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Microbiota compositions and methods for treating disorders

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11 Mar 2026
2026201833

ABSTRACT

The present invention relates to compositions for storing microbiota. The present invention also relates to dosage forms and methods of treating disorders and diseases by administering the composition to a patient in need thereof.

Microbiota Compositions and Methods for Treating Disorders

Related application

[0001A] This application is a divisional of Australian Patent Application No. 2024234064, the entire contents of which are herein incorporated by reference.

Field of the Invention

[0001B] The present invention relates to compositions for storing microbiota. The present invention also relates to dosage forms and methods of treating disorders and diseases by administering the composition to a patient in need thereof.

Background

[0002] The following discussion of the background art is intended to facilitate an understanding of the present invention only. The discussion is not an acknowledgement or admission that any of the material referred to is or was part of the common general knowledge as at the priority date of the application.

[0003] ***Intestinal Microbiota***

[0004] The human intestinal microbiota consists of trillions of microorganisms including at least 100 prevalent and at least 1000 less common bacterial species, harbouring over 100-fold more genes than those present in the human genome. The intestinal microbiota is composed predominantly of bacteria, yet also contains archaea, fungi, yeast, protozoa, and viruses. The microbiota performs vital functions essential to health maintenance, including food processing, digestion of complex indigestible polysaccharides and synthesis of vitamins, and it secretes bioactive metabolites with diverse functions, ranging from inhibition of pathogens, metabolism of toxic compounds to modulation of host metabolism.

[0005] ***Cultured microbiome therapies***

[0006] Cultured microbiome therapies are composed of one or more microorganisms that may be administered to a patient with the aim of treating or preventing disease or potentiating the effect of another therapy. Cultured microbiome therapies are now being developed as a treatment for many diseases. However, these efforts have been challenged by the loss of viable microbes which limits stability, storage, transport and delivery conditions and thereby limiting the clinical use of these therapies. There is a challenge to sample, store, ship and deliver a viable and effective microbiota sample to the patient. There is also a need in the art for effective treatments of diseases associated with loss of gut microbes or dysbiosis.

[0007] ***Storing Microbiota***

[0008] Efforts have been made to store microbiota in freeze dried or lyophilized forms. Freeze drying, also known as lyophilization, is a low temperature dehydration process that

11 Mar 2026
2026201833

involves freezing the product and lowering pressure, removing the ice by sublimation. This is in contrast to dehydration by most conventional methods that evaporate water using heat. Freeze drying microbiota is a useful method for long-term preservation. However, one of the major challenges is the loss of cell viability due to freeze-thaw damage and the impact of osmotic pressure changes on the functional and structural integrity of the microorganisms. Attempts have been reported in the prior art to develop suitable cryoprotectants to protect the functional and structural integrity of the microorganisms during the freeze drying and thawing processes however many of these attempts have failed. There is a need in the art for an effective cryoprotectant which maintains cell viability during the freeze drying and thawing processes and maintains the functional and structural integrity of the microorganisms.

[0009] It is an objective of the invention to overcome one or more problems foreshadowed by the prior art.

Summary of the Invention

[0010] In a first aspect, the invention broadly resides in a composition for preventing or treating a disease or disorder in a subject in need thereof, said composition comprising at least one strain of a microorganism,

wherein the microorganism is selected from the group consisting of: bacteria, yeast or archaea; and

an excipient.

[0011] In a preferred embodiment, the excipient is a cryoprotectant. In a preferred embodiment, the excipient is inulin or an analog or variant thereof. In a preferred embodiment, the inulin is selected from the group consisting of: *alpha*-D-glucopyranosyl-[beta-D-fructofuranosyl](n-1)-D-fructofuranosides; *beta*-D-fructopyranosyl-[D-fructofuranosyl](n-1)-D-fructofuranosides; fructo-oligosaccharides; fructo-oligosaccharides containing between 2 and 70 fructose units; fructo-oligosaccharides containing between 1 and 500 fructose units; fructo-oligosaccharides containing between 1 and 300 fructose units; fructo-oligosaccharides containing between 1 and 200 fructose units; fructo-oligosaccharides containing between 1 and 100 fructose units; or an analog or variant or combination thereof.

[0012] In a preferred embodiment, the excipient is maltodextrin or an analog or variant thereof. In a preferred embodiment, the maltodextrin is selected from the group consisting of: a maltodextrin having a length selected from the group consisting of: 3 to 17 glucose units; corn syrup with a length of 20 glucose units or more; corn syrup solid; modified corn starch;

modified rice starch; modified tapioca starch; modified wheat starch; or an analog or variant or combination thereof.

[0013] In a further preferred embodiment, the composition comprises inulin or an analog of variant thereof at a concentration selected from the group consisting of: 0.01% w/v to 20% w/v; 0.1% w/v to 20% w/v; 0.1% w/v to 10% w/v; 1% w/v to 10% w/v; 2% w/v to 9% w/v; 3% w/v to 8% w/v; 4% w/v to 7% w/v; 4% w/v to 6% w/v; 1% w/v; 2% w/v; 3% w/v; 4% w/v; 5% w/v; 6% w/v; 7% w/v; 8% w/v; 9% w/v; and 10% w/v.

[0014] In a further preferred embodiment, the composition comprises maltodextrin or an analog of variant thereof at a concentration selected from the group consisting of: 0.01% w/v to 20% w/v; 0.01% w/v to 20% w/v; 0.1% w/v to 10% w/v; 1% w/v to 10% w/v; 2% w/v to 9% w/v; 3% w/v to 8% w/v; 4% w/v to 7% w/v; 4% w/v to 6% w/v; 1% w/v; 2% w/v; 3% w/v; 4% w/v; 5% w/v; 6% w/v; 7% w/v; 8% w/v; 9% w/v; and 10% w/v.

[0015] In a further preferred embodiment, the composition comprises inulin and maltodextrin.

[0016] In a preferred embodiment, the composition comprises inulin and maltodextrin at a concentration selected from the group consisting of: inulin (1% w/v) and maltodextrin (1% w/v); inulin (2% w/v) and maltodextrin (2% w/v); inulin (3% w/v) and maltodextrin (3% w/v); inulin (4% w/v) and maltodextrin (4% w/v); inulin (5% w/v) and maltodextrin (5% w/v); inulin (6% w/v) and maltodextrin (6% w/v); inulin (7% w/v) and maltodextrin (7% w/v); inulin (8% w/v) and maltodextrin (8% w/v); inulin (9% w/v) and maltodextrin (9% w/v); and inulin (10% w/v) and maltodextrin (10% w/v).

[0017] In a preferred embodiment, the composition comprises inulin and maltodextrin at a concentration selected from the group consisting of: (1) inulin at a concentration selected from the group consisting of: 1% w/v; 2% w/v; 3% w/v; 4% w/v; 5% w/v; 6% w/v; 7% w/v; 8% w/v; 9% w/v; and 10% w/v; AND (2) maltodextrin at a concentration selected from the group consisting of: 1% w/v; 2% w/v; 3% w/v; 4% w/v; 5% w/v; 6% w/v; 7% w/v; 8% w/v; 9% w/v; and 10% w/v.

[0018] In a preferred embodiment, the composition comprises inulin and maltodextrin at a concentration selected from the group consisting of: inulin (1% w/v) and maltodextrin (1% w/v); inulin (2% w/v) and maltodextrin (2% w/v); inulin (3% w/v) and maltodextrin (3% w/v); inulin (4% w/v) and maltodextrin (4% w/v); inulin (5% w/v) and maltodextrin (5% w/v); inulin (6% w/v) and maltodextrin (6% w/v); inulin (7% w/v) and maltodextrin (7% w/v); inulin (8% w/v) and maltodextrin (8% w/v); inulin (9% w/v) and maltodextrin (9% w/v); and inulin (10% w/v) and maltodextrin (10% w/v).

[0019] In a preferred embodiment, the composition is in lyophilized form.

[0020] In a preferred embodiment, the composition is in liquid form.

[0021] In a preferred embodiment, the excipient is selected from the group consisting of: inulin; inulin and maltodextrin; inulin and dextran 70k; inulin and pectin; inulin and sucrose; inulin and trehalose; inulin and maltodextrin and sucrose; inulin and maltodextrin and dextran 70k; inulin and maltodextrin and pectin; inulin and maltodextrin and sucrose; and inulin and maltodextrin and pectin.

[0022] In a preferred embodiment, the excipient is selected from the group consisting of: inulin (10% w/v); inulin (5% w/v); inulin (5% w/v) and maltodextrin (5% w/v); inulin (5% w/v) and dextran 70k (5% w/v); inulin (5% w/v) and pectin (5% w/v); inulin (5% w/v) and sucrose (5% w/v); inulin (5% w/v) and trehalose (5% w/v); inulin (5% w/v) and maltodextrin (5% w/v) and sucrose (5% w/v); inulin (5% w/v) and maltodextrin (5% w/v) and dextran 70k(5% w/v); inulin (5% w/v) and maltodextrin (5% w/v) and pectin (5% w/v); inulin (5% w/v) and maltodextrin (5% w/v) and sucrose (5% w/v); and inulin (5% w/v) and maltodextrin (5% w/v) and pectin (5% w/v).

[0023] In a preferred embodiment, the composition has a moisture concentration less than a concentration selected from the group consisting of: 1% w/v; 2% w/v; 3% w/v; 4% w/v; 5% w/v; 6% w/v; 7% w/v; 8% w/v; 9% w/v; and 10% w/v.

[0024] In a preferred embodiment, the composition has a moisture concentration selected from the group consisting of: between 1 and 5% w/v; between 0.5 and 2% w/v; between 0.9 and 1.2% w/v; between 0.99 and 1.09% w/v; and 1.093% w/v.

[0025] In a preferred embodiment, the composition has a Young's Modulus measure selected from the group consisting of: between 1 and 5; between 2 and 4; between 2.1 and 3.9; between 2.5 and 3.8; 2; 3; 4; 5; 2.98; 2.19; 3.51; 3.23; less than 2; less than 3; less than 4; less than 5; greater than 2; greater than 3; greater than 4; greater than 5.

[0026] In a preferred embodiment, the composition has a Max Stress (kPa) at the fractur point measure selected from the group consisting of: between 20 and 40; between 21 and 39; between 20 and 30; between 20 and 29; between 25 and 30; 20; 21; 22; 23; 24; 25; 26; 27; 28; 29; 30; 29.36; 29.01; 22.68; less than 20; less than 21; less than 22; less than 23; less than 24; less than 25; less than 26; less than 27; less than 28; less than 29; less than 30; greater than 20; greater than 21; greater than 22; greater than 23 greater than 24; greater than 25; greater than 26; greater than 27; greater than 28; greater than 29; and greater than 30.

[0027] In a preferred embodiment, the composition comprises further excipients and carriers.

[0028] In a preferred embodiment, the microorganism is a faecal or colonic microorganism.

[0029] In a preferred embodiment, the microorganism is non-inflammatory.

[0030] In a preferred embodiment, the microorganism is cultured from a faecal or colonic biopsy sample.

[0031] In a preferred embodiment, the consortia comprises a community of microorganism cells derived from a stool or biopsy of one or more human donors.

[0032] In a preferred embodiment, the community of microorganism cells comprises cultured microorganism cells.

[0033] In a preferred embodiment, the cultured microorganism cells are derived from a multiple of human donors.

[0034] In a preferred embodiment, the community of microorganism cells comprises uncultured microorganism cells.

[0035] In a preferred embodiment, the uncultured microorganism cells are derived from a single human donor.

[0036] In a preferred embodiment, the composition is a faecal transplant microbiota composition.

[0037] In a preferred embodiment, the composition comprises a purified or reconstituted faecal bacterial mixture.

[0038] In a preferred embodiment, the composition is lyophilized.

[0039] In a preferred embodiment, the composition is a liquid.

[0040] In a preferred embodiment, after at least 4 weeks of storage at a storage temperature, the composition is capable of maintaining at least 50% cell viability relative to the initial cell viability immediately prior to storage.

[0041] In a preferred embodiment, after at least 4 weeks of storage at a storage temperature, the composition is capable of maintaining about 60% to about 80% cell viability relative to the initial cell viability immediately prior to the start of said storage.

2026201833 11 Mar 2026

[0042] In a preferred embodiment, after at least 2, 4, 8, 12, 16, or 20 weeks of storage at a storage temperature, the composition is capable of maintaining at least about 5%, 10%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% cell viability relative to the initial cell viability immediately prior to storage.

[0043] In a preferred embodiment, after at least 8, 12, 16, 20, 50, 75, 100, 150, or 200 weeks of storage at a storage temperature, the composition maintains at least 50% cell viability relative to the initial cell viability immediately prior to storage.

[0044] In a preferred embodiment, after at least 12 weeks of at a storage temperature, the composition maintains between 30% and 90%, between 40% and 90%, between 50% and 90%, between 60% and 90%, between 70% and 90%, between 80% and 90%, between 40% and 80%, between 50% and 70%, between 55% and 65%, between 30% and 40%, between 40% and 50%, between 50% and 60%, between 60% and 70%, or between 70% and 80% cell viability, relative to the initial cell viability immediately prior to storage.

[0045] In a preferred embodiment, the storage temperature is selected from the group consisting of: ambient temperature or below; -70°C; -50°C; -47°C; -30°C; -20°C; -8°C; -4°C; below zero; 2°C; 4°C; 8°C; between 2 and 8°C; 18°C; 25°C; room temperature; and ambient temperature.

[0046] In a preferred embodiment, the cell viability is measured by a method selected from the group consisting of; using imaging assays that measure membrane permeability; using a combination of membrane permeant and impermeant DNA dyes stains; SYTO and propidium idodide are used to stain and differentiate live and dead bacteria; live cell determination is combined with fluorescent Gram staining; a colorimetric method; bacterial cell viability is assessed by using a BactoBox or other impedance flow cytometry tools; bacterial cell viability is assessed by counting the number of colonies on an agar plate; cell viability is evaluated via molecular viability analyses.

[0047] In a preferred embodiment, the composition comprises a prebiotic.

[0048] In a preferred embodiment, the composition comprises a carrier.

[0049] In a preferred embodiment, the composition comprises an insoluble fibre, a buffer, an osmotic agent, an antifoaming agent, and/or a preservative.

[0050] In a preferred embodiment, the composition comprises a chemostat medium.

[0051] In a preferred embodiment, the composition comprises a saline composition.

[0052] In a preferred embodiment, the composition comprises a resistant starch.

11 Mar 2026
2026201833

[0053] In a preferred embodiment, the composition is lyophilized with pharmaceutically acceptable excipients.

[0054] In a preferred embodiment, the composition comprises a further stabiliser and/or further cryoprotectant.

[0055] In a preferred embodiment, the further cryoprotectant is selected from the group consisting of: trehalose; mannitol; sucrose; glycerol; sorbitol; DMSO; propylene glycol; ethylene glycol; saccharose; galactose-lactose; and any combination thereof.

[0056] In a preferred embodiment, the further cryoprotectant further comprises a compound selected from the group consisting of: glycerol; polyethylene glycol (PEG); glycerin; erythritol; arabitol; xylitol; sorbitol; glucose; lactose; ribose; and any combination thereof.

[0057] In a preferred embodiment, the said further cryoprotectant is trehalose at a concentration of 2% to 15% in said lyophilized formulation.

[0058] In a preferred embodiment, the further cryoprotectant is trehalose at a concentration of at least 5% in said lyophilized formulation.

[0059] In a preferred embodiment, the further cryoprotectant is trehalose at a concentration of at least 10% in said lyophilized formulation.

[0060] In a preferred embodiment, the composition is a pharmaceutical composition.

[0061] In a preferred embodiment, the at least one strain of microorganism is diluted with an inert powdered diluent.

[0062] In a preferred embodiment, the composition comprises one or more pharmaceutically acceptable carriers or excipients.

[0063] In a preferred embodiment, the said composition is formulated as a geltab, pill, enema, microcapsule, capsule, or tablet.

[0064] In a preferred embodiment, the capsule or tablet is enteric-coated, pH dependant, slow-release, and/or gastro-resistant.

[0065] In a preferred embodiment, the composition is adapted for administration orally or rectally.

[0066] In a preferred embodiment, the composition comprises one or more, two or more, three or more, four or more, or five or more isolated, purified, or cultured microorganisms.

[0067] In a preferred embodiment, the microorganism is a member of the phylum, family, genus, species taxa selected from those listed in the groups consisting of: Table 1, Table 2, Table 3, Table 4, Table 5, Table 13, Table 14, Table 15 and any combination thereof.

[0068] For example, the composition comprises one or more microorganisms selected from Table 3.

[0069] In another example, the composition comprises one or more microorganisms selected from the BB265 complex consortium as listed in Table 4.

[0070] For example, the composition comprises one or more microorganisms selected from the 143 isolate BB265 complex consortium listed in Table 5.

[0071] In a preferred embodiment, the composition is further supplemented with at least one microorganism from those listed in the groups consisting of: Table 1, Table 2, Table 3, Table 4, Table 5, Table 13, Table 14, Table 15 and any combination thereof.

[0072] In a preferred embodiment, the composition lacks one or more of the microorganisms selected from those listed in the groups consisting of: Table 1, Table 2, Table 3, Table 4, Table 5, Table 13, Table 14, Table 15 and any combination thereof.

[0073] Please note that in Tables 1 to 5, * indicates taxa identified in the analyses considered for inclusion in BB265 but not present in the BB265 complex consortium.

[0074] **Table 1 - List of taxa by phylum.**

Actinomycetota (formerly known as Actinobacteria)
Bacillota (formerly known as Firmicutes)
Bacteroidota (formerly known as Bacteroidetes)
*Campylobacterota
*Pseudomonadota (formerly known as Proteobacteria)
*Thermodesulfobacteriota
Verrucomicrobiota

[0075] **Table 2 - List of taxa by genus.**

*Absiella	Enorma	*Megasphaera
*Acidaminococcaceae (family)	Enterocloster	Merdibacter
*Acidaminococcus	*Enterococcus	*Mesosutterella
*Adlercreutzia	Erysipelatoclostridium	*Mitsuokella
Agathobaculum	*Escherichia	*Mogibacterium
Akkermansia	*Ethanoligenens	*Muribaculum

Alistipes	*Eubacterium*	**Negativibacillus*
**Alterileibacterium*	**Evtepia*	**Novisyntrophococcus*
**Amedibacillus*	*Faecalibacillus*	*Odoribacter*
Amedibacterium	*Faecalibacterium*	**Olsenella*
**Aminipila*	**Faecalibaculum*	**Oscillibacter*
Anaerobutyricum	*Finegoldia*	**Oscillospiraceae (family)*
**Anaerococcus*	**Flavonifractor*	**Oscillospiraceae incertae sedis (unclassified rank, Oscillospiraceae family)*
**Anaerocolumna*	*Flintibacter*	*Parabacteroides*
Anaerofustis	**Fusicatenibacter*	*Paraclostridium*
Anaerostipes	*Gemmiger*	**Paraprevotella*
Anaerotignum	**Gordonibacter*	**Parolsenella*
Anaerotruncus	**Granulicatella*	**Peptacetobacter*
**Angelakisella*	**Herbinix*	**Peptoniphilus*
Bacillus	*Holdemanella*	**Peptostreptococcus*
Bacteroides	**Holdemania*	**Phascolarctobacterium*
Barnesiella	**Hoylesella*	*Phocaeicola*
Bifidobacterium	*Hungatella*	*Porphyromonas*
**Bilophila*	**Intestinibacter*	*Prevotella*
Blautia	**Intestinibaculum*	**Pseudobutyrivibrio*
Butyricimonas	*Intestinimonas*	**Pusillibacter*
**Butyrivibrio*	*Lachnoanaerobaculum*	*Pusillimonas*
**Campylobacter*	**Lachnoclostridium*	*Romboutsia*
**Caproicibacter*	*Lachnospira*	*Roseburia*
**Caproicibacterium*	**Lachnospiraceae incertae sedis (unclassified rank, Lachnospiraceae family)*	**Ruminiclostridium*
**Caproiciproducens*	*Lacrimispora*	*Ruminococcus*
**Casaltella*	*Lactococcus*	*Ruthenibacterium*
**Catenibacterium*	*Ligilactobacillus*	**Schaalia*
Christensenella	**Longibaculum*	**Segatella*
Clostridium	*Longicatena*	**Selenomonas*
Collinsella	**Luoshenia*	*Sellimonas*
Coprobacillus	**Mageeibacillus*	**Senegalimassilia*
Coprobacter	**Maliibacterium*	**Slackia*
Coprococcus	**Marvinbryantia*	**Sodaliphilus*
**Desulfovibrio*	*Massilimicrobiota*	*Solibaculum*
Dorea	**Massiliprevotella*	*Streptococcus*
**Duodenibacillus*	**Massilistercora*	**Subdoligranulum*
**Dysosmobacter*	**Mediterraneibacter*	*Thomasclavelia*
**Eggerthella*	*Megamonas*	**Tyzzerella*
		**Veillonella*
		Vescimonas

[0076] **Table 3 – Broad list of families (bacterial, archaeal, and viral).**

Acanthopleuribacteraceae	Cyclobacteriaceae	Lentisphaeraceae	Pseudoalteromonadaceae
Acaryochloridaceae	Cyclonatronaceae	Leptolyngbyaceae	Pseudobdellovibrionaceae
Acetobacteraceae	Cytophagaceae	Leptospiraceae	Pseudomonadaceae
Acetomicrobiaceae	Deferribacteraceae	Leptotrichiaceae	Pseudonocardiaceae
Acholeplasmataceae	Dehalococcoidaceae	Lichenihabitantaceae	Psychromonadaceae
Acidaminococcaceae	Deinococcaceae	Limnochordaceae	Puniceicoccaceae
Acidiferrobacteraceae	Demequinaceae	Lipothrixviridae	Pyrodictiaceae
Acidilobaceae	Demerecviridae	Lispiviridae	Quadriviridae
Acidilutibacteraceae	Dermabacteraceae	Listeriaceae	Reichenbachiellaceae
Acidimicrobiaceae	Dermacoccaceae	Litoricolaceae	Reoviridae
Acidithiobacillaceae	Dermatophilaceae	Litorivicinaceae	Retroviridae
Acidobacteriaceae	Dermocarpellaceae	Luteoviridae	Rhabdochlamydiaceae
Acidothermaceae	Desulfallaceae	Magnetococcaceae	Rhabdoviridae
Ackermannviridae	Desulfarculaceae	Malacoherpesviridae	Rhizobiaceae
Actinomycetaceae	Desulfatibacillaceae	Maliibacteriaceae	Rhodanobacteraceae
Actinopolymorphaceae	Desulfitobacteriaceae	Mangrovivirgaceae	Rhodobacteraceae
Actinopolysporaceae	Desulfobaccaceae	Maricaulaceae	Rhodocyclaceae
Adenoviridae	Desulfobacteraceae	Marinifilaceae	Rhodospirillaceae
Adomaviridae	Desulfobulbaceae	Marinilabiliaceae	Rhodothermaceae
Aerococcaceae	Desulfocapsaceae	Marinobacteraceae	Rickettsiaceae
Aeromonadaceae	Desulfococcaceae	Mariprofundaceae	Rikenellaceae
Akkermansiaceae	Desulfohalobiaceae	Marivirgaceae	Rivulariaceae
Alcaligenaceae	Desulfolunaceae	Marnaviridae	Roniviridae
Alcanivoracaceae	Desulfomicrobiaceae	Marseilleviridae	Roseiflexaceae
Alicyclobacillaceae	Desulfomonilaceae	Matonaviridae	Roseobacteraceae
Aliusviridae	Desulfosarcinaceae	Mayoviridae	Rountreeviridae
Alloherpesviridae	Desulfosudaceae	Medioniviridae	Ruaniaceae
Alphaflexiviridae	Desulfotomaculaceae	Megabirnaviridae	Rubrobacteraceae
Alphasatellitidae	Desulfovibrionaceae	Melioribacteraceae	Rudiviridae
Alphatetraviridae	Desulfurellaceae	Merismopediaceae	Saccharospirillaceae
Alteromonadaceae	Desulfurobacteriaceae	Mesoaciditogaceae	Salasmaviridae
Amalgaviridae	Desulfurococcaceae	Mesoniviridae	Salinibacteraceae
Aminithiophilaceae	Desulfuromonadaceae	Metamycoplasmataceae	Salinisphaeraceae
Aminobacteriaceae	Dethiosulfovibrionaceae	Methanobacteriaceae	Salinivirgaceae
Amoebophilaceae	Devosiaceae	Methanocaldococcaceae	Sandaracinaceae
Amorphaceae	Dicistroviridae	Methanocellaceae	Sanguibacteraceae
Ampullaviridae	Dictyoglomaceae	Methanococcaceae	Saprospiraceae
Anaerohalosphaeraceae	Dietziaceae	Methanocorpusculaceae	Schitoviridae
Anaerolineaceae	Dissulfurispiraceae	Methanomassiliicoccaceae	Schleiferiaceae
Anaeromyxobacteraceae	Drexlerviridae	Methanomicrobiaceae	Scytonemataceae
Anaplasmataceae	Dysgonomonadaceae	Methanopyraceae	Secoviridae
Anelloviridae	Ectothiorhodospiraceae	Methanoregulaceae	Sedimentisphaeraceae
Aphanizomenonaceae	Eggerthellaceae	Methanosarcinaceae	Segniliparaceae
Aphanothecaceae	Egibacteraceae	Methanospirillaceae	Selenomonadaceae
Aquificaceae	Egicoccaceae	Methanothermaceae	Shewanellaceae
Archaeoglobaceae	Elioraeaceae	Methanotrichaceae	Silvanigrellaceae
Archangiaceae	Elusimicrobiaceae	Methylacidiphilaceae	Simkaniaceae
Arcobacteraceae	Emcibacteraceae	Methylobacteriaceae	Sinhaliviridae
Ardenticatenaceae	Endomicrobiaceae	Methylococcaceae	Sinobacteraceae
Arenaviridae	Endornaviridae	Methylocystaceae	Siphoviridae
Arteriviridae	Endozoicomonadaceae	Methylophilaceae	Sirenicapillariaceae
Artoviridae	Enterobacteriaceae	Microbacteriaceae	Smacoviridae
Ascoviridae	Enterococcaceae	Microbulbiferaceae	Sneathiellaceae
Asfarviridae	Entomoplasmataceae	Micrococcaceae	Solemoviridae
Aspiviridae	Erwiniaceae	Microcoleaceae	Solinviviridae
Astroviridae	Erysipelotrichaceae	Microcystaceae	Sphaerobacteraceae
Atopobiaceae	Erythrobacteraceae	Micromonosporaceae	Sphaerochaetaceae
Atribacteraceae	Eubacteriaceae	Microvenatoraceae	Sphaerolipoviridae
Aurantimonadaceae	Eubacteriales Family XIII. Incertae Sedis	Microviridae	Sphaerotilaceae
Autographiviridae	Euroniviridae	Miltoncostaeaceae	Sphingobacteriaceae
Azonexaceae	Euzebyaceae	Mimiviridae	Sphingomonadaceae

Azospirillaceae	Fastidiosibacteraceae	Minwuiaceae	Sphingosinicellaceae
Bacillaceae	Ferrimonadaceae	Mitoviridae	Spiraviridae
Bacilladnaviridae	Ferroplasmaceae	Mononiviridae	Spirochaetaceae
Bacillales Family X. Incertae Sedis	Ferrovaceae	Moorellaceae	Spiroplasmataceae
Bacteriovoracaceae	Fervidicoccaceae	Moraxellaceae	Spirosomaceae
Bacteroidaceae	Fervidobacteriaceae	Morganellaceae	Spongiibacteraceae
Baculoviridae	Fibrobacteraceae	Moritellaceae	Sporichthyaceae
Baekduiaceae	Filoviridae	Mucispirillaceae	Sporolactobacillaceae
Barnesiellaceae	Fimbriimonadaceae	Muribaculaceae	Sporomusaceae
Bartonellaceae	Fimoviridae	Mycobacteriaceae	Staphylococcaceae
Bdellovibrionaceae	Finnlakeviridae	Mycoplasmataceae	Stappiaceae
Beijerinckiaceae	Flammeovirgaceae	Mycoplasmoidaceae	Stellaceae
Benyviridae	Flaviviridae	Mymonaviridae	Steroidobacteraceae
Bernardetiaceae	Flavobacteriaceae	Myoviridae	Sterolibacteriaceae
Betaflexiviridae	Flexistipitaceae	Myriaviridae	Streptococcaceae
Beutenbergiaceae	Fluviibacteraceae	Myxococcaceae	Streptomycetaceae
Bicaudaviridae	Fontisphaeraceae	Nairoviridae	Streptosporangiaceae
Bidnaviridae	Francisellaceae	Nakamurellaceae	Succinivibrionaceae
Bifidobacteriaceae	Frankiaceae	Nanghoshaviridae	Sulfolobaceae
Birnaviridae	Fulvivirgaceae	Nanhypoviridae	Sulfuricellaceae
Blastochloridaceae	Fusariviridae	Nannocystaceae	Sulfurimonadaceae
Blattabacteriaceae	Fuselloviridae	Nanoviridae	Sulfurospirillaceae
Bogoriellaceae	Fusobacteriaceae	Narnaviridae	Sulfurovaceae
Bornaviridae	Gallionellaceae	Natranaerobiaceae	Sunviridae
Borreliaceae	Geminicoccaceae	Natranaerofabaceae	Sutterellaceae
Boseaceae	Geminiviridae	Natrialbaceae	Symbiobacteriaceae
Brachyspiraceae	Geminocystaceae	Nautiliaceae	Synechococcaceae
Bradymonadaceae	Gemmataceae	Neisseriaceae	Synergistaceae
Bradyrhizobiaceae	Gemmatimonadaceae	Nimaviridae	Syntrophaceae
Breoghaniaceae	Genomoviridae	Nitratiruptoraceae	Syntrophobacteraceae
Brevibacteriaceae	Geoalkalibacteraceae	Nitrobacteraceae	Syntrophomonadaceae
Breznakiellaceae	Geobacteraceae	Nitrosomonadaceae	Syntrophotaleaceae
Bromoviridae	Geodermatophilaceae	Nitrosopumilaceae	Tannerellaceae
Brucellaceae	Geovibrionaceae	Nitrososphaeraceae	Tectiviridae
Bruguierivoracaceae	Globuloviridae	Nitrospinaceae	Tenuifilaceae
Bryobacteraceae	Gloeobacteraceae	Nitrospiraceae	Tepidanaerobacteraceae
Budviciaceae	Gloeomargaritaceae	Nocardiaceae	Tepidiformaceae
Burkholderiaceae	Glycomycetaceae	Nocardioidaceae	Tepidimicrobiaceae
Caedimonadaceae	Gomontiellaceae	Nocardiopsaceae	Tepidisphaeraceae
Caldilineaceae	Gordoniaceae	Nodaviridae	Terasakiellaceae
Caldisericaceae	Gottschalkiaceae	Nostocaceae	Thalassobaculaceae
Caldisphaeraceae	Granulosicoccaceae	Nudiviridae	Thalassospiraceae
Calditerrivibrionaceae	Gresnaviridae	Nyamiviridae	Thermaceae
Calditrichaceae	Guelinviridae	Oceanospirillaceae	Thermincolaceae
Caliciviridae	Hafniaceae	Oculatellaceae	Thermoactinomycetaceae
Calotrichaceae	Hahellaceae	Odoribacteraceae	Thermoanaerobacteraceae
Campylobacteraceae	Halanaerobiaceae	Oleiphilaceae	Thermoanaerobacterales Family III. Incertae Sedis
Candidatus Absconditicoccaceae	Halieaceae	Opitutaceae	Thermoanaerobacterales Family IV. Incertae Sedis
Candidatus Babeliaceae	Haliscomenobacteraceae	Orbaceae	Thermococcaceae
Candidatus Brocadiaceae	Haloarculaceae	Ornithinimicrobiaceae	Thermodesulfatatoraceae
Candidatus Chazhemtobacteraceae	Halobacteriaceae	Orthomyxoviridae	Thermodesulfobacteriacea e
Candidatus Chromulinivoraceae	Halobacteriovoraceae	Oscillatoriaceae	Thermodesulfobiaceae
Candidatus Cloacimonadaceae	Halobacteroidaceae	Oscillospiraceae	Thermodesulfovibrionacea e
Candidatus Comchoanobacteraceae	Haloferacaceae	Ovaliviridae	Thermofilaceae
Candidatus Deianiraeaceae	Halomonadaceae	Oxalobacteraceae	Thermoguttaceae

Candidatus Desulfofervidaceae	Halorubraceae	Paenibacillaceae	Thermohalobacteraceae
Candidatus Izemoplasmataceae	Halothermotrichaceae	Paludibacteraceae	Thermoleophilaceae
Candidatus Methanoliparaceae	Halothiobacillaceae	Papillomaviridae	Thermomicrobiaceae
Candidatus Methanomethylophilaceae	Halspiviridae	Parachlamydiaceae	Thermomonosporaceae
Candidatus Micrarchaeaceae	Hantaviridae	Paracoccaceae	Thermoplasmataceae
Candidatus Midichloriaceae	Hapalosiphonaceae	Paraconexibacteraceae	Thermoplasmatales
Candidatus Nanohalobiaceae	Helicobacteraceae	Paramyxoviridae	Thermoproteaceae
Candidatus Nanopelagicaceae	Heliobacteriaceae	Partitiviridae	Thermosediminibacteraceae
Candidatus Nanosynbacteraceae	Hepadnaviridae	Parvibaculaceae	Thermospiraceae
Candidatus Nitrosocaldaceae	Hepeviridae	Parvicellaceae	Thermostichaceae
Candidatus Paracaedibacteraceae	Herelleviridae	Parvoviridae	Thermosynechococcaceae
Candidatus Saccharimonadaceae	Herpesviridae	Parvularculaceae	Thermotogaceae
Candidatus Uabimicrobiaceae	Hippeaceae	Pasteurellaceae	Thermotomaculaceae
Capillimicrobiaceae	Holosporaceae	Paulinoviridae	Thermovirgaceae
Cardiobacteriaceae	Hominidae	Pectobacteriaceae	Thioalkalibacteraceae
Carnobacteriaceae	Hoyosellaceae	Pelagibacteraceae	Thioalkalispiraceae
Casimicrobiaceae	Hydrogenimonaceae	Peptococcaceae	Thiobacillaceae
Catenulisporaceae	Hydrogenimonadaceae	Peptoniphilaceae	Thiotrichaceae
Caulimoviridae	Hydrogenophilaceae	Peptostreptococcaceae	Thiovulaceae
Caulobacteraceae	Hydrogenothermaceae	Peribunyaviridae	Tichowtungiaceae
Celerinatantimonadaceae	Hyellaceae	Persicobacteraceae	Tissierellaceae
Cellulomonadaceae	Hymenobacteraceae	Petrotogaceae	Tobaniviridae
Cellulosilyticaceae	Hyphomicrobiaceae	Phasmaviridae	Togaviridae
Cellvibrionaceae	Hyphomonadaceae	Phenuiviridae	Tolecusatellitidae
Chamaesiphonaceae	Hypoviridae	Phototrophicaceae	Tolypothrichaceae
Chaseviridae	Hytrosaviridae	Phreatobacteraceae	Tombusviridae
Chelatococcaceae	Iamiaceae	Phycisphaeraceae	Tospoviridae
Chitinophagaceae	Ichthyobacteriaceae	Phycodnaviridae	Totiviridae
Chlamydiaceae	Idiomarinaceae	Phyllobacteriaceae	Treponemataceae
Chlorobiaceae	Iflaviridae	Picobirnaviridae	Trichocoleusaceae
Chloroflexaceae	Ignatzschineriaceae	Picornaviridae	Tristromaviridae
Chloroherpetonaceae	Ignavibacteriaceae	Picrophilaceae	Tropherymataceae
Christensenellaceae	Ilumatobacteraceae	Pirellulaceae	Trueperaceae
Chromatiaceae	Immundisolibacteraceae	Piscirickettsiaceae	Tsukamurellaceae
Chromobacteriaceae	Inoviridae	Pithoviridae	Turicibacteraceae
Chroococcaceae	Intrasporangiaceae	Planctomycetaceae	Turriviridae
Chroococcidiopsidaceae	Iridoviridae	Planococcaceae	Tymoviridae
Chrysiogenaceae	Isosphaeraceae	Plasmaviridae	Usitatibacteraceae
Chrysoviridae	Jatrophihabitantaceae	Plectroviridae	Vallitaleaceae
Chthonomonadaceae	Jiangellaceae	Pleolipoviridae	Veillonellaceae
Chuviridae	Jonesiaceae	Pleomorphomonadaceae	Verrucomicrobiaceae
Circoviridae	Kaistiaceae	Pneumoviridae	Vibrionaceae
Closteroviridae	Kangiellaceae	Podoviridae	Vicinamibacteraceae
Clostridiaceae	Kiloniellaceae	Polyangiaceae	Virgaviridae
Clostridiales Family XVI. Incertae Sedis	Kineosporiaceae	Polycipiviridae	Vulgatibacteraceae
Clostridiales Family XVII. Incertae Sedis	Kiritimatiellaceae	Polydnaviridae	Waddliaceae
Cohaesibacteraceae	Kitaviridae	Polymycoviridae	Weeksellaceae

Coleofasciculaceae	Kofleriaceae	Polyomaviridae	Wenzhouxiangellaceae
Colwelliaceae	Koleobacteraceae	Porphyromonadaceae	Woeseiaceae
Comamonadaceae	Kordiimonadaceae	Portogloboviridae	Wupedeviridae
Conexibacteraceae	Kosmotogaceae	Pospiviroidae	Xanthobacteraceae
Conexivisphaeraceae	Kribbellaceae	Potyviridae	Xanthomonadaceae
Coprobacillaceae	Ktedonosporobacteraceae	Poxviridae	Xinmoviridae
Coprothermobacteraceae	Kytococcaceae	Prevotellaceae	Yersiniaceae
Coriobacteriaceae	Labilitrichaceae	Prochlorococcaceae	Yueviridae
Coronaviridae	Lachnospiraceae	Prochlorotrichaceae	Zhaonellaceae
Corynebacteriaceae	Lacipirellulaceae	Prolixibacteraceae	Zobellviridae
Coxiellaceae	Lactobacillaceae	Promicromonosporaceae	Zoogloeaceae
Cremegaviridae	Lavidaviridae	Propionibacteriaceae	Zooshikellaceae
Crocinitomicaceae	Lawsonellaceae	Proteinivoraceae	Zymomonadaceae
Cuniculiplasmataceae	Legionellaceae	Pseudanabaenaceae	

[0077] **Table 4 - List of taxa by Species / 16S identification including 16S sequences for each species in the BB265 complex consortium or identified as sulphidogens.**

**[Clostridium] hylemonae*	**Blautia liquoris*	**Lachnoanaerobaculum gingivalis*
**[Clostridium] innocuum*	**Blautia luti*	*Lachnoanaerobaculum umeaense*
**[Clostridium] leptum*	*Blautia massiliensis*	**Lachnoclostridium phocaeense*
**[Clostridium] methylpentosum*	**Blautia obeum*	**Lachnoclostridium phytofermentans*
**[Clostridium] scindens*	**Blautia producta*	**Lachnoclostridium sp. YL32*
**[Clostridium] spiroforme*	**Blautia provencensis*	*Lachnospira eligens*
**[Eubacterium] eligens*	**Blautia pseudococcoides*	*Lacrimispora saccharolytica*
**[Eubacterium] rectale*	**Blautia sp. SC05B48*	**Lactobacillus ruminis*
**[Eubacterium] siraeum*	*Blautia stercoris*	**Lactococcus garvieae*
**[Ruminococcus] faecis*	*Blautia wexlerae*	*Lactococcus lactis*
**[Ruminococcus] gnavus*	**Bryantella formatexigens*	*Lactococcus petauri*
**[Ruminococcus] torques*	*Butyricimonas faecalis*	*Ligilactobacillus ruminis*
**Absiella argi*	**Butyricimonas phoceensis*	**Longibaculum sp. KGMB06250*
**Absiella dolichum*	*Butyricimonas virosa*	*Longicatena caecimuris*
**Absiella tortuosum*	**Butyrivibrio crossotus*	**Luoshenia tenuis*
**Acidaminococcaceae sp.*	**Butyrivibrio hungatei*	**Mageeibacillus indolicus*
**Acidaminococcus fermentans*	**Butyrivibrio proteoclasticus*	*Massilimicrobiota merdigallinarum*
**Acidaminococcus intestini*	**Campylobacter coli*	*Massilimicrobiota timonensis*
**Adlercreutzia equolifaciens*	**Campylobacter jejuni*	**Massiliprevotella massiliensis*
**Adlercreutzia hattorii*	**Caproicibacter fermentans*	**Massilistercora timonensis*
**Agathobaculum butyriciproducens*	**Caproicibacterium amylolyticum*	*Megamonas funiformis*
**Agathobaculum sp.*	**Caproiciproducens sp. NJN-50*	**Megamonas rupellensis*
Agathobaculum sp003481705	**Casaltella massiliensis*	**Megasphaera elsdenii*
Akkermansia muciniphila	**Catenibacterium mitsuokai*	**Megasphaera stantonii*
**Alistipes communis*	*Christensenella minuta*	**Merdibacter massiliensis*
Alistipes dispar	*Christensenella sp. Marseille-P3954*	*Merdibacter merdipullorum*
Alistipes finegoldii	**Clostridium asparagiforme*	**Mesosutterella multiformis*

Alistipes ihumii	**Clostridium bartlettii*	**Mitsuokella multacida*
Alistipes indistinctus	**Clostridium beijerinckii*	**Mogibacterium diversum*
**Alistipes megaguti*	**Clostridium bolteae*	**Muribaculum intestinale*
Alistipes onderdonkii	*Clostridium bornimense*	**Negativibacillus massiliensis*
Alistipes putredinis	**Clostridium botulinum*	**Novisyntrophococcus fermenticellae*
Alistipes senegalensis	*Clostridium butyricum*	*Odoribacter splanchnicus*
Alistipes shahii	**Clostridium cadaveris*	**Olsenella sp. GAM18*
**Alistipes sp. dk3624*	**Clostridium hathewayi*	**Olsenella uli*
**Alterileibacterium massiliense*	*Clostridium hylemonae*	**Oscillibacter sp. PEA192*
**Alterileibacterium sp.*	**Clostridium innocuum*	**Oscillibacter valericigenes*
Amedibacterium intestinale	*Clostridium leptum*	*Oscillospiraceae bacterium sp.*
**Aminipila luticellarii*	*Clostridium methylpentosum*	**Oscillospiraceae sp.*
Anaerobutyricum hallii	**Clostridium nexile*	*Parabacteroides distasonis*
**Anaerococcus vaginalis*	*Clostridium paraputrificum*	*Parabacteroides goldsteinii*
**Anaerocolumna chitinilytica*	**Clostridium pasteurianum*	*Parabacteroides johnsonii*
**Anaerocolumna sedimenticola*	**Clostridium perfringens*	*Parabacteroides merdae*
**Anaerofustis sp.*	**Clostridium saccharobutylicum*	*Paraclostridium bifermentans*
Anaerofustis stercorihominis	**Clostridium saccharoperbutylacetonicum*	**Paraclostridium dentum*
Anaerostipes caccae	*Clostridium scindens*	**Paraprevotella xylaniphila*
Anaerostipes hadrus	**Clostridium sp. BNL1100*	**Parolsenella catena*
Anaerostipes hominis	**Clostridium sp. C1*	**Peptacetobacter hiranonis*
**Anaerostipes rhamnosivorans*	**Clostridium sp. SY8519*	**Peptoniphilus grossensis*
**Anaerotignum lactatifermentans*	**Clostridium spiroforme*	**Peptoniphilus obesi*
Anaerotignum propionicum	**Clostridium sporogenes*	**Peptostreptococcus sp.*
**Anaerotignum sp.*	**Clostridium tetani*	**Phascolarctobacterium faecium*
Anaerotruncus colihominis	*Collinsella aerofaciens*	**Phascolarctobacterium sp.*
Anaerotruncus rubiinfantis	*Collinsella bouchesdurhonensis*	**Phascolarctobacterium succinatutens*
**Angelakisella massiliensis*	**Collinsella stercoris*	*Phocaeicola coprocola*
Bacillus cereus	*Coprobacillus cateniformis*	*Phocaeicola coprophilus*
**Bacillus mobilis*	**Coprobacter fastidiosus*	*Phocaeicola dorei*
Bacteroides caccae	*Coprobacter secundus*	**Phocaeicola salanitronis*
**Bacteroides caecimuris*	*Coprococcus catus*	*Phocaeicola vulgatus*
Bacteroides cellulosilyticus	*Coprococcus comes*	*Porphyromonas asaccharolytica*
**Bacteroides coprocola*	*Coprococcus eutactus*	**Prevotella buccae*
**Bacteroides coprophilus*	**Desulfovibrio piger*	**Prevotella buccalis*
**Bacteroides dorei*	**Dorea formicigenerans*	*Prevotella copri*
Bacteroides eggerthii	*Dorea longicatena*	**Prevotella intermedia*
Bacteroides faecis	**Duodenibacillus sp.*	**Prevotella melaninogenica*
Bacteroides finegoldii	**Dysosmobacter welbionis*	**Pseudobutyrivibrio xylanivorans*
**Bacteroides fragilis*	**Eggerthella guodeyinii*	**Pusillibacter faecalis*
**Bacteroides helcogenes*	**Eggerthella lenta*	*Pusillimonas faecalis*
**Bacteroides heparinolyticus*	*Enorma massiliensis*	**Romboutsia sp. CE17*
**Bacteroides humanifaecis*	**Enorma shizhengliae*	*Romboutsia timonensis*

Bacteroides intestinalis	**Enorma sp.*	*Roseburia hominis*
**Bacteroides kribbi*	*Enterocloster aldenensis*	*Roseburia intestinalis*
**Bacteroides luhongzhouii*	**Enterococcus faecium*	*Roseburia inulinivorans*
Bacteroides nordii	*Erysipelatoclostridium ramosum*	**Ruminiclostridium cellulolyticum*
Bacteroides ovatus	**Escherichia coli*	**Ruminococcus albus*
**Bacteroides pectinophilus*	**Escherichia fergusonii*	*Ruminococcus bicirculans*
**Bacteroides plebeius*	**Ethanoligenens harbinense*	*Ruminococcus bromii*
**Bacteroides rodentium*	**Eubacterium (Lachnospira) eligens*	**Ruminococcus faecis*
Bacteroides salyersiae	*Eubacterium callanderi*	**Ruminococcus flavefaciens*
**Bacteroides sp. A1C1*	*Eubacterium limosum*	**Ruminococcus gauvreauii*
**Bacteroides sp. CACC 737*	**Eubacterium maltosivorans*	*Ruminococcus gnavus*
**Bacteroides sp. CBA7301*	*Eubacterium rectale*	**Ruminococcus lactaris*
**Bacteroides sp. HF-162*	*Eubacterium siraeum*	**Ruminococcus sp. JE7A12*
**Bacteroides sp. KGMB10229*	**Eubacterium sp. c-25*	*Ruminococcus torques*
Bacteroides stercoris	**Eubacterium sp. NSJ-61*	*Ruthenibacterium lactatiformans*
Bacteroides thetaiotaomicron	*Eubacterium ventriosum*	**Schaalia odontolytica*
Bacteroides uniformis	**Evtepia gabavorous*	**Selenomonas ruminantium*
**Bacteroides vulgatus*	*Faecalibacillus intestinalis*	*Sellimonas intestinalis*
Bacteroides xylanisolvens	**Faecalibacterium duncaniae*	**Senegalimassilia anaerobia*
**Bacteroides zhangwenhongi*	*Faecalibacterium prausnitzii*	**Slackia exigua*
**Bacteroides zoogleoformans*	**Faecalibaculum rodentium*	**Slackia heliotrinireducens*
Barnesiella intestinihominis	*Finegoldia magna*	**Slackia isoflavoniconvertens*
**Barnesiella viscericola*	**Flavonifractor plautii*	**Sodaliphilus pleomorphus*
Bifidobacterium adolescentis	*Flintibacter sp. KGMB00164*	*Solibaculum mannosilyticum*
Bifidobacterium bifidum	**Fusicatenibacter sp.*	**Solobacterium moorei*
**Bifidobacterium breve*	*Gemmiger formicilis*	*Streptococcus infantarius*
**Bifidobacterium catenulatum*	**Gordonibacter urolithinfaciens*	**Streptococcus lutetiensis*
**Bifidobacterium faecale*	**Granulicatella adiacens*	**Streptococcus pasteurianus*
Bifidobacterium longum	**Herbinix luporum*	*Streptococcus salivarius*
Bifidobacterium pseudocatenulatum	**Holdemanella biformis*	**Streptococcus sanguinis*
**Bilophila wadsworthia*	*Holdemanella porci*	**Streptococcus suis*
**Blautia argi*	**Holdemania filiformis*	**Streptococcus thermophilus*
**Blautia hansenii*	*Hungatella effluvii*	**Subdoligranulum variabile*
**Blautia hydrogenotrophica*	**Intestinibaculum porci*	*Thomasclavelia spiroformis*
**Blautia intestinalis*	*Intestinimonas butyriciproducens*	**Veillonella dispar*

[0078] **Table 5 – List of isolates comprising the 143 isolate BB265 complex consortium.**

Isolate ID	**Species**
bb0002	*Bifidobacterium longum*
bb0004	*Bacteroides uniformis*
bb0009	*Bacteroides faecis*

bb0012	*Collinsella aerofaciens*
bb0019	*Phocaeicola vulgatus*
*bb0028	*Coprococcus eutactus*
bb0040	*Collinsella aerofaciens*
bb0045	*Faecalibacillus intestinalis*
bb0058	*Bacteroides xylanisolvens*
bb0074	*Bacteroides caccae*
*bb0076	*Collinsella aerofaciens*
bb0085	*Bifidobacterium adolescentis*
bb0094	*Bacteroides xylanisolvens*
*bb0099	*Streptococcus salivarius*
bb0101	*Parabacteroides merdae*
bb0106	*Faecalibacterium prausnitzii*
bb0109	*Roseburia inulinivorans*
bb0117	*Bacteroides ovatus*
bb0126	*Phocaeicola dorei*
bb0129	*Bacteroides nordii*
bb0130	*Odoribacter splanchnicus*
bb0133	*Coprococcus catus*
bb0135	*Ruminococcus bromii*
bb0138	*Ruminococcus bicirculans*
bb0139	*Phocaeicola vulgatus*
bb0141	*Enterocloster aldenensis*
bb0142	*Roseburia hominis*
bb0147	*Parabacteroides merdae*
bb0149	*Coprococcus comes*
*bb0150	*Parabacteroides distasonis*
bb0153	*Blautia massiliensis*
bb0162	*Ruminococcus torques*
bb0163	*Bifidobacterium pseudocatenulatum*
bb0168	*Bacteroides thetaiotaomicron*
bb0171	*Blautia massiliensis*
bb0176	*Alistipes senegalensis*
bb0179	*Bacteroides uniformis*
bb0181	*Bacteroides finegoldii*
bb0182	*Ruthenibacterium lactatiformans*
bb0183	*Coprobacter secundus*
bb0185	*Bifidobacterium bifidum*
bb0186	*Bacteroides thetaiotaomicron*
bb0189	*Bacteroides xylanisolvens*
*bb0192	*Bacteroides eggerthii*
*bb0196	*Bacteroides cellulosilyticus*
*bb0197	*Alistipes finegoldii*
bb0198	*Eubacterium limosum*

bb0203	*Blautia stercoris*
bb0204	*Solibaculum mannosilyticum*
*bb0214	*Ruminococcus bicirculans*
bb0216	*Ruminococcus bromii*
bb0217	*Bacteroides stercoris*
*bb0229	*Alistipes onderdonkii*
bb0230	*Phocaeicola coprophilus*
bb0231	*Bacteroides intestinalis*
bb0233	*Prevotella copri*
bb0235	*Ruminococcus gnavus*
bb0244	*Clostridium butyricum*
bb0245	*Clostridium butyricum*
*bb0284	*Bacteroides uniformis*
*bb0287	*Alistipes shahii*
bb0288	*Hungatella effluvii*
bb0290	*Clostridium hylemonae*
bb0296	*Bacteroides salyersiae*
bb0312	*Lachnospira eligens*
bb0313	*Anaerostipes hadrus*
bb0317	*Vescimonas coprocola*
bb0318	*Gemmiger formicilis*
bb0322	*Bacteroides finegoldii*
bb0324	*Vescimonas coprocola*
bb0331	*Flintibacter sp. KGMB00164*
bb0334	*Butyricimonas virosa*
bb0341	*Alistipes dispar*
bb0345	*Alistipes putredinis*
*bb0347	*Alistipes indistinctus*
bb0350	*Pusillimonas faecalis*
*bb0352	*Blautia massiliensis*
bb0366	*Akkermansia muciniphila*
bb0375	*Butyricimonas faecalis*
bb0379	*Clostridium scindens*
*bb0384	*Parabacteroides goldsteinii*
bb0388	*Parabacteroides johnsonii*
bb0398	*Erysipelatoclostridium ramosum*
bb0401	*Roseburia intestinalis*
*bb0405	*Massilimicrobiota timonensis*
bb0406	*Massilimicrobiota merdigallinarum*
bb0408	*Ligilactobacillus ruminis*
bb0415	*Romboutsia timonensis*
bb0417	*Streptococcus infantarius*
*bb0418	*Streptococcus salivarius*
*bb0422	*Eubacterium ventriosum*

bb0424	*Eubacterium siraeum*
bb0425	*Sellimonas intestinalis*
*bb0427	*Thomasclavelia spiroformis*
bb0428	*Dorea longicatena*
bb0431	*Alistipes ihumii*
bb0433	*Lactococcus lactis*
bb0434	*Anaerotruncus colihominis*
bb0435	*Blautia wexlerae*
bb0436	*Holdemanella porci*
bb0447	*Intestinimonas butyriciproducens*
*bb0450	*Clostridium paraputrificum*
bb0452	*Paraclostridium bifermentans*
bb0457	*Merdibacter merdipullorum*
bb0464	*Agathobaculum sp003481705*
bb0468	*Ruthenibacterium lactatiformans*
bb0469	*Intestinimonas butyriciproducens*
bb0470	*Christensenella sp. Marseille-P3954*
bb0471	*Anaerotruncus rubiinfantis*
bb0472	*Oscillospiraceae bacterium*
bb0473	*Megamonas funiformis*
*bb0475	*Anaerofustis stercorihominis*
bb0476	*Bifidobacterium adolescentis*
*bb0479	*Coprobacillus cateniformis*
bb0480	*Anaerotignum propionicum*
bb0481	*Enorma massiliensis*
bb0483	*Eubacterium rectale*
bb0485	*Clostridium leptum*
bb0489	*Collinsella bouchesdurhonensis*
*bb0490	*Eubacterium callanderi*
bb0501	*Amedibacterium intestinale*
bb0502	*Anaerostipes caccae*
*bb0503	*Anaerobutyricum hallii*
bb0507	*Porphyromonas asaccharolytica*
bb0508	*Bifidobacterium adolescentis*
bb0509	*Bifidobacterium adolescentis*
bb0510	*Bifidobacterium pseudocatenulatum*
bb0512	*Bacillus cereus*
bb0513	*Lactococcus lactis*
bb0514	*Lactococcus petauri*
bb0517	*Finegoldia magna*
bb0518	*Megamonas funiformis*
bb0519	*Clostridium methylpentosum*
*bb0521	*Clostridium bornimense*
*bb0523	*Anaerostipes hominis*

11 Mar 2026
2026201833

*bb0525	*Erysipelatoclostridium ramosum*
*bb0526	*Erysipelatoclostridium ramosum*
bb0529	*Barnesiella intestinihominis*
*bb0531	*Longicatena caecimuris*
*bb0536	*Christensenella minuta*
bb0538	*Lacrimispora saccharolytica*
bb0539	*Phocaeicola coprocola*
bb0541	*Lachnoanaerobaculum umeaense*

[0079] In a preferred embodiment, the *Enterococcus sp.* is selected from the group consisting of: *Enterococcus faecalis*; *Enterococcus faecium*; *Enterococcus* sp. CC00149 deposited under V19/018754 on 9 September 2019 at the National Measurement Institute, Australia; *Enterococcus* sp. CC00259 deposited under V19/018755 on 9 September 2019 at the National Measurement Institute, Australia; *Enterococcus* sp. CC00620 deposited under V21/013048 on 29 June 2021 at the National Measurement Institute, Australia; *Enterococcus* sp. CC00064 deposited under V21/013046 on 29 June 2021 at the National Measurement Institute, Australia; *Enterococcus* sp. CC00619 deposited under V21/013047 on 29 June 2021 at the National Measurement Institute, Australia; *Enterococcus* sp. CC00262 deposited under V20/006238 on 18 March 2020 at the National Measurement Institute, Australia; and *Enterococcus* sp. CC0002 deposited under V21/014119 on 20 July 2021 at the National Measurement Institute, Australia.

[0080] In a preferred embodiment, the *Lactobacillus sp.* is selected from the group consisting of: *Lactobacillus rhamnosus* (such as strain GG (ATCC 53103), CGMCC 1.3724 or SP1 (DSM 21690)); *Lactococcus lactis*; *Lactococcus cremoris*; *Lactococcus diacetylactis*; *Lactobacillus paracasei*; *Lactobacillus reuteri* (such as strain ATCC 55730 or DSM 17938); *Lactobacillus acidophilus*; *Lactobacillus murinus; Lactobacillus helveticus*; *Lactobacillus bulgaricus; Lactobacillus casei*; *Lactobacillus salivarius*; *Lactobacillus plantarum*; *Lactobacillus fermentum; Lactobacillus taiwanensis; Lactobacillus animalis; Lactobacillus johnsonii* (such as strain NCC533; CNCM 1-1225); and *Lactobacillus gasseri.*

[0081] In a preferred embodiment, the *Bifidobacterium sp.* is selected from the group consisting of: *Bifidobacterium lactis* (such as strain BB-12, BI-04 or CNCM 1-3446 (Bb12)); *Bifidobacterium longum* (such as strain NCC3001, ATCC BAA-999 (BB536)); *Bifidobacterium breve* (such as strain Bb-03, M-16V or R0070); *Bifidobacterium infantis*; *Bifidobacterium animalis*; *Bifidobacterium bifidum;* and *Bifidobacterium adolescentis.*

11 Mar 2026
2026201833

[0082] In a preferred embodiment, the Streptococcus sp. is selected from the group consisting of: Streptococcus thermophilus, Streptococcus thermophilus ST-21 and Streptococcus salivarius.

[0083] In a preferred embodiment, the Clostridium sp. is selected from the group consisting of: Clostridium difficile, Clostridium hylemonae; Clostridium scindens and Flavinofractor plautii.

[0084] In a preferred embodiment, the microorganism is a yeast.

[0085] In a preferred embodiment, the yeast is *Saccharomyces boulardii.*

[0086] In a preferred embodiment, the microorganism is an archaea.

[0087] In a preferred embodiment, the archaea is selected from the group consisting of: *Methanobrevibacter spp; Methanobrevibacter smithii*; and *Methanosphaera sp;* M*ethanosphaera stadtmaniae.*

[0088] In a preferred embodiment, the composition comprises a faecal microbiota comprising a Shannon Diversity Index of greater than or equal to 0.3, greater than or equal to 0.4, greater than or equal to 0.5, greater than or equal to 0.6, greater than or equal to 0.7, greater than or equal to 0.8, greater than or equal to 0.9, 5 greater than or equal to 1.0, greater than or equal to 1.1, greater than or equal to 1.2, greater than or equal to 1.3, greater than or equal to 1.4, greater than or equal to 1.5, greater than or equal to 1.6, greater than or equal to 1.7, greater than or equal to 1.8, greater than or equal to 1.9, greater than or equal to 2.0, greater than or equal to 2.1, greater than or equal to 2.2, greater than or equal to 2.3, greater than or equal to 2.4, greater than or equal to 2.5, greater than or equal to 3.0, greater than or equal to 3.1, greater than or equal to 3.2, greater than or equal to 3.3, greater than or equal to 3.4, greater than or equal to 3.5, greater than or equal to 3.6, greater than or equal to 3.7, greater than or equal to 3.8, greater than or equal to 3.9, greater than or equal to 4.0, greater than or equal to 4.1, greater than or equal to 4.2, greater than or equal to 4.3, greater than or equal to 4.4, greater than or equal to 4.5, or greater than or equal to 5.0.

[0089] In a preferred embodiment, the composition comprises faecal microbiota comprising a Shannon Diversity Index of between 0.1 and 3.0, between 0.1 and 2.5, between 0.1 and 2.4, between 0.1 and 2.3, between 0.1 and 2.2, between 0.1 and 2.1, between 0.1 and 2.0, between 0.4 and 2.5, between 0.4 and 3.0, between 0.5 and 5.0, 20 between 0.7 and 5.0, between 0.9 and 5.0, between 1.1 and 5.0, between 1.3 and 5.0, between 1.5 and 5.0, between 1.7 and 5.0, between 1.9 and 5.0, between 2.1 and 5.0, between 2.3 and 5.0, between 2.5 and 5.0, between 2.7 and 5.0, between 2.9 and 5.0,

between 3.1 and 5.0, between 3.3 and 5.0, between 3.5 and 5.0, between 3.7 and 5.0, between 3.9 and 5.0, or between 4.1 and 5.0.

[0090] In a preferred embodiment, the Shannon Diversity Index is calculated at a level selected from the group consisting of: phylum level, family level, genus level, and species level.

[0091] In a preferred embodiment, the composition comprises a preparation of flora in proportional content that resembles a normal healthy human faecal flora.

[0092] In a preferred embodiment, the composition comprises faecal bacteria from at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 different families.

[0093] In a preferred embodiment, the composition comprises faecal microbiota comprising no greater than 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% weight non-living material/weight biological material.

[0094] In a preferred embodiment, the composition comprises faecal microbiota comprising no greater than 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% weight non-living material/weight biological material.

[0095] In a preferred embodiment, the composition comprises articles of non-living material and/or particles of biological material of a faecal sample that passes through a sieve, a column, or a similar filtering device having a sieve, exclusion, or particle filter size of 2.0 mm, 1.0 mm, 0.5 mm, 10 0.25 mm, 0.212 mm, 0.101 mm, 0.180 mm, 0.150 mm, 0.125 mm, 0.106 mm, 0.090 mm, 0.075 mm, 0.063 mm, 0.053 mm, 0.045 mm, 0.038 mm, 0.032 mm, 0.025 mm, 0.020 mm, 0.01 mm, or 0.2 mm.

[0096] In a preferred embodiment, the composition comprises substantially isolated or a purified faecal flora or entire (or substantially entire) microbiota that is (or comprises) an isolate of faecal flora that is at least about 90%, 91 %, 92 %, 93 %, 94%, 95%, 9 6%, 97%, 9 8%, 99%, 99.5%, 99.6 %, 99.7 %, 99.8% or 99.9% isolated or pure, or having no more than about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1.0% or more non-faecal floral material; or, a substantially isolated, purified, or substantially entire microbiota as described in WO 2012/122478, or as described in WO 2012/016287.

[0097] In a preferred embodiment, the composition comprises a weight ratio between faecal-derived non-living material and faecal-derived biological material of no greater than about 0.1%, 0. 2%, 0. 3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 5%, 8%, 10%, 15%, 20%, 30%, 40%, or 50%.

[0098] In a preferred embodiment, every 200 mg of the composition comprises a pharmacologically active dose of microorganism cells or spores selected from the group consisting of: 10^3 to 10^{14}; 10^4 to 10^{14}; 10^5 to 10^{14};10^6 to 10^{14}; 10^7 to 10^{14}; 10^8 to 10^{14}; 10^4 to 10^{13}; 10^5 to 10^{12}; 10^6 to 10^{11}; 10^7 to 10^{10}; 10^8 to 10^9; 10^3 to 10^{13}; 10^3 to 10^{12}; 10^3 to 10^{11}; 10^3 to 10^{10}; 10^3 to 10^9; 10^3 to 10^8; 10^3 to 10^7; 10^3 to 10^6; 10^3 to 10^5, and 10^3 to 10^4 colony forming units (cfu) or total cell count.

[0099] In a preferred embodiment, the composition comprises a pharmacologically active dose of microorganism cells or spores selected from the group consisting of: from 10 million cfu/mL to 100 billion cfu/mL, from 10 million to 50 million cfu/mL, more preferably from 50 million to 100 million cfu/mL, from 100 million to 500 million cfu/mL, from 500 million to 1 billion cfu/mL, from 1 billion to 5 billion cfu/mL, from 5 billion to 10 billion cfu/mL, from 10 billion to 15 billion cfu/mL, from 15 billion to 20 billion cfu/mL, from 20 billion to 25 billion cfu/mL, from 25 billion to 30 billion cfu/mL, from 30 billion to 35 billion cfu/mL, from 35 billion to 40 billion cfu/mL, from 40 billion to 45 billion cfu/mL, from 45 billion to 50 billion cfu/mL, from 50 billion to 55 billion cfu/mL, from 55 billion to 60 billion cfu/mL, from 60 billion to 65 billion cfu/mL, from 65 billion to 70 billion cfu/mL, from 70 billion to 75 billion cfu/mL, from 75 billion to 80 billion cfu/mL, from 80 billion to 85 billion cfu/mL, from 85 billion to 90 billion cfu/mL, from 90 billion to 95 billion cfu/mL, from 95 billion to 100 billion cfu/mL.

[00100] In a preferred embodiment, the composition comprises a pharmacologically active dose of microorganism cells or spores wherein the concentration of the microorganism cells or spores as a dry microbial body, is selected from the group consisting of: between 5 to 50 w/w %, 1 to 75 w/w %, 0.1 to 100 w/w % and 1 to 100 w/w %.

[00101] In a preferred embodiment, the disease or disorder is gastrointestinal tract mucosal inflammation.

[00102] In a preferred embodiment, the disease or disorder is characterized by reduced gut microbial diversity.

[00103] In a preferred embodiment, the disease or disorder is characterized by reduced gut microbial function.

[00104] In a preferred embodiment, the disease or disorder is characterized by loss of gut microbial ecology.

[00105] In a preferred embodiment, the disease or disorder is dysbiosis.

[00106] In a preferred embodiment, the dysbiosis is associated with one or more of disorders selected from the group consisting of: inflammatory bowel disease (IBD), pouchitis,

11 Mar 2026
2026201833

irritable bowel syndrome (IBS), an enteric bacterial infection, a metabolic disease, a neuropsychiatric disorder, an autoimmune disease, an allergic disorder, hepatic encephalopathy, or a cancer.

[00107] In a preferred embodiment, the disease or disorder is a malignancy or cancer.

[00108] In a preferred embodiment, the disease or disorder is a hepatic or liver disease.

[00109] In a preferred embodiment, the disease or disorder is a gastrointestinal disorder.

[00110] In a preferred embodiment, the gastrointestinal disorder is an inflammatory bowel disease.

[00111] In a preferred embodiment, the inflammatory bowel disease is selected from the group consisting of: ulcerative colitis; Crohn's disease; gastroenteritis; colitis; and pouchitis.

[00112] In a preferred embodiment, the gastrointestinal disorder is selected from the group consisting of: irritable bowel syndrome; an ulcer of the gastrointestinal tract; a cancer of the gastrointestinal tract.

[00113] In a preferred embodiment, the composition reduces endogenous sulphide levels in the colon of a patient in need thereof.

[00114] In a preferred embodiment, the composition reduces sulphide and nitric oxide load on epithelial cells leading to a metabolic lesion via inhibition of cellular respiration.

[00115] In a preferred embodiment, the composition: reduces relative abundance and or metabolic activity of sulphidogenic microbiota; reduce sulphide levels in the colon directly through consumption/assimilation; reduce sulphide levels in the colon via metabolic substrate competition; and or reduce sulphide levels in the colon by consuming hydrogen.

[00116] In a preferred embodiment, the composition reduces the relative abundance and or metabolic activity of sulphidogenic microbiota by reducing metabolizable sulphur substrates; reduce sulphur amino acid release (methionine, cysteine, homocysteine, taurine) into the colon by reducing protein fermentation.

[00117] In a preferred embodiment, the composition induces colonocyte apoptosis in lesions to break an induced stable inflammatory state driven.

[00118] In a preferred embodiment, the wherein the composition drives down undesired inflammation.

[00119] In a preferred embodiment, the composition prevents or reduces activation of the mucosa immune system in a natural killer T-cell driven IL-13 and IL-5 dependent, TH2 mediated, immune response.

[00120] In a preferred embodiment, the composition decreases inflammation in the subject when measured by a parameter selected from the group consisting of: TNFα signalling via NF-κB; IFNα signalling; IFNɣ signalling; IL6 JAK STAT3 signalling; activation of pro-apoptotic pathways; initiation of unfolded protein response.

[00121] In a preferred embodiment, the composition down regulates genes associated with pro-apoptotic pathways and the unfolded protein response, including genes selected from the group consisting of: CHAC1, CEBPB, TRIB3, PPP1R15A, DDIT3, ATF4 and XBP1.

[00122] In a preferred embodiment, the composition does not contain an excipient selected from the group consisting of: inulin HP-Gel (Orafti) (10% w/v); inulin (5% w/v) without maltodextrin; inulin (10% w/v) without maltodextrin; inulin (15% w/v) without maltodextrin; trehalose (2.5% w/v) and inulin (2.5% w/v); trehalose (5% w/v) and inulin (5% w/v); trehalose (7.5% w/v) and inulin (7.5% w/v); inulin (15% w/v) and tocopheral (10µL/L); inulin (15% w/v) and tocopheral (100µL/L); inulin (15% w/v) and ascorbic acid (4mg/L); and inulin (15% w/v) and ascorbic acid (40mg/L).

[00123] In a preferred embodiment, the micro-organism is not *Lactobacillus acidophilus* MJLA1.

[00124] In a preferred embodiment, the composition does not contain an excipient selected from the group consisting of: inulin HP-Gel (Orafti) (10% w/v); inulin (5% w/v) without maltodextrin; inulin (10% w/v) without maltodextrin; inulin (15% w/v) without maltodextrin; trehalose (2.5% w/v) and inulin (2.5% w/v); trehalose (5% w/v) and inulin (5% w/v); trehalose (7.5% w/v) and inulin (7.5% w/v); inulin (15% w/v) and tocopheral (10µL/L); inulin (15% w/v) and tocopheral (100µL/L); inulin (15% w/v) and ascorbic acid (4mg/L); and inulin (15% w/v) and ascorbic acid (40mg/L); and wherein the micro-organism is not *Lactobacillus acidophilus* MJLA1.

[00125] In a preferred embodiment, the composition contains an excipient selected from the group consisting of: inulin HP-Gel (Orafti) (10% w/v); inulin (5% w/v) without maltodextrin; inulin (10% w/v) without maltodextrin; inulin (15% w/v) without maltodextrin; trehalose (2.5% w/v) and inulin (2.5% w/v); trehalose (5%) and inulin (5% w/v); trehalose (7.5% w/v) and inulin (7.5%); inulin (15% w/v) and tocopheral (10µL/L); inulin (15% w/v) and tocopheral (100µL/L); inulin (15% w/v) and ascorbic acid (4mg/L); and inulin (15% w/v) and ascorbic acid (40mg/L).

2026201833 11 Mar 2026

[00126] In a preferred embodiment, the micro-organism is *Lactobacillus acidophilus* MJLA1.

[00127] In a preferred embodiment, the composition contains an excipient selected from the group consisting of: inulin HP-Gel (Orafti) (10% w/v); inulin (5% w/v) without maltodextrin; inulin (10% w/v) without maltodextrin; inulin (15% w/v) without maltodextrin; trehalose (2.5% w/v) and inulin (2.5% w/v); trehalose (5% w/v) and inulin (5% w/v); trehalose (7.5% w/v) and inulin (7.5% w/v); inulin (15% w/v) and tocopheral (10µL/L); inulin (15% w/v) and tocopheral (100µL/L); inulin (15% w/v) and ascorbic acid (4mg/L); and inulin (15% w/v) and ascorbic acid (40mg/L); and wherein the micro-organism is *Lactobacillus acidophilus* MJLA1.

[00128] In a preferred embodiment, the composition does not contain an excipient selected from the group consisting of: inulin (2% w/v); maltodextrin (2% w/v); sucrose (2% w/v).

[00129] In a preferred embodiment, the composition does not contain an excipient selected from the group consisting of: inulin (10% w/v); maltodextrin (10% w/v); and sucrose (10% w/v).

[00130] In a preferred embodiment, the micro-organism is not *Lactobacillus plantarum*.

[00131] In a preferred embodiment, the composition contains an excipient selected from the group consisting of: inulin (2% w/v); maltodextrin (2% w/v); sucrose (2% w/v).

[00132] In a preferred embodiment, the composition contains an excipient selected from the group consisting of: inulin (10% w/v); maltodextrin (10% w/v); and sucrose (10% w/v).

[00133] In a preferred embodiment, the micro-organism is *Lactobacillus plantarum*.

[00134] In a further aspect, the invention is a biotherapeutic composition comprising the composition of the first aspect of the invention, together with an acceptable diluent or carrier.

[00135] In a preferred embodiment, the carrier is 0.9% sterile saline.

[00136] In a further aspect, the invention is a pharmaceutical composition comprising the composition of the first aspect of the invention, together with a pharmaceutically acceptable diluent or carrier.

[00137] In a further aspect, the invention is a method of treating and/or preventing a disease or disorder in a patient in need thereof said method comprising administering to the subject an effective amount of the composition of the first aspect of the invention.

11 Mar 2026
2026201833

[00138] In a preferred embodiment, the disease or disorder is gastrointestinal tract mucosal inflammation.

[00139] In a preferred embodiment, the disease or disorder is characterized by reduced gut microbial diversity.

[00140] In a preferred embodiment, the disease or disorder is characterized by reduced gut microbial function.

[00141] In a preferred embodiment, the disease or disorder is characterized by loss of gut microbial ecology.

[00142] In a preferred embodiment, the disease or disorder is dysbiosis.

[00143] In a preferred embodiment, the dysbiosis is associated with one or more of disorders selected from the group consisting of: inflammatory bowel disease (IBD), pouchitis, irritable bowel syndrome (IBS), an enteric bacterial infection, a metabolic disease, a neuropsychiatric disorder, an autoimmune disease, an allergic disorder, hepatic encephalopathy, or a cancer.

[00144] In a preferred embodiment, the disease or disorder is a malignancy or cancer.

[00145] In a preferred embodiment, the disease or disorder is a hepatic or liver disease. For example, the disease or disorder is primary sclerosing cholangitis.

[00146] In a preferred embodiment, the disease or disorder is a gastrointestinal disorder.

[00147] In a preferred embodiment, the gastrointestinal disorder is an inflammatory bowel disease.

[00148] In a preferred embodiment, the inflammatory bowel disease is selected from the group consisting of: ulcerative colitis; Crohn's disease; gastroenteritis; colitis; and pouchitis.

[00149] In a preferred embodiment, the gastrointestinal disorder is selected from the group consisting of: irritable bowel syndrome; an ulcer of the gastrointestinal tract; a cancer of the gastrointestinal tract.

[00150] In a preferred embodiment, the composition is administered orally or rectally.

[00151] In a preferred embodiment, the composition is administered in conjunction with or to support an immunotherapy,

[00152] In a preferred embodiment, the composition is administered to the patient using a dosing regimen selected from the group consisting of: hourly; every 2 hours; every 3 hours; every 4 hours; every 5 hours; every 6 hours; every 12 hours; once daily; twice daily;

every 2 days; every 3 days; every 4 days; every 5 days; every 6 days; weekly; twice weekly; every 2 weeks; every 3 weeks; every 4 weeks; every 5 weeks; every 6 weeks; once monthly; twice monthly; every 2 months; every 3 months; every 4 months; every 5 months; every 6 months; yearly; twice yearly; every 2 years; every 3 years; every 4 years; and every 5 years.

[00153] In a preferred embodiment, the composition reduces endogenous sulphide levels in the colon of a patient in need thereof.

[00154] In a preferred embodiment, the composition reduces sulphide and nitric oxide load on epithelial cells leading to a metabolic lesion via inhibition of cellular respiration.

[00155] In a preferred embodiment, the composition reduces nitric oxide production and/or reduces nitric oxide levels in the colon.

[00156] In a preferred embodiment, the composition: reduces relative abundance and or metabolic activity of sulphidogenic microbiota; reduce sulphide levels in the colon directly through consumption/assimilation; reduce sulphide levels, relative abundance and or metabolic activity of sulphidogenic microbiota via metabolic substrate competition; and/or reduce sulphide levels, relative abundance and or metabolic activity of sulphidogenic microbiota by consuming hydrogen.

[00157] In a preferred embodiment, the composition reduces the relative abundance and/or metabolic activity of sulphidogenic microbiota by reducing metabolizable sulphur substrates in the colon; and/or reduces the relative abundance and/or metabolic activity of sulphidogenic microbiota by reducing sulphur amino acid release (methionine, cysteine, homocysteine, taurine) into the colon by reducing protein fermentation.

[00158] In a preferred embodiment, the composition induces colonocyte apoptosis in lesions to break an induced stable inflammatory state.

[00159] In a preferred embodiment, the method drives down undesired inflammation.

[00160] In a preferred embodiment, the composition prevents or reduces activation of the mucosa immune system in a natural killer T-cell driven IL-13 and IL-5 dependent, TH2 mediated, immune response.

[00161] In a preferred embodiment, the method decreases inflammation in the subject when measured by a parameter selected from the group consisting of: TNFα signalling via NF-κB; IFNα signalling; IFNɣ signalling; IL6 JAK STAT3 signalling; activation of pro-apoptotic pathways; initiation of unfolded protein response.

[00162] In a preferred embodiment, the method down regulates genes associated with pro-apoptotic pathways and the unfolded protein response, including genes selected from the group consisting of: CHAC1, CEBPB, TRIB3, PPP1R15A, DDIT3, ATF4 and XBP1.

[00163] In a further aspect, the invention is a method of preparing the biotherapeutic composition of the invention, the method comprising mixing the composition of the first aspect of the invention with an acceptable diluent or carrier.

[00164] In a further aspect, the invention is a method of preparing the pharmaceutical composition of the invention, the method comprising mixing the composition of the first aspect of the invention with a pharmaceutically acceptable excipient, diluent or carrier.

[00165] In a preferred embodiment, the excipient, diluent or carrier is sterilised.

[00166] In a preferred embodiment, the cryoprotectant is sterilised.

[00167] In a further aspect, the invention is the use of the composition of the first aspect of the invention in the manufacture of a medicament for reducing or preventing a disease or disorder in a subject.

[00168] In a further aspect, the invention is a dosage form comprising the composition of the first aspect of the invention.

[00169] A kit comprising the dosage form of the invention together with instructions for its use.

[00170] Further features of the present invention are more fully described in the following description of several non-limiting embodiments thereof. This description is included solely for the purposes of exemplifying the present invention. It should not be understood as a restriction on the broad summary, disclosure or description of the invention as set out above.

Brief Description of the Drawings

[00171] Below is a brief description of each of the figures and drawings.

[00172] Figure 1 shows the results of MicroPress analysis on triplicate samples of lyophilised FMT cake (analysis 1, 2 and 3) when using a 5% inulin and 5% maltodextrin cryoprotectant formulation. MicroPress analysis was used to quantitatively determine the strength and physical characteristics of the lyophilized cake *in situ*.

[00173] Figure 2 shows the mean fold loss in CFU/mL of intermediate product when plated anaerobically on non-selective media post lyophilization for FMT prepared with 8 different cryoprotectant formulations. Error bars represent 1 S.D.

[00174] Figure 3 shows the mean CFU/mL of intermediate product when plated anaerobically on non-selective media post lyophilization for FMT prepared with 8 different cryoprotectant formulations. Error bars represent 1 S.D.

[00175] Figure 4 shows a bar plot of intact cell counts (ICCs) per gram of stool for 8 batches of donor faecal material individually, when pooled, and when comparing neat stool to the pooled intermediate product (stool homogenized in the cryoprotectant formulation). ICCs were determined using a BactoBox (SBT Instruments).

[00176] Figure 5 shows a bar plot of intact cell counts (ICCs) per gram of stool for neat stool (8 individual batches pooled), intermediate product (pooled stool homogenized in the cryoprotectant formulation), lyophilized product (post milling), and encapsulated lyophilized product 'T0', as determined by BactoBox (SBT Instruments).

[00177] Figure 6 shows a bar plot comparing the change in the intact cell count (ICCs) per gram of stool of the encapsulated lyophilized product as a factor of storage time (1 week, 2 weeks, 4 weeks, 2 months, 6 months) and temperature (-80 °C, -20 °C, 4-8 °C, and 20-25 °C). ICCs were determined using a BactoBox (SBT Instruments)

Detailed Description of the Invention

[00178] For convenience, the following sections generally outline the various meanings of the terms used herein. Following this discussion, general aspects regarding compositions, use of medicaments and methods of the invention are discussed, followed by specific examples demonstrating the properties of various embodiments of the invention and how they can be employed.

[00179] Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. The invention includes all such variations and modifications. The invention also includes all of the steps, features, formulations and compounds referred to or indicated in the specification, individually or collectively and any and all combinations or any two or more of the steps or features.

[00180] Each document, reference, patent application or patent cited in this text is expressly incorporated herein in their entirety by reference, which means that it should be read and considered by the reader as part of this text. That the document, reference, patent application or patent cited in this text is not repeated in this text is merely for reasons of conciseness. None of the cited material or the information contained in that material should, however, be understood to be common general knowledge.

2026201833 11 Mar 2026

[00181] Manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and can be employed in the practice of the invention.

[00182] The present invention is not to be limited in scope by any of the specific embodiments described herein. These embodiments are intended for the purpose of exemplification only. Functionally equivalent products, formulations and methods are clearly within the scope of the invention as described herein.

1. DEFINITIONS

[00183] The meaning of certain terms and phrases used in the specification, examples, and appended claims, are provided below. If there is an apparent discrepancy between the usage of a term in the art and its definition provided herein, the definition provided within the specification shall prevail.

[00184] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about." The term "about" when used in connection with percentages can mean ±1%.

[00185] The invention described herein may include one or more range of values (e.g. size, concentration etc.). A range of values will be understood to include all values within the range, including the values defining the range, and values adjacent to the range which lead to the same or substantially the same outcome as the values immediately adjacent to that value which defines the boundary to the range. For example, a person skilled in the field will understand that a 10% variation in upper or lower limits of a range can be totally appropriate and is encompassed by the invention. More particularly, the variation in upper or lower limits of a range will be 5% or as is commonly recognised in the art, whichever is greater.

[00186] In this application, the use of the singular also includes the plural unless specifically stated otherwise. In this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including", as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise. Also, the use of the term "portion" can include part of a moiety or the entire moiety.

[00187] Throughout this specification, unless the context requires otherwise, the word "comprise" or variations such as "comprises" or "comprising", will be understood to imply the

2026201833 11 Mar 2026

inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

[00188] "Therapeutically effective amount" as used herein with respect to methods of treatment and in particular drug dosage, shall mean that dosage that provides the specific pharmacological response for which the drug is administered in a significant number of subjects in need of such treatment. It is emphasized that "therapeutically effective amount," administered to a particular subject in a particular instance will not always be effective in treating the diseases described herein, even though such dosage is deemed a "therapeutically effective amount" by those skilled in the art. It is to be further understood that drug dosages are, in particular instances, measured as oral dosages, or with reference to drug levels as measured in blood. Amounts effective for such a use will depend on: the desired therapeutic effect; the potency of the biologically active material; the desired duration of treatment; the stage and severity of the disease being treated; the weight and general state of health of the patient; and the judgment of the prescribing physician. Treatment dosages need to be titrated to optimize safety and efficacy. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the indication for which the active agent is being used, the route of administration, and the size (body weight, body surface or organ size) and condition (the age and general health) of the patient. Accordingly, the clinician may titre the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1 μg/kg to up to about 100 mg/kg or more, depending on the factors mentioned above. In other embodiments, the dosage may range from 0.1 μg/kg up to about 100 mg/kg; or 1 μg/kg up to about 100 mg/kg; or 5 μg/kg up to about 100 mg/kg.

[00189] The frequency of dosing will depend upon the pharmacokinetic parameters of the active agent and the formulation used. Typically, a clinician will administer the composition until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as two or more doses (which may or may not contain the same amount of the desired molecule) over time, or as a continuous infusion via an implantation device or catheter. Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. Appropriate dosages may be ascertained through use of appropriate dose-response data.

[00190] As used herein, a "carrier" can be any solvents, diluents, excipients or other vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or

emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired.

[00191] As used herein, the term "pharmaceutically acceptable carrier" component can refer to a component that is not biologically or otherwise undesirable, i.e., the component may be incorporated into a composition of the invention and administered to a subject as described herein without causing any significant undesirable biological effects or interacting in a deleterious manner with any of the other components of the formulation in which it is contained. The component has generally met the required standards of toxicological and manufacturing.

[00192] As used herein the term “subject” generally includes mammals such as: humans; farm animals such as sheep, goats, pigs, cows, horses, llamas; companion animals such as dogs and cats; primates; birds, such as chickens, geese and ducks; fish; and reptiles. The subject is preferably human.

[00193] As used herein, the “gastrointestinal tract” refers to the tract from the mouth to the anus which includes all the organs of the digestive system such as the esophagus, stomach, pancreas, liver, gallbladder, small intestine (including the ileum), caecum, large intestine, colon and rectum. Strains of the invention are at least useful for conditions of the terminal ileum, caecum or rectum.

[00194] As used herein, a “non-inflammatory strain” refers to a strain of the invention which, when present in the gastrointestinal tract of a subject, preferably a human, is associated with a non-inflamed state. Non-inflammatory strains of the invention have little or no cytotoxicity against mammalian epithelial cells in culture. In an embodiment, the strain results in less than 15%, less than 10% or less than 5% of cell death of the mammalian epithelial cells in culture.

[00195] As used herein, an “inflammatory strain” refers to a strain of the invention which, when present in the gastrointestinal tract of a subject, preferably a human, is associated with an inflamed state. Inflammatory strains of the invention have cytotoxicity against mammalian epithelial cells in culture, such as Caco2 cells. In an embodiment, the strain results at least 40%, at least 45% or at least 50% of cell death of the mammalian epithelial cells in culture..

[00196] As used herein, the term “bacteriotherapy” refers to the use of a bacterial isolate to treat or prevent a disease or a condition, or provide a health benefit, in a subject.

[00197] As used herein, the term “biotherapeutic” refers to a microorganism, such as bacterial isolate, that is useful for treating or preventing a disease or a condition, or provide a health benefit, in a subject.

[00198] The term "biotherapeutic composition" as used herein, refers to a formulation comprising a biotherapeutic preparation formulated together with one or more additional formulary ingredients to obtain a finished formulation suitable for delivery to a subject.

[00199] As used herein, the terms "treat," "treating," "treatment" and grammatical variations thereof mean subjecting an individual subject to a protocol, regimen, process or remedy, in which it is desired to obtain a physiologic response or outcome in that subject. Since every treated subject may not respond to a particular treatment protocol, regimen, process or remedy, treating does not require that the desired physiologic response or outcome be achieved in each and every subject or subject population. Accordingly, a given subject or subject population may fail to respond or respond inadequately to treatment.

[00200] As used herein, the term “prevent”, “prevented”, or “preventing” when used with respect to the treatment of mucosal inflammation in the gastrointestinal refers to a prophylactic treatment which increases the resistance of a subject to mucosal inflammation in the gastrointestinal, in other words, decreases the likelihood that the subject will develop mucosal inflammation in the gastrointestinal as well as a treatment after mucosal inflammation in the gastrointestinal has begun in order to fight the inflammation, e.g., reduce or eliminate it altogether or prevent it from becoming worse.

[00201] As used herein, the term “reducing”, or variations thereof refer to a reduction but not necessarily a complete abolition of gastrointestinal tract mucosal inflammation in a subject.

[00202] As used herein, the term "sample" refers to a collection of biological material obtained from a subject or a subject's surrounding environment, such as soil or water in the area that the subject inhabits. In some embodiments, the sample is obtained directly from the subject. For example, the sample can be a faecal sample or obtained during a colonoscopy. The sample may be in a form taken directly from the subject or surrounding environment, or it may be at least partially purified to remove at least some non-nucleic acid material. The purification may be slight, for instance amounting to no more than the concentration of the solids, or cells, of the sample into a smaller volume or the separation of cells from some or all of the remainder of the sample. In some embodiments, nucleic acids are isolated from the sample. Such isolated preparations include reverse transcription products and/or PCR amplification products of the nucleic acids in the sample. In some embodiments, the predominant nucleic acid is DNA. The nucleic acid preparations can be pure or partially purified nucleic acid preparations. Techniques for the isolation of nucleic acid from samples, including complex samples, are numerous and well known in the art.

2026201833 11 Mar 2026

[00203] The unit “cfu” refers to "colony forming unit", which is the number of viable microbial cells as revealed by microbiological counts on agar plates.

[00204] The unit “% w/v” refers to weight/volume percentage concentration. It is also known as mass/volume percentage concentration. Weight/volume percentage concentration is also abbreviated as w/v (%) or w/v% or (w/v)% or %(w/v) or %w/v. Mass/volume percentage concentration is also abbreviated as m/v (%) or m/v% or (m/v)% or %(m/v) or %m/v.

[00205] Other definitions for selected terms used herein may be found within the detailed description of the invention and apply throughout. Unless otherwise defined, all other scientific and technical terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which the invention belongs.

[00206] Features of the invention will now be discussed with reference to the following non-limiting description and examples.

2. EMBODIMENTS

Composition

[00207] The present invention provides a composition for preventing or treating a disorder in a subject in need thereof, said composition comprising at least one strain of bacteria or archaea or fungi.

[00208] In a further preferred embodiment, the composition is selected from the group consisting of: a therapeutic composition; a pharmaceutical composition; a cosmetic composition; and a veterinary composition.

[00209] Preferably the compositions are combined with a pharmaceutically acceptable carrier or diluent to produce a pharmaceutical composition (which may be for human or animal use). Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions. See, e.g., Remington's Pharmaceutical Sciences, 19th

Ed. (1995, Mack Publishing Co., Easton, Pa.) and Remington's The Science and Practice of Pharmacy, 23rd Edition. (2020, Mack Publishing Co., Easton, Pa.) which are herein incorporated by reference.

[00210] The composition can contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, colour, isotonicity, odour, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. Suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulphite or sodium hydrogen-sulphite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates or other organic acids); bulking agents (such as mannitol or glycine); chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin), fillers; monosaccharides, disaccharides; and other carbohydrates (such as glucose, mannose, or dextrins); proteins (such as serum albumin, gelatin or immunoglobulins); colouring, flavouring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapol); stability enhancing agents (sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides, preferably sodium or potassium chloride), delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants.

[00211] The optimal composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format, and desired dosage. Such compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the biotherapeutic actives of the invention. The preferred form of the pharmaceutical composition depends on the intended mode of administration and therapeutic application.

[00212] The primary vehicle or carrier in a composition is aqueous in nature. For example, a suitable vehicle or carrier may be water for injection, physiological saline solution, possibly supplemented with other materials. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Other exemplary pharmaceutical

compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute therefor. In one embodiment of the present invention, pharmaceutical compositions may be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents in the form an aqueous solution.

[00213] The formulation components are present in concentrations that are acceptable to the site of administration. For example, buffers are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 5 to about 8.

[00214] Additional compositions will be evident to those skilled in the art, including formulations of the invention in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. Additional examples of sustained-sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, for example, films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides, copolymers of L-glutamic acid and gamma ethyl-L-glutamate, ethylene vinyl acetate or poly-D(-)-3-hydroxybutyric acid. Sustained-release compositions may also include liposomes, which can be prepared by any of several methods known in the art.

[00215] The composition to be used for in vivo administration can be filtered to remove undesirable components. This may be accomplished by filtration through filtration membranes. In addition, the compositions generally are placed into a sealed container to reduce exposure to oxygen. Once the pharmaceutical composition has been formulated, it may be stored in sealed containers.

[00216] The term "% sequence homology ", as used here, may for example be calculated as follows. The query sequence is aligned to the target sequence using the CLUSTAL W algorithm (Thompson et al, Nucleic Acids Research, 22: 4673-4680 (1994)). A comparison is made over the window corresponding to one of the aligned sequences, for example the shortest. The window may in some instances be defined by the target sequence. In other instances, the window may be defined by the query sequence. The amino acid residues at each position are compared, and the percentage of positions in the query sequence that have identical correspondences in the target sequence is reported as % sequence homology.

[00217] In an embodiment, the % identity of a polynucleotide is determined by GAP (Needleman and Wunsch, 1970) analysis (GCG program) with a gap creation penalty=5, and a gap extension penalty=0.3. Preferably, the GAP analysis aligns two sequences over their entire length.

[00218] The bacterial strains for use in the present invention can be cultured using microbiology techniques as detailed in, for instance; Browne et al. Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation. *Nature* (2016) Volume 533, pages 543–546 (2016); Handbook of Microbiological Media, Fourth Edition (2010) Ronald Atlas, CRC Press; Maintaining Cultures for Biotechnology and Industry (1996) Jennie C. Hunter-Cevera, Academic Press. As well as detailed in the Examples using yeast extract, casitone and fatty acid (YCFA) medium.

[00219] In yet a further preferred embodiment, the composition further comprises water.

[00220] In yet a further preferred embodiment, the composition is a liquid, such as an aqueous solution.

[00221] In yet a further preferred embodiment, further comprises a pharmaceutically acceptable carrier.

[00222] In yet a further preferred embodiment, the composition retains its effective biological activity for a period selected from the group consisting of; greater than 24 hours; greater than 36 hours; and greater than 48 hours. Preferably, the composition is stable for periods selected from the group consisting of: 6 months, 1 year and 2 years. In one example, the composition is stable at temperatures selected from the group consisting of: -4°C, 4°C, 18°C and 25°C.

[00223] Pharmaceutical and therapeutic compositions are within the scope of the invention.

[00224] The therapeutic composition of the invention may comprise a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the isolated bacteria present in the therapeutic composition. The precise nature of the pharmaceutically acceptable excipient or other material will depend on the route of administration, which may be, for example, oral or rectal. Many methods for the preparation of therapeutic compositions are known to those skilled in the art (see e.g. Robinson ed., Sustained and Controlled Release Drug Delivery Systems, Marcel Dekker, Inc., New York, 1978).

2026201833 11 Mar 2026

[00225] The therapeutic composition of the invention may comprise a prebiotic, a carrier, insoluble fibre, a buffer, an osmotic agent, an anti-foaming agent and/or a preservative.

[00226] The therapeutic composition may be made or provided in chemostat medium. Alternatively, the therapeutic composition may be made or provided in saline, e.g., 0.9% saline. It will be understood that any carrier or solution which does not impair viability of the bacteria present in the therapeutic composition and is compatible with administration to an individual may be used.

[00227] The therapeutic composition may be made or provided under reduced atmosphere, i.e., in the absence of oxygen. A synthetic stool preparation may be made or provided under N2, CO2, H2, or a mixture thereof, optionally with controlled levels of partial pressure of N2:CO2:H2.

[00228] The therapeutic composition may be made or provided under an oxygen containing atmosphere.

[00229] The therapeutic composition may be for oral or rectal administration to the individual. Where the therapeutic composition is for oral administration, the therapeutic composition may be in the form of a capsule, or a tablet. Where the therapeutic composition is for rectal administration, the therapeutic composition may be in the form of an enema or suppository. The preparation of suitable capsules, tablets, suppository and enema is well-known in the art. The capsule or tablet may comprise a coating to protect the capsule or tablet from stomach acid. For example, the capsule or tablet may be enteric-coated, pH dependent, slow-release, and/or gastro-resistant. Such capsules and tablets are used, for example, to minimize dissolution of the capsule or tablet in the stomach but allow dissolution in the small intestine.

[00230] Orally dosed formulations, for example, can, in addition to the viable microorganisms comprise, inert compression aids, such as microcrystalline cellulose or oligosaccharide, flow aids, such as a silica gel, or a lubricant of, for example magnesium stearate (vegetable source) or stearic acid (vegetable source).

[00231] A composition disclosed herein can be used as, for example, a food supplement, an edible product or pharmaceutical product. When it is a food supplement, the composition can further comprise a conventional food supplement filler and/or an extender. The composition disclosed herein can also be included in any edible products, such as dairy products, including for example, a milk product, milk, yogurt, curd, ice-cream, dressing, and cheese, beverage products, meat products, and baked goods

[00232] Suppository formulations, for example, either for rectal use, can in addition to the compositions, comprise, for example, cocoa butter, polyethylene glycol, glycerine or gelatine.

[00233] The composition may comprise a disintegrant, a glidant, and/or a lubricant. Disintegrants aid in the breakup of the compacted mass when placed in a fluid environment. The disintegrant may be any suitable disintegrant such as for example, a disintegrant selected from the group consisting of sodium croscarmellose, crospovidone, gellan gum, hydroxypropyl cellulose, starch, and sodium starch glycolate. The glidant may be any suitable glidant such as for example, a glidant selected from the group consisting of silicon dioxide, colloidal silicon dioxide, and talc. Lubricants are generally always used in the manufacture of dosage forms by direct compression in order to prevent the compacted powder mass from sticking to the equipment during the tableting or encapsulation process. The lubricant may be any suitable lubricant such as for example, a lubricant selected from the group consisting of calcium stearate, magnesium stearate, stearic acid, sodium stearyl fumerate, and vegetable based fatty acids. In the composition and method of the present invention, the carrier, may be present in the composition in a range of approximately 30% w/w to approximately 98% w/w; this weight percentage is a cumulative weight percentage taking into consideration all ingredients present in the carrier.

[00234] Coatings can be used to control the solubility of the composition. Examples of coatings include carrageenan, cellulose acetate phthalate, ethylcelulose, gellan gum, matodextrin, methacrylates, methylcellulose, microcrystalline cellulose, and shellac.

[00235] The composition may comprise one or more preservatives. Exemplary preservatives include antioxidants, chelating agents, antifungal preservatives, alcohol preservatives, acidic preservatives, and other preservatives.

[00236] Exemplary antioxidants include alpha tocopherol, ascorbic acid, acorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabi sulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabi sulfite, and sodium sulfite.

[00237] Exemplary chelating agents include ethylenediaminetetraacetic acid (EDTA) and salts and hydrates thereof (e.g., sodium edetate, disodium edetate, trisodium edetate, calcium disodium edetate, dipotassium edetate, and the like), citric acid and salts and hydrates thereof (e.g., citric acid monohydrate), fumaric acid and salts and hydrates thereof, malic acid and salts and hydrates thereof, phosphoric acid and salts and hydrates thereof, and tartaric acid and salts and hydrates thereof. Exemplary antimicrobial preservatives

include benzalkonium chloride, benzethonium chloride, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride, chlorhexidine, chlorobutanol, chlorocresol, chloroxylenol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, and thimerosal.

[00238] Exemplary antifungal preservatives include butyl paraben, methyl paraben, ethyl paraben, propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and sorbic acid. Exemplary alcohol preservatives include ethanol, polyethylene glycol, phenol, phenolic compounds, bisphenol, chlorobutanol, hydroxybenzoate, and phenylethyl alcohol.

[00239] Exemplary acidic preservatives include vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, acetic acid, dehydroacetic acid, ascorbic acid, sorbic acid, and phytic acid.

[00240] Other preservatives include tocopherol, tocopherol acetate, deteroxime mesylate, cetrimide, butylated hydroxyanisol (BHA), butylated hydroxytoluened (BHT), ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), sodium bisulfite, sodium metabi sulfite, potassium sulfite, potassium metabi sulfite, Glydant Plus, Phenonip, methylparaben, Germall 115, Germaben II, Neolone, Kathon, and Euxyl.

[00241] The therapeutic composition may be lyophilized. The lyophilized therapeutic composition may comprise one or more stabilisers and/or cryoprotectants. The lyophilized therapeutic composition may be reconstituted using a suitable diluent prior to administration to the individual.

[00242] A therapeutic composition according to the present invention may be administered alone or in combination with other treatments, concurrently or sequentially or as a combined preparation with another therapeutic agent or agents, for the treatment of dysbiosis, or a disease associated with dysbiosis as described herein. For example, a strain of the invention may be used in combination with an existing therapeutic agent for inflammatory bowel disease, irritable bowel syndrome, a metabolic disease, a neuropsychiatric disorder, an autoimmune disease, an allergic disorder, a cancer, or hepatic encephalopathy.

[00243] For example, where the therapeutic composition is for the treatment of a dysbiosis associated with cancer, the therapeutic composition may optionally be administered in combination with a cancer immunotherapy, such as an immune check-point inhibitor, to the individual. Examples of check-point inhibitors which may be employed in this context include Programmed cell death protein 1 (PD-1) inhibitors, Programmed death-

ligand 1 (PD-L1) inhibitors, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors. Manipulation of the gut microbiota in combination with immune check-point inhibitor treatment has been shown to improve efficacy of immune check-point inhibitors in treating cancer. In a preferred embodiment, the cancer in this context is lung cancer or melanoma.

[00244] In another embodiment, the composition of the invention further comprise immunomodulating compounds. In other embodiments, the immunomodulating compound is a cytokine, chemokine, or complement component that enhances expression of immune system accessory or adhesion molecules, their receptors, or combinations thereof. In some embodiments, the immunomodulating compound include interleukins, for example interleukins 1 to 15, interferons alpha, beta or gamma, tumour necrosis factor, granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), chemokines such as neutrophil activating protein (NAP), macrophage chemoattractant and activating factor (MCAF), RANTES, macrophage inflammatory peptides MIP-1a and MIP-1b, complement components, or combinations thereof. In other embodiments, the immunomodulating compound stimulate expression, or enhanced expression of OX40, OX40L (gp34), lymphotactin, CD40, CD40L, B7.1, B7.2, TRAP, ICAM-1, 2 or 3, cytokine receptors, or combination thereof.

[00245] In another embodiment, the immunomodulatory compound induces or enhances expression of co-stimulatory molecules that participate in the immune response, which include, in some embodiments, CD40 or its ligand, CD28, CTLA-4 or a B7 molecule. In another embodiment, the immunomodulatory compound induces or enhances expression of a heat stable antigen (HSA), chondroitin sulfate-modified MHC invariant chain (Ii-CS), or an intracellular adhesion molecule 1 (ICAM-1).

[00246] The therapeutic compositions of the invention may be administered to an individual, preferably a human individual. Administration may be in a "therapeutically effective amount", this being sufficient to show benefit to the individual. Such benefit may be at least amelioration of at least one symptom. Thus "treatment" of a specified disease refers to amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated, the particular patient being treated, the clinical condition of the individual patient, the cause of the dysbiosis, the site of delivery of the composition, the type of therapeutic composition, the method of administration, the scheduling of administration and other factors known to medical practitioners. Prescription of treatment, e.g. decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors and may depend on the

severity of the symptoms and/or progression of a disease being treated. A therapeutically effective amount or suitable dose of a therapeutic composition of the invention can be determined by comparing its *in vitro* activity and *in vivo* activity in an animal model or phase 0 human study. Methods for extrapolation of effective dosages in mice and other test animals to humans are known. The precise dose will depend upon a number of factors, including whether the therapeutic composition is for prevention or for treatment.

[00247] Formulary ingredients can be contacted with the preparation and mixed or prepared until a formulation is obtained. As will be clear to those of skill in the art, formulation conditions will generally be such that viable microorganisms are retained. In particular high temperatures, for example temperatures in excess of 40°C are avoided.

[00248] The amount of viable microorganisms included in a composition can vary and can be adjusted and optimized as will be appreciated by those of skill in the art. Such optimization may, for example, be achieved by preparing a series of different doses of a viable microorganism. The bacterial concentration in the composition can be, for example, from 10 million cfu/mL to 100 billion cfu/mL, from 10 million to 50 million cfu/mL, more preferably from 50 million to 100 million cfu/mL, from 100 million to 500 million cfu/mL, from 500 million to 1 billion cfu/mL, from 1 billion to 5 billion cfu/mL, from 5 billion to 10 billion cfu/mL, from 10 billion to 15 billion cfu/mL, from 15 billion to 20 billion cfu/mL, from 20 billion to 25 billion cfu/mL, from 25 billion to 30 billion cfu/mL, from 30 billion to 35 billion cfu/mL, from 35 billion to 40 billion cfu/mL, from 40 billion to 45 billion cfu/mL, from 45 billion to 50 billion cfu/mL, from 50 billion to 55 billion cfu/mL, from 55 billion to 60 billion cfu/mL, from 60 billion to 65 billion cfu/mL, from 65 billion to 70 billion cfu/mL, from 70 billion to 75 billion cfu/mL, from 75 billion to 80 billion cfu/mL, from 80 billion to 85 billion cfu/mL, from 85 billion to 90 billion cfu/mL, from 90 billion to 95 billion cfu/mL, from 95 billion to 100 billion cfu/mL.

[00249] In an embodiment, the strain of the invention can be administered at, for example, a dosage of 0.01 to 100 x 1011 cells/body, 0.1 to 10 x 1011 cells/body or 0.3 to 5 x 1011 cells/body. Furthermore, for example, the amount ingested per day as the microorganism can be 0.01 to 100 x 1011 cells/60 kg body weight, 0.1 to 10 x 1011 cells/60 kg body weight or 0.3 to 5 x 1011 cells/60 kg body weight.

[00250] The content of the at least one strain of bacteria or archaea or fungi contained in the orally ingested composition of the present invention may be determined as appropriate depending on its application form. As a dry microbial body it can be, for example, 5 to 50 w/w %, 1 to 75 w/w %, 0.1 to 100 w/w % or 1 to 100 w/w %.

[00251] In an embodiment, the composition is a controlled release composition. As used herein, the term "controlled-release" refers to release or administration of a strain of the invention from a given dosage form in a controlled fashion in order to achieve the desired pharmacokinetic profile *in vivo*. An aspect of "controlled" delivery is the ability to manipulate the formulation and/or dosage form in order to establish the desired kinetics of release.

[00252] Procedures for preparing tablets, caplets, capsules and other forms of compositions of the invention are known to those of ordinary skill in the art and include without limitation wet granulation, dry granulation, and direct compression (for tablets and caplets).

[00253] Wet and dry granulation is used to manufacture tablets, caplets, or capsules. With granulation techniques, a chilsonation is used to manufacture the powder for the dosage forms. A chilsonator houses grooved, rotating rollers that are pressed tightly against one another by hydraulic pressure. Raw materials are placed into the hopper of the chilsonator and are fed by a system of horizontal and vertical screws into the rollers. As materials pass through the grooves in the rollers, it is compacted under very high pressure and emerges from the chilsonator as dense sheets. The sheets are milled into a fine granular powder using a Fitz mill and then passed through a screen to produce a uniform free flowing granule. The chilsonation process results in a finished powder that is two to four times denser than the starting material, a feature that permits the ingredients to be fashioned into the desired dosage form.

[00254] With dry granulation, the powder may be incorporated into a gelatin capsule or it may be mixed with gelatin to form a tablet or caplet. With wet granulation, the powder is moistened thus creating large "chunks" of material that are subsequently dried and milled to convert the chunks to particles of a desired size for the manufacturing process. Once the particles of a desired size are obtained, the particles are incorporated into a gelatin capsule or mixed with gelatin to form a tablet or caplet.

[00255] General considerations in formulation and/or manufacture can be found, for example, in Remington's The Science and Practice of Pharmacy, 23rd Edition. (2020, Mack Publishing Co., Easton, Pa.) which is incorporated by reference.

Prebiotics

[00256] A composition of the invention can comprise a prebiotic. Because prebiotics have a chemical structure that resists digestion through the alimentary tract, they reach the colon as intact molecules where they are able to elicit systemic physiological functions and

act as fermentable substrates for colonic microflora. Where a prebiotic is combined with a biotherapeutic, the resulting composition is sometimes referred to as a "synbiotic."

[00257] Examples of suitable prebiotics include, but are not limited to, oligosaccharide such as fructooligosaccharides, P95 Nutraflora®, for example, galactooligosaccharides, xylooligosaccharides, isomaltooligosaccharides, human milk oligosaccharides, inulin oligosaccharides, mannan oligosaccharides, pyrodextrin, levan, maltotriose, pectic oligosaccharides, bimuno-galactooligosaccharides, arabinoxylan, fucoidan and resistant starches. Fructooligosaccharides can be extracted from, for example, chicory, artichokes, asparagus, dandelions, dahlias, endive, garlic, leeks, lettuce, and onions.

[00258] In an embodiment, the prebiotic comprises amino acids such as one or more or all of alanine, aspartic acid, glutamic acid, glycine, leucine, isoleucine, proline, serine, threonine and valine.

[00259] In an embodiment, the prebiotic comprises simple sugars which can be a monosaccharide (such as glucose, galactose or fructose) and/or a disaccharide (such as sucrose maltose or lactose).

[00260] In an embodiment, the prebiotic comprises from about 5% (w/w) to about 50% (w/w), about 7.5% (w/w) to about 30% (w/w) or about 10% (w/w) to about 15% (w/w) of the composition.

Other Microorganisms

[00261] In order to obtain the desired health benefit to the subject, it may be advantageous to include one or more additional biotherapeutic microorganisms in the composition. Thus, the composition may comprise more than one species/strain of microorganisms in addition to the strain of the invention, such as two, three, four, five or a higher plurality of species/strains of microorganisms. Non-limiting examples of biotherapeutics are suitable strains of those selected from Table 3. It is to be understood that the foregoing list is intended only to be illustrative and not a limiting representation of the biotherapeutics that may be included in the composition of the present invention. In this respect, any additional biotherapeutic species may also be used in the compositions of the present invention.

[00262] In an embodiment, the *Enterococcus sp.* includes *Enterococcus faecalis* and/or *Enterococcus faecium*.

[00263] In an embodiment, the *Lactobacillus sp.* is selected from the group consisting of *Lactobacillus rhamnosus* (such as strain GG (ATCC 53103), CGMCC 1.3724 or SP1 (DSM

21690)), *Lactococcus lactis*, *Lactococcus cremoris*, *Lactococcus diacetylactis*, *Lactobacillus paracasei*, *Lactobacillus reuteri* (such as strain ATCC 55730 or DSM 17938), *Lactobacillus acidophilus*, *Lactobacillus murinus, Lactobacillus helveticus*, *Lactobacillus bulgaricus, Lactobacillus casei*, *Lactobacillus salivarius*, *Lactobacillus plantarum*, *Lactobacillus fermentum, Lactobacillus taiwanensis, Lactobacillus animalis, Lactobacillus johnsonii* (such as strain NCC533; CNCM 1-1225) and *Lactobacillus gasseri*.

[00264] In an embodiment, the *Bifidobacterium sp.* is selected from the group consisting of *Bifidobacterium lactis* (such as strain BB-12, BI-04 or CNCM 1-3446 (Bb12)), *Bifidobacterium longum* (such as strain NCC3001, ATCC BAA-999 (BB536)), *Bifidobacterium breve* (such as strain Bb-03, M-16V or R0070), *Bifidobacterium infantis*, *Bifidobacterium animalis*, *Bifidobacterium bifidum, Bifidobacterium catenulatum, Bifidobacterium dentium, Bifidobacterium pseudocatenulatum* and *Bifidobacterium adolescentis*.

[00265] In an embodiment, the *Streptococcus sp.* includes *Streptococcus thermophilus* such as *Streptococcus thermophilus* ST-21, *Streptococcus pasteurianus* and *Streptococcus salivarius*.

[00266] In an embodiment, the *Clostridia* includes *Clostridium difficile*, *Clostridium butyricum*, *Clostridium hylemonae*, *Clostridium scindens, Clostridium sp. C1, Clostridium spiroforme* and *Flavinofractor plautii*.

[00267] Some yeasts are also useful as biotherapeutics and are sometimes included in the compositions. One non-limiting example of a yeast used in biotherapeutics is *Saccharomyces boulardii*.

[00268] Some archaea are also useful as biotherapeutics and are sometimes included in the compositions. Non-limiting examples of an archaea used in biotherapeutics is *Methanobrevibacter spp,* including *Methanobrevibacter smithii* and *Methanosphaera sp,* including M*ethanobrevibacter stadtmanae.*

Dosage Form

[00269] Dosage forms are within the scope of the invention. In a preferred embodiment, the invention provides a dosage form comprising the composition as described in the first aspect of this invention. Preferably, the dosage form is stored in a sealed and sterile container.

Method for treating

[00270] Methods for treating a disease or disorder are within the scope of the invention.

11 Mar 2026
2026201833

[00271] Methods for treating a gastrointestinal disorder are also within the scope of the invention.

[00272] In a preferred embodiment, the invention provides a method for treating a disorder associated with loss of gut microbiota or dysbiosis, wherein said method comprises the administration to a patient in need thereof a therapeutically effective amount of the composition as described in the first aspect of this invention.

[00273] Faecal Microbiota Transplantation

[00274] Faecal Microbiota Transplantation (FMT) is the administration of human colonic microbiota into the bowel of a patient and while originally designed to treat *Clostridium difficile* infection, FMT is now being explored as a treatment for many other disorders, including ulcerative colitis. However, these efforts have been challenged by the loss of viable microbiota samples due to poor sampling, storage, shipping and delivery conditions which results in losses in viable cells. There is a challenge to sample, store, ship and deliver a viable and effective microbiota sample to the patient. There is also a need in the art for effective treatments of diseases associated with loss of gut microbes or dysbiosis.

[00275] Cultured or Second generation microbiome based therapies

[00277] Clinical trials have demonstrated the efficacy and safety of FMT for a number of diseases including but not limited to *C. difficile* infection, ulcerative colitis and irritable bowel syndrome. Thus, there is the need for the identification of microbes for use in defined cultured microbiome based therapies. Cultured therapies have the advantage over FMT of being produced in a bioreactor (not human donor derived) and so can be more consistent in composition, more scalable in production and with a more predictable safety profile. The ideal second generation microbiome based therapies are originally cultured and isolated from human faecal material. Many of these organisms are highly sensitive to an oxygen environment and will not survive in storage without freezing at very low temperatures or lyophilizing (freeze drying). There is a challenge to sample, store, ship and deliver a viable and effective microbiota sample to the patient. There is also a need in the art for effective treatments of diseases associated with loss of gut microbes or dysbiosis.

[00278] In a further preferred embodiment, the dosage form is administered at an amount to at least partially treat a gastrointestinal disorder.

[00279] A subject that can be treated with the invention will include humans as well as other mammals and animals.

[00280] The effect of the administered therapeutic composition can be monitored by standard diagnostic procedures.

[00281] Methods of the invention can be used to treat or prevent a dysbiosis of the gastrointestinal tract in a subject. "Dysbiosis" in the context of the present invention refers to a state in which the normal diversity and/or function of the microbiota or microbiome, in particular the human gastrointestinal microbiota, is disrupted. Any disruption from the normal state of the microbiota in a healthy individual can be considered a dysbiosis, even if the dysbiosis does not result in a detectable decrease in health in the individual. In a preferred embodiment, the dysbiosis may be associated with one or more pathological symptoms. For example, "dysbiosis" may refer to a decrease in the microbial diversity of the microbiota. In addition, or alternatively, "dysbiosis" may refer to an increase in the abundance of one or more bacteria, e.g. one or more pathogenic bacteria, in the microbiota of an individual relative to the abundance of said bacterium or bacteria in the microbiota of a healthy individual, i.e. an individual without a dysbiosis. The pathogenic bacteria present during dysbiosis are often Proteobacteria and resistant to one or more antibiotics. Examples of Proteobacteria include *Escherichia, Salmonella, Campylobacter, Vibrio, Helicobacter,* and *Yersinia* species.

[00282] The dysbiosis may be a dysbiosis associated with an enteric bacterial infection, such as an infection of the gastrointestinal tract with a pathogenic bacterium. Many bacteria capable of causing infections of the gastrointestinal tract in humans are known and include: gram positive bacteria, and gram-negative bacteria. The pathogenic bacterium is preferably a pathogenic species of the genus *Clostridium, Escherichia, Enterococcus, Klebsiella, Enterobacter, Proteus, Salmonella, Shigella, Staphylococcus, Vibrio, Aeromonas, Campylobacter, Plesiomonas, Bacillus, Helicobacter, Listeria, or Yersinia.* Preferred examples of such pathogenic bacteria include *Clostridium difficile, Clostridium perfringens, Clostridium botulinum, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus, Campylobacter fetus, Campylobacter jejuni, Aeromonas hydrophila, Plesiomonas shigelloides, Bacillus cereus, Helicobacter pylori, Listeria monocytogenes,* and *Yersinia enterocolitica*. More preferably, the pathogenic bacterium is a pathogenic species of the genus *Clostridium* or *Escherichia*. Most preferably, the pathogenic bacterium is *Clostridioides difficile* or *Escherichia coli*.

[00283] Methods of the invention can be used to reduce or prevent gastrointestinal tract mucosal inflammation in a subject using compositions of the invention.

[00284] In an embodiment, the subject has, or is susceptible to having, an inflammatory bowel disease (IBD). Inflammatory bowel disease (IBD) is an increasingly prevalent,

11 Mar 2026
2026201833

currently incurable condition believed to be caused by an abnormal immune response to the resident gut microbiome in genetically susceptible patients. The term IBD encompasses both Ulcerative Colitis (UC), Crohn's disease (CD) and pouchitis. UC is characterised by chronic non-granulomatous inflammation that is limited to the colonic mucosa, typically involving the rectum and a variable proximal extent of the colon in continuity. CD is characterised by transmural, often granulomatous, inflammation that can involve any part of the gastrointestinal tract from the mouth to the anus.

[00285] As used herein, the term "inflammatory bowel diseases (IBD)" has its general meaning in the art and refers to a group of inflammatory diseases of the colon and small intestine such as revised in the World Health Organisation Classification K20-K93 (ICD-10) such as Crohn disease (such as granulomatous enteritis; Crohn disease of small intestine; Crohn disease of large intestine; granulomatous and regional Colitis; Crohn disease of colon, large bowel and rectum; Crohn disease of both small and large intestine), Ulcerative colitis (such as Ulcerative (chronic) pancolitis; backwash ileitis; Ulcerative (chronic) proctitis; Ulcerative (chronic) rectosigmoiditis; Inflammatory polyps; Left sided colitis; left hemicolitis) and noninfective gastroenteritis and colitis (Gastroenteritis and colitis due to radiation; Toxic gastroenteritis and colitis; Allergic and dietetic gastroenteritis and colitis; Food hypersensitivity gastroenteritis or colitis; indeterminate colitis; specified noninfective gastroenteritis and colitis such as Collagenous colitis; Eosinophilic gastritis or gastroenteritis; Lymphocytic colitis Microscopic colitis (collagenous colitis or lymphocytic colitis); Noninfective gastroenteritis and colitis such as Diarrhoea; Enteritis; Ileitis; Jejunitis; Sigmoiditis) and postprocedural disorders of digestive system such as pouchitis. In an embodiment, the IBD is paediatric IBD.

[00286] In an embodiment, the subject has, or is susceptible to having, irritable bowel syndrome (IBS) in all its forms as detailed in the Rome IV criteria. These include but are not limited to diarrhoea predominant irritable bowel syndrome IBS-D, constipation predominant irritable bowel syndrome IBS-C or mixed irritable bowel syndrome IBS-M. This includes subjects whose symptoms respond to the low FODMAP diet or gluten free diet or other dietary restrictions.

[00287] In an embodiment, the subject has, or is susceptible to having, a malignancy or cancer. These include but are not limited to melanoma, lung cancer, bowel cancer, gastric cancer, oesphageal cancer, oral cancer, hepatocellular cancer, haematalogical malignancy, breast cancer, lymphoma, sarcoma, germ cell tumor, carcinoma, renal cancer, prostate cancer, pancreatic cancer, ovarian cancer, thyroid cancer, brain malignancy, skin cancer, melanoma, bladder cancer or testicular cancer.

[00288] In an embodiment, the subject has, or is susceptible to having, a hepatic or liver disease. These include but are not limited to cirrhosis, hepatic encephalopathy, alcoholic hepatitis, infective hepatitis, autoimmune hepatitis, or ascites.

[00289] In an embodiment, the subject has a disease or is susceptible to having a disease associated with dysbiosis or reduced gut microbial diversity or reduced gut microbial ecology.

[00290] In a further aspect, the present invention also relates to a faecal microbiota transplant composition comprising the strain or strains of the invention. The term "faecal microbiota transplant composition" has its general meaning in the art and refers to any composition that can restore the faecal microbiota.

[00291] Administration to humans includes administration by a medical professional and self-administration. In general, in order to achieve a health benefit, multiple doses of the composition are administered, for example daily for a period of at least one week, at least two weeks, at least three weeks, at least six weeks, at least nine weeks, or at least twelve weeks. In one embodiment, the compositions can be administered for the remaining duration of a subject's life.

Device

[00292] Devices are within the scope of the invention. In a preferred embodiment, the invention provides a device, wherein the device comprises: (1) the composition as described in the first aspect of this invention; and (2) an applicator, container or material.

Use of a composition in the manufacture of a medicament

[00293] Uses are within the scope of this invention. In a preferred embodiment, the invention provides the use of a composition in the manufacture of a medicament for treating a gastrointestional disorder.

Method for stabilising

[00294] Methods for stabilizing the compositions of the invention are within the scope of the invention.

[00295] In a further preferred embodiment, the said method protects the compositions of the invention against degradation.

[00296] In yet a further preferred embodiment, the compositions of the invention retains its effective biological activity for a period selected from the group consisting of; greater than 24 hours; greater than 36 hours; greater than 48 hours.

[00297] The addition of approved pharmaceutical excipients to stabilise the compositions of the invention solutions is preferred from a safety standpoint, as the simpler methodology is likely to produce a less variable outcome and the choice of excipient can be limited to those with Generally Regarded as Safe (GRAS) status. Excipients for the stabilisation of protein solutions can be classified into four broad categories: salts, sugars, polymers or protein/amino acids, based on their chemical properties and mechanism of action. Salts (e.g. chlorides, nitrates) stabilise the tertiary structure of proteins by shielding charges through ionic interactions. Sugars (e.g. glycerol, sorbitol, fructose, trehalose) increase the surface tension and viscosity of the solution to prevent protein aggregation. Similarly, polymers (e.g. polyethylene glycol, cellulose derivatives) stabilise the protein tertiary structure by increasing the viscosity of the solution to prevent protein aggregation and intra- and inter-molecular electrostatic interactions between amino acids in the protein. Proteins (e.g. human serum albumin) are able to stabilise the structure of other proteins through ionic, electrostatic and hydrophobic interactions. Similarly, small amino acids with no net charge, such as alanine and glycine, stabilise proteins through the formation of weak electrostatic interactions.

[00298] As discussed above, the medicaments of the present invention may include one or more pharmaceutically acceptable carriers. The use of such media and agents for the manufacture of medicaments is well known in the art. Except insofar as any conventional media or agent is incompatible with the pharmaceutically acceptable material, use thereof in the manufacture of a pharmaceutical composition according to the invention is contemplated. Pharmaceutical acceptable carriers according to the invention may include one or more of the following examples:

a. surfactants and polymers, including, however not limited to polyethylene glycol (PEG), polyvinylpyrrolidone , polyvinylalcohol, crospovidone, polyvinylpyrrolidone-polyvinylacrylate copolymer, cellulose derivatives, HPMC, hydroxypropyl cellulose, carboxymethylethyl cellulose, hydroxypropylmethyl cellulose phthalate, polyacrylates and polymethacrylates, urea, sugars, polyols, and their polymers, emulsifiers, sugar gum, starch, organic acids and their salts, vinyl pyrrolidone and vinyl acetate; and/or

b. binding agents such as various celluloses and cross-linked polyvinylpyrrolidone, microcrystalline cellulose; and/or (3) filling agents such as lactose monohydrate, lactose anhydrous, microcrystalline cellulose and various starches; and/or

c. filling agents such as lactose monohydrate, lactose anhydrous, mannitol, microcrystalline cellulose and various starches; and/or

d. lubricating agents such as agents that act on the increased ability of the dosage form to be ejected from the packaging cavity, and/or

e. sweeteners such as any natural or artificial sweetener including sucrose, xylitol, sodium saccharin, cyclamate, aspartame, and acesulfame K; and/or

f. flavouring agents; and/or

g. preservatives such as potassium sorbate, methylparaben, propylparaben, benzoic acid and its salts, other esters of parahydroxybenzoic acid such as butylparaben, alcohols such as ethyl or benzyl alcohol, phenolic chemicals such as phenol, or quarternary compounds such as benzalkonium chloride; and/or

h. buffers; and/or

i. diluents such as pharmaceutically acceptable inert fillers, such as microcrystalline cellulose, lactose, dibasic calcium phosphate, saccharides, and/or mixtures of any of the foregoing; and/or

j. absorption enhancer such as glyceryl trinitrate; and/or

k. other pharmaceutically acceptable excipients.

[00299] Medicaments of the invention suitable for use in animals and in particular in human beings typically must be sterile and stable under the conditions of manufacture and storage.

Methods for Detection

[00300] A strain of the invention can be detected using a wide variety of known techniques. Conveniently the strain is detected using a nucleic acid based detection system.

[00301] In an embodiment, nucleic acid sequencing is used. Illustrative non-limiting examples of nucleic acid sequencing techniques include, but are not limited to, chain terminator (Sanger) sequencing and dye terminator sequencing. In some embodiments, the technology provided herein finds use in a Second Generation (a.k.a. Next Generation or Next-Gen), Third Generation (a.k.a. Next-Next-Gen), or Fourth Generation (a.k.a. N3-Gen) sequencing technology including, but not limited to, pyrosequencing, sequencing-by-ligation, single molecule sequencing, sequence-by-synthesis (SBS), massive parallel clonal, massive

parallel single molecule SBS, massive parallel single molecule real-time, massive parallel single molecule real-time nanopore technology.

[00302] In some embodiments, hybridization is employed in a detection method of the invention. Illustrative non-limiting examples of nucleic acid hybridization techniques include, but are not limited to, *in situ* hybridization (ISH), microarray, and Southern or Northern blot. In one embodiment, a FISH assay is used. In other embodiments, nucleic acid amplification is used. Nucleic acids may be amplified prior to or simultaneous with detection. Conducting one or more amplification reactions may comprise one or more PCR-based amplifications, non-PCR based amplifications, or a combination thereof. Illustrative non-limiting examples of nucleic acid amplification techniques include, but are not limited to, polymerase chain reaction (PCR), reverse transcription polymerase chain reaction (RT-PCR), nested PCR, linear amplification, multiple displacement amplification (MDA), real-time SDA, rolling circle amplification, circle-to-circle amplification transcription-mediated amplification (TMA), ligase chain reaction (LCR), strand displacement amplification (SDA), and nucleic acid sequence based amplification (NASBA). Those of ordinary skill in the art will recognize that certain amplification techniques (e.g., PCR) require that RNA be reversed transcribed to DNA prior to amplification (e.g., RT-PCR), whereas other amplification techniques directly amplify RNA (e.g., TMA and NASBA).

[00303] Non-amplified or amplified nucleic acids can be detected by any conventional means. For example, the nucleic acids can be detected by hybridization with a detectably labeled probe and measurement of the resulting hybrids. In another example, the nucleic acids are detected by sequencing. Illustrative non-limiting examples of detection methods are described herein.

[00304] Evaluation of an amplification process in "real-time" involves determining the amount of amplicon in the reaction mixture either continuously or periodically during the amplification reaction and using the determined values to calculate the amount of target sequence initially present in the sample. A variety of methods for determining the amount of initial target sequence present in a sample based on real-time amplification are well known in the art. These include methods disclosed in U.S. Pat. Nos. 6,303,305 and 6,541,205. Another method for determining the quantity of target sequence initially present in a sample, but which is not based on a real-time amplification, is disclosed in U.S. Pat. No. 5,710,029.

[00305] Amplification products may be detected in real-time through the use of various self-hybridizing probes, most of which have a stem-loop structure. Such self-hybridizing probes are labeled so that they emit differently detectable signals, depending on whether the probes are in a self-hybridized state or an altered state through hybridization to a target

sequence. By way of non-limiting example, "molecular torches" are a type of self-hybridizing probe that includes distinct regions of self-complementarity (referred to as "the target binding domain" and "the target closing domain") which are connected by a joining region (e.g., non-nucleotide linker) and which hybridize to each other under predetermined hybridization assay conditions. In a preferred embodiment, molecular torches contain single-stranded base regions in the target binding domain that are from 1 to about 20 bases in length and are accessible for hybridization to a target sequence present in an amplification reaction under strand displacement conditions. Under strand displacement conditions, hybridization of the two complementary regions, which may be fully or partially complementary, of the molecular torch is favored, except in the presence of the target sequence, which will bind to the single-stranded region present in the target binding domain and displace all or a portion of the target closing domain. The target binding domain and the target closing domain of a molecular torch include a detectable label or a pair of interacting labels (e g., luminescent/quencher) positioned so that a different signal is produced when the molecular torch is self-hybridized than when the molecular torch is hybridized to the target sequence, thereby permitting detection of probe:target duplexes in a test sample in the presence of unhybridized molecular torches. Molecular torches and a variety of types of interacting label pairs are disclosed in U.S. Pat. No. 6,534,274, herein incorporated by reference in its entirety.

[00306] Another example of a detection probe having self-complementarity is a "molecular beacon." Molecular beacons include nucleic acid molecules having a target complementary sequence, an affinity pair (or nucleic acid arms) holding the probe in a closed conformation in the absence of a target sequence present in an amplification reaction, and a label pair that interacts when the probe is in a closed conformation. Hybridization of the target sequence and the target complementary sequence separates the members of the affinity pair, thereby shifting the probe to an open conformation. The shift to the open conformation is detectable due to reduced interaction of the label pair, which may be, for example, a fluorophore and a quencher (e.g., DABCYL and 25 EDANS). Molecular beacons are disclosed in U.S. Pat. Nos. 5,925,517 and 6,150,097.

[00307] In an embodiment, the method includes quantifying the amount of strain present in the sample

[00308] The present invention will now be described with reference to the following non-limiting Examples. The description of the Examples is in no way limiting on the preceding paragraphs of this specification, however, is provided for exemplification of the methods and compositions of the invention.

Examples

[00309] It will be apparent to persons skilled in the milling and pharmaceutical arts that numerous enhancements and modifications can be made to the above-described processes without departing from the basic inventive concepts. For example, in some applications the biologically active material may be pretreated and supplied to the process in the pretreated form. All such modifications and enhancements are considered to be within the scope of the present invention, the nature of which is to be determined from the foregoing description and the appended claims. Furthermore, the following Examples are provided for illustrative purposes only, and are not intended to limit the scope of the processes or compositions of the invention.

A EXAMPLE 1 – FORMULATION STUDIES

A.1 STUDY AIM

[00310] The aim of this experiment was to carry out a development freeze-drying cycle of a fecal suspension sample. Eight formulations of excipients were added to the samples with a negative and a positive control. Specific objectives were: (1) To gather data on the chosen excipients and excipient concentrations in order to achieve an acceptable freeze-dried product that does not kill a majority of the cells; and (2) To achieve a moisture content of below 10% w/w

A.2 MATERIALS, METHODS AND RESULTS

A.2.1 EQUIPMENT

[00311] The following equipment was used in the preparation of the formulations and freeze-drying steps.

Table 6 – Equipment List

Equipment	ID number (BTL no.)	Cross-referencing SOP
Freeze dryer – SP Scientific - VirTis Genesis 25L Pilot Lyophiliser with Encore control system		LAB-QAD-315
4dp Analytical balance	BTL0086	LAB-QAD-314
TA Instruments Q100 mDSC	BTL0047	LAB-QAD-305
Freeze-drying microscopy (FDM)	BTL0075	LAB-QAD-302
MicroPress Cake Mechanical Properties	BTL0192	LAB-QAD-316
Power crimper		BTL0041

Solvent/ Metrohm Oven Karl Fischer Titrator	BTL0130	LAB-QAD-304

Consumables	**Lot Number**	**Supplier**
Rubber stoppers	#3218003691	Adelphi
50 ml vials	#6105794619	Adelphi
Crimp caps	#20200499	Adelphi
0.101 mm filter	LABPEFT -1520A	Bio-Strategy

A.2.2 EXCIPIENTS AND METHODS

[00312] Excipient solutions were prepared at 2X working concentration, so that when the 2X excipient mixture was added to the FMT sample the correct final concentration is achieved. Excipient stock solution was added w/v to the FMT solution to make up the final volume of 3ml per vial. 17 vials per formulation (formulations no. 1 to 8) were prepared with the fill volume of 3ml. 4 vials per mix were prepared for positive and negative controls.

Table 7: List of Raw Excipients

Material	**Supplier**	**Batch/lot no**
Inulin	Sigma	SLCJ4642
Dextran 70K	Sigma	#BCCF0270
Maltodextrin	Sigma	00444837
Skim Milk (Powdered)	N/A	EST570KB22103
Pectin	Sigma	SLCK5577
Sucrose	Sigma	19I1256989

Table 8. Final Excipient Concentration

Excipient **Formulation No.**	**Inulin (% w/v)**	**Sucrose (% w/v)**	**Maltodextrin (% w/v)**	**Dextran 70K (% w/v)**	**Pectin (% w/v)**	**Skim Milk (% w/v)**
1 (To be probed)	10.0%					
2	5.0%		5.0%			
3	5.0%			5.0%		
4	5.0%				5.0%	
5		10.0%				
6		5.0%	5.0%			
7		5.0%		5.0%		
8 (To be probed)		5.0%			5.0%	
9 – Negative Control (To be probed)						
10 – Positive Control						10%

[00313] The material was filtered using a 0.101 mm filter. During processing of samples and after cryoprotectants and saline are added, the stool was homogenized and filtered. Effectively filtering out large plant matter.

[00314] 0.9% sterile saline was used as a carrier for the cryoprotectant samples.

[00315] A research batch record (BB374) was created to document the production of excipients and the 10 different formulations that were produced. There were 16 vials of each cryoprotectant formulation and 4 each of the positive and negative controls was required. 1 vial of each formulation including controls was kept by the inventors for testing purposes. (126 vials to be sent to BioPharma Process Systems Ltd, Biopharma House, Winnall Valley Road, Winchester SO23 0LD, United Kingdom.

[00316] The team reported the following outcomes for the different cryoprotectants being trialed. Inulin on its own dissolved well when mixed by hand and was a very fine powder. Sucrose has larger crystals than inulin and required a smaller volume to reach the same weight. Dissolved under vortex but not by hand. Maltodextrin and Dextran 70K were similar to inulin in texture but these clumped together when trying to dissolve by hand and required vortex to dissolve. Pectin is a very fine powder and was difficult to weigh out within the chamber. When used in a formulation, it resulted in coagulation to a gel-like state which was impossible to filter sterilize

A.3 ANALYSES

[00317] Analysis included:

[00318] Lyostat analysis: Two 2ul samples were analysed using Lyostat analysis positive control skim milk powder and 10 % inulin. Lyostat analysis is microscopy looking at the freezing line of the product. This is an indication of what temperatures the lyophilizer can run at.

[00319] Appearance: The appearance of freeze-dried product was assessed visually on a scale of 1-5 (1= worst 5= best) with photographs taken.

[00320] Moisture Content: Moisture content from 3 vials of 2 formulations was analysed by Karl Fischer titration.

[00321] Mechanical Properties: Two formulations were selected for mechanical property analysis using MicroPress. Micro Press comprises of a load cell with an actuator indenter. This indenter comes down breaching surface of cake. As it presses down the pressure applied shows on the corresponding graph. The lower the max stress (kPa) the more brittle the lyophilized cake is.

[00322] mDSC: Two formulations were selected for analysis by solid state mDSC.

[00323] Plating CFU: Plate CFU analysis was performed by comparing non-lyophilized samples with lyophilized samples of all formulations. Frozen non lyophilized samples were allowed to thaw for 1.5 to 2 hours at room temperature outside the anaerobic chamber. Lyophilized samples were re-hydrated with the same amount of liquid that was removed during the lyophilization process and allowed to rest for 1 hour. Lyophillised samples were vortexed for 2 minutes to dissolve lyophillised product into solution. 1 ml was then aliquoted into a 1.5mL epi tube and vortexed for 5 min. A serial dilution into PBS (100uL FMT into 900 mL PBS) ranging from 10-3 to 10-8. 50uL of dilutions were then spread plated on WCA in triplicate and allowed to soak into the agar. The plates are then inverted and incubated anaerobically at 37°C for 48 hours. After 48 hours of incubation the plates are inspected for "best" set of triplicates for colony counting 30-300 non confluent colonies. Pictures were taken of plates and colony counts performed.

A.4 RESULTS

A.4.1 LYOSTAT ANALYSIS

[00324] Good freezing structure was observed at -50.0°C for both skim milk powder (a) and 10% inulin (C). Collapse set in at -47.0°C for skim milk powder (B) while 10% inulin (D) started to collapse at -30.5°C. This indicates that a temperature below -30.5°C will be suitable for sublimation of water while using inulin as one of the cryoprotectants.

A.4.2 APPEARANCE

[00325] All formulations that used perspective cryoprotectants visually were deemed 5/5 for drying except for 10 negative control water. Partial drying was observed. Pectin was not included for analysis based on the difficulty to work with and lack of consistency.

A.4.3 MOISTURE CONTENT

[00326] Moisture content was performed on 5% inulin / 5% maltodextrin resulting in an average of 1.093% moisture indicating that using a combination of 5% inulin and 5 % maltodextrin can achieve an appropriate moisture content for storage of microbes (<5%). The results are presented in Table 9.

Table 9: Moisture Content.

Sample Number	RMC (%)	Average (%)
Sample 5/16	0.79	1.093

Sample 5/16	0.75	
Sample 14/16	1.74	

A.4.4 MECHANICAL PROPERTIES

[00327] MicroPress showed samples to be brittle. Results were compared to the negative control sample (Highly brittle). See Table 10. See also Figure 1.

Table 10 – Max Stress at fractur point.

Sample Name	Max Stress (kPa) at the fractur point	Young's Modulus	Average of young's Modulus
F2 -Analysis 1	29.36	2.1950	2.9803
F2 -Analysis 2	29.01	3.5105	
F2 -Analysis 3	22.68	3.2355	
Negative Control	N/A	0.5903	.5903

A.4.5 MDSC

[00328] mDSC results showed a Tg onset at 51.13°C. Some studies show samples should be stable ~50°C below Tg onset, suggesting 2-8°C storage conditions may be suitable for the samples.

Table 11 – mDSC

Formulation	Event Temperature	Description of events/ General comments
5% inulin (% w/v) + 5% maltodextrin (% w/v)	51.13 to 55.2°C	Step change in reversible heat flow baseline indicative of glass transition with a midpoint at 53.16 C(H)
	126.87°C	Onset of minor endothermic event in non reversible heat flow indicative of small melt with a peak at 134.52 C
	191.05°C	Onset of major endothermic event in non reversible heat flow indicative of small melt with a peak at 192.96 C

A.4.6 PLATING CFU

[00329] Pectin additions showed the smallest drop in CFU. Pectin samples also were the lowest in CFU prior to lyophilisation and are similar to most others post lyophilisation. 10% sucrose showed the second lowest reduction in CFU and the highest CFU both pre and post lyophilisation. This may be due to the sucrose acting as a nutrient that increases CFU prior to plating. 5% inulin / 5% maltodextrin showed the next smallest reduction and the second highest CFU post lyophilisation. This has been suggested to be the preferred formulation based on both the CFU and lyophilisation process. See also Figures 2 and 3.

A.5 DISCUSSION

[00330] Inulin and maltodextrin combination was chosen as an optimal cryoprotectant for lyophilization. Inulin is a rapid to moderately fermentable fiber. The inventors achieved material with less than 5% residual moisture, the lyophilized cake that is produced is brittle and can be milled for further processing, and minimal cell loss was observed via CFU plating. From the experiments presented above, inulin and maltodextrin were preferred over all others because of the benefits that both inulin and maltodextrin had on the lyophilization process. Inulin and maltodextrin also showed the smallest log fold change in CFU when comparing lyophilized material to non-lyophilized material. Inulin showed the ability to maintain a freezing structure without collapse up to -30.5°C allowing for the increase of temperature when sublimating water from the frozen lyophilized cake. When compared to trehalose, this is relatively close to the same cake collapse temperature around -30°C. The inventors have demonstrated that, in a preferred embodiment, the composition of the invention can be lyophilized faster than sucrose and create a shorter run time while still maintaining viability within the sample. Maltodextrin also aids in the stability over time in storage of lyophilized samples. It leads to the stabilization of microbes within the lyophilized matrix and aids in the control of moisture ingress into the lyophilized product.

B EXAMPLE 2 - STABILITY STUDIES

B.1 STUDY AIM

[00331] The aim of this study was to: (1) determine if the inulin/maltodextrin cryoprotectant formulation maintained intact cell counts (ICCs) during short-term storage of the intermediate (liquid) product; (2) to determine the impact of lyophilization on ICCs; (3) to evaluate the impact of the inulin/maltodextrin cryoprotectant formulation on the ICCs of an encapsulated, lyophilized FMT product over a 6-month period; and (4) to determine optimal storage temperature of lyophilized, encapsulated FMT. The principal focus was to ascertain the efficacy of the cryoprotectant formulation in preserving ICCs across time different storage conditions as a factor of time, specifically -80 °C, -20 °C, 4-8 °C, and 20-25 °C.

B.2 MATERIALS, METHODS AND RESULTS

B.2.1 *EXCIPIENT ADDITION AND LYOPHILIZATION*

[00332] Intermediate product (liquid) was processed from 8 stool donations from a single, healthy donor over a 1-month period. These intermediate products consisted of donor stool

homogenized in an excipient solution containing 5% inulin and 5% maltodextrin dissolved in 0.9% saline (NaCl) in a 1:2.6 w/v ratio. Intermediate products were pooled, and the resulting material was homogenized and filtered. Homogenized material was then stored and frozen at -80 °C until lyophilization. Samples were then lyophilized following protocols and procedures as presented in Example 1. Post lyophilization, samples were milled, encapsulated, and stored in induction sealed bottles at respective testing temperatures.

[00333] **Table 12 – List of raw excipients**

Material	Supplier	Batch/lot no
Inulin	Sigma	SLCJ4642
Maltodextrin	Sigma	00444837

B.2.2 *SAMPLING AND INTACT CELL COUNTS*

[00334] Intact cell counts (ICCs) were determined via BactoBox (SBT Instruments) from the following samples: (1) neat stool from each batch, combined in ratios as per the intermediate product (pooled); (2) pooled intermediate product (immediately pre-lyophilization); (3) post-lyophilization at multiple timepoints (milled powder, encapsulated product ('T0')), week 1, 2, 4, and months 2, and 6, at the respective temperatures. All results were normalized to ICCs per gram of stool.

B.3 RESULTS

[00335] Eight batches of intermediate product were compared to neat stool to determine whether the inulin/maltodextrin cryoprotectant formulation was sufficient as a short-term cryoprotectant for intermediate product (Figure 4). Overall, there were no statistically significant differences between the ICCs in the neat stool samples compared to the intermediate product when tested, except for Batch 23, which had significantly lower ICCs than neat stool from the same donation. When pooled neat stool was compared to pooled intermediate product, the results were comparable (neat stool 1.97×10^9 ICCs/g (SD 1.17×10^8) vs intermediate product 1.91×10^9 ICCs/g (SD 2.14×10^8, $p = 0.729$)).

[00336] To determine loss of viability due to the lyophilization process, the ICCs/g of stool in the pre-lyophilized neat stool and intermediate product were compared to post lyophilization samples post-milling and following lyophilized powder encapsulation (Figure 5). As above, there was no difference between the pre-lyophilization neat stool and intermediate product; however, there was a significant difference between both the neat stool (pooled) and intermediate product, with the milled powder and encapsulated product. The intermediate product was determined to have an average 1.91×10^9 ICCs/g (SD 2.14×10^8),

and the milled powder 9.7 x 10^8 ICCs/g (SD 6.99 x 10^7) (p = <0.0001); when compared to the intermediate product, the encapsulated product had an average 2.81 x 10^8 ICCs/g stool (SD 1.33 x 10^8) (p = 0.0007).

[00337] The encapsulated product served as 'T0' for follow-up viability studies for comparison of effects of storage time and temperature (Figure 6). There was no significant reduction in ICCs observed between T0 and any follow-up timepoints. Furthermore, storage temperature did not impact ICCs overtime

B.4 DISCUSSION

[00338] The 5% w/v inulin and 5% w/v maltodextrin cryoprotectant formulation was tested for the ability to act as a cryoprotectant for intermediate product, as well as a lyprotectant through the lyophilization of intermediate product for FMT. Inulin and maltodextrin were suitable as cryoprotectants for the timeframe tested, with comparable ICCs to neat stool. There was a significant reduction in cell viability through the lyophilization process, which was expected. This approximately 0.78-log drop in ICCs, was within the acceptable and expected range of approximately 1-log reduction in ICCs. Furthermore, ICCs were determined to be consistent throughout the 28-week period tested, regardless of storage temperature, indicating that the inulin/maltodextrin cryoprotectant formulation maintained microbial stability regardless of storage times and temperatures tested herein.

[00339] In conclusion, inulin and maltodextrin have demonstrated efficacy as cryoprotectants/lyoprotectants for FMT.

C EXAMPLE 3 - FORMULATION STUDIES FOR BB265, A COMPLEX COMMUNITY LIVE BIOTHERAPEUTIC PRODUCT

C.1 STUDY AIM

[00340] The aim of the study was to carry out a development freeze-drying cycle for BB265, a complex community biotherapeutic product. Specific objectives were: (1) to gather data on the optimal excipients and excipient concentrations as determined in Example 1 in order to achieve an acceptable freeze-dried product that does not kill a majority of the cells; and (2) to achieve a moisture content of below 10% w/w.

C.2 MATERIALS, METHODS AND RESULTS

C.2.1 *COMPOSITION OF BB265*

[00341] ***Table 13 – List of taxa in BB265 by Phylum***

Actinomycetota (formerly known as Actinobacteria)
Bacillota (formerly known as Firmicutes)
Bacteroidota (formerly known as Bacteroidetes)
Verrucomicrobiota

[00342] ***Table 14 – List of taxa in BB265 by Genus***

Agathobaculum	*Coprococcus*	*Massilimicrobiota*
Akkermansia	*Dorea*	*Megamonas*
Alistipes	*Enorma*	*Merdibacter*
Amedibacterium	*Enterocloster*	*Odoribacter*
Anaerobutyricum	*Erysipelatoclostridium*	*Oscillospiraceae*
Anaerofustis	*Eubacterium*	*Parabacteroides*
Anaerostipes	*Faecalibacillus*	*Paraclostridium*
Anaerotignum	*Faecalibacterium*	*Phocaeicola*
Anaerotruncus	*Finegoldia*	*Porphyromonas*
Bacillus	*Flintibacter*	*Prevotella*
Bacteroides	*Gemmiger*	*Pusillimonas*
Barnesiella	*Holdemanella*	*Romboutsia*
Bifidobacterium	*Hungatella*	*Roseburia*
Blautia	*Intestinimonas*	*Ruminococcus*
Butyricimonas	*Lachnoanaerobaculum*	*Ruthenibacterium*
Christensenella	*Lachnospira*	*Sellimonas*
Clostridium	*Lacrimispora*	*Solibaculum*
Collinsella	*Lactococcus*	*Streptococcus*
Coprobacillus	*Ligilactobacillus*	*Thomasclavelia*
Coprobacter	*Longicatena*	*Vescimonas*

[00343]

[00344] ***Table 15 – List of taxa in BB265 by Species***

Agathobaculum sp003481705	*Blautia wexlerae*	*Lactococcus petauri*
Akkermansia muciniphila	*Butyricimonas faecalis*	*Ligilactobacillus ruminis*
Alistipes dispar	*Butyricimonas virosa*	*Longicatena caecimuris*
Alistipes finegoldii	*Christensenella minuta*	*Massilimicrobiota merdigallinarum*
Alistipes ihumii	*Christensenella sp. Marseille-P3954*	*Massilimicrobiota timonensis*
Alistipes indistinctus	*Clostridium bornimense*	*Megamonas funiformis*
Alistipes onderdonkii	*Clostridium butyricum*	*Merdibacter merdipullorum*
Alistipes putredinis	*Clostridium hylemonae*	*Odoribacter splanchnicus*
Alistipes senegalensis	*Clostridium leptum*	*Oscillospiraceae bacterium*
Alistipes shahii	*Clostridium methylpentosum*	*Parabacteroides distasonis*
Amedibacterium intestinale	*Clostridium paraputrificum*	*Parabacteroides goldsteinii*
Anaerobutyricum hallii	*Clostridium scindens*	*Parabacteroides johnsonii*
Anaerofustis stercorihominis	*Collinsella aerofaciens*	*Parabacteroides merdae*
Anaerostipes caccae	*Collinsella bouchesdurhonensis*	*Paraclostridium bifermentans*
Anaerostipes hadrus	*Coprobacillus cateniformis*	*Phocaeicola coprocola*
Anaerostipes hominis	*Coprobacter secundus*	*Phocaeicola coprophilus*
Anaerotignum propionicum	*Coprococcus catus*	*Phocaeicola dorei*
Anaerotruncus colihominis	*Coprococcus comes*	*Phocaeicola vulgatus*
Anaerotruncus rubiinfantis	*Coprococcus eutactus*	*Porphyromonas asaccharolytica*

Bacillus cereus	*Dorea longicatena*	*Prevotella copri*
Bacteroides caccae	*Enorma massiliensis*	*Pusillimonas faecalis*
Bacteroides cellulosilyticus	*Enterocloster aldenensis*	*Romboutsia timonensis*
Bacteroides eggerthii	*Erysipelatoclostridium ramosum*	*Roseburia hominis*
Bacteroides faecis	*Eubacterium callanderi*	*Roseburia intestinalis*
Bacteroides finegoldii	*Eubacterium limosum*	*Roseburia inulinivorans*
Bacteroides intestinalis	*Eubacterium rectale*	*Ruminococcus bicirculans*
Bacteroides nordii	*Eubacterium siraeum*	*Ruminococcus bromii*
Bacteroides ovatus	*Eubacterium ventriosum*	*Ruminococcus gnavus*
Bacteroides salyersiae	*Faecalibacillus intestinalis*	*Ruminococcus torques*
Bacteroides stercoris	*Faecalibacterium prausnitzii*	*Ruthenibacterium lactatiformans*
Bacteroides thetaiotaomicron	*Finegoldia magna*	*Sellimonas intestinalis*
Bacteroides uniformis	*Flintibacter sp. KGMB00164*	*Solibaculum mannosilyticum*
Bacteroides xylanisolvens	*Gemmiger formicilis*	*Streptococcus infantarius*
Barnesiella intestinihominis	*Holdemanella porci*	*Streptococcus salivarius*
Bifidobacterium adolescentis	*Hungatella effluvii*	*Thomasclavelia spiroformis*
Bifidobacterium bifidum	*Intestinimonas butyriciproducens*	*Vescimonas coprocola*
Bifidobacterium longum	*Lachnoanaerobaculum umeaense*	
Bifidobacterium pseudocatenulatum	*Lachnospira eligens*	
Blautia massiliensis	*Lacrimispora saccharolytica*	
Blautia stercoris	*Lactococcus lactis*	

C.2.2 *EQUIPMENT*

[00345] The following equipment was used in the preparation of the formulations and freeze-drying steps.

[00346] **Table 16 – Equipment list**

Equipment	**ID number (BTL no.)**	**Cross-referencing SOP**
Freeze dryer – Genesis 25.0 EL	BTL0127	LAB-QAD-315
4dp Analytical balance	BTL0086	LAB-QAD-314
TA Instruments Q100 mDSC	BTL0047	LAB-QAD-305
Freeze-drying microscopy (FDM)	BTL0075	LAB-QAD-302
MicroPress Cake Mechanical Properties	BTL0192	LAB-QAD-316
Power crimper	BTL0041	
Solvent/ Metrohm Oven Karl Fischer Titrator	BTL0130	LAB-QAD-304
Consumables	**Lot Number**	**Supplier**
Rubber stoppers	TBC	TBC
10 ml vials	N/A	N/A
Crimp caps	#20200499	Adelphi

C.2.3 *EXIPIENTS AND METHODS*

[00347] An excipient solution containing 5.0% w/v inulin and 5.0% w/v maltodextrin dissolved in 0.9% saline (NaCl) was prepared and added to microbial pellet of a pelletized harvest of the BB265 complex consortium in a 2.6:1 v/w ratio. The mixture was then homogenized. Samples were immediately frozen at −80 °C and sent frozen to BioPharma

Process Systems Ltd, Biopharma House, Winnall Valley Road, Winchester SO23 0LD, United Kingdom for analysis.

[00348] **Table 17 – List of raw excipients**

Material	Supplier	Batch/lot no
Inulin (Orafiti® GR)	Beneo	B2027469
Maltodextrin	Satoria Agro	2305050018 (T)

C.2.4 *ANALYSES*

[00349] Analysis included:

[00350] Lyostat analysis: A 2 µL sample was analysed using Lyostat analysis. Lyostat analysis is microscopy looking at the freezing line of the product. This is an indication of what temperatures the lyophilizer can run at.

[00351] Appearance: The appearance of freeze-dried product was assessed visually on a scale of 1-5 (1= worst 5= best) with photographs taken.

[00352] Moisture Content: Moisture content from 3 vials was analysed by Karl Fischer titration.

[00353] Mechanical Properties: Mechanical property analysis was performed using MicroPress. MicroPress comprises of a load cell with an actuator indenter. This indenter comes down breaching surface of cake. As it presses down the pressure applied shows on the corresponding graph. The lower the max stress (kPa) the more brittle the lyophilized cake is.

[00354] mDSC: Modulated differential scanning calorimetry (mDSC) was used to determine the thermal properties of the formulation during lyophilization

C.3 RESULTS

[00355] *Lyostat analysis*. Good freezing structure was observed at -50.0 °C. Collapse set in at -38.5 °C, indicating that a temperature below -38.5 °C will be suitable for sublimation of water while using inulin and maltodextrin as cryoprotectants for BB265.

[00356] *Appearance*. All vials scored 5/5 for visual appearance of drying and structure postlyophilization.

[00357] *Moisture content*. The average residual moisture content was determined to be 2.13% w/w, indicating that using a combination of 5% inulin and 5% maltodextrin can achieve an appropriate moisture content for storage of microbes (<5%).

[00358] **Table 18 – Residual moisture content**

Vial no	RMC (% w/w)	Average (%)
1	2.26	
2	2.07	2.13
3	2.06	

[00359] *Mechanical properties*. MicroPress analysis indicated robustness of the samples, with the max stress at the moment of fracture point demonstrated to be 296.544 kPa, and a Young's Modulus of 4.80 *E.*

[00360] *Modulated differential scanning calorimetery (mDSC)*. mDSC results showed a Tg onset at 53.89 °C. Some studies show samples should be stable ~ 50 °C below Tg onset, suggesting 2-8 °C storage conditions may be suitable for the samples

[00361] **Table 19 – mDSC**

Formulation	Event temperature (°C)	Description of events/General comments
5% inulin + 5% maltodextrin + 0.9% saline (NaCl)	53.89 to 63.95	Step change in reversible heat flow baseline indicative of glass transition with a midpoint at 58.31°C(H)
	185.47	Onset of major endothermic event in heat flow indicative of melt with a peak at 186.68°C

C.4 DISCUSSION

[00362] Inulin and maltodextrin combination was chosen as an optimal cryoprotectant for lyophilization of FMT material and used here for assessment of cryoprotection of a complex community biotherapeutic product, BB265. The inventors achieved material with less than 5% residual moisture, the lyophilized cake that is produced is robust and can be milled for further processing. From the experiments presented above, the inulin and maltodextrin combination paralleled those results shown for FMT, indicating optimal cryoprotection parameters when used for lyophilization of BB265. The mixture demonstrated the ability to maintain a freezing structure without collapse up to -38.5 °C, allowing for the increase of temperature when sublimating water from the frozen lyophilized cake. When compared to trehalose, this is relatively close to the same cake collapse temperature around -30 °C

11 Mar 2026
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CLAIMS

1. A composition for preventing or treating a disease or disorder in a subject in need thereof, said composition comprising at least one strain of a microorganism,

wherein the microorganism is selected from the group consisting of: bacteria, yeast or archaea;

and

an excipient.

2. The composition of claim 1, wherein the excipient is a cryoprotectant.

3. The composition of any one of claims 1 to 2, wherein the excipient is inulin or an analog or variant thereof.

4. The composition of claim 3, wherein the inulin is selected from the group consisting of: *alpha*-D-glucopyranosyl-[beta-D-fructofuranosyl](n-1)-D-fructofuranosides; *beta*-D-fructopyranosyl-[D-fructofuranosyl](n-1)-D-fructofuranosides; fructo-oligosaccharides; fructo-oligosaccharides containing between 2 and 70 fructose units; fructo-oligosaccharides containing between 1 and 500 fructose units; fructo-oligosaccharides containing between 1 and 300 fructose units; fructo-oligosaccharides containing between 1 and 200 fructose units; fructo-oligosaccharides containing between 1 and 100 fructose units; or an analog or variant or combination thereof.

5. The composition of any one of claims 1 to 4, wherein the excipient is maltodextrin or an analog or variant thereof.

6. The composition of any one of claim 5, wherein the maltodextrin is selected from the group consisting of: a maltodextrin having a length selected from the group consisting of: 3 to 17 glucose units; corn syrup with a length of 20 glucose units or more; corn syrup solid; modified corn starch; modified rice starch; modified tapioca starch; modified wheat starch; or an analog or variant or combination thereof.

7. The composition of any one of claims 1 to 6, wherein the composition comprises inulin or an analog of variant thereof at a concentration selected from the group consisting of: 0.01%

w/v to 20% w/v; 0.1% w/v to 20% w/v; 0.1% w/v to 10% w/v; 1% w/v to 10% w/v; 2% w/v to 9% w/v; 3% w/v to 8% w/v; 4% w/v to 7% w/v; 4% w/v to 6% w/v; 1% w/v; 2% w/v; 3% w/v; 4% w/v; 5% w/v; 6% w/v; 7% w/v; 8% w/v; 9% w/v; and 10% w/v.

8. The composition of any one of claims 1 to 7, wherein the composition comprises maltodextrin or an analog of variant thereof at a concentration selected from the group consisting of: 0.01% w/v to 20% w/v; 0.01% w/v to 20% w/v; 0.1% w/v to 10% w/v; 1% w/v to 10% w/v; 2% w/v to 9% w/v; 3% w/v to 8% w/v; 4% w/v to 7% w/v; 4% w/v to 6% w/v; 1% w/v; 2% w/v; 3% w/v; 4% w/v; 5% w/v; 6% w/v; 7% w/v; 8% w/v; 9% w/v; and 10% w/v.

9. The composition of any one of claims 1 to 8, wherein the composition comprises inulin and maltodextrin.

10. The composition of any one of claims 1 to 9, wherein the composition comprises inulin and maltodextrin at a concentration selected from the group consisting of: inulin (1% w/v) and maltodextrin (1% w/v); inulin (2% w/v) and maltodextrin (2% w/v); inulin (3% w/v) and maltodextrin (3% w/v); inulin (4% w/v) and maltodextrin (4% w/v); inulin (5% w/v) and maltodextrin (5% w/v); inulin (6% w/v) and maltodextrin (6% w/v); inulin (7% w/v) and maltodextrin (7% w/v); inulin (8% w/v) and maltodextrin (8% w/v); inulin (9% w/v) and maltodextrin (9% w/v); and inulin (10% w/v) and maltodextrin (10% w/v).

11. The composition of any one of claims 1 to 10, wherein the composition comprises inulin and maltodextrin at a concentration selected from the group consisting of: (1) inulin at a concentration selected from the group consisting of: 1% w/v; 2% w/v; 3% w/v; 4% w/v; 5% w/v; 6% w/v; 7% w/v; 8% w/v; 9% w/v; and 10% w/v; AND (2) maltodextrin at a concentration selected from the group consisting of: 1% w/v; 2% w/v; 3% w/v; 4% w/v; 5% w/v; 6% w/v; 7% w/v; 8% w/v; 9% w/v; and 10% w/v.

12. The composition of any one of claims 1 to 11, wherein the composition comprises inulin and maltodextrin at a concentration selected from the group consisting of: inulin (1% w/v) and maltodextrin (1% w/v); inulin (2% w/v) and maltodextrin (2% w/v); inulin (3% w/v) and maltodextrin (3% w/v); inulin (4% w/v) and maltodextrin (4% w/v); inulin (5% w/v) and maltodextrin (5% w/v); inulin (6% w/v) and maltodextrin (6% w/v); inulin (7% w/v) and maltodextrin (7% w/v); inulin (8% w/v) and maltodextrin (8% w/v); inulin (9% w/v) and maltodextrin (9% w/v); and inulin (10% w/v) and maltodextrin (10% w/v).

11 Mar 2026
2026201833

13. A biotherapeutic composition comprising the composition of any one of claims 1 to 12, together with an acceptable diluent or carrier.

14. A pharmaceutical composition comprising the composition of any one of claims 1 to 12, together with a pharmaceutically acceptable diluent or carrier.

15. A method of treating and/or preventing a disease or disorder in a patient in need thereof said method comprising administering to the subject an effective amount of a composition according to any one of claims 1 to 12.

16. A method of preparing the biotherapeutic composition according claim 13, the method comprising mixing the composition according to any one of claims 1 to 12 with an acceptable diluent or carrier.

17. A method of preparing the pharmaceutical composition according to claim 14, the method comprising mixing the composition according to any one of claims 1 to 12 with a pharmaceutically acceptable excipient, diluent or carrier.

18. Use of the composition according to any one of claims 1 to 12 in the manufacture of a medicament for reducing or preventing a disease or disorder in a subject.

19. A dosage form comprising the composition according to any one of claims 1 to 12.

20. A kit comprising the dosage form of claim 19 together with instructions for its use.

2026201833 11 Mar 2026

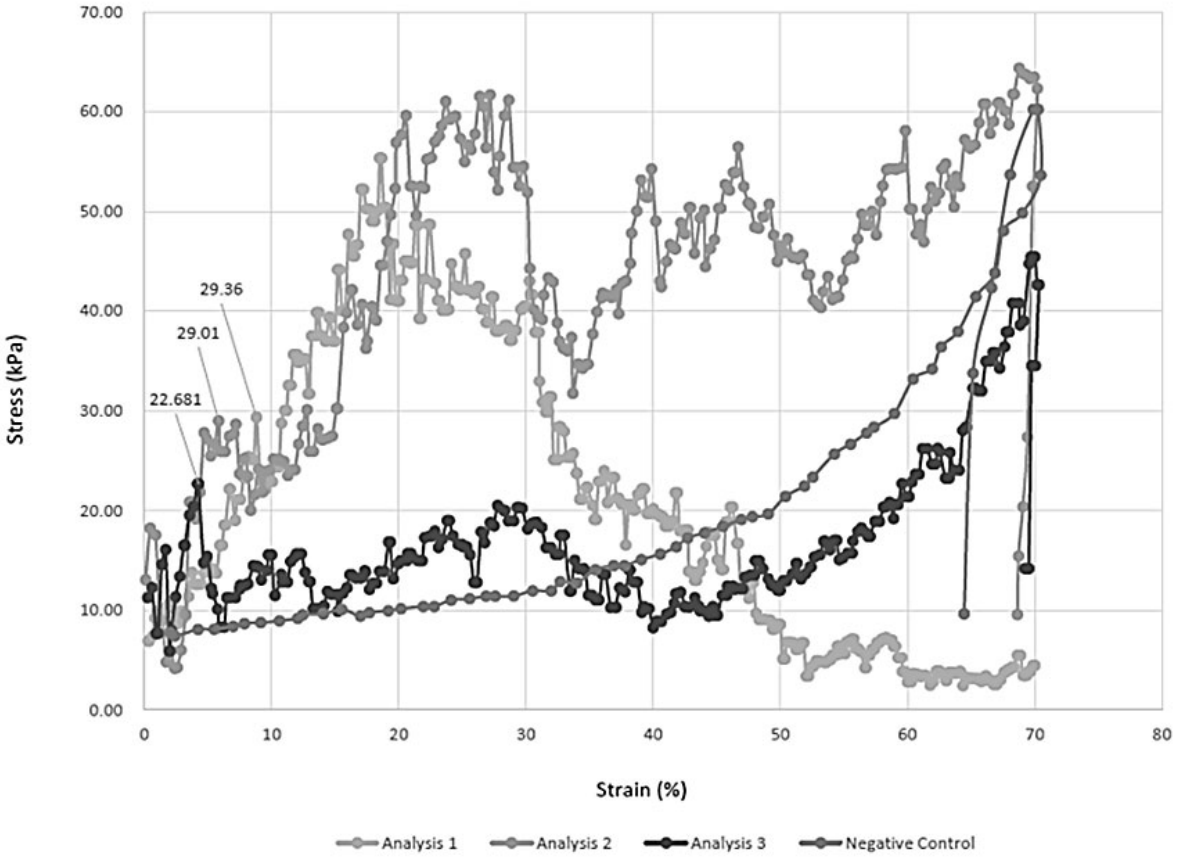

FIGURE 1

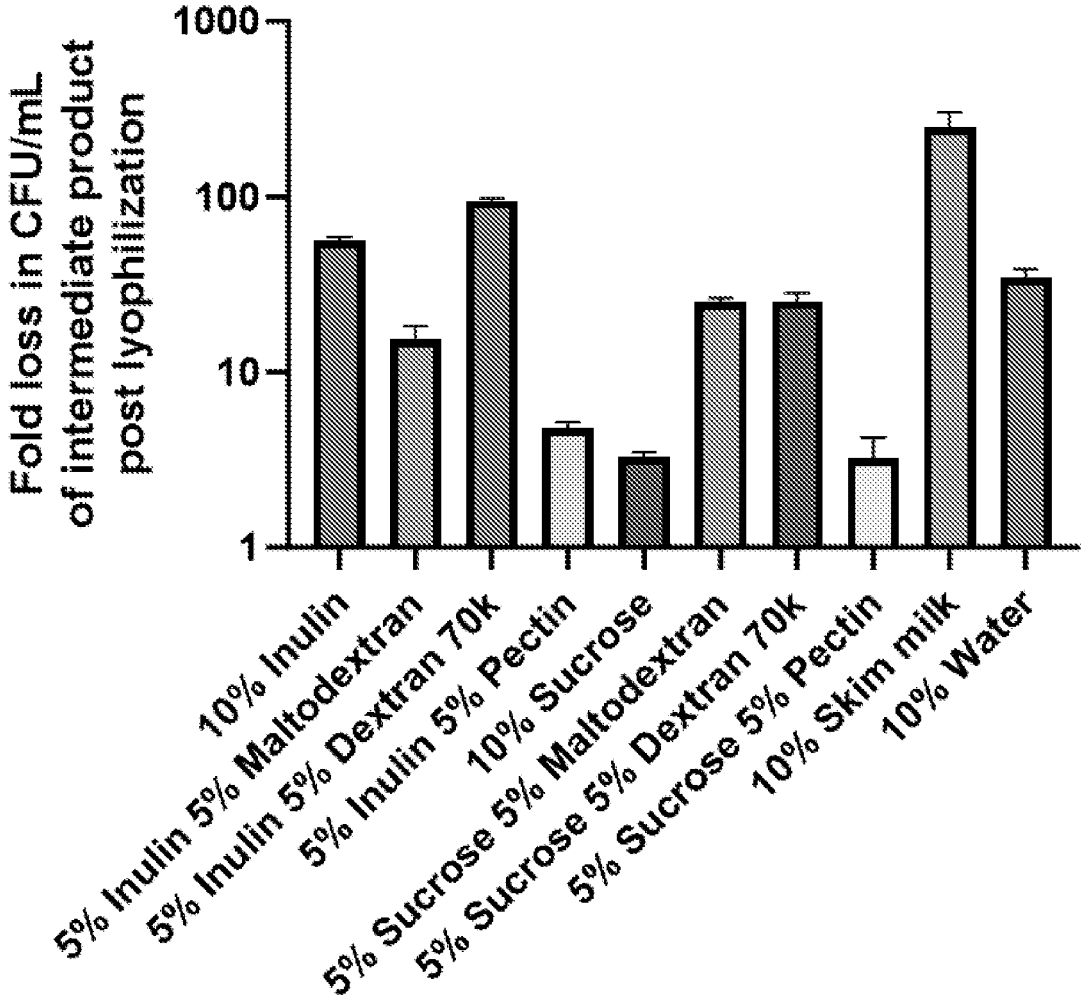

FIGURE 2

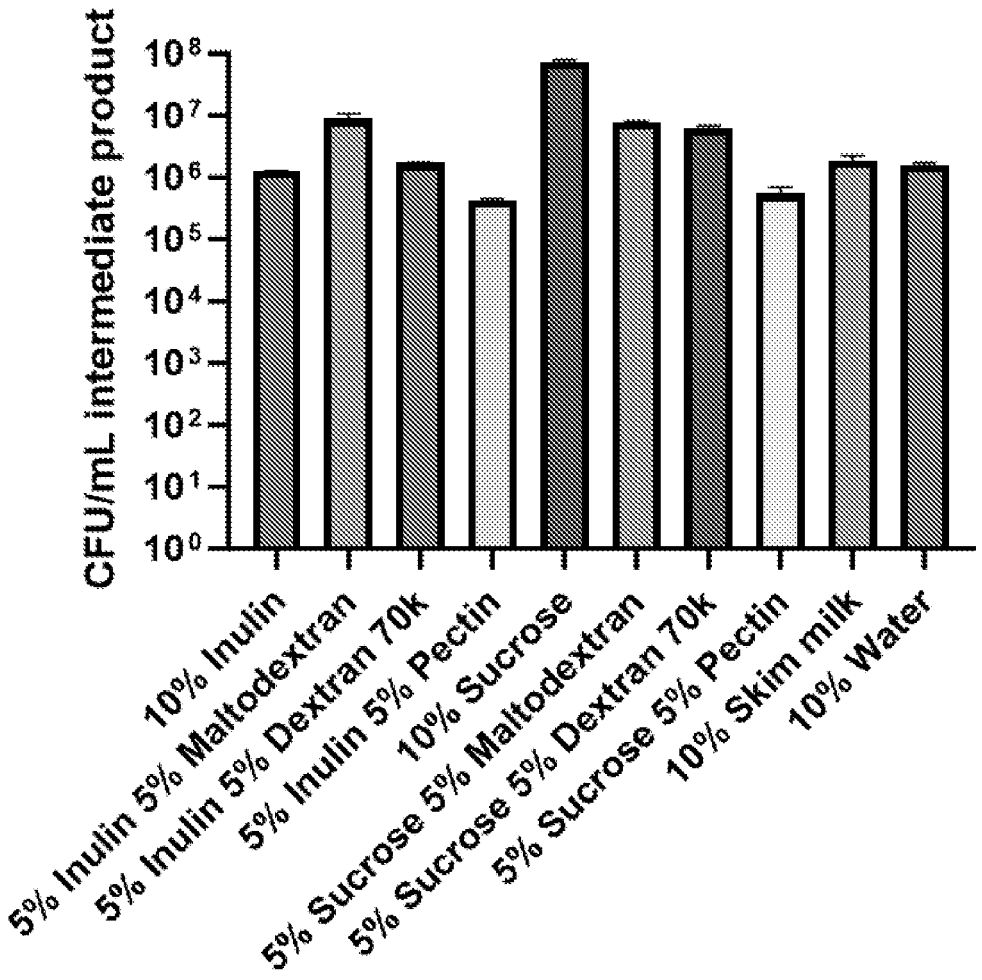
CFU/mL intermediate product
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10% Inulin
5% Inulin 5% Maltodextran
5% Inulin 5% Dextran 70k
5% Inulin 5% Pectin
10% Sucrose
5% Sucrose 5% Maltodextran
5% Sucrose 5% Dextran 70k
5% Sucrose 5% Pectin
10% Skim milk
10% Water

FIGURE 3

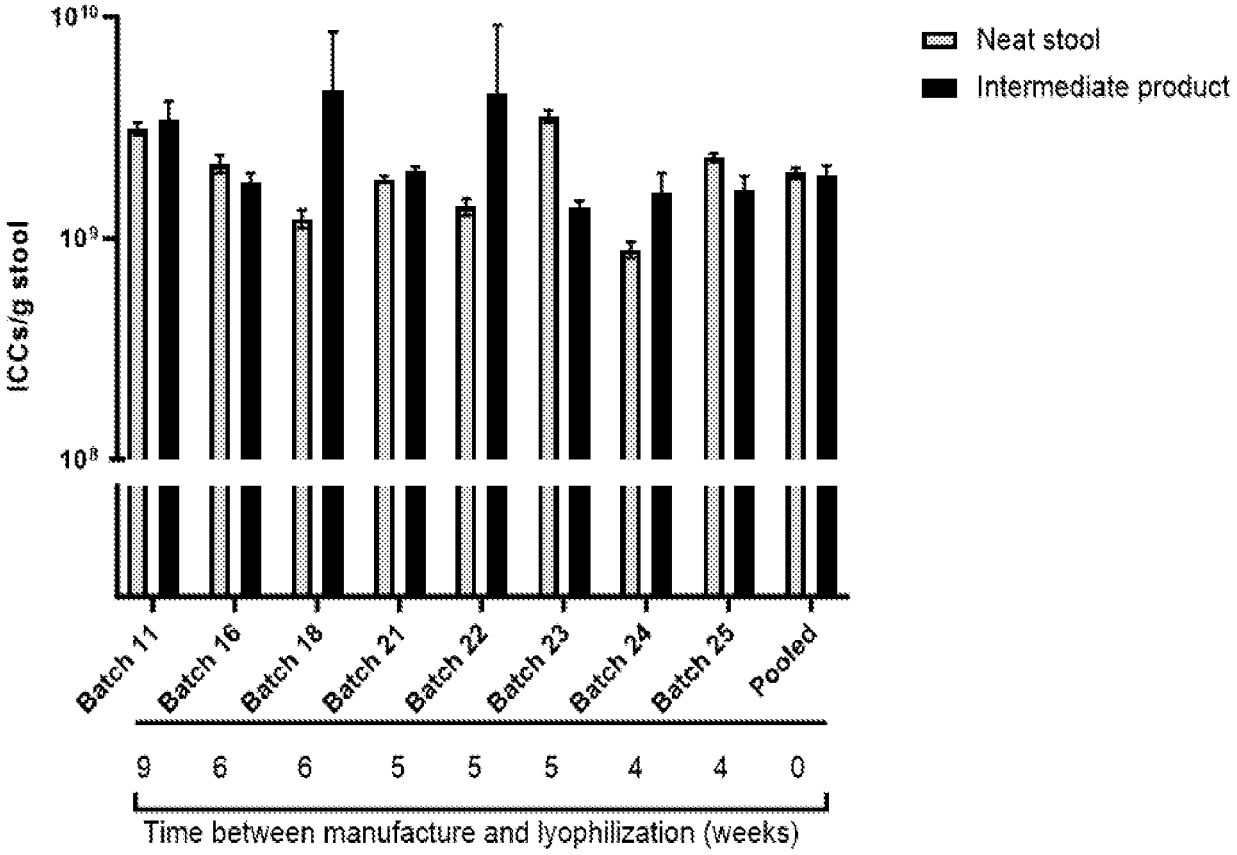

FIGURE 4

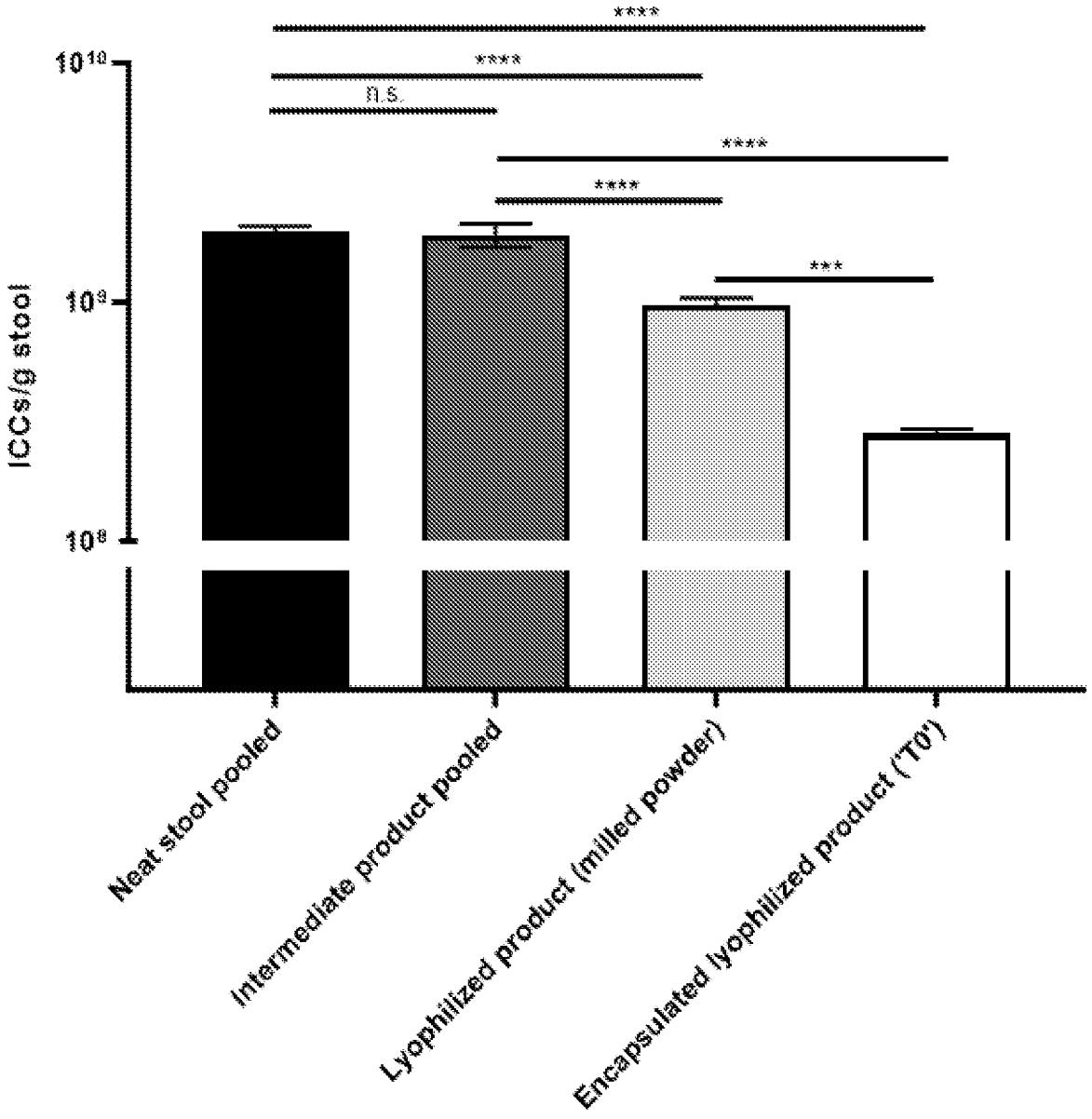
****
****
n.s.
****
****
***
10^{10}
10^{9}
10^{8}
ICCs/g stool
Neat stool pooled
Intermediate product pooled
Lyophilized product (milled powder)
Encapsulated lyophilized product ('T0')

FIGURE 5

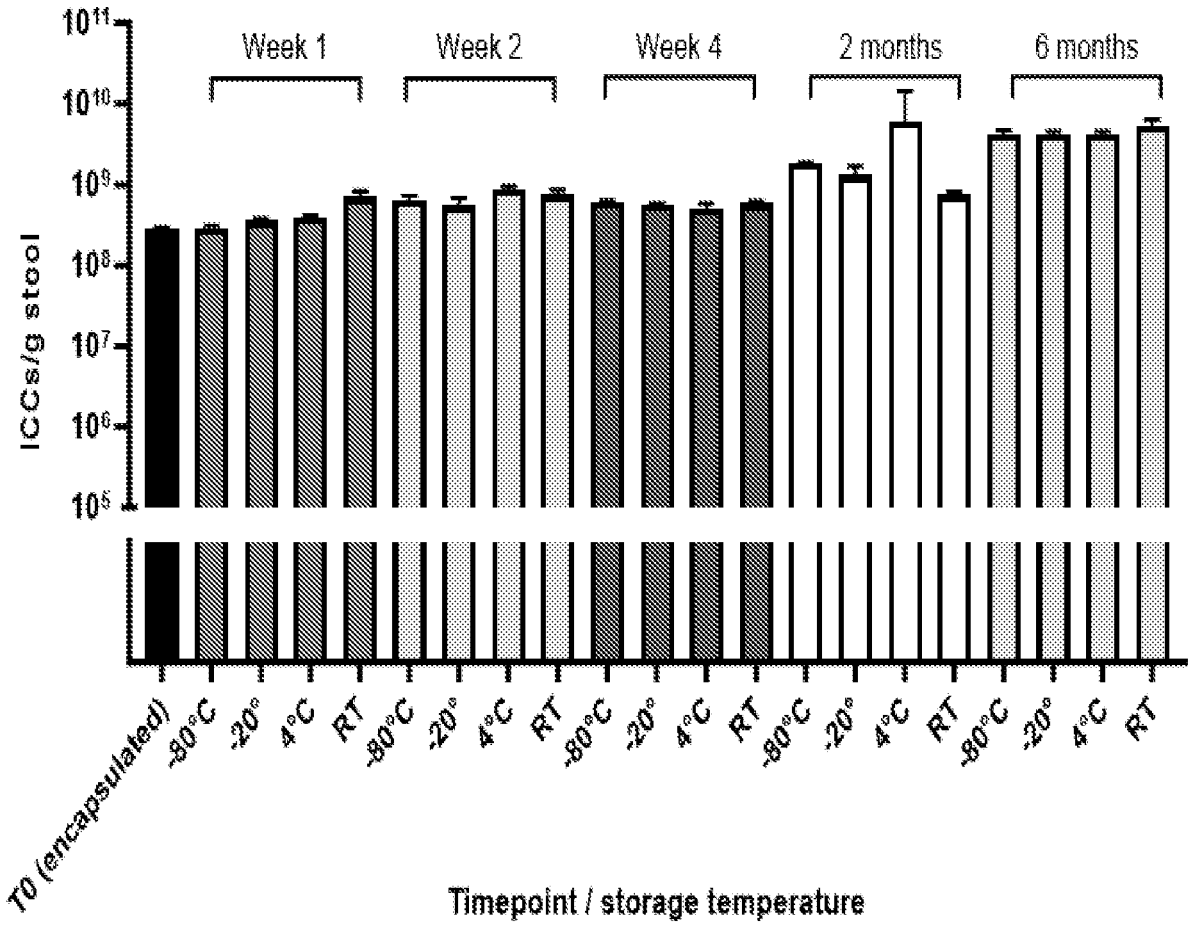

FIGURE 6