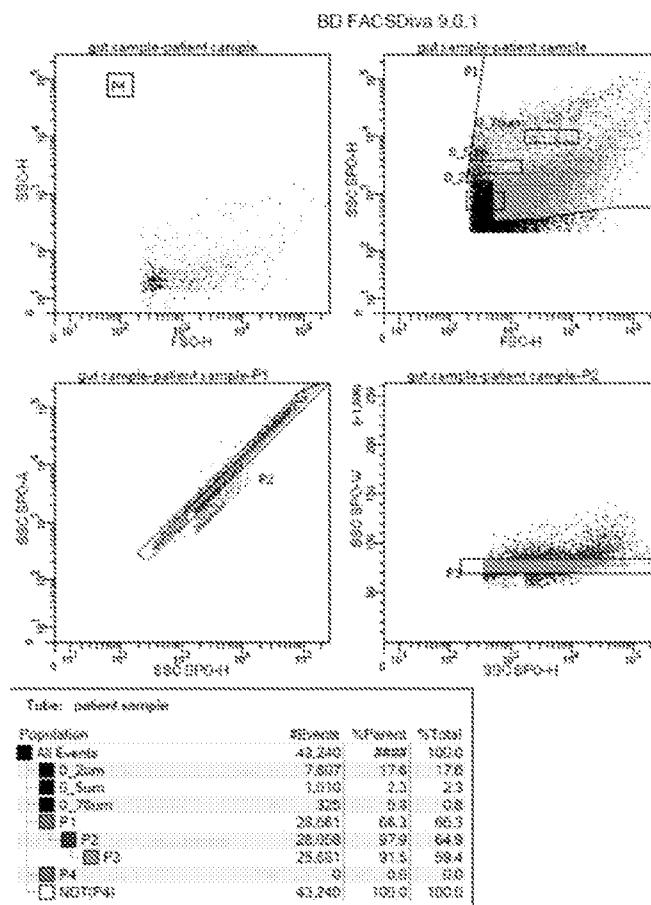


FIGURE 4

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G

FIGURE 7
CONT'D

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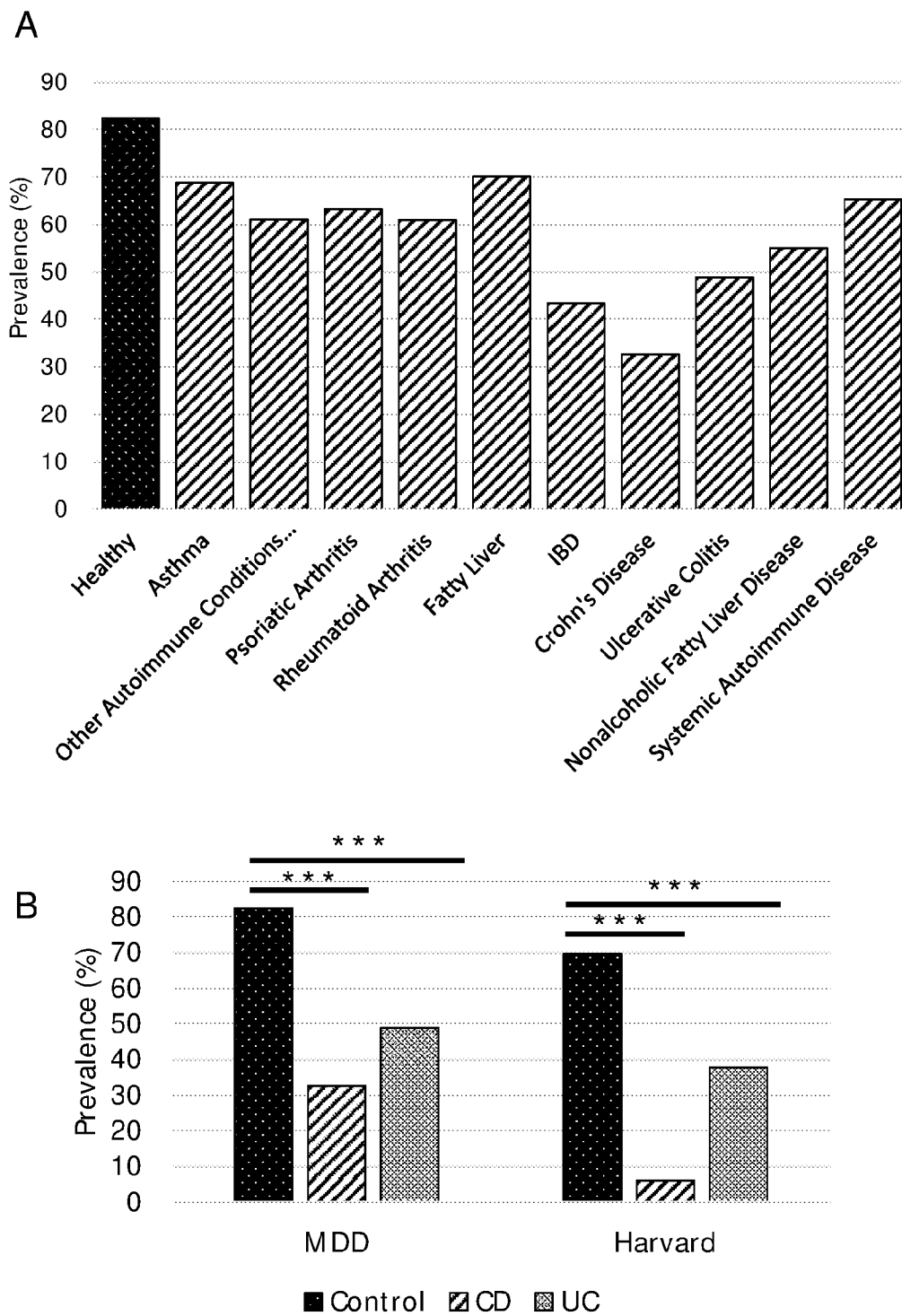
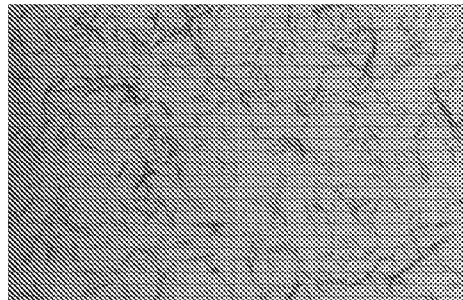


FIGURE 8

12/25

A



B

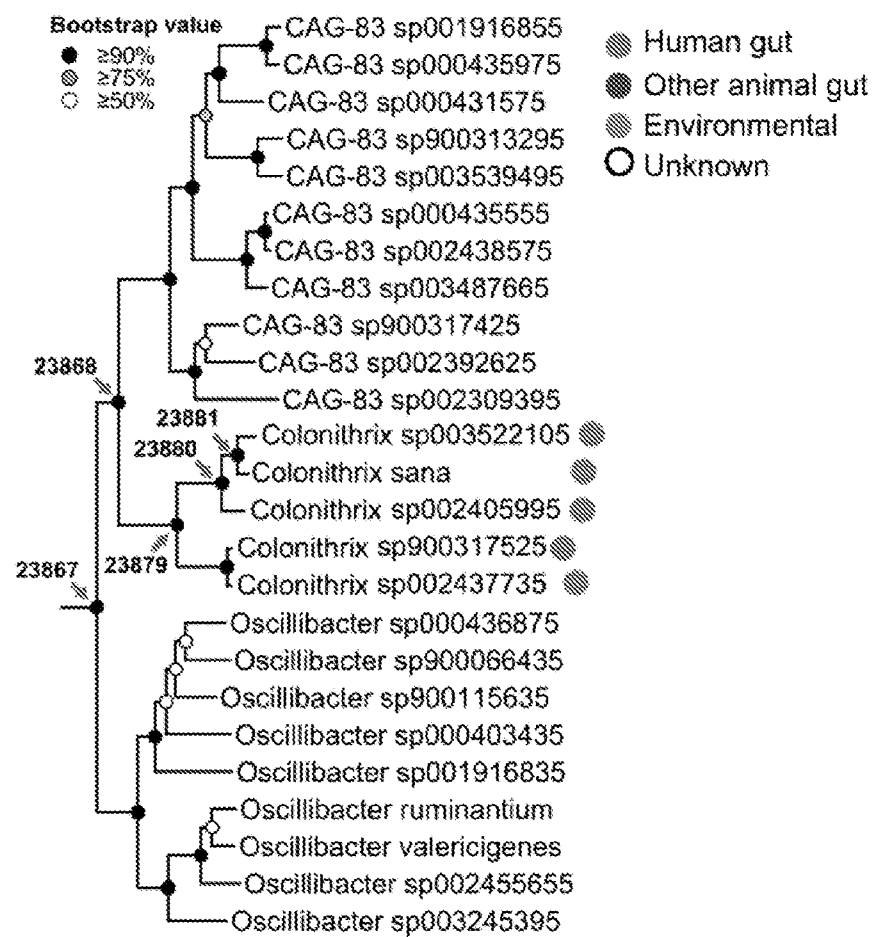
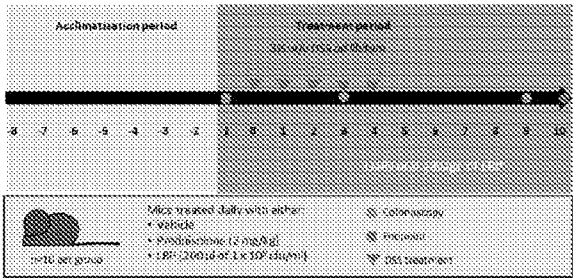
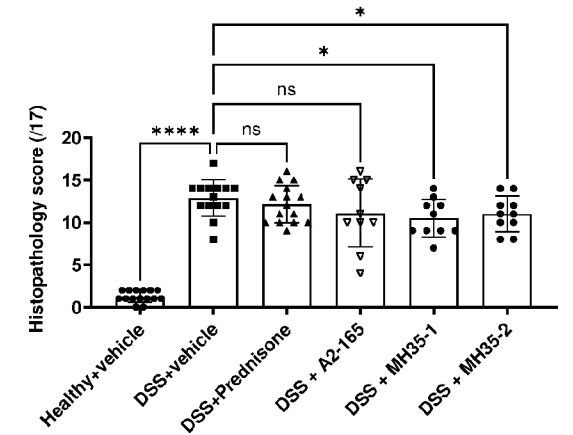


FIGURE 9

A



B



C

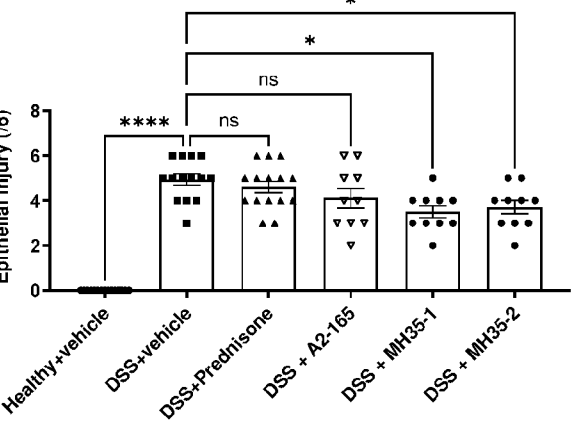
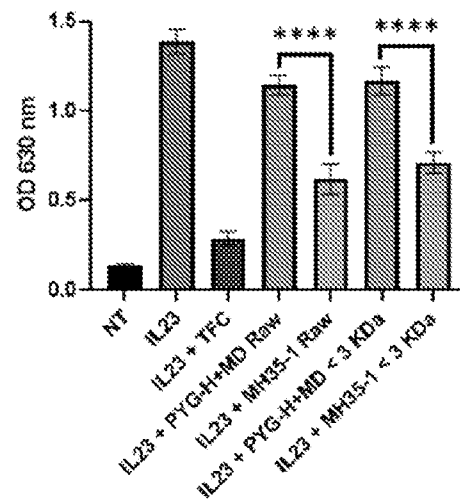


FIGURE 10

14/25

A



B

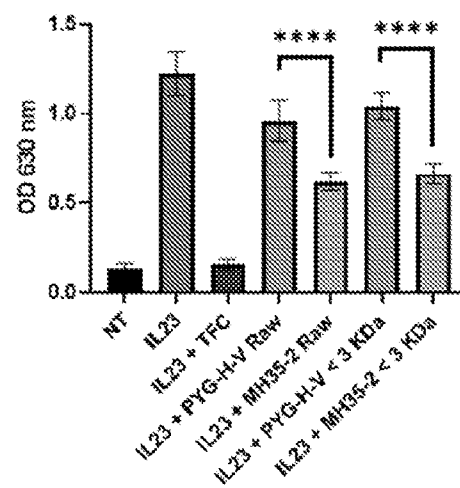


FIGURE 11

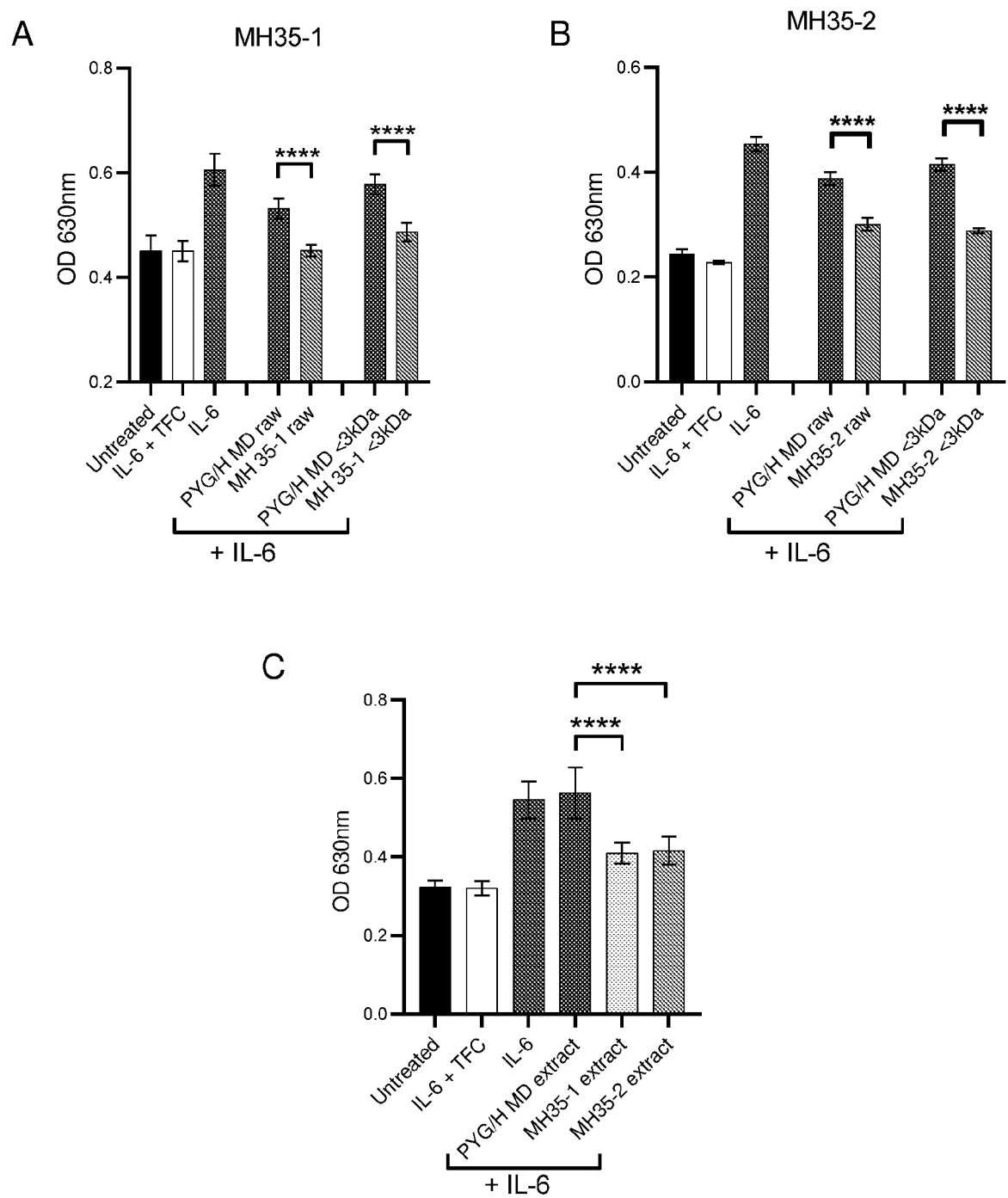


FIGURE 12

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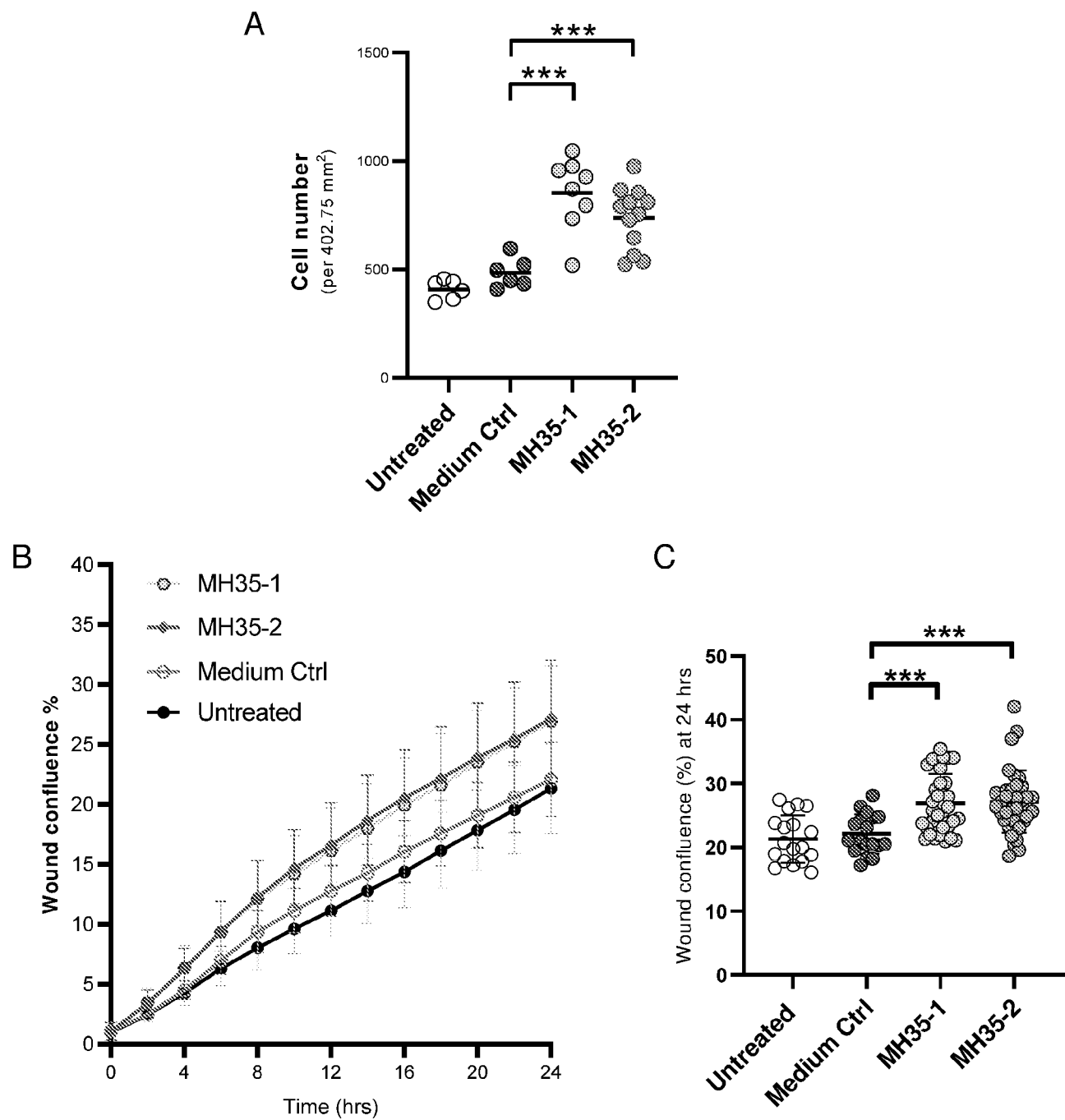


FIGURE 13

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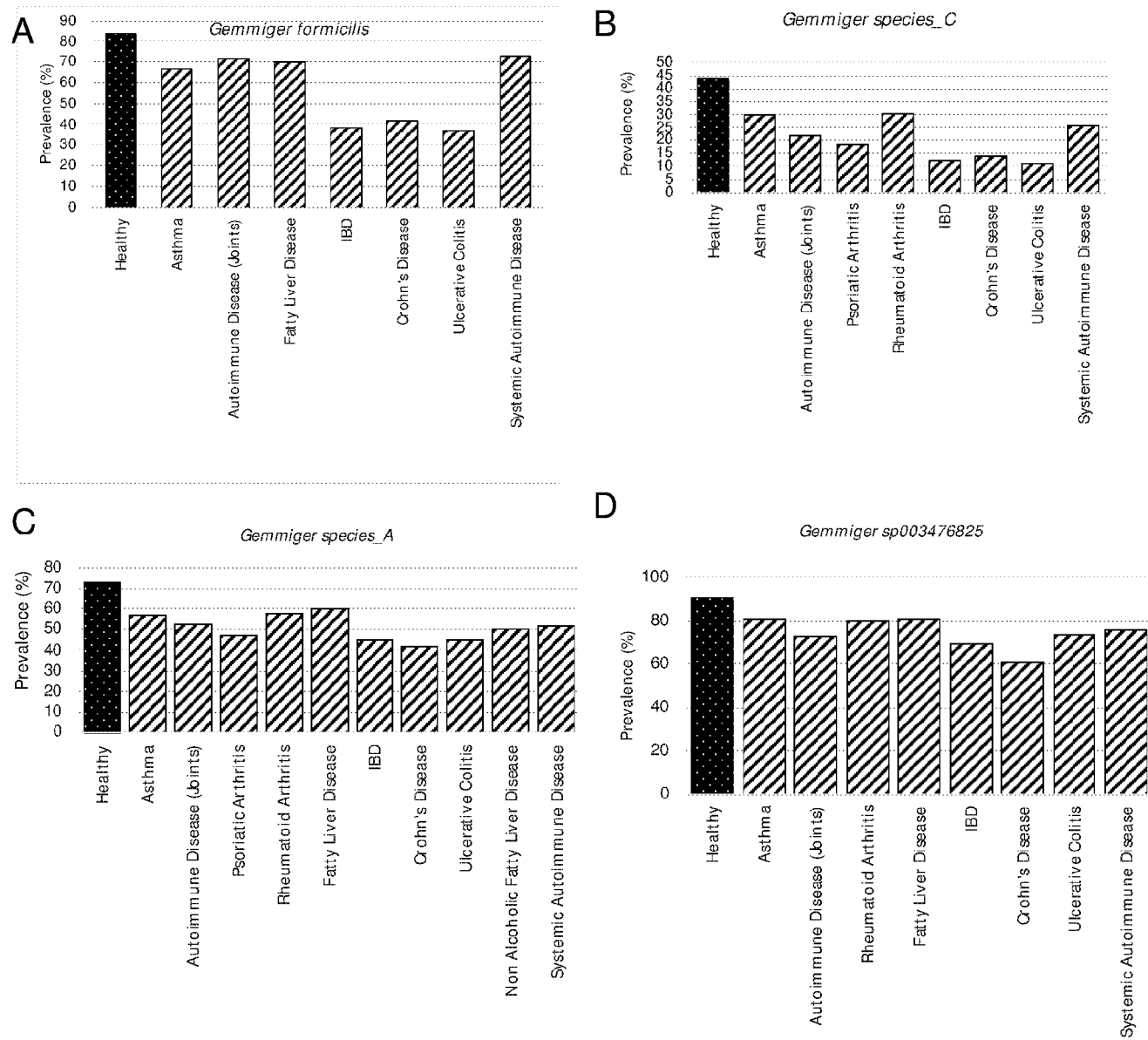
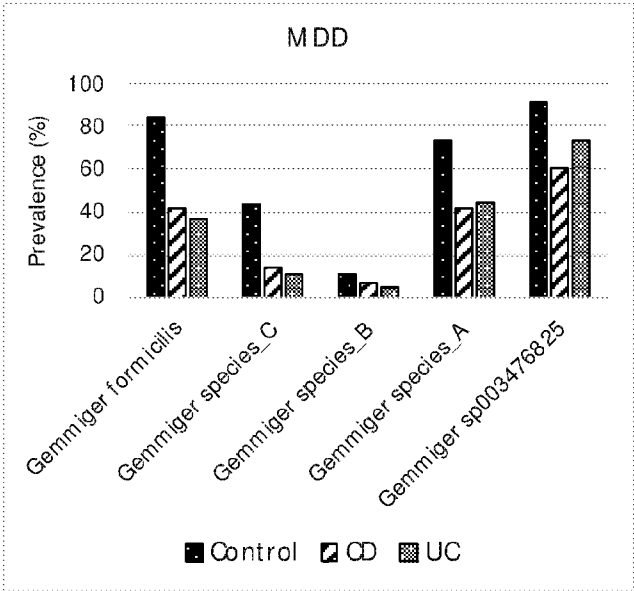


FIGURE 14

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E



F

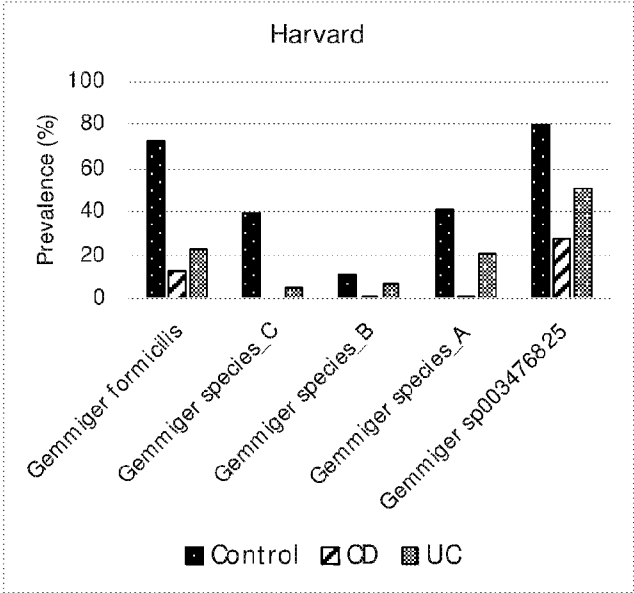
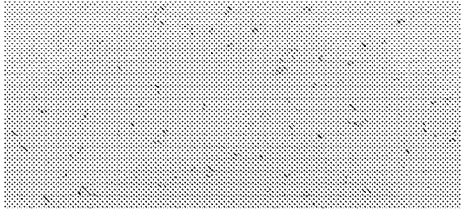


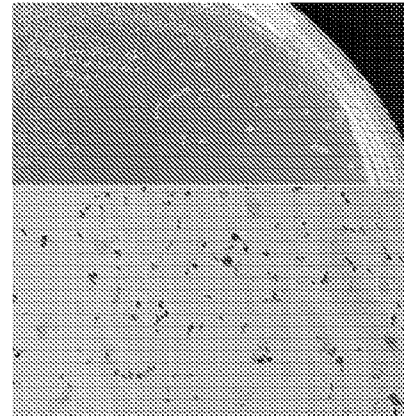
FIGURE 14 CON'T

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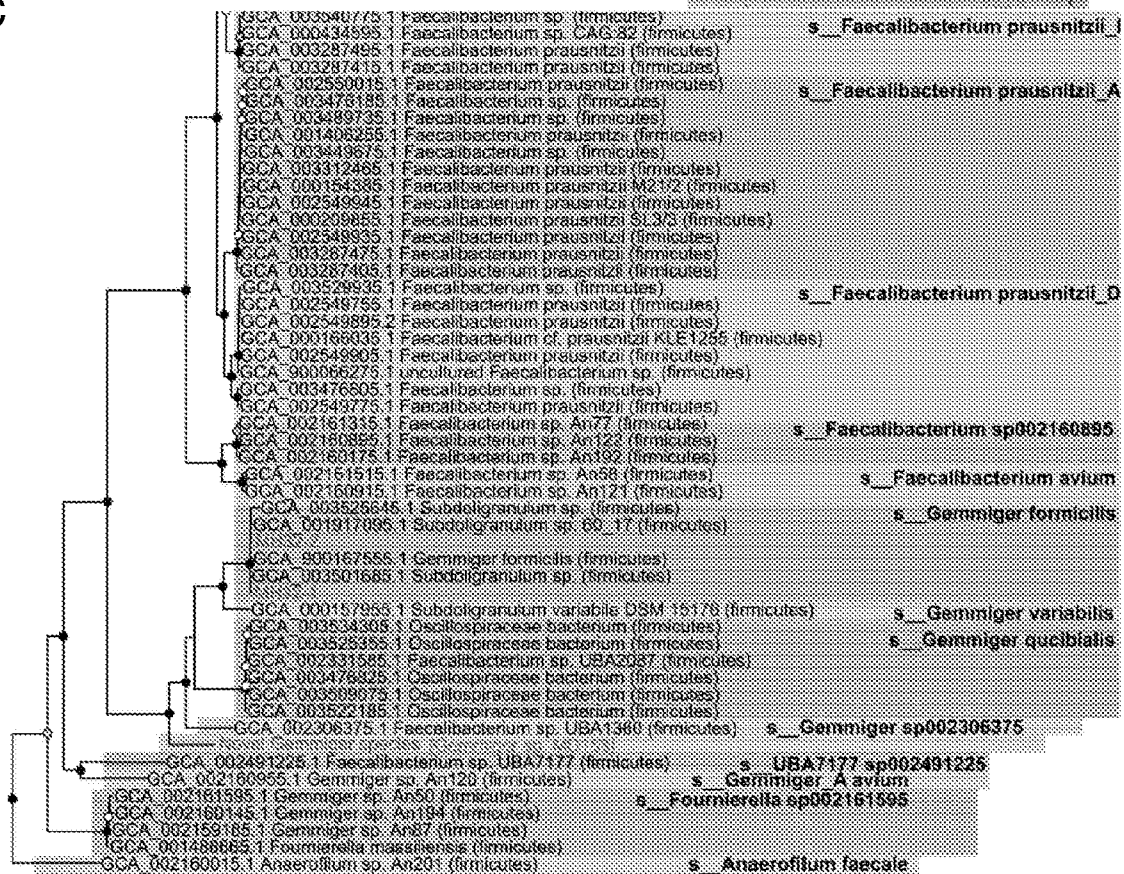
A



B



C



D

Bootstrap value

- 100%
- 75%
- 50%

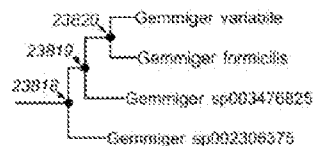


FIGURE 15

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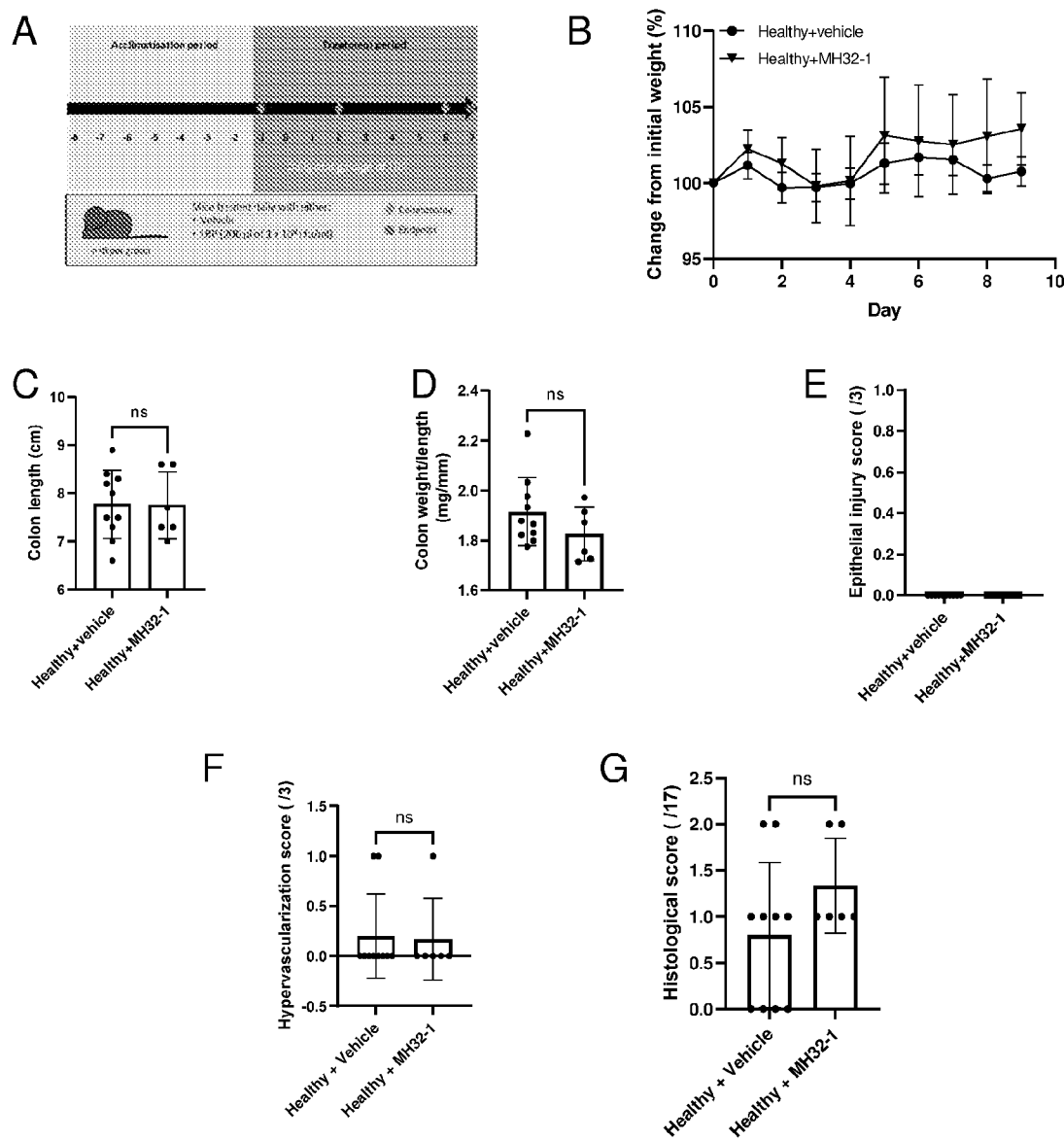


FIGURE 16

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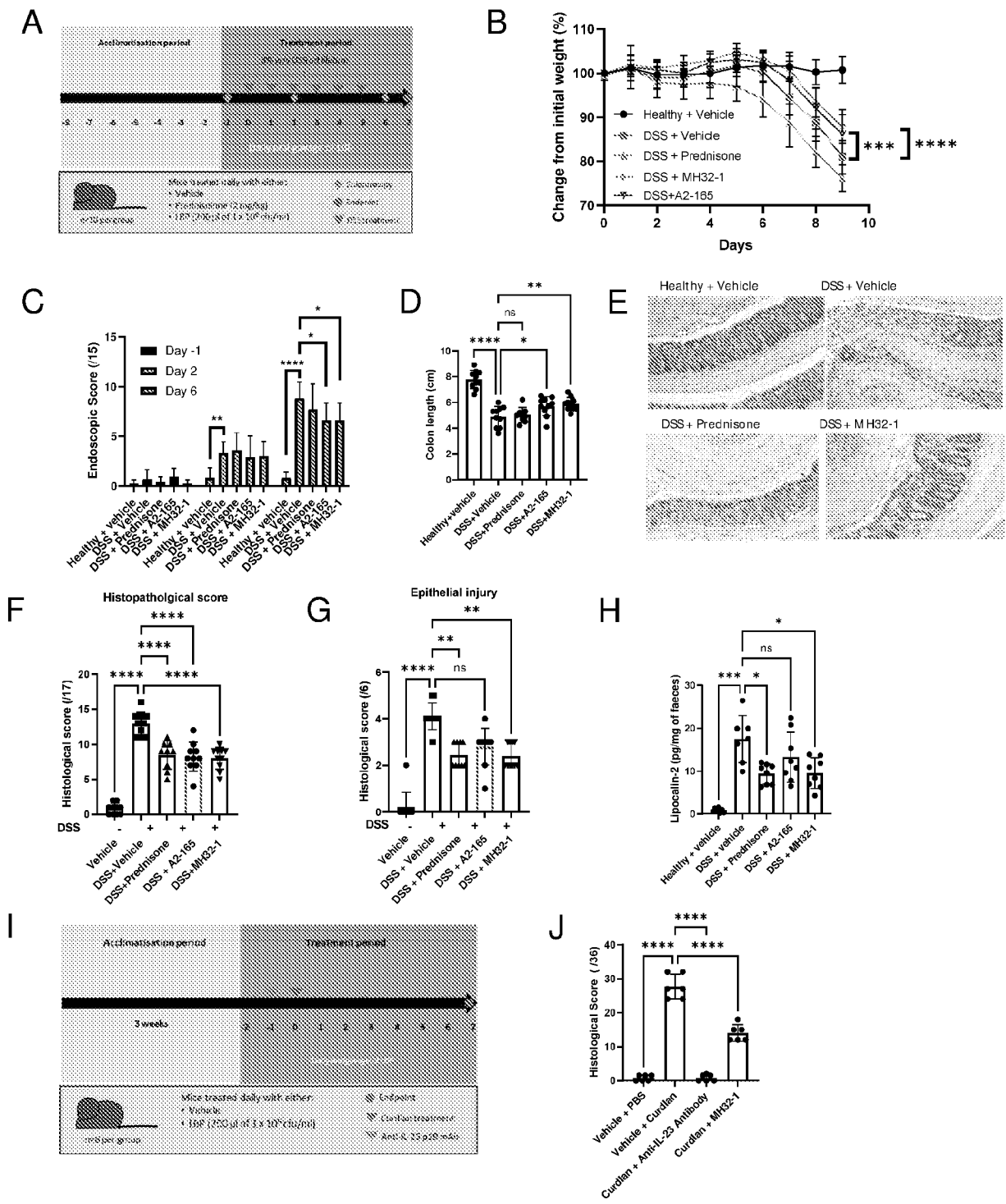


FIGURE 17

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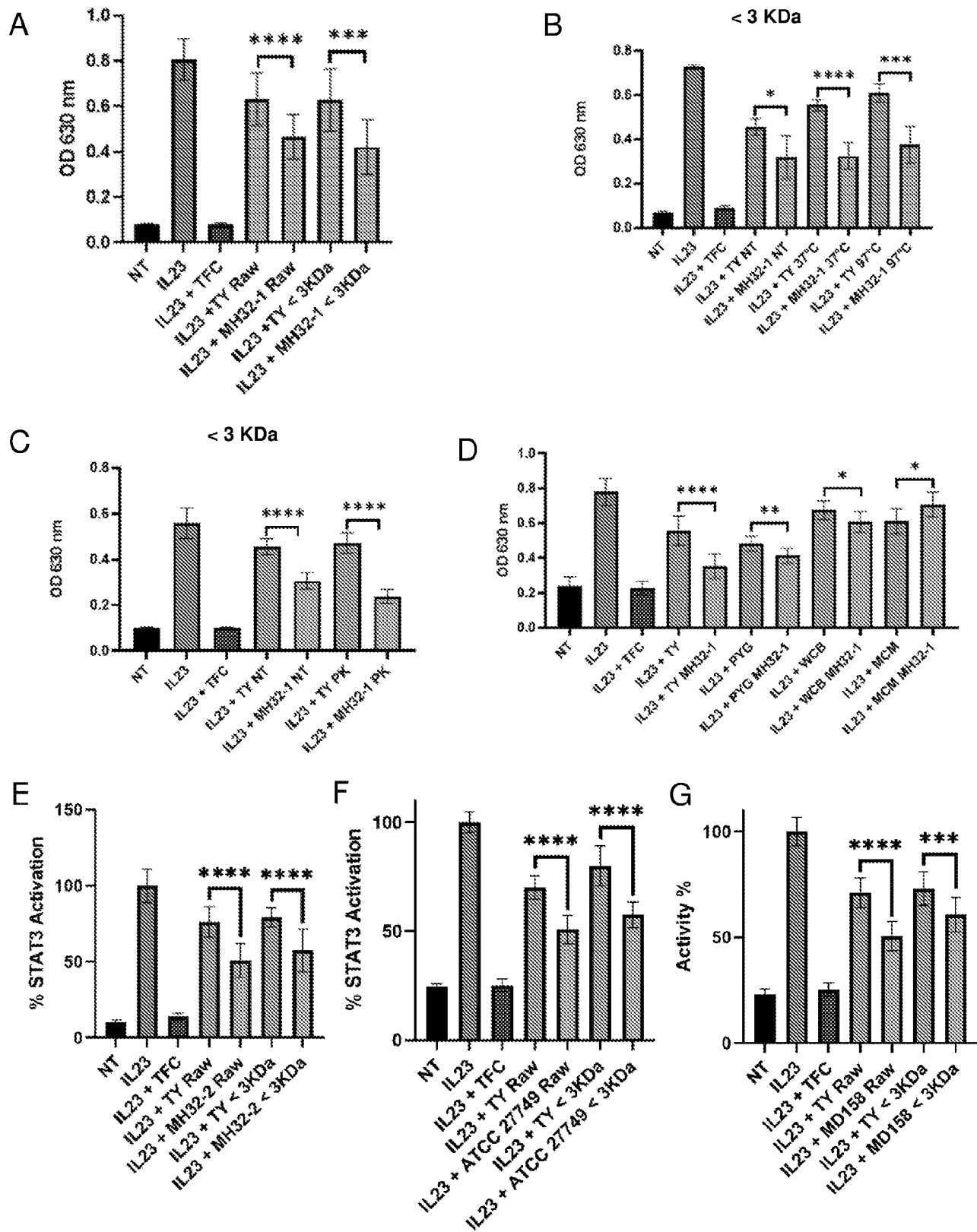
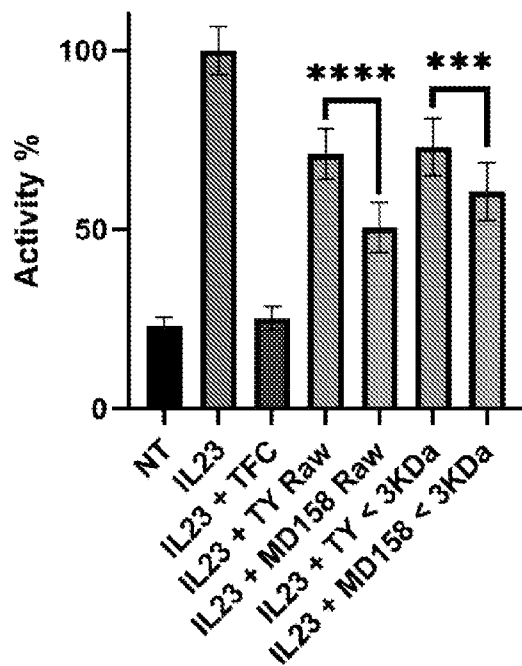


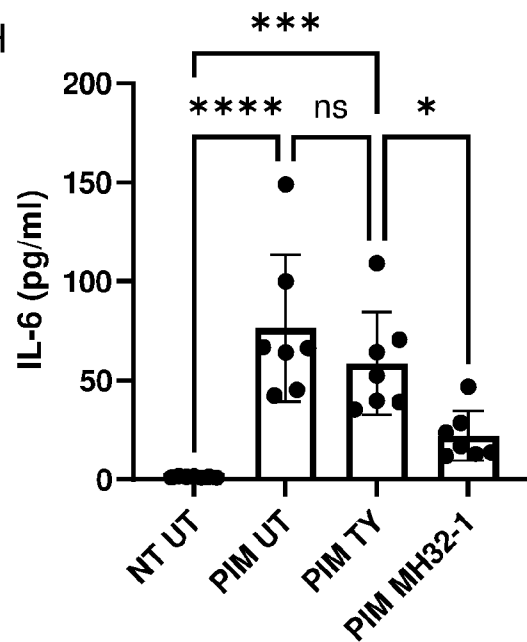
FIGURE 18

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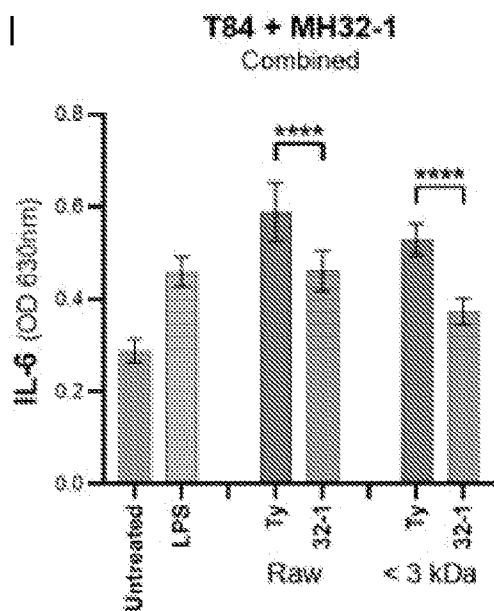
G



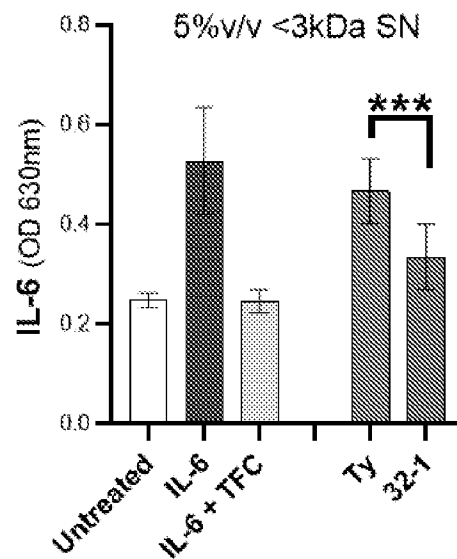
H



I



J

FIGURE 18
CONT'D

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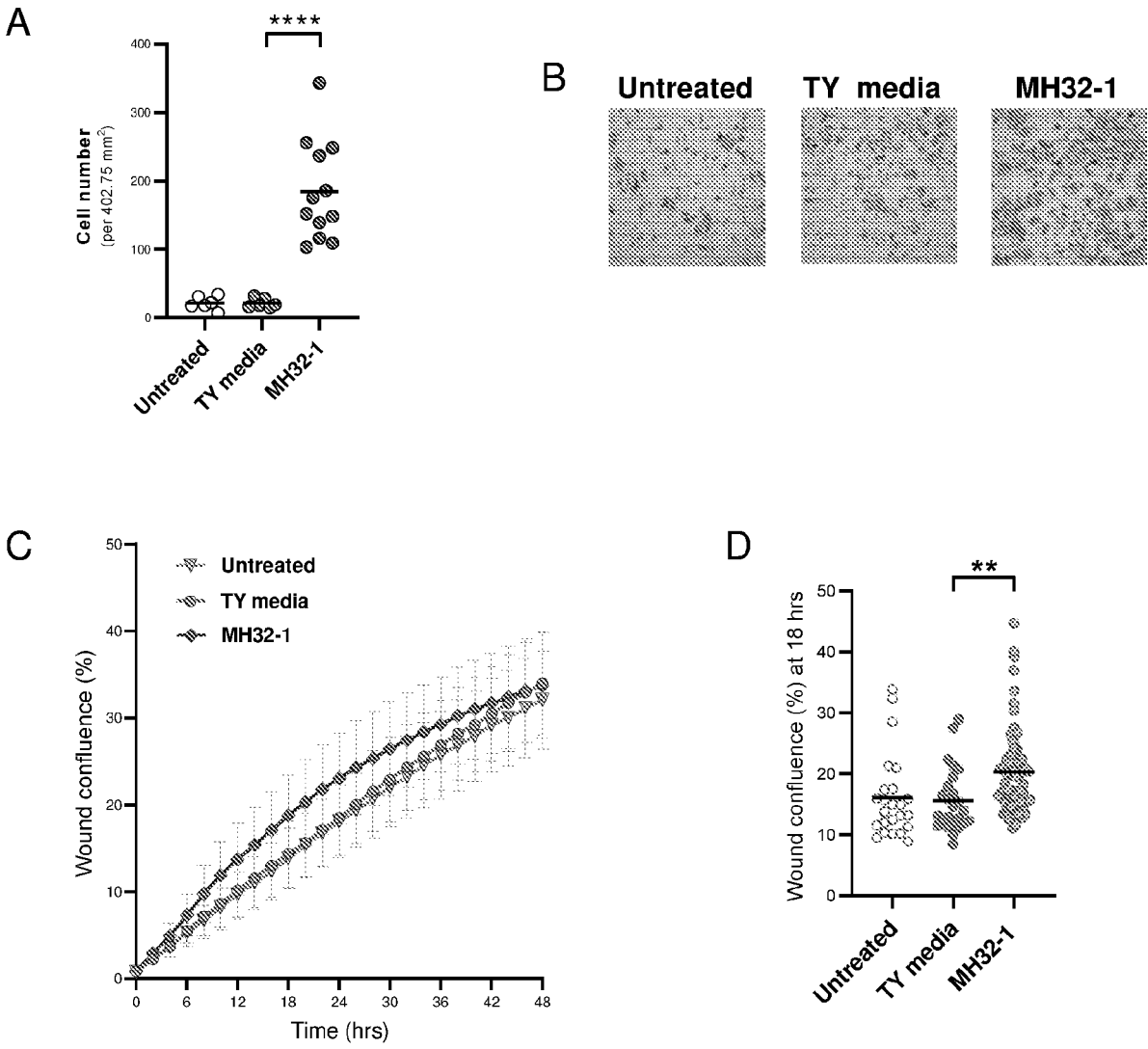
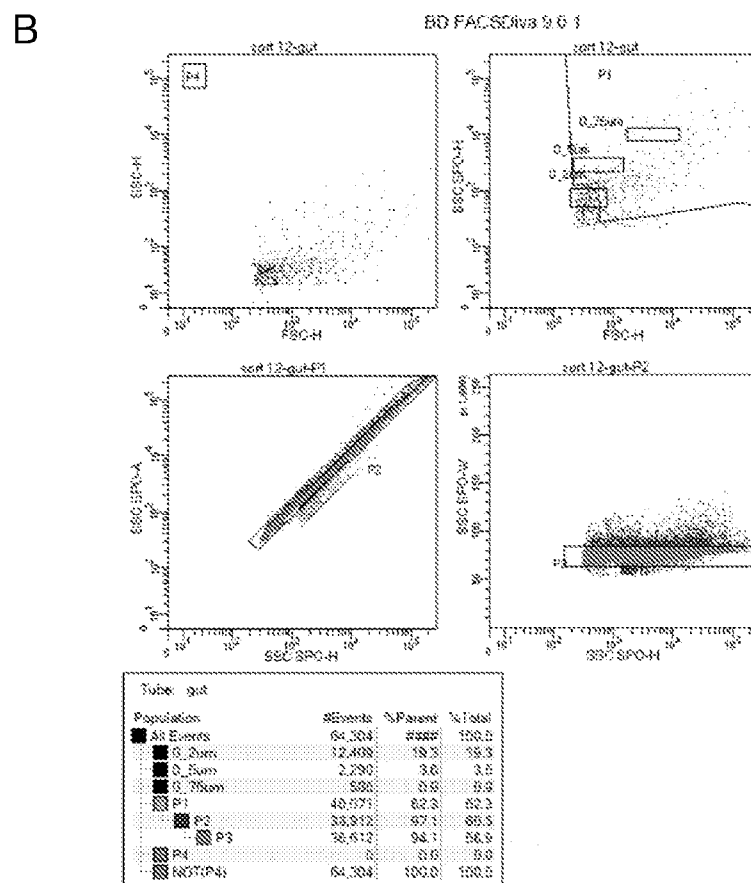


FIGURE 19



SUBSTITUTE SHEETS (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2022/050726

A. CLASSIFICATION OF SUBJECT MATTER

C12N 1/20 (2006.01) C12Q 1/689 (2018.01) C12R 1/01 (2006.01) A61K 35/00 (2006.01) A61K 35/741 (2015.01)
A61P 1/00 (2006.01) A61P 29/00 (2006.01) A61P 37/00 (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

BIOSIS, EMBASE, HCAPLUS, MEDLINE (STAT3, JAK2, TYK, IL-23, Alistipes, shahii, metabolite, agent, molecule, supernatant, short chain fatty acid, butyrate, acetate, fumarate, ethanol, and like terms), EPODOC, WPIAP, and all English language databases (Alistipes shahii, inflammatory bowel disease, Crohn's disease, ulcerative colitis, gut barrier function, mucosa, inflammation, autoimmune, treat, prevent, ameliorate, STAT3, JAK2, TYK, IL-23, attenuate, impair, inhibit, block, butyrate, acetate, fumarate, ethanol, corticosteroids, and like terms), ESPACENET, PubMed, DOCDB, DWPI, IP Australia internal databases (applicant and inventor search), Google (butyrate, acetate, STAT3, IL-8, inflammation), Google Patents (Alistipes, autoimmune, inflammation).

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

☒ Further documents are listed in the continuation of Box C☒ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"D" document cited by the applicant in the international application	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
31 October 2022

Date of mailing of the international search report
31 October 2022

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
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Authorised officer

Melissa Adams
AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No. +61 2 6283 3112

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. PCT/AU2022/050726
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/086210 A1 (Epiva Biosciences, Inc) 02 June 2016 Paragraphs 5, 9–12, 51, 100, 183–185, 195, 197, 261, 285, 348, 384, 385, 400, 402, 404, 406, 435, 458, 459, 475, 518, 574, 579, 580; Table 1, 1A, 1B, 1F; SEQ ID NOs 129–136 define Alistipes strains, SEQ ID NO: 132 has >96% identity to SEQ ID NOs 1–3 and 44, SEQ ID NO: 134 has >98% identity to SEQ ID NOs 2–3, SEQ ID NO: 135 has >94% identity to SEQ ID NOs 1–3 and 44	7–22, 24–33, 42–54, 58–65, 67–69, 71–94, 97–113, 115–130
X	WO 2014/082050 A1 (Seres Health, Inc.) 30 May 2014 Paragraphs 13, 16, 17, 26, 95, 96, 118, 119, 133, 140, 144, 146, 149; Claim 62; Table 9; SEQ ID NO: 134 has >96% identity to SEQ ID NOs 1–3, 7, 8 and 44	5–19, 25, 26, 77–82, 86–113, 115–130
X	WO 2021/108728 A1 (Seres Therapeutics, Inc.) 03 June 2021 Paragraphs 7, 25, 38–40, 126, 140, 186, 204, 228, 251, 254, 257, 262, 266–269, 273, 295, 394, 397, 398 436; Examples 8, 9, 12; Table 4; Figures 18, 19; SEQ ID NO: 112 has >97% identity to SEQ ID NOs 1–3	7–22, 24–31, 33–63, 65, 67–69, 71–94, 97–113, 115–130
A	Chen J, et al., "Effects of intestinal microbial–elaborated butyrate on oncogenic signaling pathways", Nutrients, 2019, vol. 11, no. 5, article 1026 Figures 1, 8	20–24, 48–66
A	Cotton JA, et al., "Interleukin-8 in gastrointestinal inflammation and malignancy: induction and clinical consequences", International Journal of Interferon, Cytokine and Mediator Research, 2016, vol. 8, p. 13–34 Page 25	20–24, 48–66
Form PCT/ISA/210 (fifth sheet) (July 2019)		

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1–130

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Supplemental Box**Continuation of: Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1 to 130 are directed to cells, cultures, compositions, methods and products comprising bacteria of the *Alistipes* genus;
- Claims 131 to 260 are directed to cells, cultures, compositions, methods and products comprising bacteria of the *Colonithrix* genus;
- Claims 261 to 390 are directed to cells, cultures, compositions, methods and products comprising bacteria of the *Gemmiger* genus.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a priori*.

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13^{ter}.1(a)).
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☒ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

The attached sequence listing could not be validated for compliance with ST.26 standard due to the inclusion of additional characters within a non-mandatory field. However, the attached sequence listing file appears to be in the required XML format. Consequently, it was possible to conduct a full search and opinion based on the information in the attached sequence listing file.

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/AU2022/050726	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2016/086210 A1	02 June 2016	WO 2016086210 A1	02 Jun 2016
		AU 2015353425 A1	13 Jul 2017
		AU 2015353425 B2	08 Apr 2021
		AU 2021204574 A1	29 Jul 2021
		CA 2973223 A1	02 Jun 2016
		CN 108135944 A	08 Jun 2018
		EP 3223834 A2	04 Oct 2017
		EP 3223834 B1	06 May 2020
		EP 3747450 A1	09 Dec 2020
		HK 1244683 A1	17 Aug 2018
		MA 41020 A	03 Oct 2017
		MA 52257 A	21 Apr 2021
		US 2016143961 A1	26 May 2016
		US 9603878 B2	28 Mar 2017
		US 2016143962 A1	26 May 2016
		US 9610307 B2	04 Apr 2017
		US 2018071344 A1	15 Mar 2018
		US 10869903 B2	22 Dec 2020
		US 2016199424 A1	14 Jul 2016
		US 10980845 B2	20 Apr 2021
		US 2016193258 A1	07 Jul 2016
		US 2016235792 A1	18 Aug 2016
		US 2016271188 A1	22 Sep 2016
		US 2018015130 A1	18 Jan 2018
		US 2018169153 A1	21 Jun 2018
		US 2019336543 A1	07 Nov 2019
		US 2020061129 A1	27 Feb 2020
		US 2022323511 A1	13 Oct 2022
		WO 2016086205 A2	02 Jun 2016
		WO 2016086206 A1	02 Jun 2016
		WO 2016086208 A1	02 Jun 2016
		WO 2016086209 A1	02 Jun 2016
WO 2014/082050 A1	30 May 2014	WO 2014082050 A1	30 May 2014
		AU 2013347805 A1	09 Jul 2015
		AU 2013347805 B2	05 Apr 2018

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

Form PCT/ISA/210 (Family Annex)(July 2019)

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.
Form PCT/ISA/210 (Family Annex)(July 2019)

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/AU2022/050726	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
		AU 2018204406 A1	05 Jul 2018
		AU 2018204406 B2	05 Dec 2019
		AU 2020201598 A1	19 Mar 2020
		AU 2020201598 B2	24 Mar 2022
		AU 2022204478 A1	14 Jul 2022
		BR 112015011933 A2	15 May 2018
		CA 2892297 A1	30 May 2014
		CN 104955466 A	30 Sep 2015
		EP 2953472 A1	16 Dec 2015
		EP 3628161 A1	01 Apr 2020
		HK 1218836 A1	17 Mar 2017
		JP 2015537042 A	24 Dec 2015
		JP 6506173 B2	24 Apr 2019
		JP 2019135240 A	15 Aug 2019
		JP 6978463 B2	08 Dec 2021
		JP 2022028787 A	16 Feb 2022
		KR 20150108357 A	25 Sep 2015
		KR 102426653 B1	28 Jul 2022
		KR 20220108205 A	02 Aug 2022
		MX 2015006491 A	03 Dec 2015
		NZ 709392 A	28 Oct 2016
		RU 2015124366 A	10 Jan 2017
		SG 10201704035T A	29 Jun 2017
		SG 11201503966P A	29 Jun 2015
		US 2014147425 A1	29 May 2014
		US 8906668 B2	09 Dec 2014
		US 2014199281 A1	17 Jul 2014
		US 9028841 B2	12 May 2015
		US 2015190435 A1	09 Jul 2015
		US 9533014 B2	03 Jan 2017
		US 2019350988 A1	21 Nov 2019
		US 10864235 B2	15 Dec 2020
		US 2021169946 A1	10 Jun 2021
		US 11389490 B2	19 Jul 2022
		US 2021169947 A1	10 Jun 2021
		US 11458173 B2	04 Oct 2022
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.			

INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/AU2022/050726	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2021/108728 A1	03 June 2021	US 2021169948 A1	10 Jun 2021
		US 11458174 B2	04 Oct 2022
		US 2021169949 A1	10 Jun 2021
		US 11464812 B2	11 Oct 2022
		US 2017165302 A1	15 Jun 2017
		WO 2021108728 A1	03 Jun 2021
		AU 2020394211 A1	14 Jul 2022
		BR 112022010411 A2	23 Aug 2022
		CA 3159711 A1	03 Jun 2021
		CN 115175575 A	11 Oct 2022
		EP 4064862 A1	05 Oct 2022
		KR 20220120573 A	30 Aug 2022
		End of Annex	
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2019)			