METHODS AND COMPOSITIONS RELATING TO MICROBIAL TREATMENT AND DIAGNOSIS OF DISORDERS

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/073,912, filed on October 31, 2014, which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] The body of an individual is inhabited by trillions of microbes across various locations, often referred to as microbiomes. Microbiomes can play a key role in many health conditions and diseases. Despite the interrelation between microbiomes and health, the complexity of the various microbiomes, as well as difficulties in characterizing, categorizing, and analyzing microbiome constituents has made understanding microbiomes challenging. Consequently, these challenges have presented hurdles in the development of diagnostic and therapeutic applications for microbiome-related health conditions and diseases. The present disclosure provides methods, systems, compositions, and kits to address the need for microbiome-related treatment of health conditions and disease.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on October 30, 2015, is named 46790-702.601.txt and is 36,254,267 bytes in size.

SUMMARY OF THE INVENTION

[0003a] In one aspect, provided herein is a composition comprising:

a) a first isolated and purified microbe comprising a 16S rRNA sequence comprising at least 97% sequence identity to a 16S rRNA sequence of *Akkermansia muciniphila*;

b) a second isolated and purified microbe comprising a 16S rRNA sequence comprising at least 97% sequence identity to a 16S rRNA sequence of *Eubacterium hallii*; and

a third isolated and purified microbe comprising a 16S rRNA sequence comprising at least 97% sequence identity to a 16S rRNA sequence of *Clostridium beijerinckii.*

[0003b] In one aspect, provided herein is a composition comprising a microbe comprising a 16S rRNA sequence that has at least 97% sequence identity to a 16S rRNA sequence of *Akkermansia muciniphila*, wherein the microbe has been cultured in culture medium comprising a substantially plant-based culture medium.

[0003c] In one aspect, provided herein is a use of the composition as described herein in the manufacture of a medicament for treating a metabolic disorder.

[0003d] In one aspect, provided herein is a method of producing a microbial composition, the method comprising:

a) culturing *Akkermansia muciniphila* in a culture medium comprising substantially a plant-based culture medium, thereby generating cultured Akkermansia muciniphila; and

b) formulating the cultured *Akkermansia muciniphila* into a unit dosage form, thereby producing the microbial composition.

[0003e] In one aspect, provided herein is a use of a composition in the manufacture of a medicament for treating a subject suffering from type 2 diabetes, insulin insensitivity, type 1 diabetes, or obesity, the composition comprising a first population of isolated and purified primary fermenter microbes that produces an intermediate molecule and a second population of isolated and purified secondary fermenter microbes that converts the intermediate molecule to butyrate, wherein: the first population of isolated and purified primary fermenter microbes comprises at least one microbe selected from the group consisting of *Akkermansia muciniphila, Bifidobacterium adolescentis, Bifidobacterium infantis*, and *Bifidobacterium longum*; and the second population of isolated and purified secondary fermenter microbes comprises at least one microbe selected from the group consisting of *Clostridium beijerinckii, Clostridium butyricum, Clostridium indolis, Eubacterium hallii*, and *Faecalibacterium prausnitzii.*

[0003f] In one aspect, provided herein is a composition comprising a first population of isolated and purified primary fermenter microbes that produces an intermediate molecule and a second population of isolated and purified secondary fermenter microbes that converts the intermediate molecule to butyrate, wherein: the first population of isolated and purified primary fermenter microbes comprises at least one microbe selected from the group consisting of *Akkermansia muciniphila, Bifidobacterium adolescentis, Bifidobacterium infantis*, and *Bifidobacterium longum*; and the second population of isolated and purified secondary fermenter microbes comprises at least one microbe selected from the group consisting of *Clostridium beijerinckii, Clostridium butyricum, Clostridium indolis,*

Eubacterium hallii, and *Faecalibacterium prausnitzii*, or the composition as described herein, for use in therapy.

[0004] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe that alters glucagon-like peptide-1 (GLP-1) production, and a pharmaceutically-acceptable carrier.

[0005] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe that encodes for an enzyme selected from the group consisting of: butyrate kinase, butyrate coenzyme A, butyrate coenzyme a transferase, and any combination thereof, and a pharmaceutically-acceptable carrier.

[0006] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe that is capable of producing butyrate, and a pharmaceutically-acceptable carrier.

[0007] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Akkermansia muciniphilia*, and a pharmaceutically-acceptable carrier.

[0008] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Anaerostipes caccae*, and a pharmaceutically-acceptable carrier.

[0009] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified

microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Bifidobacterium adolescentis*, and a pharmaceutically-acceptable carrier.

[0010] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Bifidobacterium bifidum*, and a pharmaceutically-acceptable carrier.

[0011] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Bifidobacterium infantis*, and a pharmaceutically-acceptable carrier.

[0012] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Bifidobacterium longum*, and a pharmaceutically-acceptable carrier.

[0013] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Butyrivibrio fibrisolvens*, and a pharmaceutically-acceptable carrier.

[0014] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Clostridium acetobutylicum*, and a pharmaceutically-acceptable carrier.

[0015] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Clostridium beijerinckii*, and a pharmaceutically-acceptable carrier.

[0016] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Clostridium butyricum*, and a pharmaceutically-acceptable carrier.

[0017] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Clostridium colinum*, and a pharmaceutically-acceptable carrier.

[0018] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Clostridium indolis*, and a pharmaceutically-acceptable carrier.

[0019] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Enterococcus faecium*, and a pharmaceutically-acceptable carrier.

[0020] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85%

sequence identity to a rRNA sequence of *Eubacterium hallii*, and a pharmaceutically-acceptable carrier.

[0021] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Eubacterium rectale*, and a pharmaceutically-acceptable carrier.

[0022] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Faecalibacterium prausnitzii*, and a pharmaceutically-acceptable carrier.

[0023] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Fibrobacter succinogenes*, and a pharmaceutically-acceptable carrier.

[0024] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Lactobacillus acidophilus*, and a pharmaceutically-acceptable carrier.

[0025] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Lactobacillus brevis*, and a pharmaceutically-acceptable carrier.

[0026] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective

amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Lactobacillus bulgaricus*, and a pharmaceutically-acceptable carrier.

[0027] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Lactobacillus casei*, and a pharmaceutically-acceptable carrier.

[0028] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Lactobacillus caucasicus*, and a pharmaceutically-acceptable carrier.

[0029] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Lactobacillus fermentum*, and a pharmaceutically-acceptable carrier.

[0030] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Lactobacillus helveticus*, and a pharmaceutically-acceptable carrier.

[0031] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Lactobacillus lactis*, and a pharmaceutically-acceptable carrier.

[0032] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Lactobacillus plantarum*, and a pharmaceutically-acceptable carrier.

[0033] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Lactobacillus reuteri*, and a pharmaceutically-acceptable carrier.

[0034] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Lactobacillus rhamnosus*, and a pharmaceutically-acceptable carrier.

[0035] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Roseburia cecicola*, and a pharmaceutically-acceptable carrier.

[0036] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Roseburia inulinivorans*, and a pharmaceutically-acceptable carrier.

[0037] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85%

sequence identity to a rRNA sequence of *Ruminococcus flavefaciens*, and a pharmaceutically-acceptable carrier.

[0038] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Ruminococcus gnavus*, and a pharmaceutically-acceptable carrier.

[0039] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Streptococcus cremoris*, and a pharmaceutically-acceptable carrier.

[0040] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Streptococcus faecium*, and a pharmaceutically-acceptable carrier.

[0041] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Streptococcus infantis*, and a pharmaceutically-acceptable carrier.

[0042] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Streptococcus mutans*, and a pharmaceutically-acceptable carrier.

[0043] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective

amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Streptococcus thermophilus*, and a pharmaceutically-acceptable carrier.

[0044] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Clostridium aminophilum*, and a pharmaceutically-acceptable carrier.

[0045] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Clostridium orbiscindens*, and a pharmaceutically-acceptable carrier.

[0046] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Oscillospira guilliermondii*, and a pharmaceutically-acceptable carrier.

[0047] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Ruminococcus obeum*, and a pharmaceutically-acceptable carrier.

[0048] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Anaerofustis stercorihominis*, and a pharmaceutically-acceptable carrier.

[0049] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Anaerostipes hadrus*, and a pharmaceutically-acceptable carrier.

[0050] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Anaerotruncus colihominis*, and a pharmaceutically-acceptable carrier.

[0051] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Clostridium sporogenes*, and a pharmaceutically-acceptable carrier.

[0052] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Clostridium tetani*, and a pharmaceutically-acceptable carrier.

[0053] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Coprococcus*, and a pharmaceutically-acceptable carrier.

[0054] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85%

sequence identity to a rRNA sequence of *Coprococcus eutactus*, and a pharmaceutically-acceptable carrier.

[0055] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Eubacterium cylindroides*, and a pharmaceutically-acceptable carrier.

[0056] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Eubacterium dolichum*, and a pharmaceutically-acceptable carrier.

[0057] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Eubacterium ventriosum*, and a pharmaceutically-acceptable carrier.

[0058] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Roseburia faeccis*, and a pharmaceutically-acceptable carrier.

[0059] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Roseburia hominis*, and a pharmaceutically-acceptable carrier.

[0060] In some examples, this disclosure comprises a method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective

amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of *Roseburia intestinalis*, and a pharmaceutically-acceptable carrier.

[0061] A method of treating a metabolic disorder in a subject in need thereof, the method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising a population of isolated and purified microbe, wherein at least one of said microbes comprises a microbe with at least about 85% sequence identity to a rRNA sequence of a microbe selected from the group consisting of: *Akkermansia muciniphila, Anaerostipes caccae, Bifidobacterium adolescentis, Bifidobacterium bifidum , Bifidobacterium infantis, Bifidobacterium longum, Butyrivibrio fibrisolvens, Clostridium acetobutylicum, Clostridium aminophilum, Clostridium beijerinckii, Clostridium butyricum, Clostridium colinum, Clostridium indolis, Clostridium orbiscindens, Enterococcus faecium, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Fibrobacter succinogenes, Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus caucasicus, Lactobacillus fermentum, Lactobacillus helveticus, Lactobacillus lactis, Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus rhamnosus, Oscillospira guilliermondii, Roseburia cecicola, Roseburia inulinivorans, Ruminococcus flavefaciens, Ruminococcus gnavus, Ruminococcus obeum, Streptococcus cremoris, Streptococcus faecium, Streptococcus infantis, Streptococcus mutans, Streptococcus thermophilus, Anaerofustis stercorihominis*, *Anaerostipes hadrus*, *Anaerotruncus colihominis*, *Clostridium sporogenes*, *Clostridium tetani*, *Coprocoaccus*, *Coprococcus eutactus*, *Eubacterium cylindroides*, *Eubacterium dolichum*, *Eubacterium ventriosum*, *Roseburia faeccis*, *Roseburia hominis*, *Roseburia intestinalis*, and any combination thereof.

[0062] The method of any of the preceding examples, wherein said treating results in a subject with an altered microbiome.

[0063] The method of any of the preceding examples, wherein said treating results in a subject with an altered gut microbiome.

[0064] The method of any of the preceding examples, wherein the pharmaceutical composition further comprises a second population of isolated and purified microbe. In some examples, the method may further comprise a second population of isolated and purified microbe, wherein said second population comprises a microbe with at least about 85% sequence identity to a rRNA sequence of a microbe selected from the group consisting of:

Akkermansia muciniphila, Anaerostipes caccae, Bifidobacterium adolescentis, Bifidobacterium bifidum , Bifidobacterium infantis, Bifidobacterium longum, Butyrivibrio fibrisolvens, Clostridium acetobutylicum, Clostridium aminophilum, Clostridium beijerinckii, Clostridium butyricum, Clostridium colinum, Clostridium indolis, Clostridium orbiscindens, Enterococcus faecium, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Fibrobacter succinogenes, Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus caucasicus, Lactobacillus fermentum, Lactobacillus helveticus, Lactobacillus lactis, Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus rhamnosus, Oscillospira guilliermondii, Roseburia cecicola, Roseburia inulinivorans, Ruminococcus flavefaciens, Ruminococcus gnavus, Ruminococcus obeum, Streptococcus cremoris, Streptococcus faecium, Streptococcus infantis, Streptococcus mutans, Streptococcus thermophilus, Anaerofustis stercorihominis, *Anaerostipes hadrus*, *Anaerotruncus colihominis*, *Clostridium sporogenes*, *Clostridium tetani*, *Coprococcus*, *Coprococcus eutactus*, *Eubacterium cylindroides*, *Eubacterium dolichum*, *Eubacterium ventriosum*, *Roseburia faeccis*, *Roseburia hominis*, *Roseburia intestinalis*, and any combination thereof.

[0065] The method of any of the preceding examples, wherein said metabolic disorder is obesity.

[0066] The method of any of the preceding examples, wherein said metabolic disorder is insulin insensitivity.

[0067] The method of any of the preceding examples, wherein said metabolic disorder is Type 2 Diabetes Mellitus.

[0068] The method of any of the preceding examples, wherein said treating results in the subject losing weight as compared to a pre-treatment level.

[0069] The method of any of the preceding examples, wherein said treating results in the subject having increased insulin sensitivity as compared to a pre-treatment level.

[0070] The method of any of the preceding examples, wherein said treating results in the subject having reduced symptoms associated with the metabolic disorder as compared to a pre-treatment level.

[0071] The method of any of the preceding examples, wherein said subject is a subject enrolled in a clinical study.

[0072] The method of any of the preceding examples, wherein said at least about 85% sequence identity is selected from the group consisting of: at least about 96%, at least about

97%, at least about 98%, at least about 99%, at least about 99.5%, and at least about 99.5% sequence identity to a rRNA sequence.

[0073] The method of any of the preceding examples, wherein said pharmaceutical composition is substantially free of fecal matter obtained from a subject.

[0074] The method of any of the preceding examples, wherein said at least one of said microbes comprises a population of said microbes.

[0075] The method of any of the preceding examples, wherein said rRNA sequence is a 16S rRNA sequence.

[0076] The method of any of the preceding examples, wherein said rRNA sequence is a 23S rRNA sequence.

[0077] The method of any of the preceding examples, wherein said rRNA sequence is both a 16S rRNA sequence and a 23S rRNA sequence.

[0078] The method of any of the preceding examples, wherein the pharmaceutical composition is formulated for oral delivery.

[0079] The method of any of the preceding examples, wherein the pharmaceutical composition is formulated for anal delivery.

[0080] The method of any of the preceding examples, wherein the pharmaceutical composition is formulated as a pill.

[0081] The method of any of the preceding examples, wherein the pharmaceutical composition is formulated as a capsule.

[0082] The method of any of the preceding examples, wherein the pharmaceutical composition is formulated in a liquid form suitable for administration via an enema.

[0083] The method of any of the preceding examples, wherein the pharmaceutical composition is formulated as a suppository.

[0084] The method of any of the preceding examples, wherein the pharmaceutical composition is formulated in a liquid form suitable for delivery via injection.

[0085] The method of any of the preceding examples, wherein the pharmaceutical composition further comprises a probiotic. In some examples, the pharmaceutical composition may further comprise a probiotic, said probiotic is selected from the group consisting of: *Akkermansia muciniphila, Anaerostipes caccae, Bifidobacterium adolescentis, Bifidobacterium bifidum , Bifidobacterium infantis, Bifidobacterium longum, Butyrivibrio fibrisolvens, Clostridium acetobutylicum, Clostridium aminophilum, Clostridium beijerinckii, Clostridium butyricum, Clostridium colinum, Clostridium indolis, Clostridium orbiscindens, Enterococcus faecium, Eubacterium hallii, Eubacterium rectale, Faecalibacterium*

prausnitzii, Fibrobacter succinogenes, Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus caucasicus, Lactobacillus fermentum, Lactobacillus helveticus, Lactobacillus lactis, Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus rhamnosus, Oscillospira guilliermondii, Roseburia cecicola, Roseburia inulinivorans, Ruminococcus flavefaciens, Ruminococcus gnavus, Ruminococcus obeum, Streptococcus cremoris, Streptococcus faecium, Streptococcus infantis, Streptococcus mutans, Streptococcus thermophilus, Anaerofustis stercorihominis, *Anaerostipes hadrus*, *Anaerotruncus colihominis*, *Clostridium sporogenes*, *Clostridium tetani*, *Coprococcus*, *Coprococcus eutactus*, *Eubacterium cylindroides*, *Eubacterium dolichum*, *Eubacterium ventriosum*, *Roseburia faeccis*, *Roseburia hominis*, *Roseburia intestinalis*, and any combination thereof.

[0086] The method of any of the preceding examples, wherein the pharmaceutical composition further comprises a prebiotic. In some examples, the pharmaceutical composition may further comprise a prebiotic, said prebiotic is selected from the group consisting of: complex carbohydrates, complex sugars, resistant dextrins, resistant starch, amino acids, peptides, nutritional compounds, biotin, polydextrose, fructooligosaccharide (FOS), galactooligosaccharides (GOS), inulin, starch, lignin, psyllium, chitin, chitosan, gums (e.g. guar gum), high amylose cornstarch (HAS), cellulose, β-glucans, hemi-celluloses, lactulose, mannooligosaccharides, mannan oligosaccharides (MOS), oligofructose-enriched inulin, oligofructose, oligodextrose, tagatose, trans-galactooligosaccharide, pectin, resistant starch, xylooligosaccharides (XOS), and any combination thereof. In some examples, said prebiotic is inulin.

[0087] The method of any of the preceding examples, wherein the pharmaceutical composition is co-administered with an antibiotic.

[0088] The method of any of the preceding examples, wherein the pharmaceutical composition is administered after an antibiotic. In some examples, the method may comprise administering the pharmaceutical composition after an antibiotic, wherein the pharmaceutical composition is administered at least one hour after an antibiotic. In some examples, the method may comprise administering the pharmaceutical composition after an antibiotic, wherein the pharmaceutical composition is administered at least 2 hours after an antibiotic. In some examples, the method may comprise administering the pharmaceutical composition after an antibiotic, wherein the pharmaceutical composition is administered at least 12 hours after an antibiotic. In some examples, the method may comprise administering the pharmaceutical composition after an antibiotic, wherein the pharmaceutical composition is

administered at least 1 day after an antibiotic. In some examples, the method may comprise administering the pharmaceutical composition after an antibiotic, wherein the pharmaceutical composition is administered at least 1 week after an antibiotic. In some examples, the method may comprise administering the pharmaceutical composition after an antibiotic, wherein the pharmaceutical composition is administered at least 2 weeks after an antibiotic.

[0089] The method of any of the preceding examples, wherein the pharmaceutical composition is administered after completion of an antibiotic regimen by the subject.

[0090] The method of any of the preceding examples, wherein the pharmaceutical composition is formulated as a dietary supplement.

[0091] The method of any of the preceding examples, wherein the pharmaceutical composition is a biologic product.

[0092] The method of any of the preceding examples, further comprising determining the sequence of a population of the subject's microbiome by sequencing. In some examples, the method may further comprise determining the sequence of the subject's microbiome by sequencing, said sequencing comprises sequencing the 16S rRNA. In some examples, the method may further comprise determining the sequence of the subject's microbiome by sequencing, said sequencing comprises sequencing the 23S rRNA. In some examples, the method may further comprise determining the sequence of the subject's microbiome by sequencing, said sequencing comprises sequencing the 23S and 16S rRNA. In some examples, the method may further comprise determining the sequence of the subject's microbiome by sequencing, said sequencing comprises Complete Biome Test resolution. In some examples, said sequencing comprises long-read sequencing. In some examples, the method may further comprise determining the sequence of the subject's microbiome by sequencing,

wherein the determining the sequence of the population of the subject's microbiome is performed before treating the subject with the pharmaceutical composition. In some examples, the method may further comprise determining the sequence of the subject's microbiome by sequencing, wherein the determining the sequence of the population of the subject's microbiome is performed after treating the subject with the pharmaceutical composition.

[0093] The method of any of the preceding examples, further comprising transmitting data via machine-readable code.

[0094] The method of any of the preceding examples, further comprising computing data via machine-readable code.

[0095] The method of any of the preceding examples, further comprising storing data via machine-readable code.

[0096] The method of any of the preceding examples, wherein the subject is a mammal.

[0097] The method of any of the preceding examples, wherein the subject is a laboratory mammal.

[0098] The method of any of the preceding examples, wherein the subject is a human.

[0099] The method of any of the preceding examples, wherein said method further comprises a companion diagnostic.

[00100] A method of producing the microbes of any of the preceding examples, the method comprising genetically-modifying the microbes to generate recombinant microbes. In some examples, the method may comprise genetically-modifying the microbes to generate recombinant microbes, wherein an operon controls growth of the recombinant microbe.

[00101] The method of any of the preceding examples, wherein the pharmaceutical composition is formulated as an enteric-coated pill. In some examples, the method may comprise formulating the pharmaceutical composition as an enteric-coated pill, wherein the enteric-coating is formed by a pH sensitive polymer. In some examples, the method may comprise formulating the pharmaceutical composition as an enteric-coated pill, wherein the enteric-coating is formed by a pH sensitive polymer, wherein the polymer is eudragit FS30D.

[00102] The method of any of the preceding examples, wherein the pharmaceutical composition is formulated for delivery of the microbes to the subject's ileum region.

[00103] The method of any of the preceding examples, wherein the pharmaceutical composition is formulated for delivery of the microbes to the subject's colon region.

[00104] The method of any of the preceding examples, wherein the pharmaceutical composition is formulated for delivery of the microbes to the subject's ileum and colon region.

[00105] The method of any of the preceding examples, wherein the pharmaceutical composition is delivered to the subject's ileum region.

[00106] The method of any of the preceding examples, wherein the pharmaceutical composition is delivered to the subject's colon region.

[00107] The method of any of the preceding examples, wherein the pharmaceutical composition is delivered to the subject's ileum and colon region.

[00108] The method of any of the preceding examples, wherein the pharmaceutical composition is administered before food intake. In some examples, the method may comprise administering the pharmaceutical composition before food intake, wherein the pharmaceutical composition is administered at least one hour before food intake. In some examples, the method may comprise administering the pharmaceutical composition before food intake, wherein the pharmaceutical composition is administered at least 2 hours before food intake. In some examples, the method may comprise administering the pharmaceutical composition before food intake, wherein the pharmaceutical composition is administered at least 3 hours before food intake. In some examples, the method may comprise administering the pharmaceutical composition before food intake, wherein the pharmaceutical composition is administered at least 4 hours before food intake.

[00109] The method of any of the preceding examples, wherein said microbes are administered with food intake.

[00110] The method of any of the preceding examples, wherein said microbes comprise a synergistic stability in the pharmaceutical composition as compared to individual strains.

CLAIMS

WHAT IS CLAIMED IS:

1. A composition comprising:
    a) a first isolated and purified microbe comprising a 16S rRNA sequence comprising at least 97% sequence identity to a 16S rRNA sequence of *Akkermansia muciniphila*;
    b) a second isolated and purified microbe comprising a 16S rRNA sequence comprising at least 97% sequence identity to a 16S rRNA sequence of *Eubacterium hallii*; and
    c) a third isolated and purified microbe comprising a 16S rRNA sequence comprising at least 97% sequence identity to a 16S rRNA sequence of *Clostridium beijerinckii*.

2. The composition of claim 1, wherein the first isolated and purified microbe is the *Akkermansia muciniphila*, the second isolated and purified microbe is the *Eubacterium hallii*, and the isolated and purified third microbe is the *Clostridium beijerinckii*.

3. The composition of claim 1 or claim 2, wherein the composition comprises a therapeutically-effective amount of the first isolated and purified microbe, the second isolated and purified microbe, and the third isolated and purified microbe.

4. The composition of any one of claims 1-3, further comprising *Clostridium butyricum*.

5. The composition of any one of claims 1-4, further comprising *Bifidobacterium infantis*.

6. The composition of any one of claims 1-5, wherein the composition is formulated for oral delivery.

7. The composition of any one of claims 1-6, wherein the composition is in a powdered form.

8. The composition of any one of claims 1-7, wherein the composition comprises an enteric coating.

9. The composition of any one of claims 1-8, wherein the composition is formulated as a pill, tablet, capsule, or liquid capsule.

10. The composition of any one of claims 1-6, wherein the composition is in a form of a food or a drink.

11. The composition of any one of claims 1-10, further comprises a prebiotic.

12. The composition of claim 11, wherein the prebiotic is inulin.

13. The composition of claim 11, wherein the prebiotic comprises a complex carbohydrate, complex sugar, resistant dextrin, resistant starch, amino acid, peptide, nutritional compound, biotin, polydextrose, fructooligosaccharide (FOS), galactooligosaccharides (GOS), starch, lignin, psyllium, chitin, chitosan, gum, high amylose cornstarch (HAS), cellulose, β-glucan, hemi-cellulose, lactulose, mannooligosaccharide, mannan oligosaccharide (MOS), oligofructose-enriched inulin, oligofructose, oligodextrose, tagatose, trans-galactooligosaccharide, pectin, xylooligosaccharides (XOS), or any combination thereof.

14. A composition comprising a microbe comprising a 16S rRNA sequence that has at least 97% sequence identity to a 16S rRNA sequence of *Akkermansia muciniphila*, wherein the microbe has been cultured in culture medium comprising a substantially plant-based culture medium.

15. The composition of claim 14, wherein the substantially plant-based culture medium comprises yeast extract, a plant-derived peptone, a plant-derived hydrolysate, or a combination thereof.

16. The composition of claim 14 or claim 15, wherein the substantially plant-based culture medium comprises one or more vitamins, one or more salts, a buffer, or a combination thereof

17. The composition of claim 14, wherein the substantially plant-based culture medium comprises PYGveg, one or more vitamins, one or more salts, and a buffer.

18. The composition of any one of claims 1-17, wherein the composition comprises at least 1 x 10^5 CFUs of the *Akkermansia muciniphila*.

19. Use of the composition of any one of claims 1-18 in the manufacture of a medicament for treating a metabolic disorder.

20. The use of claim 19, wherein the metabolic disorder comprises insulin resistance.

21. The use of claim 19, wherein the metabolic disorder comprises type 2 diabetes.

22. The use of claim 19, wherein the metabolic disorder comprises type 1 diabetes.

23. The use of claim 19, wherein the metabolic disorder comprises obesity.

24. The use of any one of claims 19-23, wherein the composition is to be administered before, after, or concurrent to an antibiotic regimen.

25. The use of any one of claims 19-24, wherein the composition lowers blood glucose levels in a subject that is administered the composition.

26. A method of producing a microbial composition, the method comprising:
    a) culturing *Akkermansia muciniphila* in a culture medium comprising substantially a plant-based culture medium, thereby generating cultured Akkermansia muciniphila; and
    b) formulating the cultured *Akkermansia muciniphila* into a unit dosage form, thereby producing the microbial composition.

27. The method of claim 26, wherein the microbial composition is suitable for oral administration.

28. The method of claim 26 or claim 27, wherein the substantially plant-based culture medium comprises yeast extract, a plant-derived peptone, a plant-derived hydrolysate, or a combination thereof.

29. The method of any one of claims 26-28, wherein the culture medium comprises one or more vitamins, one or more salts, a buffer, or a combination thereof

30. The method of any one of claims 26-28, wherein the culture medium comprises PYGveg, one or more vitamins, one or more salts, and a buffer.

31. The method of any one of claims 26-30, wherein the unit dosage form further comprises a second microbe that comprises a 16S rRNA sequence comprising at least 97% sequence identity to a 16S rRNA sequence of *Eubacterium hallii*.

32. The method of claim 31, wherein the second microbe is the *Eubacterium hallii*.

33. Use of a composition in the manufacture of a medicament for treating a subject suffering from type 2 diabetes, insulin insensitivity, type 1 diabetes, or obesity, the composition comprising a first population of isolated and purified primary fermenter microbes that produces an intermediate molecule and a second population of isolated and purified secondary fermenter microbes that converts the intermediate molecule to butyrate, wherein:

    the first population of isolated and purified primary fermenter microbes comprises at least one microbe selected from the group consisting of *Akkermansia muciniphila, Bifidobacterium adolescentis, Bifidobacterium infantis*, and *Bifidobacterium longum*; and

    the second population of isolated and purified secondary fermenter microbes comprises at least one microbe selected from the group consisting of *Clostridium beijerinckii, Clostridium butyricum, Clostridium indolis, Eubacterium hallii*, and *Faecalibacterium prausnitzii*.

34. A composition comprising a first population of isolated and purified primary fermenter microbes that produces an intermediate molecule and a second population of isolated and purified secondary fermenter microbes that converts the intermediate molecule to butyrate, wherein:

the first population of isolated and purified primary fermenter microbes comprises at least one microbe selected from the group consisting of *Akkermansia muciniphila, Bifidobacterium adolescentis, Bifidobacterium infantis*, and *Bifidobacterium longum*; and

the second population of isolated and purified secondary fermenter microbes comprises at least one microbe selected from the group consisting of *Clostridium beijerinckii, Clostridium butyricum, Clostridium indolis, Eubacterium hallii*, and *Faecalibacterium prausnitzii*, or the composition of any one of claims 1-18, for use in therapy.