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(71) Applicant: SERES HEALTH, INC. [US/US]; 161 First Street, Suite 1A, Cambridge, MA 02142 (US).

(72) Inventors: VON MALTZAHN, Geoffrey; c/o Seres Health, Inc., 161 First Street, Suite 1A, Cambridge, MA 02142 (US). HENN, Matthew, R.; c/o Seres Health, Inc., 161 First Street, Suite 1A, Cambridge, MA 02142 (US). COOK, David, N.; c/o Seres Health, Inc., 161 First Street, Suite 1A, Cambridge, MA 02142 (US). BARRY, David, A.; c/o Seres Health, Inc., 161 First Street, Suite 1A, Cambridge, MA 02142 (US). AFEYAN, Noubar, B.; c/o Seres Health, Inc., 161 First Street, Suite 1A, Cambridge, MA 02142 (US). GOODMAN, Brian; c/o Seres Health, Inc., 161 First Street, Suite 1A, Cambridge, MA 02142 (US). LOMBARDO, Mary-Jane; c/o Seres Health, Inc., 161 First Street, Suite 1A, Cambridge, MA 02142 (US).

VULIC, Marin; c/o Seres Health, Inc., 161 First Street, Suite 1A, Cambridge, MA 02142 (US).

(74) Agents: HUBL, Susan, T. et al.; Fenwick & West LLP, Silicon Valley Center, 801 California Street, Mountain View, CA 94041 (US).

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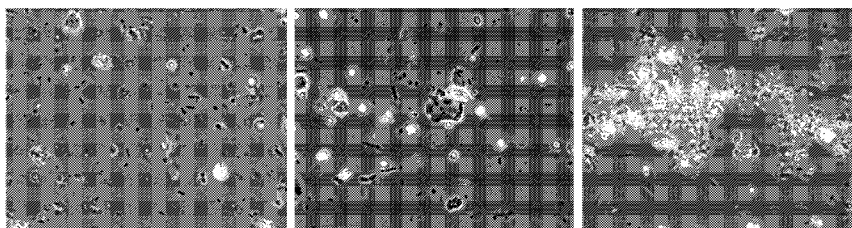


FIG. 3

(57) Abstract: Methods and compositions for populating the gastrointestinal tract of a subject are described. Methods include administering to a subject a therapeutic composition comprising a purified population of spore-forming bacteria produced by providing a fecal material and subjecting the material to a treatment step resulting in purification of spore-forming bacteria. Methods include administering an amount effective to engraft and/or augment in the gastrointestinal tract in order to treat or prevent a dysbiosis in the mammalian subject.

TITLE

[001] Methods of Populating a Gastrointestinal Tract

RELATED APPLICATIONS

[002] This application is related to U.S. Provisional Application No. 61/760,584, filed on Feb. 4, 2013, and to U.S. Provisional Application No. 61/760,585, filed on Feb. 4, 2013, and to U.S. Provisional Application No. 61/760,574, filed on Feb. 4, 2013, and to U.S. Provisional Application No. 61/760,606, filed on Feb. 4, 2013, and to U.S. Provisional Application No. 61/926,928, filed on Jan. 13, 2014, which are each incorporated by reference in its entirety.

REFERENCE TO A SEQUENCE LISTING

[003] This application includes a Sequence Listing submitted electronically as a text file named 25967PCT_CRF_sequencelisting.txt, created on February 4, 2013, with a size of 911,074 bytes. The sequence listing is incorporated by reference.

BACKGROUND

[004] Mammals are colonized by microbes in the gastrointestinal (GI) tract, on the skin, and in other epithelial and tissue niches such as the oral cavity, eye surface and vagina. The gastrointestinal tract harbors an abundant and diverse microbial community. It is a complex system, providing an environment or niche for a community of many different species or organisms, including diverse strains of bacteria. Hundreds of different species may form a commensal community in the GI tract in a healthy person, and this complement of organisms evolves from the time of birth to ultimately form a functionally mature microbial population by about 3 years of age. Interactions between microbial strains in these populations and between microbes and the host, *e.g.*, the host immune system, shape the community

structure, with availability of and competition for resources affecting the distribution of microbes. Such resources may be food, location and the availability of space to grow or a physical structure to which the microbe may attach. For example, host diet is involved in shaping the GI tract flora.

[005] A healthy microbiota provides the host with multiple benefits, including colonization resistance to a broad spectrum of pathogens, essential nutrient biosynthesis and absorption, and immune stimulation that maintains a healthy gut epithelium and an appropriately controlled systemic immunity. In settings of 'dysbiosis' or disrupted symbiosis, microbiota functions can be lost or deranged, resulting in increased susceptibility to pathogens, altered metabolic profiles, or induction of proinflammatory signals that can result in local or systemic inflammation or autoimmunity. Thus, the intestinal microbiota plays a significant role in the pathogenesis of many diseases and disorders, including a variety of pathogenic infections of the gut. For instance, subjects become more susceptible to pathogenic infections when the normal intestinal microbiota has been disturbed due to use of broad-spectrum antibiotics. Many of these diseases and disorders are chronic conditions that significantly decrease a subject's quality of life and can be ultimately fatal.

[006] Manufacturers of probiotics have asserted that their preparations of bacteria promote mammalian health by preserving the natural microflora in the GI tract and reinforcing the normal controls on aberrant immune responses. See, e.g., U.S. Patent No. 8,034,601. Probiotics, however, have been limited to a very narrow group of genera and a correspondingly limited number of species; as such, they do not adequately replace the missing natural microflora of the GI tract in many situations.

[007] Thus, there is a need for methods of populating a subject's gastrointestinal tract with a wider selection of microbiota in order to alter a dysbiosis. There is also a need to design microbial compositions of isolated bacterial strains with a plurality of beneficial properties based on current understanding of bacterial strains and analysis of the properties that would enhance the utility and commercialization of a microbial composition.

[008] Therefore, there exists a need for durable, efficient, and effective compositions and methods for treatment of GI diseases by way of restoring or enhancing microbiota functions and for addressing these and other shortcomings in the art.

SUMMARY OF THE INVENTION

[009] Disclosed herein are methods of populating the gastrointestinal tract of a mammalian subject, including the step of orally administering to the mammalian subject an effective amount of a therapeutic composition comprising a purified population of spore-forming bacteria produced by the steps of a) providing a fecal material and b) subjecting the material to a treatment step resulting in purification of spore-forming bacteria, wherein the purified population is present in an amount effective to engraft and/or augment in the gastrointestinal tract in order to treat or prevent a dysbiosis in the mammalian subject.

[010] Disclosed herein are methods for populating the gastrointestinal tract of a mammalian subject, comprising the step of orally administering to the subject an effective amount of a therapeutic composition comprising a purified population of spore-forming bacteria in an amount effective to engraft and/or augment in the gastrointestinal tract in order to treat or prevent a dysbiosis in a mammalian subject.

[011] Also described are therapeutic compositions comprising a purified population of spore-forming bacteria or bacteria having a substantial activity of spore-forming bacteria in an amount effective to populate the gastrointestinal tract of a mammalian subject to whom the therapeutic composition is administered. In some embodiments, the population is effective to i) treat a gastrointestinal dysbiosis, and/or ii) engraft at least one type of bacteria present in the therapeutic composition but not present in a mammalian subject prior to treatment; and/or iii) augment at least one type of bacteria not present in the therapeutic composition in a mammalian subject to whom the therapeutic composition is administered.

[012] Also provided are therapeutic compositions comprising a purified population of spore-forming bacteria in an amount effective to populate the gastrointestinal tract of a mammalian subject to whom the therapeutic composition is administered, wherein the purified population is obtained by the steps of a) providing a fecal material comprising spore-forming bacteria and b) subjecting the spore-forming bacteria to a treatment step resulting in purification of the spore-forming bacteria.

[013] Also provided are methods for production of a composition comprising a population of bacterial spores suitable for therapeutic administration to a mammalian subject in need thereof, comprising the steps of: (a) providing a fecal material obtained from a mammalian donor subject; and (b) subjecting the fecal material to at least one purification step under conditions such that a purified population of spore-forming bacteria is produced from the fecal material. Also provided are methods of treating or preventing a dysbiosis in a human subject, comprising administering to the human subject the therapeutic compositions of the invention.

[014] The invention also includes therapeutic compositions comprising a purified bacterial spore population comprising at least about 1×10^3 , 1×10^4 , 1×10^5 , or 1×10^6 spores, wherein the composition does not exceed about 1 gram in weight, formulated for oral administration to populate the gastrointestinal tract in a mammalian subject in need thereof, in order to i) engraft in the mammalian recipient and/or ii) augment a bacterial population in the mammalian recipient and thereby treat or prevent a dysbiosis.

[015] The invention includes kits comprising in one or more containers a fecal material collection apparatus and a solvent solution, and instructions for use thereof for generating purified populations of spore-forming bacteria.

[016] The invention includes methods of populating the gastrointestinal tract of a human subject, comprising the step of administering to the human subject a therapeutic composition comprising a purified population of spore-forming bacteria, under conditions such that a microbial population present in the gastrointestinal tract and/or a microbial population outside the gastrointestinal tract is modulated.

[017] The invention also includes methods of populating a bacterial population in the gastrointestinal tract of a human subject, comprising the step of administering to the human subject a therapeutic composition comprising a purified population of spore-forming bacteria, under conditions such that at least i) a subset of the spore-forming bacteria sustainably engraft within the gastrointestinal tract, or ii) at least one type of bacteria not present in the therapeutic composition is augmented within the gastrointestinal tract.

[018] In some embodiments, the invention includes methods of populating the gastrointestinal tract of a mammalian subject, comprising the step of orally administering to the subject an effective amount of a bacterial composition

comprising at least a first type of isolated bacteria and a second type of isolated bacteria, wherein the first type and the second type are not identical, under conditions such that the first type of isolated bacteria and the second type of isolated bacteria functionally populate the gastrointestinal tract of the subject.

[019] In other embodiments, the invention includes methods of populating the gastrointestinal tract of a mammalian subject, comprising the step of orally administering to the subject an effective amount of a therapeutic composition comprising a purified population of spore-forming bacteria produced by the steps of a) providing a fecal material and b) subjecting the material to a treatment step resulting in purification of spore-forming bacteria, wherein the purified population is present in an amount effective to treat or prevent a dysbiosis in a mammalian recipient subject to whom the therapeutic composition is administered.

[020] The invention also includes methods of populating the gastrointestinal tract of a mammalian subject, comprising the step of orally administering to the subject an effective amount of a therapeutic composition comprising a purified population of spore-forming bacteria, in an amount effective to treat or prevent a dysbiosis in a mammalian recipient subject to whom the therapeutic composition is administered.

BRIEF DESCRIPTION OF THE DRAWINGS

[021] Figure 1A provides a schematic of 16S rRNA gene and denotes the coordinates of hypervariable regions 1-9 (V1-V9), according to an embodiment of the invention. Coordinates of V1-V9 are 69-99, 137-242, 433-497, 576-682, 822-879, 986-1043, 1117-1173, 1243-1294, and 1435-1465 respectively, based on numbering using *E. coli* system of nomenclature defined by Brosius *et al.*, *Complete nucleotide sequence of a 16S ribosomal RNA gene (16S rRNA) from Escherichia coli*, PNAS 75(10):4801-4805 (1978).

[022] Figure 1B highlights in bold the nucleotide sequences for each hypervariable region in the exemplary reference *E. coli* 16S sequence described by Brosius *et al.*

[023] Figure 2 shows a photograph of a CsCl gradient demonstrating the spore separation from other residual habitat material, according to an embodiment of the invention.

[024] Figure 3 shows three phase contrast images demonstrating the progressive enrichment of spores from a fecal suspension (ethanol treated, CsCl purified spore preparation, and an ethanol treated, CsCl purified, sucrose purified spore preparation).

[025] Figure 4 shows a set of survival curves demonstrating efficacy of the spore population in a hamster prophylaxis model of *C. difficile*. (Circles are ethanol-treated spores + Vancomycin (orange); Squares are ethanol-treated, gradient purified spores + Vancomycin (light blue); X's are vancomycin control (green); and Diamonds are the vehicle control (dark blue)).

[026] Figure 5 provides a set of survival curves demonstrating efficacy of the spore population in a hamster relapse prevention model of *C. difficile*. (Opaque diamonds are ethanol-treated spores + Vancomycin (blue diamond); squares are ethanol-treated, gradient purified spores + Vancomycin (red squares); X's are vancomycin control; and Clear Diamonds are vehicle control (green)).

[027] Figure 6 demonstrates the cell viability under a variety of ethanol and heat treatments for varying lengths of time.

[028] Figure 7 shows the strong correlation and linear correspondence between the measurement of DPA concentration by a coupled fluorescence assay and the viable spore colony forming units.

[029] Figure 8 demonstrates the effect on various germination treatments on the ability to cultivate vegetative bacteria from a spore population.

[030] Figure 9 demonstrates the increase in bacterial diversity from using a germinant treatment to grow vegetative bacteria from spore populations.

[031] Figure 10 demonstrates the role of heat activation at various temperatures on spores from three different donor fecal samples.

[032] Figure 11 demonstrates that a lysozyme treatment with heat activation improves germination at most temperatures.

[033] Figure 12 demonstrates the microbial diversity measured in the ethanol treated spore treatment sample and patient pre- and post-treatment samples. Total microbial diversity is defined using the Chao1 Alpha-Diversity Index and is measured at the same genomic sampling depths to confirm adequate sequence coverage to assay the microbiome in the target samples. The patient pretreatment (purple) harbored a microbiome that was significantly reduced in total diversity as compared to the ethanol treated spore treatment (red) and patient post treatment at days 5 (blue), 14 (orange), and 25 (green).

[034] Figure 13 demonstrates how the patient microbial ecology is shifted by treatment with an ethanol treated spore treatment from a dysbiotic state to a state of health. Principle coordinates analysis based on the total diversity and structure of the microbiome (Bray Curtis Beta Diversity) of the patient pre- and post-treatment delineates that the combination of engraftment of the OTUs from the spore treatment and the augmentation of the patient microbial ecology leads to a microbial ecology that is distinct from both the pretreatment microbiome and the ecology of the ethanol treated spore treatment.

[035] Figure 14 demonstrates the augmentation of bacteroides species in patients treated with the spore population. Comparing the number of Bacteroides colonies from fecal suspensions pre-treatment and in week 4 post treatment reveals an increase of 4 logs or greater. Colonies were enumerated by serial dilution and plating on Bacteroides Bile Esculin agar which is highly selective for the *B. fragilis* group. Species were determined by 16S full-length sequence identification.

[036] Figure 15 demonstrates the increase in number of species engrafting and species augmenting in patient's microbiomes after treatment with an ethanol-treated spore population. Relative abundance of species that engrafted or augmented as described were determined based on the number of 16S sequence reads. Each plot is from a different patient treated with the ethanol-treated spore population for recurrent *C. difficile*.

[037] The figures depict various embodiments of the present invention for purposes of illustration only. One skilled in the art will readily recognize from the following discussion that alternative embodiments of the structures and methods illustrated herein may be employed without departing from the principles of the invention described herein.

DETAILED DESCRIPTION

DEFINITIONS

[038] "Microbiota" refers to the community of microorganisms that occur (sustainably or transiently) in and on an animal subject, typically a mammal such as a human, including eukaryotes, archaea, bacteria, and viruses (including bacterial viruses i.e., phage).

[039] "Microbiome" refers to the genetic content of the communities of microbes that live in and on the human body, both sustainably and transiently, including

eukaryotes, archaea, bacteria, and viruses (including bacterial viruses (i.e., phage)), wherein “genetic content” includes genomic DNA, RNA such as ribosomal RNA, the epigenome, plasmids, and all other types of genetic information.

[040] “Microbial Carriage” or simply “Carriage” refers to the population of microbes inhabiting a niche within or on humans. Carriage is often defined in terms of relative abundance. For example, OTU1 comprises 60% of the total microbial carriage, meaning that OTU1 has a relative abundance of 60% compared to the other OTUs in the sample from which the measurement was made. Carriage is most often based on genomic sequencing data where the relative abundance or carriage of a single OTU or group of OTUs is defined by the number of sequencing reads that are assigned to that OTU/s relative to the total number of sequencing reads for the sample.

[041] “Microbial Augmentation” or simply “augmentation” refers to the establishment or significant increase of a population of microbes that are (i) absent or undetectable (as determined by the use of standard genomic and microbiological techniques) from the administered therapeutic microbial composition, (ii) absent, undetectable, or present at low frequencies in the host niche (for example: gastrointestinal tract, skin, anterior-nares, or vagina) before the delivery of the microbial composition, and (iii) are found after the administration of the microbial composition or significantly increase, for instance 2-fold, 5-fold, 1×10^2 , 1×10^3 , 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , or greater than 1×10^8 , in cases where they were present at low frequencies. The microbes that comprise an augmented ecology can be derived from exogenous sources such as food and the environment, or grow out from micro-niches within the host where they reside at low frequency. The administration of the therapeutic microbial composition induces an environmental shift in the target niche

that promotes favorable conditions for the growth of these commensal microbes. In the absence of treatment with a therapeutic microbial composition, the host can be constantly exposed to these microbes; however, sustained growth and the positive health effects associated with the stable population of increased levels of the microbes comprising the augmented ecology are not observed.

[042] “Microbial Engraftment” or simply “engraftment” refers to the establishment of OTUs comprising a therapeutic microbial composition in a target niche that are absent in the treated host prior to treatment. The microbes that comprise the engrafted ecology are found in the therapeutic microbial composition and establish as constituents of the host microbial ecology upon treatment. Engrafted OTUs can establish for a transient period of time, or demonstrate long-term stability in the microbial ecology that populates the host post treatment with a therapeutic microbial composition. The engrafted ecology can induce an environmental shift in the target niche that promotes favorable conditions for the growth of commensal microbes capable of catalyzing a shift from a dysbiotic ecology to one representative of a health state.

[043] “Ecological Niche” or simply “Niche” refers to the ecological space in which an organism or group of organisms occupies. Niche describes how an organism or population or organisms responds to the distribution of resources, physical parameters (e.g., host tissue space) and competitors (e.g., by growing when resources are abundant, and when predators, parasites and pathogens are scarce) and how it in turn alters those same factors (e.g., limiting access to resources by other organisms, acting as a food source for predators and a consumer of prey).

[044] “Dysbiosis” refers to a state of the microbiota or microbiome of the gut or other body area, including mucosal or skin surfaces in which the normal diversity

and/or function of the ecological network is disrupted. Any disruption from the preferred (e.g., ideal) state of the microbiota can be considered a dysbiosis, even if such dysbiosis does not result in a detectable decrease in health. This state of dysbiosis may be unhealthy, it may be unhealthy under only certain conditions, or it may prevent a subject from becoming healthier. Dysbiosis may be due to a decrease in diversity, the overgrowth of one or more pathogens or pathobionts, symbiotic organisms able to cause disease only when certain genetic and/or environmental conditions are present in a patient, or the shift to an ecological network that no longer provides a beneficial function to the host and therefore no longer promotes health.

[045] The term “pathobiont” refer to specific bacterial species found in healthy hosts that may trigger immune-mediated pathology and/or disease in response to certain genetic or environmental factors. Chow et al., (2011) *Curr Op Immunol*. Pathobionts of the intestinal microbiota and inflammatory disease. **23**: 473-80. Thus, a pathobiont is a pathogen that is mechanistically distinct from an acquired infectious organism. Thus, the term “pathogen” includes both acquired infectious organisms and pathobionts.

[046] The terms “pathogen”, “pathobiont” and “pathogenic” in reference to a bacterium or any other organism or entity includes any such organism or entity that is capable of causing or affecting a disease, disorder or condition of a host organism containing the organism or entity.

[047] “Phylogenetic tree” refers to a graphical representation of the evolutionary relationships of one genetic sequence to another that is generated using a defined set of phylogenetic reconstruction algorithms (e.g. parsimony, maximum likelihood, or Bayesian). Nodes in the tree represent distinct ancestral sequences and the

confidence of any node is provided by a bootstrap or Bayesian posterior probability, which measures branch uncertainty.

[048] “Operational taxonomic units,” “OTU” (or plural, “OTUs”) refer to a terminal leaf in a phylogenetic tree and is defined by a nucleic acid sequence, e.g., the entire genome, or a specific genetic sequence, and all sequences that share sequence identity to this nucleic acid sequence at the level of species. In some embodiments the specific genetic sequence may be the 16S sequence or a portion of the 16S sequence. In other embodiments, the entire genomes of two entities are sequenced and compared. In another embodiment, select regions such as multilocus sequence tags (MLST), specific genes, or sets of genes may be genetically compared. In 16S embodiments, OTUs that share $\geq 97\%$ average nucleotide identity across the entire 16S or some variable region of the 16S are considered the same OTU (see e.g. Claesson MJ, Wang Q, O’Sullivan O, Greene-Diniz R, Cole JR, Ross RP, and O’Toole PW. 2010. Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. *Nucleic Acids Res* **38**: e200. Konstantinidis KT, Ramette A, and Tiedje JM. 2006. The bacterial species definition in the genomic era. *Philos Trans R Soc Lond B Biol Sci* **361**: 1929–1940.). In embodiments involving the complete genome, MLSTs, specific genes, or sets of genes OTUs that share $\geq 95\%$ average nucleotide identity are considered the same OTU (see e.g. Achtman M, and Wagner M. 2008. Microbial diversity and the genetic nature of microbial species. *Nat. Rev. Microbiol.* **6**: 431–440. Konstantinidis KT, Ramette A, and Tiedje JM. 2006. The bacterial species definition in the genomic era. *Philos Trans R Soc Lond B Biol Sci* **361**: 1929–1940.). OTUs are frequently defined by comparing sequences between organisms. Generally, sequences with less than 95% sequence identity are not

considered to form part of the same OTU. OTUs may also be characterized by any combination of nucleotide markers or genes, in particular highly conserved genes (e.g., “house-keeping” genes), or a combination thereof. Such characterization employs, e.g., WGS data or a whole genome sequence.

[049] Table 1 below shows a List of Operational Taxonomic Units (OTU) with taxonomic assignments made to Genus, Species, and Phylogenetic Clade. Clade membership of bacterial OTUs is based on 16S sequence data. Clades are defined based on the topology of a phylogenetic tree that is constructed from full-length 16S sequences using maximum likelihood methods familiar to individuals with ordinary skill in the art of phylogenetics. Clades are constructed to ensure that all OTUs in a given clade are: (i) within a specified number of bootstrap supported nodes from one another, and (ii) within 5% genetic similarity. OTUs that are within the same clade can be distinguished as genetically and phylogenetically distinct from OTUs in a different clade based on 16S-V4 sequence data, while OTUs falling within the same clade are closely related. OTUs falling within the same clade are evolutionarily closely related and may or may not be distinguishable from one another using 16S-V4 sequence data. Members of the same clade, due to their evolutionary relatedness, play similar functional roles in a microbial ecology such as that found in the human gut. Compositions substituting one species with another from the same clade are likely to have conserved ecological function and therefore are useful in the present invention. All OTUs are denoted as to their putative capacity to form spores and whether they are a Pathogen or Pathobiont (see Definitions for description of “Pathobiont”). NIAID Priority Pathogens are denoted as ‘Category-A’, ‘Category-B’, or ‘Category-C’, and Opportunistic Pathogens are denoted as ‘OP’. OTUs that are not pathogenic or for which their ability to exist as a pathogen is unknown are

denoted as 'N'. The 'SEQ ID Number' denotes the identifier of the OTU in the Sequence Listing File and 'Public DB Accession' denotes the identifier of the OTU in a public sequence repository.

[050] Table 2 contains bacterial OTUs identified from the 16s analysis of the ethanol treated spore population before and after a CsCl gradient purification.

[051] "Residual habitat products" refers to material derived from the habitat for microbiota within or on a human or animal. For example, microbiota live in feces in the gastrointestinal tract, on the skin itself, in saliva, mucus of the respiratory tract, or secretions of the genitourinary tract (i.e., biological matter associated with the microbial community). Substantially free of residual habitat products means that the bacterial composition no longer contains the biological matter associated with the microbial environment on or in the human or animal subject and is 100% free, 99% free, 98% free, 97% free, 96% free, or 95% free of any contaminating biological matter associated with the microbial community. Residual habitat products can include abiotic materials (including undigested food) or it can include unwanted microorganisms. Substantially free of residual habitat products may also mean that the bacterial composition contains no detectable cells from a human or animal and that only microbial cells are detectable. In one embodiment, substantially free of residual habitat products may also mean that the bacterial composition contains no detectable viral (including bacterial viruses (i.e., phage)), fungal, mycoplasmal contaminants. In another embodiment, it means that fewer than $1 \times 10^{-2}\%$, $1 \times 10^{-3}\%$, $1 \times 10^{-4}\%$, $1 \times 10^{-5}\%$, $1 \times 10^{-6}\%$, $1 \times 10^{-7}\%$, $1 \times 10^{-8}\%$ of the viable cells in the bacterial composition are human or animal, as compared to microbial cells. There are multiple ways to accomplish this degree of purity, none of which are limiting. Thus, contamination may be reduced by isolating desired constituents through multiple

steps of streaking to single colonies on solid media until replicate (such as, but not limited to, two) streaks from serial single colonies have shown only a single colony morphology. Alternatively, reduction of contamination can be accomplished by multiple rounds of serial dilutions to single desired cells (e.g., a dilution of 10^{-8} or 10^{-9}), such as through multiple 10-fold serial dilutions. This can further be confirmed by showing that multiple isolated colonies have similar cell shapes and Gram staining behavior. Other methods for confirming adequate purity include genetic analysis (e.g. PCR, DNA sequencing), serology and antigen analysis, enzymatic and metabolic analysis, and methods using instrumentation such as flow cytometry with reagents that distinguish desired constituents from contaminants.

[052] “Clade” refers to the OTUs or members of a phylogenetic tree that are downstream of a statistically valid node in a phylogenetic tree. The clade comprises a set of terminal leaves in the phylogenetic tree that is a distinct monophyletic evolutionary unit and that share some extent of sequence similarity.

[053] 16s Sequencing, 16s, 16s-rRNA, 16s-NGS: In microbiology, “16S sequencing” or “16S-rRNA” or “16S” refers to sequence derived by characterizing the nucleotides that comprise the 16S ribosomal RNA gene(s). The bacterial 16S rDNA is approximately 1500 nucleotides in length and is used in reconstructing the evolutionary relationships and sequence similarity of one bacterial isolate to another using phylogenetic approaches. 16S sequences are used for phylogenetic reconstruction as they are in general highly conserved, but contain specific hypervariable regions that harbor sufficient nucleotide diversity to differentiate genera and species of most bacteria.

[054] The “V1-V9 regions” of the 16S rRNA refers to the first through ninth hypervariable regions of the 16S rRNA gene that are used for genetic typing of

bacterial samples. These regions in bacteria are defined by nucleotides 69-99, 137-242, 433-497, 576-682, 822-879, 986-1043, 1117-1173, 1243-1294 and 1435-1465 respectively using numbering based on the *E. coli* system of nomenclature. Brosius et al., Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*, PNAS 75(10):4801-4805 (1978). In some embodiments, at least one of the V1, V2, V3, V4, V5, V6, V7, V8, and V9 regions are used to characterize an OTU. In one embodiment, the V1, V2, and V3 regions are used to characterize an OTU. In another embodiment, the V3, V4, and V5 regions are used to characterize an OTU. In another embodiment, the V4 region is used to characterize an OTU. A person of ordinary skill in the art can identify the specific hypervariable regions of a candidate 16S rRNA by comparing the candidate sequence in question to a reference sequence and identifying the hypervariable regions based on similarity to the reference hypervariable regions, or alternatively, one can employ Whole Genome Shotgun (WGS) sequence characterization of microbes or a microbial community.

[055] The term “subject” refers to any animal subject including humans, laboratory animals (e.g., primates, rats, mice), livestock (e.g., cows, sheep, goats, pigs, turkeys, and chickens), and household pets (e.g., dogs, cats, and rodents). The subject may be suffering from a dysbiosis, including, but not limited to, an infection due to a gastrointestinal pathogen or may be at risk of developing or transmitting to others an infection due to a gastrointestinal pathogen.

[056] The term “phenotype” refers to a set of observable characteristics of an individual entity. As example an individual subject may have a phenotype of “health” or “disease”. Phenotypes describe the state of an entity and all entities within a phenotype share the same set of characteristics that describe the phenotype. The

phenotype of an individual results in part, or in whole, from the interaction of the entities genome and/or microbiome with the environment.

[057] The term “Network Ecology” refers to a consortium of OTUs that co-occur in some number of subjects. As used herein, a “network” is defined mathematically by a graph delineating how specific nodes (i.e. OTUs) and edges (connections between specific OTUs) relate to one another to define the structural ecology of a consortium of OTUs. Any given Network Ecology will possess inherent phylogenetic diversity and functional properties. A Network Ecology can also be defined in terms of function where for example the nodes would be comprised of elements such as, but not limited to, enzymes, clusters of orthologous groups (COGS; <http://www.ncbi.nlm.nih.gov/books/NBK21090/>), or KEGG pathways (www.genome.jp/kegg/).

[058] Network Class, Core Network, Core Network Ecology: The terms “Network Class”, “Core Network” and “Core Network Ecology” refer to a group of network ecologies that in general are computationally determined to comprise ecologies with similar phylogenetic and/or functional characteristics. A Core Network therefore contains important biological features, defined either phylogenetically or functionally, of a group (i.e., a cluster) of related network ecologies. One representation of a Core Network Ecology is a designed consortium of microbes, typically non-pathogenic bacteria, that represents core features of a set of phylogenetically or functionally related network ecologies seen in many different subjects. In many occurrences, a Core Network, while designed as described herein, exists as a Network Ecology observed in one or more subjects. Core Network ecologies are useful for reversing or reducing a dysbiosis in subjects where the underlying, related Network Ecology has been disrupted.

[059] The term “Keystone OTU” refers to one or more OTUs that are common to many network ecologies and are members of networks ecologies that occur in many subjects (i.e. are pervasive). Due to the ubiquitous nature of Keystone OTUs, they are central to the function of network ecologies in healthy subjects and are often missing or at reduced levels in subjects with disease. Keystone OTUs may exist in low, moderate, or high abundance in subjects.

[060] The term “non-Keystone OTU” refers to an OTU that is observed in a Network Ecology and is not a keystone OTU.

[061] The term “Phylogenetic Diversity” refers to the biodiversity present in a given Network Ecology or Core Network Ecology based on the OTUs that comprise the network. Phylogenetic diversity is a relative term, meaning that a Network Ecology or Core Network that is comparatively more phylogenetically diverse than another network contains a greater number of unique species, genera, and taxonomic families. Uniqueness of a species, genera, or taxonomic family is generally defined using a phylogenetic tree that represents the genetic diversity all species, genera, or taxonomic families relative to one another. In another embodiment phylogenetic diversity may be measured using the total branch length or average branch length of a phylogenetic tree.

[062] A “spore” or a population of “spores” includes bacteria (or other single-celled organisms) that are generally viable, more resistant to environmental influences such as heat and bacteriocidal agents than vegetative forms of the same bacteria, and typically capable of germination and out-growth. “Spore-formers” or bacteria “capable of forming spores” are those bacteria containing the genes and other necessary abilities to produce spores under suitable environmental conditions.

[063] A “spore population” refers to a plurality of spores present in a composition. Synonymous terms used herein include spore composition, spore preparation, ethanol treated spore fraction and spore ecology. A spore population may be purified from a fecal donation, e.g. via ethanol or heat treatment, or a density gradient separation or any combination of methods described herein to increase the purity, potency and/or concentration of spores in a sample. Alternatively, a spore population may be derived through culture methods starting from isolated spore former species or spore former OTUs or from a mixture of such species, either in vegetative or spore form.

[064] In one embodiment, the spore preparation comprises spore forming species wherein residual non-spore forming species have been inactivated by chemical or physical treatments including ethanol, detergent, heat, sonication, and the like; or wherein the non-spore forming species have been removed from the spore preparation by various separations steps including density gradients, centrifugation, filtration and/or chromatography; or wherein inactivation and separation methods are combined to make the spore preparation. In yet another embodiment, the spore preparation comprises spore forming species that are enriched over viable non-spore formers or vegetative forms of spore formers. In this embodiment, spores are enriched by 2-fold, 5-fold, 10-fold, 50-fold, 100-fold, 1000-fold, 10,000-fold or greater than 10,000-fold compared to all vegetative forms of bacteria. In yet another embodiment, the spores in the spore preparation undergo partial germination during processing and formulation such that the final composition comprises spores and vegetative bacteria derived from spore forming species.

[065] The term “isolated” encompasses a bacterium or other entity or substance that has been (1) separated from at least some of the components with which it was

associated when initially produced (whether in nature or in an experimental setting), and/or (2) produced, prepared, purified, and/or manufactured by the hand of man. Isolated bacteria include those bacteria that are cultured, even if such cultures are not monocultures. Isolated bacteria may be separated from at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more of the other components with which they were initially associated. In some embodiments, isolated bacteria are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components. The terms "purify," "purifying" and "purified" refer to a bacterium or other material that has been separated from at least some of the components with which it was associated either when initially produced or generated (e.g., whether in nature or in an experimental setting), or during any time after its initial production. A bacterium or a bacterial population may be considered purified if it is isolated at or after production, such as from a material or environment containing the bacterium or bacterial population, or by passage through culture, and a purified bacterium or bacterial population may contain other materials up to about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or above about 90% and still be considered "isolated." In some embodiments, purified bacteria and bacterial populations are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. In the instance of bacterial compositions provided herein, the one or more bacterial types present in the composition can be independently purified from one or more other bacteria produced

and/or present in the material or environment containing the bacterial type. Bacterial compositions and the bacterial components thereof are generally purified from residual habitat products.

[066] “Inhibition” of a pathogen encompasses the inhibition of any desired function or activity of the bacterial compositions of the present invention.

Demonstrations of pathogen inhibition, such as decrease in the growth of a pathogenic bacterium or reduction in the level of colonization of a pathogenic bacterium are provided herein and otherwise recognized by one of ordinary skill in the art. Inhibition of a pathogenic bacterium’s “growth” may include inhibiting the increase in size of the pathogenic bacterium and/or inhibiting the proliferation (or multiplication) of the pathogenic bacterium. Inhibition of colonization of a pathogenic bacterium may be demonstrated by measuring the amount or burden of a pathogen before and after a treatment. An “inhibition” or the act of “inhibiting” includes the total cessation and partial reduction of one or more activities of a pathogen, such as growth, proliferation, colonization, and function.

[067] A “germinant” is a material or composition or physical-chemical process capable of inducing vegetative growth of a bacterium that is in a dormant spore form, or group of bacteria in the spore form, either directly or indirectly in a host organism and/or in vitro.

[068] A “sporulation induction agent” is a material or physical-chemical process that is capable of inducing sporulation in a bacterium, either directly or indirectly, in a host organism and/or in vitro.

[069] To “increase production of bacterial spores” includes an activity or a sporulation induction agent. “Production” includes conversion of vegetative bacterial cells into spores and augmentation of the rate of such conversion, as well as

decreasing the germination of bacteria in spore form, decreasing the rate of spore decay *in vivo*, or *ex vivo*, or to increasing the total output of spores (e.g. via an increase in volumetric output of fecal material).

[070] The “colonization” of a host organism includes the non-transitory residence of a bacterium or other microscopic organism. As used herein, “reducing colonization” of a host subject’s gastrointestinal tract (or any other microbiotal niche) by a pathogenic bacterium includes a reduction in the residence time of the pathogen in the gastrointestinal tract as well as a reduction in the number (or concentration) of the pathogen in the gastrointestinal tract or adhered to the luminal surface of the gastrointestinal tract. Measuring reductions of adherent pathogens may be demonstrated, e.g., by a biopsy sample, or reductions may be measured indirectly, e.g., by measuring the pathogenic burden in the stool of a mammalian host.

[071] A “combination” of two or more bacteria includes the physical co-existence of the two bacteria, either in the same material or product or in physically connected products, as well as the temporal co-administration or co-localization of the two bacteria.

[072] A “cytotoxic” activity or bacterium includes the ability to kill a bacterial cell, such as a pathogenic bacterial cell. A “cytostatic” activity or bacterium includes the ability to inhibit, partially or fully, growth, metabolism, and/or proliferation of a bacterial cell, such as a pathogenic bacterial cell.

[073] Non-comestible products: To be free of “non-comestible products” means that a bacterial composition or other material provided herein does not have a substantial amount of a non-comestible product, e.g., a product or material that is inedible, harmful or otherwise undesired in a product suitable for administration, e.g.,

oral administration, to a human subject. Non-comestible products are often found in preparations of bacteria from the prior art.

[074] As used herein the term "vitamin" is understood to include any of various fat-soluble or water-soluble organic substances (non-limiting examples include *vitamin A*, Vitamin B1 (thiamine), Vitamin B2 (riboflavin), Vitamin B3 (*niacin* or niacinamide), Vitamin B5 (pantothenic acid), Vitamin B6 (pyridoxine, pyridoxal, or pyridoxamine, or pyridoxine hydrochloride), Vitamin B7 (biotin), Vitamin B9 (folic acid), and Vitamin B12 (various cobalamins; commonly cyanocobalamin in vitamin supplements), vitamin C, vitamin D, vitamin E, vitamin K, K1 and K2 (i.e. MK-4, MK-7), folic acid and biotin) essential in minute amounts for normal growth and activity of the body and obtained naturally from plant and animal foods or synthetically made, pro-vitamins, derivatives, analogs.

[075] As used herein, the term "minerals" is understood to include boron, calcium, chromium, copper, iodine, iron, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, selenium, silicon, tin, vanadium, zinc, or combinations thereof.

[076] As used herein, the term "antioxidant" is understood to include any one or more of various substances such as beta-carotene (a *vitamin A* precursor), vitamin C, vitamin E, and selenium) that inhibit oxidation or reactions promoted by Reactive Oxygen Species ("ROS") and other radical and non-radical species. Additionally, antioxidants are molecules capable of slowing or preventing the oxidation of other molecules. Non-limiting examples of antioxidants include astaxanthin, carotenoids, coenzyme Q10 ("CoQ10"), flavonoids, glutathione, Goji (wolfberry), hesperidin, lactowolfberry, lignan, lutein, lycopene, polyphenols, selenium, vitamin A, vitamin C, vitamin E, zeaxanthin, or combinations thereof.

COMPOSITIONS OF THE INVENTION

Bacterial Compositions

[077] Provided are bacteria and combinations of bacteria of the human gut microbiota with the capacity to meaningfully provide functions of a healthy microbiota when administered to mammalian hosts. Without being limited to a specific mechanism, it is thought that such compositions inhibit the growth, proliferation, and/or colonization of one or a plurality of pathogenic bacteria in the dysbiotic microbial niche, so that a healthy, diverse and protective microbiota colonizes and populates the intestinal lumen to establish or reestablish ecological control over pathogens or potential pathogens (e.g., some bacteria are pathogenic bacteria only when present in a dysbiotic environment). In some embodiments, bacterial composition engraft in the host gut are detectable 1, 2, 3, 4, 5, or 6 days, 1 or 2 weeks, 1, 3 or 6 months, 1 year or greater than 1 year after the administration of composition when the bacteria compositions were not present before treatment. In some embodiments, the bacteria compositions delivered shift the gut environment to a state allowing other healthy commensal bacteria to augment the delivered population. In this manner, augmentation of the commensal bacteria not directly delivered increases the overall diversity of the subsequent microbiome with healthy commensal bacteria and improves host health.

[078] Inhibition of pathogens includes those pathogens such as *C. difficile*, *Salmonella* spp., enteropathogenic *E coli*, multi-drug resistant bacteria such as *Klebsiella*, and *E. coli*, Carbapenem-resistant Enterobacteriaceae (CRE), extended spectrum beta-lactam resistant *Enterococci* (ESBL), and vancomycin-resistant Enterococci (VRE). In particular, provided are synergistic combinations that treat, prevent, delay or reduce the symptoms of diseases, disorders and conditions associated

with a dysbiosis. Representative diseases, disorders and conditions potentially associated with a dysbiosis, which are suitable for treatment with the compositions and methods as described herein, are provided in Tables 3 and 4.

[079] As used herein, a “type” or more than one “types” of bacteria may be differentiated at the genus level, the species level, the sub-species level, the strain level or by any other taxonomic method, as described herein and otherwise known in the art.

[080] Bacterial compositions may comprise two types of bacteria (termed “binary combinations” or “binary pairs”) or greater than two types of bacteria. For instance, a bacterial composition may comprise at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or at least 40, at least 50 or greater than 50 types of bacteria, as defined by species or operational taxonomic unit (OTU), or otherwise as provided herein.

[081] In another embodiment, the number of types of bacteria present in a bacterial composition is at or below a known value. For example, in such embodiments the bacterial composition comprises 50 or fewer types of bacteria, such as 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, or 10 or fewer, or 9 or fewer types of bacteria, 8 or fewer types of bacteria, 7 or fewer types of bacteria, 6 or fewer types of bacteria, 5 or fewer types of bacteria, 4 or fewer types of bacteria, or 3 or fewer types of bacteria. In another embodiment, a bacterial composition comprises from 2 to no more than 40, from 2 to no more than 30, from 2 to no more than 20, from 2 to no more than 15, from 2 to no more than 10, or from 2

to no more than 5 types of bacteria. In some embodiments, bacterial compositions are provided with the ability to exclude pathogenic bacteria. Exemplary bacterial compositions are demonstrated to reduce the growth rate of one pathogen, *C. difficile*, as provided in the Examples, wherein the ability of the bacterial compositions is demonstrated by assessing the antagonism activity of a combination of OTUs or strains towards a given pathogen using in vitro assays.

Bacterial Compositions Described by Species

[082] Bacterial compositions may be prepared comprising at least two types of isolated bacteria, chosen from the species in Table 1.

[083] In one embodiment, the bacterial composition comprises at least one and preferably more than one of the following: *Enterococcus faecalis* (previously known as *Streptococcus faecalis*), *Clostridium innocuum*, *Clostridium ramosum*, *Bacteroides ovatus*, *Bacteroides vulgatus*, *Bacteroides thetaiotaomicron*, *Escherichia coli* (1109 and 1108-1), *Clostridium bifermentans*, and *Blautia producta* (previously known as *Peptostreptococcus productus*). In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[084] In one embodiment, the bacterial composition comprises at least one and preferably more than one of the following: *Enterococcus faecalis* (previously known as *Streptococcus faecalis*), *Clostridium innocuum*, *Clostridium ramosum*, *Bacteroides ovatus*, *Bacteroides vulgatus*, *Bacteroides thetaiotaomicron*, *Escherichia coli* (1109 and 1108-1), *Clostridium bifermentans*, and *Blautia producta* (previously known as *Peptostreptococcus productus*). In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[085] In another embodiment, the bacterial composition comprises at least one and preferably more than one of the following: *Acidaminococcus intestinalis*, *Bacteroides ovatus*, two strains of *Bifidobacterium adolescentis*, two strains of *Bifidobacterium longum*, *Blautia producta*, *Clostridium cocleatum*, *Collinsella aerofaciens*, two strains of *Dorea longicatena*, *Escherichia coli*, *Eubacterium desmolans*, *Eubacterium eligens*, *Eubacterium limosum*, four strains of *Eubacterium rectale*, *Eubacterium ventriosum*, *Faecalibacterium prausnitzii*, *Lachnospira pectinoshiza*, *Lactobacillus casei*, *Lactobacillus casei/paracasei*, *Paracateroides distasonis*, *Raoultella sp.*, one strain of *Roseburia* (chosen from *Roseburia faecalis* or *Roseburia faecis*), *Roseburia intestinalis*, two strains of *Ruminococcus torques*, two strains of *Ruminococcus obeum*, and *Streptococcus mitis*. In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[086] In yet another embodiment, the bacterial composition comprises at least one and preferably more than one of the following: *Barnesiella intestinihominis*; *Lactobacillus reuteri*; a species characterized as one of *Enterococcus hirae*, *Enterococcus faecium*, or *Enterococcus durans*; a species characterized as one of *Anaerostipes caccae* or *Clostridium indolis*; a species characterized as one of *Staphylococcus warneri* or *Staphylococcus pasteurii*; and *Adlercreutzia equolifaciens*. In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[087] In other embodiments, the bacterial composition comprises at least one and preferably more than one of the following: *Clostridium absonum*, *Clostridium argentinense*, *Clostridium baratii*, *Clostridium bartlettii*, *Clostridium bifermentans*, *Clostridium botulinum*, *Clostridium butyricum*, *Clostridium cadaveris*, *Clostridium*

camis, Clostridium celatum, Clostridium chauvoei, Clostridium clostridioforme, Clostridium cochlearium, Clostridium difficile, Clostridium fallax, Clostridium felsineum, Clostridium ghonii, Clostridium glycolicum, Clostridium haemolyticum, Clostridium hastiforme, Clostridium histolyticum, Clostridium indolis, Clostridium innocuum, Clostridium irregulare, Clostridium limosum, Clostridium malenominatum, Clostridium novyi, Clostridium oroticum, Clostridium paraputrificum, Clostridium perfringens, Clostridium piliforme, Clostridium putrefaciens, Clostridium putrificum, Clostridium ramosum, Clostridium sardiniense, Clostridium sartagoforme, Clostridium scindens, Clostridium septicum, Clostridium sordellii, Clostridium sphenoides, Clostridium spiroforme, Clostridium sporogenes, Clostridium subterminale, Clostridium symbiosum, Clostridium tertium, Clostridium tetani, Clostridium welchii, and Clostridium villosum. In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[088] In one embodiment, the bacterial composition comprises at least one and preferably more than one of the following: *Clostridium innocuum, Clostridium bifermentans, Clostridium butyricum, Bacteroides fragilis, Bacteroides thetaiotaomicron, Bacteroides uniformis*, three strains of *Escherichia coli*, and *Lactobacillus sp.* In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[089] In one embodiment, the bacterial composition comprises at least one and preferably more than one of the following: *Clostridium bifermentans, Clostridium innocuum, Clostridium butyricum*, three strains of *Escherichia coli*, three strains of *Bacteroides*, and *Blautia producta*. In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[090] In one embodiment, the bacterial composition comprises at least one and preferably more than one of the following: *Bacteroides sp.*, *Escherichia coli*, and non pathogenic *Clostridia*, including *Clostridium innocuum*, *Clostridium bifermentans* and *Clostridium ramosum*. In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[091] In one embodiment, the bacterial composition comprises at least one and preferably more than one of the following: *Bacteroides* species, *Escherichia coli* and non-pathogenic *Clostridia*, such as *Clostridium butyricum*, *Clostridium bifermentans* and *Clostridium innocuum*. In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[092] In one embodiment, the bacterial composition comprises at least one and preferably more than one of the following: *Bacteroides caccae*, *Bacteroides capillosus*, *Bacteroides coagulans*, *Bacteroides distasonis*, *Bacteroides eggerthii*, *Bacteroides forsythus*, *Bacteroides fragilis*, *Bacteroides fragilis-ryhm*, *Bacteroides gracilis*, *Bacteroides levii*, *Bacteroides macacae*, *Bacteroides merdae*, *Bacteroides ovatus*, *Bacteroides pneumosintes*, *Bacteroides putredinis*, *Bacteroides pyogenes*, *Bacteroides splanchnicus*, *Bacteroides stercoris*, *Bacteroides tectum*, *Bacteroides thetaiotaomicron*, *Bacteroides uniformis*, *Bacteroides ureolyticus*, and *Bacteroides vulgatus*. In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[093] In one embodiment, the bacterial composition comprises at least one and preferably more than one of the following: *Bacteroides*, *Eubacteria*, *Fusobacteria*, *Propionibacteria*, *Lactobacilli*, *anaerobic cocci*, *Ruminococcus*, *Escherichia coli*, *Gemmiger*, *Desulfomonas*, and *Peptostreptococcus*. In an alternative embodiment,

at least one of the preceding species is not substantially present in the bacterial composition.

[094] In one embodiment, the bacterial composition comprises at least one and preferably more than one of the following: *Bacteroides fragilis* ss. *Vulgatus*, *Eubacterium aerofaciens*, *Bacteroides fragilis* ss. *Thetaiotaomicron*, *Blautia producta* (previously known as *Peptostreptococcus productus* II), *Bacteroides fragilis* ss. *Distasonis*, *Fusobacterium prausnitzii*, *Coprococcus eutactus*, *Eubacterium aerofaciens* III, *Blautia producta* (previously known as *Peptostreptococcus productus* I), *Ruminococcus bronii*, *Bifidobacterium adolescentis*, *Gemmiger formicilis*, *Bifidobacterium longum*, *Eubacterium siraeum*, *Ruminococcus torques*, *Eubacterium rectale* III-H, *Eubacterium rectale* IV, *Eubacterium eligens*, *Bacteroides eggerthii*, *Clostridium leptum*, *Bacteroides fragilis* ss. A, *Eubacterium bifforme*, *Bifidobacterium infantis*, *Eubacterium rectale* III-F, *Coprococcus comes*, *Bacteroides capillosus*, *Ruminococcus albus*, *Eubacterium formicigenerans*, *Eubacterium hallii*, *Eubacterium ventriosum* I, *Fusobacterium russii*, *Ruminococcus obeum*, *Eubacterium rectale* II, *Clostridium ramosum* I, *Lactobacillus leichmanii*, *Ruminococcus cailidus*, *Butyrivibrio crossotus*, *Acidaminococcus fermentans*, *Eubacterium ventriosum*, *Bacteroides fragilis* ss. *fragilis*, *Bacteroides* AR, *Coprococcus catus*, *Eubacterium hadrum*, *Eubacterium cylindroides*, *Eubacterium ruminantium*, *Eubacterium* CH-1, *Staphylococcus epidermidis*, *Peptostreptococcus* BL, *Eubacterium limosum*, *Bacteroides praeacutus*, *Bacteroides* L, *Fusobacterium mortiferum* I, *Fusobacterium naviforme*, *Clostridium innocuum*, *Clostridium ramosum*, *Propionibacterium acnes*, *Ruminococcus flavefaciens*, *Ruminococcus* AT, *Peptococcus* AU-1, *Eubacterium* AG, -AK, -AL, -AL-1, -AN; *Bacteroides fragilis* ss. *ovatus*, -ss. d, -ss. f; *Bacteroides* L-1, L-5; *Fusobacterium nucleatum*, *Fusobacterium mortiferum*, *Escherichia coli*,

Streptococcus morbillorum, *Peptococcus magnus*, *Peptococcus G*, AU-2;
Streptococcus intermedius, *Ruminococcus lactaris*, *Ruminococcus CO Gemmiger X*,
Coprococcus BH, -CC; *Eubacterium tenue*, *Eubacterium ramulus*, *Eubacterium AE*, -
 AG-H, -AG-M, -AJ, -BN-1; *Bacteroides clostridiiformis* ss. *clostridiiformis*,
Bacteroides coagulans, *Bacteroides orails*, *Bacteroides ruminicola* ss. *brevis*, -ss.
ruminicola, *Bacteroides splanchnicus*, *Desulfomonas pigra*, *Bacteroides L-4*, -N-i;
Fusobacterium H, *Lactobacillus G*, and *Succinivibrio A*. In an alternative
 embodiment, at least one of the preceding species is not substantially present in the
 bacterial composition.

Bacterial Compositions Described by Operational Taxonomic Unit (OTUs)

[095] Bacterial compositions may be prepared comprising at least two types of isolated bacteria, chosen from Table 1.

[096] In one embodiment, the OTUs can be characterized by one or more of the variable regions of the 16S sequence (V1-V9). These regions in bacteria are defined by nucleotides 69-99, 137-242, 433-497, 576-682, 822-879, 986-1043, 1117-1173, 1243-1294 and 1435-1465 respectively using numbering based on the *E. coli* system of nomenclature. (See, e.g., Brosius *et al.*, Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*, PNAS 75(10):4801-4805 (1978)). In some embodiments, at least one of the V1, V2, V3, V4, V5, V6, V7, V8, and V9 regions are used to characterize an OTU. In one embodiment, the V1, V2, and V3 regions are used to characterize an OTU. In another embodiment, the V3, V4, and V5 regions are used to characterize an OTU. In another embodiment, the V4 region is used to characterize an OTU.

Bacterial Compositions Exclusive of Certain Bacterial Species Or Strains

[097] In one embodiment, the bacterial composition does not comprise at least one of *Enterococcus faecalis* (previously known as *Streptococcus faecalis*), *Clostridium innocuum*, *Clostridium ramosum*, *Bacteroides ovatus*, *Bacteroides vulgatus*, *Bacteroides thetaiotaomicron*, *Escherichia coli* (1109 and 1108-1), *Clostridium bifermentans*, and *Blautia producta* (previously known as *Peptostreptococcus productus*).

[098] In another embodiment, the bacterial composition does not comprise at least one of *Acidaminococcus intestinalis*, *Bacteroides ovatus*, two species of *Bifidobacterium adolescentis*, two species of *Bifidobacterium longum*, *Collinsella aerofaciens*, two species of *Dorea longicatena*, *Escherichia coli*, *Eubacterium eligens*, *Eubacterium limosum*, four species of *Eubacterium rectale*, *Eubacterium ventriosum*, *Faecalibacterium prausnitzii*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Paracateroides distasonis*, *Raoultella* sp., one species of *Roseburia* (chosen from *Roseburia faecalis* or *Roseburia faecis*), *Roseburia intestinalis*, two species of *Ruminococcus torques*, and *Streptococcus mitis*.

[099] In yet another embodiment, the bacterial composition does not comprise at least one of *Barnesiella intestinihominis*; *Lactobacillus reuteri*; a species characterized as one of *Enterococcus hirae*, *Enterococcus faecium*, or *Enterococcus durans*; a species characterized as one of *Anaerostipes caccae* or *Clostridium indolis*; a species characterized as one of *Staphylococcus warneri* or *Staphylococcus pasteurii*; and *Adlercreutzia equolifaciens*.

[0100] In other embodiments, the bacterial composition does not comprise at least one of *Clostridium absonum*, *Clostridium argentinense*, *Clostridium baratii*, *Clostridium bifermentans*, *Clostridium botulinum*, *Clostridium butyricum*, *Clostridium cadaveris*, *Clostridium camis*, *Clostridium celatum*, *Clostridium chauvoei*, *Clostridium*

clostridioforme, *Clostridium cochlearium*, *Clostridium difficile*, *Clostridium fallax*, *Clostridium felsineum*, *Clostridium ghonii*, *Clostridium glycolicum*, *Clostridium haemolyticum*, *Clostridium hastiforme*, *Clostridium histolyticum*, *Clostridium indolis*, *Clostridium innocuum*, *Clostridium irregulare*, *Clostridium limosum*, *Clostridium malenominatum*, *Clostridium novyi*, *Clostridium oroticum*, *Clostridium paraputrificum*, *Clostridium perfringens*, *Clostridium piliforme*, *Clostridium putrefaciens*, *Clostridium putrificum*, *Clostridium ramosum*, *Clostridium sardiniense*, *Clostridium sartagoforme*, *Clostridium scindens*, *Clostridium septicum*, *Clostridium sordellii*, *Clostridium sphenoides*, *Clostridium spiroforme*, *Clostridium sporogenes*, *Clostridium subterminale*, *Clostridium symbiosum*, *Clostridium tertium*, *Clostridium tetani*, *Clostridium welchii*, and *Clostridium villosum*.

[0101] In another embodiment, the bacterial composition does not comprise at least one of *Clostridium innocuum*, *Clostridium bifermentans*, *Clostridium butyricum*, *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Bacteroides uniformis*, three strains of *Escherichia coli*, and *Lactobacillus sp.*

[0102] In another embodiment, the bacterial composition does not comprise at least one of *Clostridium bifermentans*, *Clostridium innocuum*, *Clostridium butyricum*, three strains of *Escherichia coli*, three strains of *Bacteroides*, and *Blautia producta* (previously known as *Peptostreptococcus productus*).

[0103] In another embodiment, the bacterial composition does not comprise at least one of *Bacteroides sp.*, *Escherichia coli*, and non pathogenic *Clostridia*, including *Clostridium innocuum*, *Clostridium bifermentans* and *Clostridium ramosum*.

[0104] In another embodiment, the bacterial composition does not comprise at least one of more than one *Bacteroides* species, *Escherichia coli* and non-

pathogenic *Clostridia*, such as *Clostridium butyricum*, *Clostridium bifermentans* and *Clostridium innocuum*.

[0105] In another embodiment, the bacterial composition does not comprise at least one of *Bacteroides caccae*, *Bacteroides capillosus*, *Bacteroides coagulans*, *Bacteroides distasonis*, *Bacteroides eggerthii*, *Bacteroides forsythus*, *Bacteroides fragilis*, *Bacteroides fragilis-ryhm*, *Bacteroides gracilis*, *Bacteroides levii*, *Bacteroides macacae*, *Bacteroides merdae*, *Bacteroides ovatus*, *Bacteroides pneumosintes*, *Bacteroides putredinis*, *Bacteroides pyogenes*, *Bacteroides splanchnicus*, *Bacteroides stercoris*, *Bacteroides tectum*, *Bacteroides thetaiotaomicron*, *Bacteroides uniformis*, *Bacteroides ureolyticus*, and *Bacteroides vulgatus*.

[0106] In another embodiment, the bacterial composition does not comprise at least one of *Bacteroides*, *Eubacteria*, *Fusobacteria*, *Propionibacteria*, *Lactobacilli*, *anaerobic cocci*, *Ruminococcus*, *Escherichia coli*, *Gemmiger*, *Desulfomonas*, and *Peptostreptococcus*.

[0107] In another embodiment, the bacterial composition does not comprise at least one of *Bacteroides fragilis* ss. *Vulgatus*, *Eubacterium aerofaciens*, *Bacteroides fragilis* ss. *Thetaiotaomicron*, *Blautia producta* (previously known as *Peptostreptococcus productus* II), *Bacteroides fragilis* ss. *Distasonis*, *Fusobacterium prausnitzii*, *Coprococcus eutactus*, *Eubacterium aerofaciens* III, *Blautia producta* (previously known as *Peptostreptococcus productus* I), *Ruminococcus bromii*, *Bifidobacterium adolescentis*, *Gemmiger formicilis*, *Bifidobacterium longum*, *Eubacterium siraeum*, *Ruminococcus torques*, *Eubacterium rectale* III-H, *Eubacterium rectale* IV, *Eubacterium eligens*, *Bacteroides eggerthii*, *Clostridium leptum*, *Bacteroides fragilis* ss. A, *Eubacterium biforme*, *Bifidobacterium infantis*, *Eubacterium rectale* III-F, *Coprococcus comes*, *Bacteroides capillosus*,

Ruminococcus albus, *Eubacterium formicigenerans*, *Eubacterium hallii*, *Eubacterium ventriosum* I, *Fusobacterium russii*, *Ruminococcus obeum*, *Eubacterium rectale* II, *Clostridium ramosum* I, *Lactobacillus leichmanii*, *Ruminococcus cailidus*, *Butyrivibrio crossotus*, *Acidaminococcus fermentans*, *Eubacterium ventriosum*, *Bacteroides fragilis* ss. *fragilis*, *Bacteroides* AR, *Coprococcus catus*, *Eubacterium hadrum*, *Eubacterium cylindroides*, *Eubacterium ruminantium*, *Eubacterium* CH-1, *Staphylococcus epidermidis*, *Peptostreptococcus* BL, *Eubacterium limosum*, *Bacteroides praeacutus*, *Bacteroides* L, *Fusobacterium mortiferum* I, *Fusobacterium naviforme*, *Clostridium innocuum*, *Clostridium ramosum*, *Propionibacterium acnes*, *Ruminococcus flavefaciens*, *Ruminococcus* AT, *Peptococcus* AU-1, *Eubacterium* AG, -AK, -AL, -AL-1, -AN; *Bacteroides fragilis* ss. *ovatus*, -ss. d, -ss. f; *Bacteroides* L-1, L-5; *Fusobacterium nucleatum*, *Fusobacterium mortiferum*, *Escherichia coli*, *Streptococcus morbillorum*, *Peptococcus magnus*, *Peptococcus* G, AU-2; *Streptococcus intermedius*, *Ruminococcus lactaris*, *Ruminococcus* CO Gemmiger X, *Coprococcus* BH, -CC; *Eubacterium tenue*, *Eubacterium ramulus*, *Eubacterium* AE, -AG-H, -AG-M, -AJ, -BN-1; *Bacteroides clostridiiformis* ss. *clostridiiformis*, *Bacteroides coagulans*, *Bacteroides orails*, *Bacteroides ruminicola* ss. *brevis*, -ss. *ruminicola*, *Bacteroides splanchnicus*, *Desulfomonas pigra*, *Bacteroides* L-4, -N-i; *Fusobacterium* H, *Lactobacillus* G, and *Succinivibrio* A.

Inhibition of Bacterial Pathogens

[0108] In some embodiments, the bacterial composition provides a protective or therapeutic effect against infection by one or more GI pathogens of interest.

[0109] A list of exemplary bacterial pathogens is provided in Table 1, demarcated by the pathogen status column.

[0110] In some embodiments, the pathogenic bacterium or pathobiont is selected from the group categorized as pathogens or pathobionts in Table 1. In another embodiment, the pathogenic bacterium is selected from the group consisting of *Yersinia*, *Vibrio*, *Treponema*, *Streptococcus*, *Staphylococcus*, *Shigella*, *Salmonella*, *Rickettsia*, *Orientia*, *Pseudomonas*, *Neisseria*, *Mycoplasma*, *Mycobacterium*, *Listeria*, *Leptospira*, *Legionella*, *Klebsiella*, *Helicobacter*, *Haemophilus*, *Francisella*, *Escherichia*, *Ehrlichia*, *Enterococcus*, *Coxiella*, *Corynebacterium*, *Clostridium*, *Chlamydia*, *Chlamydophila*, *Campylobacter*, *Burkholderia*, *Brucella*, *Borrelia*, *Bordetella*, *Bifidobacterium*, *Bacillus*, multi-drug resistant bacteria, extended spectrum beta-lactam resistant *Enterococci* (ESBL), Carbapenem-resistant Enterobacteriaceae (CRE), and vancomycin-resistant Enterococci (VRE).

[0111] In some embodiments, these pathogens include, but are not limited to, *Aeromonas hydrophila*, *Campylobacter fetus*, *Plesiomonas shigelloides*, *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, enteroaggregative *Escherichia coli*, enterohemorrhagic *Escherichia coli*, enteroinvasive *Escherichia coli*, enterotoxigenic *Escherichia coli* (such as, but not limited to, LT and/or ST), *Escherichia coli* 0157:H7, *Helicobacter pylori*, *Klebsiella pneumonia*, *Listeria monocytogenes*, *Plesiomonas shigelloides*, *Salmonella* spp., *Salmonella typhi*, *Salmonella paratyphi*, *Shigella* spp., *Staphylococcus* spp., *Staphylococcus aureus*, vancomycin-resistant *enterococcus* spp., *Vibrio* spp., *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Yersinia enterocolitica*.

[0112] In one embodiment, the pathogen of interest is at least one pathogen chosen from *Clostridium difficile*, *Salmonella* spp., pathogenic *Escherichia coli*, vancomycin-resistant *Enterococcus* spp., and extended spectrum beta-lactam resistant *Enterococci* (ESBL).

Purified Spore Populations

[0113] In some embodiments, the bacterial compositions comprise purified spore populations. Purified spore populations contain combinations of commensal bacteria of the human gut microbiota with the capacity to meaningfully provide functions of a healthy microbiota when administered to a mammalian subject. Without being limited to a specific mechanism, it is thought that such compositions inhibit the growth of a pathogen such as *C. difficile*, *Salmonella* spp., enteropathogenic *E. coli*, and vancomycin-resistant *Enterococcus* spp., so that a healthy, diverse and protective microbiota can be maintained or, in the case of pathogenic bacterial infections such as *C. difficile* infection, repopulate the intestinal lumen to reestablish ecological control over potential pathogens. Spore forming bacteria often exist in both a spore form and a vegetative form in a given population of cells. The biological activity and resistance to environmental stress can vary in each form and as a result both forms might be advantageous for different purposes. Bacterial compositions and methods described allow one to purify compositions containing spores to produce bacterial compositions that are resistant to environmental stresses such as but not limited to aerobic conditions, lack of nutrients, low pH, and temperature variations to populate the gut. In vegetative form, the proper bacterial compositions disclosed herein can produce beneficial health effects.

[0114] Disclosed herein are therapeutic compositions containing non-pathogenic, germination-competent bacterial spores, for the prevention, control, and treatment of gastrointestinal diseases, disorders and conditions and for general nutritional health. These compositions are advantageous in being suitable for safe administration to humans and other mammalian subjects and are efficacious in numerous gastrointestinal diseases, disorders and conditions and in general nutritional health.

While spore-based compositions are known, these are generally prepared according to various techniques such as lyophilization or spray-drying of liquid bacterial cultures, resulting in poor efficacy, instability, substantial variability and lack of adequate safety.

[0115] It has now been found that populations of bacterial spores can be obtained from biological materials obtained from mammalian subjects, including humans. These populations are formulated into compositions as provided herein, and administered to mammalian subjects using the methods as provided herein.

[0116] Provided herein are therapeutic compositions containing a purified population of bacterial spores. As used herein, the terms “purify”, “purified” and “purifying” refer to the state of a population (e.g., a plurality of known or unknown amount and/or concentration) of desired bacterial spores, that have undergone one or more processes of purification, e.g., a selection or an enrichment of the desired bacterial spore, or alternatively a removal or reduction of residual habitat products as described herein. In some embodiments, a purified population has no detectable undesired activity or, alternatively, the level or amount of the undesired activity is at or below an acceptable level or amount. In other embodiments, a purified population has an amount and/or concentration of desired bacterial spores at or above an acceptable amount and/or concentration. In other embodiments, the purified population of bacterial spores is enriched as compared to the starting material (e.g., a fecal material) from which the population is obtained. This enrichment may be by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99%, 99.999%, 99.9999%, or greater than 99.9999% as compared to the starting material.

[0117] In certain embodiments, the purified populations of bacterial spores have reduced or undetectable levels of one or more pathogenic activities, such as toxicity, an infection of the mammalian recipient subject, an immunomodulatory activity, an autoimmune response, a metabolic response, or an inflammatory response or a neurological response. Such a reduction in a pathogenic activity may be by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99%, 99.999%, 99.9999%, or greater than 99.9999% as compared to the starting material. In other embodiments, the purified populations of bacterial spores have reduced sensory components as compared to fecal material, such as reduced odor, taste, appearance, and umami.

[0118] Provided are purified populations of bacterial spores that are substantially free of residual habitat products. In certain embodiments, this means that the bacterial spore composition no longer contains a substantial amount of the biological matter associated with the microbial community while living on or in the human or animal subject, and the purified population of spores may be 100% free, 99% free, 98% free, 97% free, 96% free, or 95% free of any contamination of the biological matter associated with the microbial community. Substantially free of residual habitat products may also mean that the bacterial spore composition contains no detectable cells from a human or animal, and that only microbial cells are detectable, in particular, only desired microbial cells are detectable. In another embodiment, it means that fewer than $1 \times 10^{-2}\%$, $1 \times 10^{-3}\%$, $1 \times 10^{-4}\%$, $1 \times 10^{-5}\%$, $1 \times 10^{-6}\%$, $1 \times 10^{-7}\%$, $1 \times 10^{-8}\%$ of the cells in the bacterial composition are human or animal, as compared to microbial cells. In another embodiment, the residual habitat product present in the purified population is reduced at least a certain level from the fecal material obtained from the mammalian donor subject, e.g., reduced by at least about 10%, 20%, 30%,

40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99%, 99.999%, 99.9999%, or greater than 99.9999%.

[0119] In one embodiment, substantially free of residual habitat products or substantially free of a detectable level of a pathogenic material means that the bacterial composition contains no detectable viral (including bacterial viruses (i.e., phage)), fungal, or mycoplasmal or toxoplasmal contaminants, or a eukaryotic parasite such as a helminth. Alternatively, the purified spore populations are substantially free of an acellular material, e.g., DNA, viral coat material, or non-viable bacterial material.

[0120] As described herein, purified spore populations can be demonstrated by genetic analysis (e.g., PCR, DNA sequencing), serology and antigen analysis, and methods using instrumentation such as flow cytometry with reagents that distinguish desired bacterial spores from non-desired, contaminating materials.

[0121] Exemplary biological materials include fecal materials such as feces or materials isolated from the various segments of the small and large intestines. Fecal materials are obtained from a mammalian donor subject, or can be obtained from more than one donor subject, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 200, 300, 400, 500, 750, 1000 or from greater than 1000 donors, where such materials are then pooled prior to purification of the desired bacterial spores.

[0122] In alternative embodiments, the desired bacterial spores are purified from a single fecal material sample obtained from a single donor, and after such purification are combined with purified spore populations from other purifications, either from the same donor at a different time, or from one or more different donors, or both.

[0123] Preferred bacterial genera include Acetonema, Alkaliphilus, Alicyclobacillus, Amphibacillus, Ammonifex, Anaerobacter, Anaerofustis, Anaerostipes, Anaerotruncus, Anoxybacillus, Bacillus, Blautia, Brevibacillus, Bryantella, Caldicellulosiruptor, Caloramator, Candidatus, Carboxydibrachium, Carboxydotherrmus, Clostridium, Cohnella, Coprococcus, Dendrosporobacter Desulfitobacterium, Desulfosporosinus, Desulfotomaculum, Dorea, Eubacterium, Faecalibacterium, Filifactor, Geobacillus, Halobacteroides, Heliobacillus, Heliobacterium, Heliophilum, Heliorestis, Lachnoanaerobaculum, Lysinibacillus, Moorella, Oceanobacillus, Orenia (S.), Oxalophagus, Oxobacter, Paenibacillus, Pelospora, Pelotomaculum, Propionispora, Roseburia, Ruminococcus, Sarcina, Sporobacterium, Sporohalobacter, Sporolactobacillus, Sporomusa, Sporosarcina, Sporotomaculum, Subdoligranulum, Symbiobacterium, Syntrophobotulus, Syntrophospora, Terribacillus, Thermoanaerobacter, and Thermosinus.

[0124] Preferred bacterial species are provided at Table 1, demarcated as spore former or non-spore former. Where specific strains of a species are provided, one of skill in the art will recognize that other strains of the species or species in the same clade can be substituted for the named strain.

[0125] In some embodiments, spore-forming bacteria are identified by the presence of nucleic acid sequences that modulate sporulation. In particular, signature sporulation genes are highly conserved across members of distantly related genera including Clostridium and Bacillus. Traditional approaches of forward genetics have identified many, if not all, genes that are essential for sporulation (spo). The developmental program of sporulation is governed in part by the successive action of four compartment-specific sigma factors (appearing in the order

σ F, σ E, σ G and σ K), whose activities are confined to the forespore (σ F and σ G) or the mother cell (σ E and σ K).

[0126] Provided are spore populations containing more than one type of bacterium. As used herein, a “type” or more than one “types” of bacteria may be differentiated at the genus level, the species level, the sub-species level, the strain level or by any other taxonomic method, as described herein and otherwise known in the art.

[0127] In some embodiments, all or essentially all of the bacterial spores present in a purified population are obtained from a fecal material treated as described herein or otherwise known in the art. In alternative embodiments, one or more than one bacterial spores or types of bacterial spores are generated in culture and combined to form a purified spore population. In other alternative embodiments, one or more of these culture-generated spore populations are combined with a fecal material-derived spore population to generate a hybrid spore population. Bacterial compositions may contain at least two types of these preferred bacteria, including strains of the same species. For instance, a bacterial composition may comprise at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 or more than 20 types of bacteria, as defined by species or operational taxonomic unit (OTU) encompassing such species.

[0128] Thus, provided herein are methods for production of a composition containing a population of bacterial spores suitable for therapeutic administration to a mammalian subject in need thereof. And the composition is produced by generally following the steps of: (a) providing a fecal material obtained from a mammalian donor subject; and (b) subjecting the fecal material to at least one purification

treatment or step under conditions such that a population of bacterial spores is produced from the fecal material. The composition is formulated such that a single oral dose contains at least about 1×10^4 colony forming units of the bacterial spores, and a single oral dose will typically contain about 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 , 1×10^9 , 1×10^{10} , 1×10^{11} , 1×10^{12} , 1×10^{13} , 1×10^{14} , 1×10^{15} , or greater than 1×10^{15} CFUs of the bacterial spores. The presence and/or concentration of a given type of bacteria spore may be known or unknown in a given purified spore population. If known, for example the concentration of spores of a given strain, or the aggregate of all strains, is e.g., 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 , 1×10^9 , 1×10^{10} , 1×10^{11} , 1×10^{12} , 1×10^{13} , 1×10^{14} , 1×10^{15} , or greater than 1×10^{15} viable bacterial spores per gram of composition or per administered dose.

[0129] In some formulations, the composition contains at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than 90% spores on a mass basis. In some formulations, the administered dose does not exceed 200, 300, 400, 500, 600, 700, 800, 900 milligrams or 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, or 1.9 grams in mass.

[0130] The bacterial spore compositions are generally formulated for oral or gastric administration, typically to a mammalian subject. In particular embodiments, the composition is formulated for oral administration as a solid, semi-solid, gel, or liquid form, such as in the form of a pill, tablet, capsule, or lozenge. In some embodiments, such formulations contain or are coated by an enteric coating to protect the bacteria through the stomach and small intestine, although spores are generally resistant to the stomach and small intestines.

[0131] The bacterial spore compositions may be formulated to be effective in a given mammalian subject in a single administration or over multiple administrations.

For example, a single administration is substantially effective to reduce *Cl. difficile* and/or *Cl. difficile* toxin content in a mammalian subject to whom the composition is administered. Substantially effective means that *Cl. difficile* and/or *Cl. difficile* toxin content in the subject is reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99% or greater than 99% following administration of the composition.

METHODS OF THE INVENTION

In Vitro Assays Substantiating Protective Effect Of Bacterial Compositions

[0132] In one embodiment, provided is an *In Vitro* Assay utilizing competition between the bacterial compositions or subsets thereof and *C. difficile*. Exemplary embodiments of this Assay are provided herein and in the Examples.

[0133] In another embodiment, provided is an *In Vitro* Assay utilizing 10% (wt/vol) Sterile-Filtered Feces. Provided is an in vitro assay to test for the protective effect of the bacterial compositions and to screen *in vitro* for combinations of microbes that inhibit the growth of a pathogen. The assay can operate in automated high-throughput or manual modes. Under either system, human or animal feces may be re-suspended in an anaerobic buffer solution, such as pre-reduced PBS or other suitable buffer, the particulate removed by centrifugation, and filter sterilized. This 10% sterile-filtered feces material serves as the base media for the *in vitro* assay. To test a bacterial composition, an investigator may add it to the sterile-filtered feces material for a first incubation period and then may inoculate the incubated microbial solution with the pathogen of interest for a second incubation period. The resulting titer of the pathogen may be quantified by any number of methods such as those described below, and the change in the amount of pathogen is compared to standard controls including the pathogen cultivated in the absence of the bacterial

composition. The assay is conducted using at least one control. Feces from a healthy subject may be used as a positive control. As a negative control, antibiotic-treated feces or heat-treated feces may be used. Various bacterial compositions may be tested in this material and the bacterial compositions optionally compared to the positive and/or negative controls. The ability to inhibit the growth of the pathogen may be measured by plating the incubated material on *C. difficile* selective media and counting colonies. After competition between the bacterial composition and *C. difficile*, each well of the *in vitro* assay plate is serially diluted ten-fold six times, and plated on selective media, such as but not limited to cycloserine cefoxitin mannitol agar (CCMA) or cycloserine cefoxitin fructose agar (CCFA), and incubated. Colonies of *C. difficile* are then counted to calculate the concentration of viable cells in each well at the end of the competition. Colonies of *C. difficile* are confirmed by their characteristic diffuse colony edge morphology as well as fluorescence under UV light.

[0134] In another embodiment, the *in vitro* assay utilizes Antibiotic-Treated Feces. In an alternative embodiment, and instead of using 10% sterile-filtered feces, human or animal feces may be resuspended in an anaerobic buffer solution, such as pre-reduced PBS or other suitable buffer. The resuspended feces is treated with an antibiotic, such as clindamycin, or a cocktail of several antibiotics in order to reduce the ability of feces from a healthy subject to inhibit the growth of *C. difficile*; this material is termed the antibiotic-treated matrix. While not being bound by any mechanism, it is believed that beneficial bacteria in healthy subjects protects them from infection by competing out *C. difficile*. Treating feces with antibiotics kills or reduces the population of those beneficial bacteria, allowing *C. difficile* to grow in this assay matrix. Antibiotics in addition to clindamycin that inhibit the normal flora

include ceftriaxone and piperacillin-tazobactam and may be substituted for the clindamycin. The antibiotic-treated matrix is centrifuged, the supernatant removed, and the pelleted material resuspended in filter-sterilized, diluted feces in order to remove any residual antibiotic. This washed antibiotic-treated matrix may be used in the *in vitro* assay described above in lieu of the 10% sterile-filtered feces.

[0135] Alternatively, the ability to inhibit the growth of the pathogen may be measured by quantitative PCR (qPCR). Standard techniques may be followed to generate a standard curve for the pathogen of interest. Genomic DNA may be extracted from samples using commercially-available kits, such as the Mo Bio Powersoil®-htp 96 Well Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA), the Mo Bio Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA), or the QIAamp DNA Stool Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. The qPCR may be conducted using HotMasterMix (5PRIME, Gaithersburg, MD) and primers specific for the pathogen of interest, and may be conducted on a MicroAmp® Fast Optical 96-well Reaction Plate with Barcode (0.1mL) (Life Technologies, Grand Island, NY) and performed on a BioRad C1000™ Thermal Cycler equipped with a CFX96™ Real-Time System (BioRad, Hercules, CA), with fluorescent readings of the FAM and ROX channels. The C_q value for each well on the FAM channel is determined by the CFX Manager™ software version 2.1. The log₁₀(cfu/ml) of each experimental sample is calculated by inputting a given sample's C_q value into linear regression model generated from the standard curve comparing the C_q values of the standard curve wells to the known log₁₀(cfu/ml) of those samples. The skilled artisan may employ alternative qPCR modes.

In Vitro Assays Substantiating Protective Effect Of Bacterial Compositions

[0136] Also provided are *in vivo* assays establishing the protective effect of bacterial compositions. Provided is an *in vivo* mouse model to test for the protective effect of the bacterial compositions against *C. difficile*. In this model (based on Chen, et al., A mouse model of Clostridium difficile associated disease, Gastroenterology 135(6):1984-1992 (2008)), mice are made susceptible to *C. difficile* by a 7 day treatment (days -12 to -5 of experiment) with 5 to 7 antibiotics (including kanamycin, colistin, gentamycin, metronidazole and vancomycin and optionally including ampicillin and ciprofloxacin) delivered via their drinking water, followed by a single dose with Clindamycin on day -3, then challenged three days later on day 0 with 10⁴ spores of *C. difficile* via oral gavage (i.e., oro-gastric lavage). Bacterial compositions may be given either before (prophylactic treatment) or after (therapeutic treatment) *C. difficile* gavage. Further, bacterial compositions may be given after (optional) vancomycin treatment (see below) to assess their ability to prevent recurrence and thus suppress the pathogen *in vivo*. The outcomes assessed each day from day -1 to day 6 (or beyond, for prevention of recurrence) are weight, clinical signs, mortality and shedding of *C. difficile* in the feces. Weight loss, clinical signs of disease, and *C. difficile* shedding are typically observed without treatment. Vancomycin provided by oral gavage on days -1 to 4 protects against these outcomes and serves as a positive control. Clinical signs are subjective, and scored each day by the same experienced observer. Animals that lose greater than or equal to 25% of their body weight are euthanized and counted as infection-related mortalities. Feces are gathered from mouse cages (5 mice per cage) each day, and the shedding of *C. difficile* spores is detected in the feces using a selective plating assay as described for the *in vitro* assay above, or via qPCR for the toxin gene as described herein. The effects of test materials including 10% suspension of human feces (as a positive

control), bacterial compositions, or PBS (as a negative vehicle control), are determined by introducing the test article in a 0.2 mL volume into the mice via oral gavage on day -1, one day prior to *C. difficile* challenge, on day 1, 2 and 3 as treatment or post-vancomycin treatment on days 5, 6, 7 and 8. Vancomycin, as discussed above, is given on days 1 to 4 as another positive control. Alternative dosing schedules and routes of administration (e.g. rectal) may be employed, including multiple doses of test article, and 10^3 to 10^{10} of a given organism or composition may be delivered.

Methods for Determining 16S Sequences

[0137] OTUs can be defined either by full 16S sequencing of the rRNA gene, by sequencing of a specific hypervariable region of this gene (i.e. V1, V2, V3, V4, V5, V6, V7, V8, or V9), or by sequencing of any combination of hypervariable regions from this gene (e.g. V1-3 or V3-5). The bacterial 16S rDNA is approximately 1500 nucleotides in length and is used in reconstructing the evolutionary relationships and sequence similarity of one bacterial isolate to another using phylogenetic approaches. 16S sequences are used for phylogenetic reconstruction as they are in general highly conserved, but contain specific hypervariable regions that harbor sufficient nucleotide diversity to differentiate genera and species of most microbes.

[0138] Using well known techniques, in order to determine the full 16S sequence or the sequence of any hypervariable region of the 16S sequence, genomic DNA is extracted from a bacterial sample, the 16S rDNA (full region or specific hypervariable regions) amplified using polymerase chain reaction (PCR), the PCR products cleaned, and nucleotide sequences delineated to determine the genetic composition of 16S gene or subdomain of the gene. If full 16S sequencing is performed, the sequencing method used may be, but is not limited to, Sanger sequencing. If one or

more hypervariable regions are used, such as the V4 region, the sequencing can be, but is not limited to being, performed using the Sanger method or using a next-generation sequencing method, such as an Illumina (sequencing by synthesis) method using barcoded primers allowing for multiplex reactions.

[0139] OTUs can be defined by a combination of nucleotide markers or genes, in particular highly conserved genes (e.g., “house-keeping” genes), or a combination thereof, full-genome sequence, or partial genome sequence generated using amplified genetic products, or whole genome sequence (WGS). Using well defined methods DNA extracted from a bacterial sample will have specific genomic regions amplified using PCR and sequenced to determine the nucleotide sequence of the amplified products. In the whole genome shotgun (WGS) method, extracted DNA will be directly sequenced without amplification. Sequence data can be generated using any sequencing technology including, but not limited to Sanger, Illumina, 454 Life Sciences, Ion Torrent, ABI, Pacific Biosciences, and/or Oxford Nanopore.

Methods for Preparing a Bacterial Composition for Administration to a Subject

[0140] Methods for producing bacterial compositions can include three main processing steps, combined with one or more mixing steps. The steps include organism banking, organism production, and preservation.

[0141] For banking, the strains included in the bacterial composition may be (1) isolated directly from a specimen or taken from a banked stock, (2) optionally cultured on a nutrient agar or broth that supports growth to generate viable biomass, and (3) the biomass optionally preserved in multiple aliquots in long-term storage.

[0142] In embodiments that use a culturing step, the agar or broth can contain nutrients that provide essential elements and specific factors that enable growth. An example would be a medium composed of 20 g/L glucose, 10 g/L yeast extract, 10

g/L soy peptone, 2 g/L citric acid, 1.5 g/L sodium phosphate monobasic, 100 mg/L ferric ammonium citrate, 80 mg/L magnesium sulfate, 10 mg/L hemin chloride, 2 mg/L calcium chloride, 1 mg/L menadione. A variety of microbiological media and variations are well known in the art (e.g. R.M. Atlas, *Handbook of Microbiological Media* (2010) CRC Press). Medium can be added to the culture at the start, may be added during the culture, or may be intermittently/continuously flowed through the culture. The strains in the bacterial composition may be cultivated alone, as a subset of the bacterial composition, or as an entire collection comprising the bacterial composition. As an example, a first strain may be cultivated together with a second strain in a mixed continuous culture, at a dilution rate lower than the maximum growth rate of either cell to prevent the culture from washing out of the cultivation.

[0143] The inoculated culture is incubated under favorable conditions for a time sufficient to build biomass. For bacterial compositions for human use, this is often at 37°C temperature, pH, and other parameter with values similar to the normal human niche. The environment can be actively controlled, passively controlled (e.g., via buffers), or allowed to drift. For example, for anaerobic bacterial compositions (e.g., gut microbiota), an anoxic/reducing environment can be employed. This can be accomplished by addition of reducing agents such as cysteine to the broth, and/or stripping it of oxygen. As an example, a culture of a bacterial composition can be grown at 37°C, pH 7, in the medium above, pre-reduced with 1 g/L cysteine HCl.

[0144] When the culture has generated sufficient biomass, it can be preserved for banking. The organisms can be placed into a chemical milieu that protects from freezing (adding 'cryoprotectants'), drying ('lyoprotectants'), and/or osmotic shock ('osmoprotectants'), dispensing into multiple (optionally identical) containers to create a uniform bank, and then treating the culture for preservation. Containers are

generally impermeable and have closures that assure isolation from the environment. Cryopreservation treatment is accomplished by freezing a liquid at ultra-low temperatures (e.g., at or below -80°C). Dried preservation removes water from the culture by evaporation (in the case of spray drying or 'cool drying') or by sublimation (e.g., for freeze drying, spray freeze drying). Removal of water improves long-term bacterial composition storage stability at temperatures elevated above cryogenic. If the bacterial composition comprises spore forming species and results in the production of spores, the final composition can be purified by additional means, such as density gradient centrifugation preserved using the techniques described above. Bacterial composition banking can be done by culturing and preserving the strains individually, or by mixing the strains together to create a combined bank. As an example of cryopreservation, a bacterial composition culture can be harvested by centrifugation to pellet the cells from the culture medium, the supernate decanted and replaced with fresh culture broth containing 15% glycerol. The culture can then be aliquoted into 1 mL cryotubes, sealed, and placed at -80°C for long-term viability retention. This procedure achieves acceptable viability upon recovery from frozen storage.

[0145] Organism production can be conducted using similar culture steps to banking, including medium composition and culture conditions. It can be conducted at larger scales of operation, especially for clinical development or commercial production. At larger scales, there can be several subcultivations of the bacterial composition prior to the final cultivation. At the end of cultivation, the culture is harvested to enable further formulation into a dosage form for administration. This can involve concentration, removal of undesirable medium components, and/or introduction into a chemical milieu that preserves the bacterial composition and

renders it acceptable for administration via the chosen route. For example, a bacterial composition can be cultivated to a concentration of 10^{10} CFU/mL, then concentrated 20-fold by tangential flow microfiltration; the spent medium can be exchanged by diafiltering with a preservative medium consisting of 2% gelatin, 100 mM trehalose, and 10 mM sodium phosphate buffer. The suspension can then be freeze-dried to a powder and titrated.

[0146] After drying, the powder can be blended to an appropriate potency, and mixed with other cultures and/or a filler such as microcrystalline cellulose for consistency and ease of handling, and the bacterial composition formulated as provided herein.

Methods of Treating a Subject

[0147] In some embodiments, the compositions disclosed herein are administered to a patient or a user (sometimes collectively referred to as a “subject”). As used herein “administer” and “administration” encompasses embodiments in which one person directs another to consume a bacterial composition in a certain manner and/or for a certain purpose, and also situations in which a user uses a bacteria composition in a certain manner and/or for a certain purpose independently of or in variance to any instructions received from a second person. Non-limiting examples of embodiments in which one person directs another to consume a bacterial composition in a certain manner and/or for a certain purpose include when a physician prescribes a course of conduct and/or treatment to a patient, when a parent commands a minor user (such as a child) to consume a bacterial composition, when a trainer advises a user (such as an athlete) to follow a particular course of conduct and/or treatment, and when a manufacturer, distributor, or marketer recommends conditions of use to an end user, for example through

advertisements or labeling on packaging or on other materials provided in association with the sale or marketing of a product.

[0148] The bacterial compositions offer a protective and/or therapeutic effect against infection by one or more GI pathogens of interest and can be administered after an acute case of infection has been resolved in order to prevent relapse, during an acute case of infection as a complement to antibiotic therapy if the bacterial composition is not sensitive to the same antibiotics as the GI pathogen, or to prevent infection or reduce transmission from disease carriers. In some embodiments a bacterial compositions administered to the gut are used to treat disorders that are not gut-related. Altering the gut microbiome can have effects on the skin and vagina of mammalian hosts (Levkovich et al, Probiotic bacteria induce a 'glow of health', PlosOne, Vol 8, Issue 1, e53867. 2013) and can treat distal bacterial infections including mastitis (Jimenez et al. Oral Administration of Lactobacillus strains isolate from breast milk as an alternative for the treatment of Infectious mastitis during lactation. Applied and Environmental Microbiology (74) No. 15. 2008). Because the gut serves as a reservoir for bacteria for the rest of the body, the bacterial compositions described herein can be used to treat non-GI related health problems.

[0149] The present bacterial compositions can be useful in a variety of clinical situations. For example, the bacterial compositions can be administered as a complementary treatment to antibiotics when a patient is suffering from an acute infection, to reduce the risk of recurrence after an acute infection has subsided, or when a patient will be in close proximity to others with or at risk of serious gastrointestinal infections (physicians, nurses, hospital workers, family members of those who are ill or hospitalized).

[0150] The present bacterial compositions can be administered to animals, including humans, laboratory animals (e.g., primates, rats, mice), livestock (e.g., cows, sheep, goats, pigs, turkeys, chickens), and household pets (e.g., dogs, cats, rodents).

[0151] In the present method, the bacterial composition can be administered enterically, in other words, by a route of access to the gastrointestinal tract. This includes oral administration, rectal administration (including enema, suppository, or colonoscopy), by an oral or nasal tube (nasogastric, nasojejunal, oral gastric, or oral jejunal), as detailed more fully herein.

Pretreatment Protocols

[0152] Prior to administration of the bacterial composition, the patient can optionally have a pretreatment protocol to prepare the gastrointestinal tract to receive the bacterial composition. In certain embodiments, the pretreatment protocol is advisable, such as when a patient has an acute infection with a highly resilient pathogen. In other embodiments, the pretreatment protocol is entirely optional, such as when the pathogen causing the infection is not resilient, or the patient has had an acute infection that has been successfully treated but where the physician is concerned that the infection may recur. In these instances, the pretreatment protocol can enhance the ability of the bacterial composition to affect the patient's microbiome.

[0153] As one way of preparing the patient for administration of the microbial ecosystem, at least one antibiotic can be administered to alter the bacteria in the patient. As another way of preparing the patient for administration of the microbial ecosystem, a standard colon-cleansing preparation can be administered to the patient to substantially empty the contents of the colon, such as used to prepare a

patient for a colonoscopy. By “substantially emptying the contents of the colon,” this application means removing at least 75%, at least 80%, at least 90%, at least 95%, or about 100% of the contents of the ordinary volume of colon contents. Antibiotic treatment can precede the colon-cleansing protocol.

[0154] If a patient has received an antibiotic for treatment of an infection, or if a patient has received an antibiotic as part of a specific pretreatment protocol, in one embodiment, the antibiotic can be stopped in sufficient time to allow the antibiotic to be substantially reduced in concentration in the gut before the bacterial composition is administered. In one embodiment, the antibiotic can be discontinued 1, 2, or 3 days before the administration of the bacterial composition. In another embodiment, the antibiotic can be discontinued 3, 4, 5, 6, or 7 antibiotic half-lives before administration of the bacterial composition. In another embodiment, the antibiotic can be chosen so the constituents in the bacterial composition have an MIC₅₀ that is higher than the concentration of the antibiotic in the gut.

[0155] MIC₅₀ of a bacterial composition or the elements in the composition can be determined by methods well known in the art. Reller et al., Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices, Clinical Infectious Diseases 49(11):1749-1755 (2009). In such an embodiment, the additional time between antibiotic administration and administration of the bacterial composition is not necessary. If the pretreatment protocol is part of treatment of an acute infection, the antibiotic can be chosen so that the infection is sensitive to the antibiotic, but the constituents in the bacterial composition are not sensitive to the antibiotic.

Routes of Administration

[0156] The bacterial compositions of the invention are suitable for administration to mammals and non-mammalian animals in need thereof. In certain embodiments, the mammalian subject is a human subject who has one or more symptoms of a dysbiosis.

[0157] When a mammalian subject is suffering from a disease, disorder or condition characterized by an aberrant microbiota, the bacterial compositions described herein are suitable for treatment thereof. In some embodiments, the mammalian subject has not received antibiotics in advance of treatment with the bacterial compositions. For example, the mammalian subject has not been administered at least two doses of vancomycin, metronidazole and/or or similar antibiotic compound within one week prior to administration of the therapeutic composition. In other embodiments, the mammalian subject has not previously received an antibiotic compound in the one month prior to administration of the therapeutic composition. In other embodiments, the mammalian subject has received one or more treatments with one or more different antibiotic compounds and such treatment(s) resulted in no improvement or a worsening of symptoms.

[0158] In some embodiments, the gastrointestinal disease, disorder or condition is diarrhea caused by *C. difficile* including recurrent *C. difficile* infection, ulcerative colitis, colitis, Crohn's disease, or irritable bowel disease. Beneficially, the therapeutic composition is administered only once prior to improvement of the disease, disorder or condition. In some embodiments, the therapeutic composition is administered at intervals greater than two days, such as once every three, four, five or six days, or every week or less frequently than every week. In other embodiments, the preparation can be administered intermittently according to a set schedule, e.g., once a day, once weekly, or once monthly, or when the subject

relapses from the primary illness. In another embodiment, the preparation may be administered on a long-term basis to subjects who are at risk for infection with or who may be carriers of these pathogens, including subjects who will have an invasive medical procedure (such as surgery), who will be hospitalized, who live in a long-term care or rehabilitation facility, who are exposed to pathogens by virtue of their profession (livestock and animal processing workers), or who could be carriers of pathogens (including hospital workers such as physicians, nurses, and other health care professionals).

[0159] In certain embodiments, the bacterial composition is administered enterically. This preferentially includes oral administration, or by an oral or nasal tube (including nasogastric, nasojejunal, oral gastric, or oral jejunal). In other embodiments, administration includes rectal administration (including enema, suppository, or colonoscopy). The bacterial composition can be administered to at least one region of the gastrointestinal tract, including the mouth, esophagus, stomach, small intestine, large intestine, and rectum. In some embodiments, it is administered to all regions of the gastrointestinal tract. The bacterial compositions can be administered orally in the form of medicaments such as powders, capsules, tablets, gels or liquids. The bacterial compositions can also be administered in gel or liquid form by the oral route or through a nasogastric tube, or by the rectal route in a gel or liquid form, by enema or instillation through a colonoscope or by a suppository.

[0160] If the composition is administered colonoscopically and, optionally, if the bacterial composition is administered by other rectal routes (such as an enema or suppository) or even if the subject has an oral administration, the subject can have a colon-cleansing preparation. The colon-cleansing preparation can facilitate proper use of the colonoscope or other administration devices, but even when it does not

serve a mechanical purpose, it can also maximize the proportion of the bacterial composition relative to the other organisms previously residing in the gastrointestinal tract of the subject. Any ordinarily acceptable colon-cleansing preparation may be used such as those typically provided when a subject undergoes a colonoscopy.

Dosages and Schedule for Administration

[0161] In some embodiments, the bacteria and bacterial compositions are provided in a dosage form. In certain embodiments, the dosage form is designed for administration of at least one OTU or combination thereof disclosed herein, wherein the total amount of bacterial composition administered is selected from 0.1ng to 10g, 10ng to 1g, 100ng to 0.1g, 0.1mg to 500mg, 1mg to 100mg, or from 10-15mg. In other embodiments, the bacterial composition is consumed at a rate of from 0.1ng to 10g a day, 10ng to 1g a day, 100ng to 0.1g a day, 0.1mg to 500mg a day, 1mg to 100mg a day, or from 10-15mg a day, or more.

[0162] In certain embodiments, the treatment period is at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, or at least 1 year. In some embodiments the treatment period is from 1 day to 1 week, from 1 week to 4 weeks, from 1 month, to 3 months, from 3 months to 6 months, from 6 months to 1 year, or for over a year.

[0163] In one embodiment, 10^5 and 10^{12} microorganisms total can be administered to the patient in a given dosage form. In another embodiment, an effective amount can be provided in from 1 to 500 ml or from 1 to 500 grams of the bacterial composition having from 10^7 to 10^{11} bacteria per ml or per gram, or a capsule, tablet or suppository having from 1 mg to 1000 mg lyophilized powder

having from 10^7 to 10^{11} bacteria. Those receiving acute treatment can receive higher doses than those who are receiving chronic administration (such as hospital workers or those admitted into long-term care facilities).

[0164] Any of the preparations described herein can be administered once on a single occasion or on multiple occasions, such as once a day for several days or more than once a day on the day of administration (including twice daily, three times daily, or up to five times daily). In another embodiment, the preparation can be administered intermittently according to a set schedule, e.g., once weekly, once monthly, or when the patient relapses from the primary illness. In one embodiment, the preparation can be administered on a long-term basis to individuals who are at risk for infection with or who may be carriers of these pathogens, including individuals who will have an invasive medical procedure (such as surgery), who will be hospitalized, who live in a long-term care or rehabilitation facility, who are exposed to pathogens by virtue of their profession (livestock and animal processing workers), or who could be carriers of pathogens (including hospital workers such as physicians, nurses, and other health care professionals).

Patient Selection

[0165] Particular bacterial compositions can be selected for individual patients or for patients with particular profiles. For example, 16S sequencing can be performed for a given patient to identify the bacteria present in his or her microbiota. The sequencing can either profile the patient's entire microbiome using 16S sequencing (to the family, genera, or species level), a portion of the patient's microbiome using 16S sequencing, or it can be used to detect the presence or absence of specific candidate bacteria that are biomarkers for health or a particular disease state, such as markers of multi-drug resistant organisms or specific genera of concern such as

Escherichia. Based on the biomarker data, a particular composition can be selected for administration to a patient to supplement or complement a patient's microbiota in order to restore health or treat or prevent disease. In another embodiment, patients can be screened to determine the composition of their microbiota to determine the likelihood of successful treatment.

Combination Therapy

[0166] The bacterial compositions can be administered with other agents in a combination therapy mode, including anti-microbial agents and prebiotics.

Administration can be sequential, over a period of hours or days, or simultaneous.

[0167] In one embodiment, the bacterial compositions are included in combination therapy with one or more anti-microbial agents, which include anti-bacterial agents, anti-fungal agents, anti-viral agents and anti-parasitic agents.

[0168] Anti-bacterial agents can include cephalosporin antibiotics (cephalexin, cefuroxime, cefadroxil, cefazolin, cephalothin, cefaclor, cefamandole, cefoxitin, cefprozil, and ceftobiprole); fluoroquinolone antibiotics (cipro, Levaquin, floxin, tequin, avelox, and norflox); tetracycline antibiotics (tetracycline, minocycline, oxytetracycline, and doxycycline); penicillin antibiotics (amoxicillin, ampicillin, penicillin V, dicloxacillin, carbenicillin, vancomycin, and methicillin); and carbapenem antibiotics (ertapenem, doripenem, imipenem/cilastatin, and meropenem).

[0169] Anti-viral agents can include Abacavir, Acyclovir, Adefovir, Amprenavir, Atazanavir, Cidofovir, Darunavir, Delavirdine, Didanosine, Docosanol, Efavirenz, Elvitegravir, Emtricitabine, Enfuvirtide, Etravirine, Famciclovir, Foscarnet, Fomivirsen, Ganciclovir, Indinavir, Idoxuridine, Lamivudine, Lopinavir Maraviroc, MK-2048, Nelfinavir, Nevirapine, Penciclovir, Raltegravir, Rilpivirine, Ritonavir, Saquinavir, Stavudine, Tenofovir Trifluridine, Valaciclovir, Valganciclovir, Vidarabine,

Ibacinabine, Amantadine, Oseltamivir, Rimantidine, Tipranavir, Zalcitabine, Zanamivir and Zidovudine.

[0170] Examples of antifungal compounds include, but are not limited to polyene antifungals such as natamycin, rimocidin, filipin, nystatin, amphotericin B, candicin, and hamycin; imidazole antifungals such as miconazole, ketoconazole, clotrimazole, econazole, omoconazole, bifonazole, butoconazole, fenticonazole, isoconazole, oxiconazole, sertaconazole, sulconazole, and tioconazole; triazole antifungals such as fluconazole, itraconazole, isavuconazole, ravuconazole, posaconazole, voriconazole, terconazole, and albaconazole; thiazole antifungals such as abafungin; allylamine antifungals such as terbinafine, naftifine, and butenafine; and echinocandin antifungals such as anidulafungin, caspofungin, and micafungin. Other compounds that have antifungal properties include, but are not limited to polygodial, benzoic acid, ciclopirox, tolnaftate, undecylenic acid, flucytosine or 5-fluorocytosine, griseofulvin, and haloprogin.

[0171] In one embodiment, the bacterial compositions are included in combination therapy with one or more corticosteroids, mesalazine, mesalamine, sulfasalazine, sulfasalazine derivatives, immunosuppressive drugs, cyclosporin A, mercaptopurine, azathiopurine, prednisone, methotrexate, antihistamines, glucocorticoids, epinephrine, theophylline, cromolyn sodium, anti-leukotrienes, anti-cholinergic drugs for rhinitis, anti-cholinergic decongestants, mast-cell stabilizers, monoclonal anti-IgE antibodies, vaccines, and combinations thereof.

[0172] A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health. Prebiotics can include complex carbohydrates, amino acids, peptides, or other essential nutritional

components for the survival of the bacterial composition. Prebiotics include, but are not limited to, amino acids, biotin, fructooligosaccharide, galactooligosaccharides, inulin, lactulose, mannan oligosaccharides, oligofructose-enriched inulin, oligofructose, oligodextrose, tagatose, trans-galactooligosaccharide, and xylooligosaccharides.

Methods for Testing Bacterial Compositions for Populating Effect

In Vivo Assay for Determining Whether a Bacterial Composition Populates a Subject's Gastrointestinal Tract

[0173] In order to determine that the bacterial composition populates the gastrointestinal tract of a subject, an animal model, such as a mouse model, can be used. The model can begin by evaluating the microbiota of the mice. Qualitative assessments can be accomplished using 16S profiling of the microbial community in the feces of normal mice. It can also be accomplished by full genome sequencing, whole genome shotgun sequencing (WGS), or traditional microbiological techniques. Quantitative assessments can be conducted using quantitative PCR (qPCR), described below, or by using traditional microbiological techniques and counting colony formation.

[0174] Optionally, the mice can receive an antibiotic treatment to mimic the condition of dysbiosis. Antibiotic treatment can decrease the taxonomic richness, diversity, and evenness of the community, including a reduction of abundance of a significant number of bacterial taxa. Dethlefsen *et al.*, The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing, PLoS Biology 6(11):3280 (2008). At least one antibiotic can be used, and antibiotics are well known. Antibiotics can include aminoglycoside antibiotic (amikacin, arbekacin, gentamicin, kanamycin, neomycin, netilmicin, paromomycin, rhodostreptomycin, streptomycin, tobramycin, and apramycin), amoxicillin,

ampicillin, Augmentin (an amoxicillin/clavulanate potassium combination), cephalosporin (cefaclor, defadroxil, cefazolin, cefixime, fefoxitin, cefprozil, ceftazidime, cefuroxime, cephalexin), clavulanate potassium, clindamycin, colistin, gentamycin, kanamycin, metronidazole, or vancomycin. As an individual, nonlimiting specific example, the mice can be provided with drinking water containing a mixture of the antibiotics kanamycin, colistin, gentamycin, metronidazole and vancomycin at 40 mg/kg, 4.2 mg/kg, 3.5 mg/kg, 21.5 mg/kg, and 4.5 mg/kg (mg per average mouse body weight), respectively, for 7 days. Alternatively, mice can be administered ciprofloxacin at a dose of 15-20 mg/kg (mg per average mouse body weight), for 7 days.

If the mice are provided with an antibiotic, a wash out period of from one day to three days may be provided with no antibiotic treatment and no bacterial composition treatment.

[0175] Subsequently, the test bacterial composition is administered to the mice by oral gavage. The test bacterial composition may be administered in a volume of 0.2 ml containing 10^4 CFUs of each type of bacteria in the bacterial composition. Dose-response may be assessed by using a range of doses, including, but not limited to 10^2 , 10^3 , 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , and/or 10^{10} .

[0176] The mice can be evaluated using 16S sequencing, full genome sequencing, whole genome shotgun sequencing (WGS), or traditional microbiological techniques to determine whether the test bacterial composition has populated the gastrointestinal tract of the mice. For example only, one day, three days, one week, two weeks, and one month after administration of the bacterial composition to the mice, 16S profiling is conducted to determine whether the test bacterial composition has populated the gastrointestinal tract of the mice.

Quantitative assessments, including qPCR and traditional microbiological techniques such as colony counting, can additionally or alternatively be performed, at the same time intervals.

[0177] Furthermore, the number of sequence counts that correspond exactly to those in the bacterial composition over time can be assessed to determine specifically which components of the bacterial composition reside in the gastrointestinal tract over a particular period of time. In one embodiment, the strains of the bacterial composition persist for a desired period of time. In another embodiment, the components of the bacterial composition persist for a desired period of time, while also increasing the ability of other microbes (such as those present in the environment, food, etc.) to populate the gastrointestinal tract, further increasing overall diversity, as discussed below.

Ability of Bacterial compositions to Populate Different Regions of the Gastrointestinal Tract

[0178] The present bacterial compositions can also be assessed for their ability to populate different regions on the gastrointestinal tract. In one embodiment, a bacterial composition can be chosen for its ability to populate one or more than one region of the gastrointestinal tract, including, but not limited to the stomach, the small intestine (duodenum, jejunum, and ileum), the large intestine (the cecum, the colon (the ascending, transverse, descending, and sigmoid colon), and the rectum).

[0179] An *in vivo* study can be conducted to determine which regions of the gastrointestinal tract a given bacterial composition will populate. A mouse model similar to the one described above can be conducted, except instead of assessing the feces produced by the mice, particular regions of the gastrointestinal tract can be removed and studied individually. For example, at least one particular region of the gastrointestinal tract can be removed and a qualitative or quantitative determination

can be performed on the contents of that region of the gastrointestinal tract. In another embodiment, the contents can optionally be removed and the qualitative or quantitative determination may be conducted on the tissue removed from the mouse.

qPCR

[0180] As one quantitative method for determining whether a bacterial composition populates the gastrointestinal tract, quantitative PCR (qPCR) can be performed. Standard techniques can be followed to generate a standard curve for the bacterial composition of interest, either for all of the components of the bacterial composition collectively, individually, or in subsets (if applicable). Genomic DNA can be extracted from samples using commercially-available kits, such as the Mo Bio Powersoil®-htp 96 Well Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA), the Mo Bio Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA), or the QIAamp DNA Stool Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions.

[0181] In some embodiments, qPCR can be conducted using HotMasterMix (5PRIME, Gaithersburg, MD) and primers specific for the bacterial composition of interest, and may be conducted on a MicroAmp® Fast Optical 96-well Reaction Plate with Barcode (0.1mL) (Life Technologies, Grand Island, NY) and performed on a BioRad C1000™ Thermal Cycler equipped with a CFX96™ Real-Time System (BioRad, Hercules, CA), with fluorescent readings of the FAM and ROX channels. The Cq value for each well on the FAM channel is determined by the CFX Manager™ software version 2.1. The $\log_{10}(\text{cfu/ml})$ of each experimental sample is calculated by inputting a given sample's Cq value into linear regression model generated from the standard curve comparing the Cq values of the standard curve

wells to the known $\log_{10}(\text{cfu/ml})$ of those samples. The skilled artisan may employ alternative qPCR modes.

Methods for Characterization of Bacterial Compositions

[0182] In certain embodiments, provided are methods for testing certain characteristics of bacterial compositions. For example, the sensitivity of bacterial compositions to certain environmental variables is determined, e.g., in order to select for particular desirable characteristics in a given composition, formulation and/or use. For example, the constituents in the bacterial composition can be tested for pH resistance, bile acid resistance, and/or antibiotic sensitivity, either individually on a constituent-by-constituent basis or collectively as a bacterial composition comprised of multiple bacterial constituents (collectively referred to in this section as bacterial composition).

pH Sensitivity Testing

[0183] If a bacterial composition will be administered other than to the colon or rectum (i.e., for example, an oral route), optionally testing for pH resistance enhances the selection of bacterial compositions that will survive at the highest yield possible through the varying pH environments of the distinct regions of the GI tract. Understanding how the bacterial compositions react to the pH of the GI tract also assists in formulation, so that the number of bacteria in a dosage form can be increased if beneficial and/or so that the composition may be administered in an enteric-coated capsule or tablet or with a buffering or protective composition. As the pH of the stomach can drop to a pH of 1 to 2 after a high-protein meal for a short time before physiological mechanisms adjust it to a pH of 3 to 4 and often resides at a resting pH of 4 to 5, and as the pH of the small intestine can range from a pH of 6 to 7.4, bacterial compositions can be prepared that survive these varying pH ranges

(specifically wherein at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or as much as 100% of the bacteria can survive gut transit times through various pH ranges). This can be tested by exposing the bacterial composition to varying pH ranges for the expected gut transit times through those pH ranges. Therefore, as a nonlimiting example only, 18-hour cultures of bacterial compositions can be grown in standard media, such as gut microbiota medium ("GMM", see Goodman et al., Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice, PNAS 108(15):6252-6257 (2011)) or another animal-products-free medium, with the addition of pH adjusting agents for a pH of 1 to 2 for 30 minutes, a pH of 3 to 4 for 1 hour, a pH of 4 to 5 for 1 to 2 hours, and a pH of 6 to 7.4 for 2.5 to 3 hours. An alternative method for testing stability to acid is described in U.S. Patent No. 4,839,281. Survival of bacteria may be determined by culturing the bacteria and counting colonies on appropriate selective or non-selective media.

Bile Acid Sensitivity Testing

[0184] Additionally, in some embodiments, testing for bile-acid resistance enhances the selection of bacterial compositions that will survive exposures to bile acid during transit through the GI tract. Bile acids are secreted into the small intestine and can, like pH, affect the survival of bacterial compositions. This can be tested by exposing the bacterial compositions to bile acids for the expected gut exposure time to bile acids. For example, bile acid solutions can be prepared at desired concentrations using 0.05 mM Tris at pH 9 as the solvent. After the bile acid is dissolved, the pH of the solution may be adjusted to 7.2 with 10% HCl. Bacterial compositions can be cultured in 2.2 ml of a bile acid composition mimicking the concentration and type of bile acids in the patient, 1.0 ml of 10% sterile-filtered feces

media and 0.1 ml of an 18-hour culture of the given strain of bacteria. Incubations may be conducted for from 2.5 to 3 hours or longer. An alternative method for testing stability to bile acid is described in U.S. Patent No. 4,839,281. Survival of bacteria **may** be determined by culturing the bacteria and counting colonies on appropriate selective or non-selective media.

Antibiotic Sensitivity Testing

[0185] As a further optional sensitivity test, bacterial compositions can be tested for sensitivity to antibiotics. In one embodiment, bacterial compositions can be chosen so that the bacterial constituents are sensitive to antibiotics such that if necessary they can be eliminated or substantially reduced from the patient's gastrointestinal tract by at least one antibiotic targeting the bacterial composition.

Adherence to Gastrointestinal Cells

[0186] The bacterial compositions may optionally be tested for the ability to adhere to gastrointestinal cells. A method for testing adherence to gastrointestinal cells is described in U.S. Patent No. 4,839,281.

Methods for Purifying Spores

Solvent Treatments

[0187] To purify the bacterial spores, the fecal material is subjected to one or more solvent treatments. A solvent treatment is a miscible solvent treatment (either partially miscible or fully miscible) or an immiscible solvent treatment. Miscibility is the ability of two liquids to mix with each to form a homogeneous solution. Water and ethanol, for example, are fully miscible such that a mixture containing water and ethanol in any ratio will show only one phase. Miscibility is provided as a wt/wt%, or weight of one solvent in 100 g of final solution. If two solvents are fully miscible in all proportions, their miscibility is 100%. Provided as fully miscible solutions with water are alcohols, e.g., methanol, ethanol, isopropanol, butanol, etc. The alcohols can be

provided already combined with water; e.g., a solution containing 10%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or greater than 95%. Other solvents are only partially miscible, meaning that only some portion will dissolve in water. Diethyl ether, for example, is partially miscible with water. Up to 7 grams of diethyl ether will dissolve in 93 g of water to give a 7% (wt/wt%) solution. If more diethyl ether is added, a two phase solution will result with a distinct diethyl ether layer above the water. Other miscible materials include ethers, dimethoxyethane, or tetrahydrofuran. In contrast, an oil such as an alkane and water are immiscible and form two phases. Further, immiscible treatments are optionally combined with a detergent, either an ionic detergent or a non-ionic detergent. Exemplary detergents include Triton X-100, Tween 20, Tween 80, Nonidet P40, a pluronic, or a polyol.

Chromatography treatments

[0188] To purify spore populations, the fecal materials are subjected to one or more chromatographic treatments, either sequentially or in parallel. In a chromatographic treatment, a solution containing the fecal material is contacted with a solid medium containing a hydrophobic interaction chromatographic (HIC) medium or an affinity chromatographic medium. In an alternative embodiment, a solid medium capable of absorbing a residual habitat product present in the fecal material is contacted with a solid medium that adsorbs a residual habitat product. In certain embodiments, the HIC medium contains sepharose or a derivatized sepharose such as butyl sepharose, octyl sepharose, phenyl sepharose, or butyl-s sepharose. In other embodiments, the affinity chromatographic medium contains material derivatized with mucin type I, II, III, IV, V, or VI, or oligosaccharides derived from or similar to those of mucins type I, II, III, IV, V, or VI. Alternatively, the affinity

chromatographic medium contains material derivatized with antibodies that recognize spore-forming bacteria.

Mechanical Treatments

[0189] Provided herein is the physical disruption of the fecal material, particularly by one or more mechanical treatment such as blending, mixing, shaking, vortexing, impact pulverization, and sonication. As provided herein, the mechanical disrupting treatment substantially disrupts a non-spore material present in the fecal material and does not substantially disrupt a spore present in the fecal material. Mechanical treatments optionally include filtration treatments, where the desired spore populations are retained on a filter while the undesirable (non-spore) fecal components pass through, and the spore fraction is then recovered from the filter medium. Alternatively, undesirable particulates and eukaryotic cells may be retained on a filter while bacterial cells including spores pass through. In some embodiments the spore fraction retained on the filter medium is subjected to a diafiltration step, wherein the retained spores are contacted with a wash liquid, typically a sterile saline-containing solution or other diluent, in order to further reduce or remove the undesirable fecal components.

Thermal Treatments

[0190] Provided herein is the thermal disruption of the fecal material. Generally, the fecal material is mixed in a saline-containing solution such as phosphate-buffered saline (PBS) and subjected to a heated environment, such as a warm room, incubator, water-bath, or the like, such that efficient heat transfer occurs between the heated environment and the fecal material. Preferably the fecal material solution is mixed during the incubation to enhance thermal conductivity and disrupt particulate aggregates. Thermal treatments can be modulated by the temperature of the

environment and/or the duration of the thermal treatment. For example, the fecal material or a liquid comprising the fecal material is subjected to a heated environment, e.g., a hot water bath of at least about 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or greater than 100 degrees Celsius, for at least about 1, 5, 10, 15, 20, 30, 45 seconds, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 hours. In certain embodiments the thermal treatment occurs at two different temperatures, such as 30 seconds in a 100 degree Celsius environment followed by 10 minutes in a 50 degree Celsius environment. In preferred embodiments the temperature and duration of the thermal treatment are sufficient to kill or remove pathogenic materials while not substantially damaging or reducing the germination-competency of the spores.

Irradiation Treatments

[0191] Provided are methods of treating the fecal material or separated contents of the fecal material with ionizing radiation, typically gamma irradiation, ultraviolet irradiation or electron beam irradiation provided at an energy level sufficient to kill pathogenic materials while not substantially damaging the desired spore populations. For example, ultraviolet radiation at 254nm provided at an energy level below about 22,000 microwatt seconds per cm^2 will not generally destroy desired spores.

Centrifugation and Density Separation Treatments

[0192] Provided are methods of separating desired spore populations from the other components of the fecal material by centrifugation. A solution containing the fecal material is subjected to one or more centrifugation treatments, e.g., at about 1000 x g, 2000 x g, 3000 x g, 4000 x g, 5000 x g, 6000 x g, 7000 x g, 8000 x g or greater than 8000 x g. Differential centrifugation separates desired spores from undesired non-spore material; at low forces the spores are retained in solution, while

at higher forces the spores are pelleted while smaller impurities (e.g., virus particles, phage) are retained in solution. For example, a first low force centrifugation pellets fibrous materials; a second, higher force centrifugation pellets undesired eukaryotic cells, and a third, still higher force centrifugation pellets the desired spores while small contaminants remain in suspension. In some embodiments density or mobility gradients or cushions (e.g., step cushions), such as Percoll, Ficoll, Nycodenz, Histodenz or sucrose gradients, are used to separate desired spore populations from other materials in the fecal material.

[0193] Also provided herein are methods of producing spore populations that combine two or more of the treatments described herein in order to synergistically purify the desired spores while killing or removing undesired materials and/or activities from the spore population. It is generally desirable to retain the spore populations under non-germinating and non-growth promoting conditions and media, in order to minimize the growth of pathogenic bacteria present in the spore populations and to minimize the germination of spores into vegetative bacterial cells.

PHARMACEUTICAL COMPOSITIONS AND FORMULATIONS OF THE INVENTION

Formulations

[0194] Provided are formulations for administration to humans and other subjects in need thereof. Generally the bacterial compositions are combined with additional active and/or inactive materials in order to produce a final product, which may be in single dosage unit or in a multi-dose format.

[0195] In some embodiments, the composition comprises at least one carbohydrate. A "carbohydrate" refers to a sugar or polymer of sugars. The terms "saccharide," "polysaccharide," "carbohydrate," and "oligosaccharide" may be used interchangeably. Most carbohydrates are aldehydes or ketones with many hydroxyl

groups, usually one on each carbon atom of the molecule. Carbohydrates generally have the molecular formula $C_nH_{2n}O_n$. A carbohydrate can be a monosaccharide, a disaccharide, trisaccharide, oligosaccharide, or polysaccharide. The most basic carbohydrate is a monosaccharide, such as glucose, sucrose, galactose, mannose, ribose, arabinose, xylose, and fructose. Disaccharides are two joined monosaccharides. Exemplary disaccharides include sucrose, maltose, cellobiose, and lactose. Typically, an oligosaccharide includes between three and six monosaccharide units (e.g., raffinose, stachyose), and polysaccharides include six or more monosaccharide units. Exemplary polysaccharides include starch, glycogen, and cellulose. Carbohydrates can contain modified saccharide units, such as 2'-deoxyribose wherein a hydroxyl group is removed, 2'-fluororibose wherein a hydroxyl group is replaced with a fluorine, or N-acetylglucosamine, a nitrogen-containing form of glucose (e.g., 2'-fluororibose, deoxyribose, and hexose). Carbohydrates can exist in many different forms, for example, conformers, cyclic forms, acyclic forms, stereoisomers, tautomers, anomers, and isomers.

[0196] In some embodiments, the composition comprises at least one lipid. As used herein, a "lipid" includes fats, oils, triglycerides, cholesterol, phospholipids, fatty acids in any form including free fatty acids. Fats, oils and fatty acids can be saturated, unsaturated (cis or trans) or partially unsaturated (cis or trans). In some embodiments, the lipid comprises at least one fatty acid selected from lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1), margaric acid (17:0), heptadecenoic acid (17:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), octadecatetraenoic acid (18:4), arachidic acid (20:0), eicosenoic acid (20:1), eicosadienoic acid (20:2), eicosatetraenoic acid (20:4), eicosapentaenoic acid (20:5) (EPA), docosanoic acid (22:0), docosenoic acid

(22:1), docosapentaenoic acid (22:5), docosahexaenoic acid (22:6) (DHA), and tetracosanoic acid (24:0). In other embodiments, the composition comprises at least one modified lipid, for example, a lipid that has been modified by cooking.

[0197] In some embodiments, the composition comprises at least one supplemental mineral or mineral source. Examples of minerals include, without limitation: chloride, sodium, calcium, iron, chromium, copper, iodine, zinc, magnesium, manganese, molybdenum, phosphorus, potassium, and selenium. Suitable forms of any of the foregoing minerals include soluble mineral salts, slightly soluble mineral salts, insoluble mineral salts, chelated minerals, mineral complexes, non-reactive minerals such as carbonyl minerals, and reduced minerals, and combinations thereof.

[0198] In certain embodiments, the composition comprises at least one supplemental vitamin. The at least one vitamin can be fat-soluble or water soluble vitamins. Suitable vitamins include but are not limited to vitamin C, vitamin A, vitamin E, vitamin B12, vitamin K, riboflavin, niacin, vitamin D, vitamin B6, folic acid, pyridoxine, thiamine, pantothenic acid, and biotin. Suitable forms of any of the foregoing are salts of the vitamin, derivatives of the vitamin, compounds having the same or similar activity of the vitamin, and metabolites of the vitamin.

[0199] In other embodiments, the composition comprises an excipient. Non-limiting examples of suitable excipients include a buffering agent, a preservative, a stabilizer, a binder, a compaction agent, a lubricant, a dispersion enhancer, a disintegration agent, a flavoring agent, a sweetener, and a coloring agent.

[0200] In another embodiment, the excipient is a buffering agent. Non-limiting examples of suitable buffering agents include sodium citrate, magnesium carbonate, magnesium bicarbonate, calcium carbonate, and calcium bicarbonate.

[0201] In some embodiments, the excipient comprises a preservative. Non-limiting examples of suitable preservatives include antioxidants, such as alpha-tocopherol and ascorbate, and antimicrobials, such as parabens, chlorobutanol, and phenol.

[0202] In other embodiments, the composition comprises a binder as an excipient. Non-limiting examples of suitable binders include starches, pregelatinized starches, gelatin, polyvinylpyrrolidone, cellulose, methylcellulose, sodium carboxymethylcellulose, ethylcellulose, polyacrylamides, polyvinylloxazolidone, polyvinylalcohols, C₁₂-C₁₈ fatty acid alcohol, polyethylene glycol, polyols, saccharides, oligosaccharides, and combinations thereof.

[0203] In another embodiment, the composition comprises a lubricant as an excipient. Non-limiting examples of suitable lubricants include magnesium stearate, calcium stearate, zinc stearate, hydrogenated vegetable oils, sterotex, polyoxyethylene monostearate, talc, polyethyleneglycol, sodium benzoate, sodium lauryl sulfate, magnesium lauryl sulfate, and light mineral oil.

[0204] In other embodiments, the composition comprises a dispersion enhancer as an excipient. Non-limiting examples of suitable dispersants include starch, alginic acid, polyvinylpyrrolidones, guar gum, kaolin, bentonite, purified wood cellulose, sodium starch glycolate, isoamorphous silicate, and microcrystalline cellulose as high HLB emulsifier surfactants.

[0205] In some embodiments, the composition comprises a disintegrant as an excipient. In other embodiments, the disintegrant is a non-effervescent disintegrant. Non-limiting examples of suitable non-effervescent disintegrants include starches such as corn starch, potato starch, pregelatinized and modified starches thereof, sweeteners, clays, such as bentonite, micro-crystalline cellulose, alginates, sodium

starch glycolate, gums such as agar, guar, locust bean, karaya, pectin, and tragacanth. In another embodiment, the disintegrant is an effervescent disintegrant. Non-limiting examples of suitable effervescent disintegrants include sodium bicarbonate in combination with citric acid, and sodium bicarbonate in combination with tartaric acid.

[0206] In another embodiment, the excipient comprises a flavoring agent. Flavoring agents can be chosen from synthetic flavor oils and flavoring aromatics; natural oils; extracts from plants, leaves, flowers, and fruits; and combinations thereof. In some embodiments the flavoring agent is selected from cinnamon oils; oil of wintergreen; peppermint oils; clover oil; hay oil; anise oil; eucalyptus; vanilla; citrus oil such as lemon oil, orange oil, grape and grapefruit oil; and fruit essences including apple, peach, pear, strawberry, raspberry, cherry, plum, pineapple, and apricot.

[0207] In other embodiments, the excipient comprises a sweetener. Non-limiting examples of suitable sweeteners include glucose (corn syrup), dextrose, invert sugar, fructose, and mixtures thereof (when not used as a carrier); saccharin and its various salts such as the sodium salt; dipeptide sweeteners such as aspartame; dihydrochalcone compounds, glycyrrhizin; Stevia Rebaudiana (Stevioside); chloro derivatives of sucrose such as sucralose; and sugar alcohols such as sorbitol, mannitol, xylitol, and the like. Also contemplated are hydrogenated starch hydrolysates and the synthetic sweetener 3,6-dihydro-6-methyl-1,2,3-oxathiazin-4-one-2,2-dioxide, particularly the potassium salt (acesulfame-K), and sodium and calcium salts thereof.

[0208] In yet other embodiments, the composition comprises a coloring agent. Non-limiting examples of suitable color agents include food, drug and cosmetic

colors (FD&C), drug and cosmetic colors (D&C), and external drug and cosmetic colors (Ext. D&C). The coloring agents can be used as dyes or their corresponding lakes.

[0209] The weight fraction of the excipient or combination of excipients in the formulation is usually about 99% or less, such as about 95% or less, about 90% or less, about 85% or less, about 80% or less, about 75% or less, about 70% or less, about 65% or less, about 60% or less, about 55% or less, 50% or less, about 45% or less, about 40% or less, about 35% or less, about 30% or less, about 25% or less, about 20% or less, about 15% or less, about 10% or less, about 5% or less, about 2% or less, or about 1% or less of the total weight of the composition.

[0210] The bacterial compositions disclosed herein can be formulated into a variety of forms and administered by a number of different means. The compositions can be administered orally, rectally, or parenterally, in formulations containing conventionally acceptable carriers, adjuvants, and vehicles as desired. The term “parenteral” as used herein includes subcutaneous, intravenous, intramuscular, or intrasternal injection and infusion techniques. In an exemplary embodiment, the bacterial composition is administered orally.

[0211] Solid dosage forms for oral administration include capsules, tablets, caplets, pills, troches, lozenges, powders, and granules. A capsule typically comprises a core material comprising a bacterial composition and a shell wall that encapsulates the core material. In some embodiments, the core material comprises at least one of a solid, a liquid, and an emulsion. In other embodiments, the shell wall material comprises at least one of a soft gelatin, a hard gelatin, and a polymer. Suitable polymers include, but are not limited to: cellulosic polymers such as hydroxypropyl cellulose, hydroxyethyl cellulose, hydroxypropyl methyl cellulose

(HPMC), methyl cellulose, ethyl cellulose, cellulose acetate, cellulose acetate phthalate, cellulose acetate trimellitate, hydroxypropylmethyl cellulose phthalate, hydroxypropylmethyl cellulose succinate and carboxymethylcellulose sodium; acrylic acid polymers and copolymers, such as those formed from acrylic acid, methacrylic acid, methyl acrylate, ammonio methylacrylate, ethyl acrylate, methyl methacrylate and/or ethyl methacrylate (e.g., those copolymers sold under the trade name "Eudragit"); vinyl polymers and copolymers such as polyvinyl pyrrolidone, polyvinyl acetate, polyvinylacetate phthalate, vinylacetate crotonic acid copolymer, and ethylene-vinyl acetate copolymers; and shellac (purified lac). In yet other embodiments, at least one polymer functions as taste-masking agents.

[0212] Tablets, pills, and the like can be compressed, multiply compressed, multiply layered, and/or coated. The coating can be single or multiple. In one embodiment, the coating material comprises at least one of a saccharide, a polysaccharide, and glycoproteins extracted from at least one of a plant, a fungus, and a microbe. Non-limiting examples include corn starch, wheat starch, potato starch, tapioca starch, cellulose, hemicellulose, dextrans, maltodextrin, cyclodextrins, inulins, pectin, mannans, gum arabic, locust bean gum, mesquite gum, guar gum, gum karaya, gum ghatti, tragacanth gum, funori, carrageenans, agar, alginates, chitosans, or gellan gum. In some embodiments the coating material comprises a protein. In another embodiment, the coating material comprises at least one of a fat and an oil. In other embodiments, the at least one of a fat and an oil is high temperature melting. In yet another embodiment, the at least one of a fat and an oil is hydrogenated or partially hydrogenated. In one embodiment, the at least one of a fat and an oil is derived from a plant. In other embodiments, the at least one of a fat and an oil comprises at least one of glycerides, free fatty acids, and fatty acid esters.

In some embodiments, the coating material comprises at least one edible wax. The edible wax can be derived from animals, insects, or plants. Non-limiting examples include beeswax, lanolin, bayberry wax, carnauba wax, and rice bran wax. Tablets and pills can additionally be prepared with enteric coatings.

[0213] Alternatively, powders or granules embodying the bacterial compositions disclosed herein can be incorporated into a food product. In some embodiments, the food product is a drink for oral administration. Non-limiting examples of a suitable drink include fruit juice, a fruit drink, an artificially flavored drink, an artificially sweetened drink, a carbonated beverage, a sports drink, a liquid dairy product, a shake, an alcoholic beverage, a caffeinated beverage, infant formula and so forth. Other suitable means for oral administration include aqueous and nonaqueous solutions, emulsions, suspensions and solutions and/or suspensions reconstituted from non-effervescent granules, containing at least one of suitable solvents, preservatives, emulsifying agents, suspending agents, diluents, sweeteners, coloring agents, and flavoring agents.

[0214] In some embodiments, the food product can be a solid foodstuff. Suitable examples of a solid foodstuff include without limitation a food bar, a snack bar, a cookie, a brownie, a muffin, a cracker, an ice cream bar, a frozen yogurt bar, and the like.

[0215] In other embodiments, the compositions disclosed herein are incorporated into a therapeutic food. In some embodiments, the therapeutic food is a ready-to-use food that optionally contains some or all essential macronutrients and micronutrients. In another embodiment, the compositions disclosed herein are incorporated into a supplementary food that is designed to be blended into an existing meal. In one embodiment, the supplemental food contains some or all

essential macronutrients and micronutrients. In another embodiment, the bacterial compositions disclosed herein are blended with or added to an existing food to fortify the food's protein nutrition. Examples include food staples (grain, salt, sugar, cooking oil, margarine), beverages (coffee, tea, soda, beer, liquor, sports drinks), snacks, sweets and other foods.

[0216] In one embodiment, the formulations are filled into gelatin capsules for oral administration. An example of an appropriate capsule is a 250 mg gelatin capsule containing from 10 (up to 100 mg) of lyophilized powder (10^8 to 10^{11} bacteria), 160 mg microcrystalline cellulose, 77.5 mg gelatin, and 2.5 mg magnesium stearate. In an alternative embodiment, from 10^5 to 10^{12} bacteria may be used, 10^5 to 10^7 , 10^6 to 10^7 , or 10^8 to 10^{10} , with attendant adjustments of the excipients if necessary. In an alternative embodiment, an enteric-coated capsule or tablet or with a buffering or protective composition can be used.

EXAMPLES

[0217] Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

[0218] The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., T.E. Creighton, *Proteins: Structures and Molecular Properties* (W.H. Freeman and Company, 1993); A.L. Lehninger,

Biochemistry (Worth Publishers, Inc., current addition); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.); *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); Carey and Sundberg *Advanced Organic Chemistry 3rd Ed.* (Plenum Press) Vols A and B(1992).

Example 1: Species Identification

[0219] The identity of the bacterial species which grew up from a complex fraction can be determined in multiple ways. First, individual colonies can be picked into liquid media in a 96 well format, grown up and saved as 15% glycerol stocks at -80°C. Aliquots of the *cultures* can be placed into cell lysis buffer and colony PCR methods can be used to amplify and sequence the 16S rDNA gene (Example 2 and described above). Alternatively, colonies may be streaked to purity in several passages on solid media. Well separated colonies are streaked onto the fresh plates of the same kind and incubated for 48-72 hours at 37°C. The process is repeated multiple times in order to ensure purity. Pure cultures can be analyzed by phenotypic- or sequence-based methods, including 16S rDNA amplification and sequencing as described in Examples 2 and 3. Sequence characterization of pure isolates or mixed communities e.g. plate scrapes and spore fractions can also include whole genome shotgun sequencing. The latter is valuable to determine the presence of genes associated with sporulation, antibiotic resistance, pathogenicity, and virulence. Colonies can also be scraped from plates en masse and sequenced using a massively parallel sequencing method as described in Examples 2 and 3, such that individual 16S signatures can be identified in a complex mixture. Optionally, the sample can be sequenced prior to germination (if appropriate DNA

isolation procedures are used to lyse and release the DNA from spores) in order to compare the diversity of germinable species with the total number of species in a spore sample. As an alternative or complementary approach to 16S analysis, MALDI-TOF-mass spec can also be used for species identification (as reviewed in Anaerobe 22:123).

Example 2: 16s Sequencing to Determine Operational Taxonomic Unit (OTU)

Method for Determining 16S Sequence

[0220] OTUs can be defined either by full 16S sequencing of the rRNA gene, by sequencing of a specific hypervariable region of this gene (i.e. V1, V2, V3, V4, V5, V6, V7, V8, or V9), or by sequencing of any combination of hypervariable regions from this gene (e.g. V1-3 or V3-5). The bacterial 16S rDNA is approximately 1500 nucleotides in length and is used in reconstructing the evolutionary relationships and sequence similarity of one bacterial isolate to another using phylogenetic approaches. 16S sequences are used for phylogenetic reconstruction as they are in general highly conserved, but contain specific hypervariable regions that harbor sufficient nucleotide diversity to differentiate genera and species of most microbes.

[0221] Using well known techniques, in order to determine the full 16S sequence or the sequence of any hypervariable region of the 16S sequence, genomic DNA is extracted from a bacterial sample, the 16S rDNA (full region or specific hypervariable regions) amplified using polymerase chain reaction (PCR), the PCR products cleaned, and nucleotide sequences delineated to determine the genetic composition of 16S gene or subdomain of the gene. If full 16S sequencing is performed, the sequencing method used may be, but is not limited to, Sanger sequencing. If one or more hypervariable regions are used, such as the V4 region, the sequencing may be, but is not limited to being, performed using the Sanger method or using a next-

generation sequencing method, such as an Illumina (sequencing by synthesis) method using barcoded primers allowing for multiplex reactions.

[0222] In addition to the 16S rRNA gene, one can define an OTU by sequencing a selected set of genes that are known to be marker genes for a given species or taxonomic group of OTUs. These genes may alternatively be assayed using a PCR-based screening strategy. As example, various strains of pathogenic *Escherichia coli* can be distinguished using DNAs from the genes that encode heat-labile (LT1, LTIIa, and LTIIb) and heat-stable (ST1 and STII) toxins, verotoxin types 1, 2, and 2e (VT1, VT2, and VT2e, respectively), cytotoxic necrotizing factors (CNF1 and CNF2), attaching and effacing mechanisms (*eaeA*), enteroaggregative mechanisms (Eagg), and enteroinvasive mechanisms (Einv). The optimal genes to utilize for taxonomic assignment of OTUs by use of marker genes will be familiar to one with ordinary skill of the art of sequence based taxonomic identification.

Genomic DNA Extraction

[0223] Genomic DNA is extracted from pure microbial cultures using a hot alkaline lysis method. 1 µl of microbial culture is added to 9 µl of Lysis Buffer (25mM NaOH, 0.2 mM EDTA) and the mixture is incubated at 95°C for 30 minutes. Subsequently, the samples are cooled to 4°C and neutralized by the addition of 10 µl of Neutralization Buffer (40 mM Tris-HCl) and then diluted 10-fold in Elution Buffer (10 mM Tris-HCl). Alternatively, genomic DNA is extracted from pure microbial cultures using commercially available kits such as the Mo Bio Ultraclean® Microbial DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) or by standard methods known to those skilled in the art.

Amplification of 16S Sequences for Downstream Sanger Sequencing

[0224] To amplify bacterial 16S rDNA (Figure 1A), 2 µl of extracted gDNA is added to a 20 µl final volume PCR reaction. For full-length 16 sequencing the PCR reaction also contains 1x HotMasterMix (5PRIME, Gaithersburg, MD), 250 nM of 27f (AGRGTTTGATCMTGGCTCAG, IDT, Coralville, IA), and 250 nM of 1492r (TACGGYTACCTTGTTAYGACTT, IDT, Coralville, IA), with PCR Water (Mo Bio Laboratories, Carlsbad, CA) for the balance of the volume. Alternatively, other universal bacterial primers or thermostable polymerases known to those skilled in the art are used. For example primers are available to those skilled in the art for the sequencing of the the “V1-V9 regions” of the 16S rRNA (Figure 1A). These regions refer to the first through ninth hypervariable regions of the 16S rRNA gene that are used for genetic typing of bacterial samples. These regions in bacteria are defined by nucleotides 69-99, 137-242, 433-497, 576-682, 822-879, 986-1043, 1117-1173, 1243-1294 and 1435-1465 respectively using numbering based on the E. coli system of nomenclature. Brosius et al., Complete nucleotide sequence of a 16S ribosomal RNA gene from Escherichia coli, PNAS 75(10):4801-4805 (1978). In some embodiments, at least one of the V1, V2, V3, V4, V5, V6, V7, V8, and V9 regions are used to characterize an OTU. In one embodiment, the V1, V2, and V3 regions are used to characterize an OTU. In another embodiment, the V3, V4, and V5 regions are used to characterize an OTU. In another embodiment, the V4 region is used to characterize an OTU. A person of ordinary skill in the art can identify the specific hypervariable regions of a candidate 16S rRNA (in Figure 1A) by comparing the candidate sequence in question to the reference sequence (Figure 1B) and identifying the hypervariable regions based on similarity to the reference hypervariable regions.

[0225] The PCR can be performed on commercially available thermocyclers such as a BioRad MyCycler™ Thermal Cycler (BioRad, Hercules, CA). The reactions are run at 94°C for 2 minutes followed by 30 cycles of 94°C for 30 seconds, 51°C for 30 seconds, and 68°C for 1 minute 30 seconds, followed by a 7 minute extension at 72°C and an indefinite hold at 4°C. Following PCR, gel electrophoresis of a portion of the reaction products is used to confirm successful amplification of a ~1.5 kb product.

[0226] To remove nucleotides and oligonucleotides from the PCR products, 2 µl of HT ExoSap-IT (Affymetrix, Santa Clara, CA) is added to 5 µl of PCR product followed by a 15 minute incubation at 37°C and then a 15 minute inactivation at 80°C.

**Amplification of 16S Sequences For Downstream Characterization
By Massively Parallel Sequencing Technologies**

[0227] Amplification performed for downstream sequencing by short read technologies such as Illumina require amplification using primers known to those skilled in the art that additionally include a sequence-based barcoded tag. As example, to amplify the 16s hypervariable region V4 region of bacterial 16S rDNA, 2 µl of extracted gDNA is added to a 20 µl final volume PCR reaction. The PCR reaction also contains 1x HotMasterMix (5PRIME, Gaithersburg, MD), 200 nM of V4_515f_adapt

(AATGATACGGCGACCAACGAGATCTACACTATGGTAATTGTGTGCCAGCMGCCGCGGTAA, IDT, Coralville, IA), and 200 nM of barcoded 806rbc

(CAAGCAGAAGACGGCATACGAGAT_12bpGolayBarcode_AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT, IDT, Coralville, IA), with PCR Water (Mo Bio

Laboratories, Carlsbad, CA) for the balance of the volume. These primers incorporate barcoded adapters for Illumina sequencing by synthesis. Optionally,

identical replicate, triplicate, or quadruplicate reactions may be performed.

Alternatively other universal bacterial primers or thermostable polymerases known to those skilled in the art are used to obtain different amplification and sequencing error rates as well as results on alternative sequencing technologies.

[0228] The PCR amplification is performed on commercially available thermocyclers such as a BioRad MyCycler™ Thermal Cycler (BioRad, Hercules, CA). The reactions are run at 94°C for 3 minutes followed by 25 cycles of 94°C for 45 seconds, 50°C for 1 minute, and 72°C for 1 minute 30 seconds, followed by a 10 minute extension at 72°C and a indefinite hold at 4°C. Following PCR, gel electrophoresis of a portion of the reaction products is used to confirm successful amplification of a ~1.5 kb product. PCR cleanup is performed as specified in the previous example.

Sanger Sequencing of Target Amplicons From Pure Homogeneous Samples

[0229] To detect nucleic acids for each sample, two sequencing reactions are performed to generate a forward and reverse sequencing read. For full-length 16s sequencing primers 27f and 1492r are used. 40 ng of ExoSap-IT-cleaned PCR products are mixed with 25 pmol of sequencing primer and Mo Bio Molecular Biology Grade Water (Mo Bio Laboratories, Carlsbad, CA) to 15 µl total volume. This reaction is submitted to a commercial sequencing organization such as Genewiz (South Plainfield, NJ) for Sanger sequencing.

Massively Parallel Sequencing of Target Amplicons From Heterogeneous Samples

[0230] *DNA Quantification & Library Construction.* The cleaned PCR amplification products are quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies, Grand Island, NY) according to the manufacturer's instructions.

Following quantification, the barcoded cleaned PCR products are combined such that each distinct PCR product is at an equimolar ratio to create a prepared Illumina library.

[0231] *Nucleic Acid Detection.* The prepared library can be sequenced on Illumina HiSeq or MiSeq sequencers (Illumina, San Diego, CA) with cluster generation, template hybridization, iso-thermal amplification, linearization, blocking and denaturization and hybridization of the sequencing primers performed according to the manufacturer's instructions. 16SV4SeqFw (TATGGTAATTGTGTGCCAGCMGCCGCGGTAA), 16SV4SeqRev (AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT), and 16SV4Index (ATTAGAWACCCBDGTAGTCCGGCTGACTGACT) (IDT, Coralville, IA) are used for sequencing. Other sequencing technologies can be used such as but not limited to 454, Pacific Biosciences, Helicos, Ion Torrent, and Nanopore using protocols that are standard to someone skilled in the art of genomic sequencing.

Example 3: Sequence Read Annotation

Primary Read Annotation

[0232] Nucleic acid sequences can be analyzed and annotated to define taxonomic assignments using sequence similarity and phylogenetic placement methods or a combination of the two strategies. A similar approach can be used to annotate protein names, transcription factor names, and any other classification schema for nucleic acid sequences. Sequence similarity based methods include those familiar to individuals skilled in the art including, but not limited to BLAST, BLASTx, tBLASTn, tBLASTx, RDP-classifier, DNAClust, and various implementations of these algorithms such as Qiime or Mothur. These methods rely on mapping a sequence read to a reference database and selecting the match with

the best score and e-value. Common databases include, but are not limited to the Human Microbiome Project, NCBI non-redundant database, Greengenes, RDP, and Silva. Phylogenetic methods can be used in combination with sequence similarity methods to improve the calling accuracy of an annotation or taxonomic assignment. Here tree topologies and nodal structure are used to refine the resolution of the analysis. In this approach we analyze nucleic acid sequences using one of numerous sequence similarity approaches and leverage phylogenetic methods that are well known to those skilled in the art, including but not limited to maximum likelihood phylogenetic reconstruction (see e.g. Liu K, Linder CR, and Warnow T. 2011. RAXML and FastTree: Comparing Two Methods for Large-Scale Maximum Likelihood Phylogeny Estimation. PLoS ONE 6: e27731. McGuire G, Denham MC, and Balding DJ. 2001. Models of sequence evolution for DNA sequences containing gaps. Mol. Biol. Evol 18: 481–490. Wróbel B. 2008. Statistical measures of uncertainty for branches in phylogenetic trees inferred from molecular sequences by using model-based methods. J. Appl. Genet. 49: 49–67.) Sequence reads are placed into a reference phylogeny comprised of appropriate reference sequences. Annotations are made based on the placement of the read in the phylogenetic tree. The certainty or significance of the OTU annotation is defined based on the OTU's sequence similarity to a reference nucleic acid sequence and the proximity of the OTU sequence relative to one or more reference sequences in the phylogeny. As an example, the specificity of a taxonomic assignment is defined with confidence at the the level of Family, Genus, Species, or Strain with the confidence determined based on the position of bootstrap supported branches in the reference phylogenetic tree relative to the placement of the OTU sequence being interrogated.

Clade Assignments

[0233] The ability of 16S-V4 OTU identification to assign an OTU as a specific species depends in part on the resolving power of the 16S-V4 region of the 16S gene for a particular species or group of species. Both the density of available reference 16S sequences for different regions of the tree as well as the inherent variability in the 16S gene between different species will determine the definitiveness of a taxonomic annotation. Given the topological nature of a phylogenetic tree and the fact that tree represents hierarchical relationships of OTUs to one another based on their sequence similarity and an underlying evolutionary model, taxonomic annotations of a read can be rolled up to a higher level using a clade-based assignment procedure (Table 1). Using this approach, clades are defined based on the topology of a phylogenetic tree that is constructed from full-length 16S sequences using maximum likelihood or other phylogenetic models familiar to individuals with ordinary skill in the art of phylogenetics. Clades are constructed to ensure that all OTUs in a given clade are: (i) within a specified number of bootstrap supported nodes from one another (generally, 1-5 bootstraps), and (ii) within a 5% genetic similarity. OTUs that are within the same clade can be distinguished as genetically and phylogenetically distinct from OTUs in a different clade based on 16S-V4 sequence data. OTUs falling within the same clade are evolutionarily closely related and may or may not be distinguishable from one another using 16S-V4 sequence data. The power of clade based analysis is that members of the same clade, due to their evolutionary relatedness, are likely to play similar functional roles in a microbial ecology such as that found in the human gut. Compositions substituting one species with another from the same clade are likely to have conserved ecological function and therefore are useful in the present invention.

[0234] Notably, 16S sequences of isolates of a given OTU are phylogenetically placed within their respective clades, sometimes in conflict with the microbiological-based assignment of species and genus that may have preceded 16S-based assignment. Discrepancies between taxonomic assignment based on microbiological characteristics versus genetic sequencing are known to exist from the literature.

Example 4: Germinating Spores

[0235] Mixtures of bacteria can include species that are in spore form. Germinating a spore fraction increases the number of viable bacteria that will grow on various media types. To germinate a population of spores, the sample is moved to the anaerobic chamber, resuspended in prereduced PBS, mixed and incubated for 1 hour at 37C to allow for germination. Germinants can include amino-acids (e.g., alanine, glycine), sugars (e.g., fructose), nucleosides (e.g., inosine), bile salts (e.g., cholate and taurocholate), metal cations (e.g., Mg^{2+} , Ca^{2+}), fatty acids, and long-chain alkyl amines (e.g., dodecylamine, Germination of bacterial spores with alkyl primary amines” J. Bacteriology, 1961.). Mixtures of these or more complex natural mixtures, such as rumen fluid or Ovgall, can be used to induce germination. Ovgall is dehydrated bovine bile composed of fatty acids, bile acids, inorganic salts, sulfates, bile pigments, cholesterol, mucin, lecithin, glycuronic acids, porphyrins, and urea. The germination can also be performed in a growth medium like prereduced BHIS/ovgall germination medium, in which BHIS (Brain heart infusion powder (37 g/L), yeast extract (5 g/L), L-cysteine HCl (1 g/L)) provides peptides, amino acids, inorganic ions and sugars in the complex BHI and yeast extract mixtures and Ovgall provides additional bile acid germinants.

[0236] In addition, pressure may be used to germinate spores. The selection of germinants can vary with the microbe being sought. Different species require different germinants and different isolates of the same species can require different germinants for optimal germination. Finally, it is important to dilute the mixture prior to plating because some germinants are inhibitory to growth of the vegetative-state microorganisms. For instance, it has been shown that alkyl amines must be neutralized with anionic lipophiles in order to promote optimal growth. Bile acids can also inhibit growth of some organisms despite promoting their germination, and must be diluted away prior to plating for viable cells.

[0237] For example, BHIS/oxgall solution is used as a germinant and contains 0.5X BHIS medium with 0.25% oxgall (dehydrated bovine bile) where 1x BHIS medium contains the following per L of solution: 6g Brain Heart Infusion from solids, 7g peptic digest of animal tissue, 14.5g of pancreatic digest of casein, 5g of yeast extract, 5g sodium chloride, 2g glucose, 2.5g disodium phosphate, and 1g cysteine. Additionally, Ca-DPA is a germinant and contains 40mM CaCl_2 , and 40mM dipicolinic acid (DPA). Rumen fluid (Bar Diamond, Inc.) is also a germinant. Simulated gastric fluid (Ricca Chemical) is a germinant and is 0.2% (w/v) Sodium Chloride in 0.7% (v/v) Hydrochloric Acid. Mucin medium is a germinant and prepared by adding the following items to 1L of distilled sterile water: 0.4 g KH_2PO_4 , 0.53 g Na_2HPO_4 , 0.3 g NH_4Cl , 0.3 g NaCl , 0.1 g $\text{MgCl}_2 \times 6\text{H}_2\text{O}$, 0.11 g CaCl_2 , 1 ml alkaline trace element solution, 1 ml acid trace element solution, 1 ml vitamin solution, 0.5 mg resazurin, 4 g NaHCO_3 , 0.25 g $\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$. The trace element and vitamin solutions prepared as described previously (Stams et al., 1993). All compounds were autoclaved, except the vitamins, which were filter-sterilized. The basal medium was supplemented with 0.7% (v/v) clarified, sterile rumen fluid and 0.25% (v/v)

commercial hog gastric mucin (Type III; Sigma), purified by ethanol precipitation as described previously (Miller & Hoskins, 1981). This medium is referred herein as mucin medium.

[0238] Fetal Bovine Serum (Gibco) can be used as a germinant and contains 5% FBS heat inactivated, in Phosphate Buffered Saline (PBS, Fisher Scientific) containing 0.137M Sodium Chloride, 0.0027M Potassium Chloride, 0.0119M Phosphate Buffer. Thioglycollate is a germinant as described previously (Kamiya et al Journal of Medical Microbiology 1989) and contains 0.25M (pH10) sodium thioglycollate. Dodecylamine solution containing 1mM dodecylamine in PBS is a germinant. A sugar solution can be used as a germinant and contains 0.2% fructose, 0.2% glucose, and 0.2% mannitol. Amino acid solution can also be used as a germinant and contains 5mM alanine, 1mM arginine, 1mM histidine, 1mM lysine, 1mM proline, 1mM asparagine, 1mM aspartic acid, 1mM phenylalanine. A germinant mixture referred to herein as Germix 3 can be a germinant and contains 5mM alanine, 1mM arginine, 1mM histidine, 1mM lysine, 1mM proline, 1mM asparagine, 1mM aspartic acid, 1mM phenylalanine, 0.2%taurocholate, 0.2% fructose, 0.2% mannitol, 0.2% glucose, 1mM inosine, 2.5mM Ca-DPA, and 5mM KCl. BHIS medium + DPA is a germinant mixture and contains BHIS medium and 2mM Ca-DPA. Escherichia coli spent medium supernatant referred to herein as EcSN is a germinant and is prepared by growing E. coli MG1655 in SweetB/Fos inulin medium anaerobically for 48 hr, spinning down cells at 20,000rcf for 20 minutes, collecting the supernatant and heating to 60C for 40 min. Finally, the solution is filter sterilized and used as a germinant solution.

Example 5: Selection of Media for Growth

[0239] Provided are appropriate media to support growth, including preferred carbon sources. For example, some organisms prefer complex sugars such as cellobiose over simple sugars. Examples of media used in the isolation of sporulating organisms include EYA, BHI, BHIS, and GAM (see below for complete names and references). Multiple dilutions are plated out to ensure that some plates will have well isolated colonies on them for analysis, or alternatively plates with dense colonies may be scraped and suspended in PBS to generate a mixed diverse community.

[0240] Plates are incubated anaerobically or aerobically at 37 C for 48-72 or more hours, targeting anaerobic or aerobic spore formers, respectively.

[0241] Solid plate media include:

[0242] Gifu Anaerobic Medium (GAM, Nissui) without dextrose supplemented with fructooligosaccharides/inulin (0.4%), mannitol (0.4%), inulin (0.4%), or fructose (0.4%), or a combination thereof.

[0243] Sweet GAM [Gifu Anaerobic Medium (GAM, Nissui)] modified, supplemented with glucose, cellobiose, maltose, L-arabinose, fructose, fructooligosaccharides/inulin, mannitol and sodium lactate)

[0244] Brucella Blood Agar (BBA, Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010)

[0245] PEA sheep blood (Anaerobe Systems; 5% Sheep Blood Agar with Phenylethyl Alcohol)

[0246] Egg Yolk Agar (EYA) (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010)

- [0247] Sulfite polymyxin milk agar (Mevissen-Verhage et al., J. Clin. Microbiol. 25:285-289 (1987))
- [0248] Mucin agar (Derrien et al., IJSEM 54: 1469-1476 (2004))
- [0249] Polygalacturonate agar (Jensen & Canale-Parola, Appl. Environ. Microbiol. 52:880-997 (1986))
- [0250] M2GSC (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010)
- [0251] M2 agar (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010) supplemented with starch (1%), mannitol (0.4%), lactate (1.5g/L) or lactose (0.4%)
- [0252] Sweet B - Brain Heart Infusion agar (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010) supplemented with yeast extract (0.5%), hemin, cysteine (0.1%), maltose (0.1%), cellobiose (0.1%), soluble starch (sigma, 1%), MOPS (50mM, pH 7).
- [0253] PY-salicin (peptone-yeast extract agar supplemented with salicin) (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010).
- [0254] Modified Brain Heart Infusion (M-BHI) [[sweet and sour]] contains the following per L: 37.5g Brain Heart Infusion powder (Remel), 5g yeast extract, 2.2g meat extract, 1.2g liver extract, 1g cystein HCl, 0.3g sodium thioglycolate, 10mg hemin, 2g soluble starch, 2g FOS/Inulin, 1g cellobiose, 1g L-arabinose, 1g mannitol, 1 Na-lactate, 1mL Tween 80, 0.6g MgSO₄x7H₂O, 0.6g CaCl₂, 6g (NH₄)₂SO₄, 3g KH₂PO₄, 0.5g K₂HPO₄, 33mM Acetic acid, 9mM propionic acid, 1mM Isobutyric acid, 1mM isovaleric acid, 15g agar, and after autoclaving add 50mL of 8% NaHCO₃ solution and 50mL 1M MOPS-KOH (pH 7).

[0255] Noack-Blaut Eubacterium agar (See Noack *et al. J. Nutr.* (1998) 128:1385-1391)

[0256] BHIS az1/ge2 - BHIS az/ge agar (Reeves *et. al. Infect. Immun.* 80:3786-3794 (2012)) [Brain Heart Infusion agar (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010) supplemented with yeast extract 0.5%, cysteine 0.1%, 0.1% cellobiose, 0.1% inulin, 0.1% maltose, aztreonam 1 mg/L, gentamycin 2 mg/L]

[0257] BHIS CInM az1/ge2- BHIS CInM [Brain Heart Infusion agar (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010) supplemented with yeast extract 0.5%, cysteine 0.1%, 0.1% cellobiose, 0.1% inulin, 0.1% maltose, aztreonam 1 mg/L, gentamycin 2 mg/L]

Example 6: Purification and Isolation of a Spore Forming Fraction From Feces

[0258] To purify and selectively isolate efficacious spores from fecal material a donation is first blended with saline using a homogenization device (e.g., laboratory blender) to produce a 20% slurry (w/v). 100% ethanol is added for an inactivation treatment that lasts 10 seconds to 1 hour. The final alcohol concentration can range from 30-90%, preferably 50-70%. High speed centrifugation (3200 rcf for 10min) is performed to remove solvent and the pellet is retained and washed. Subsequently, once the washed pellet is resuspended, a low speed centrifugation step (200 rcf for 4 min) is performed to remove large particulate vegetative matter and the supernatant containing the spores is retained. High speed centrifugation (3200 rcf for 10 min) is performed on the supernatant to concentrate the spore material. The pellet is then washed and resuspended to generate a 20% slurry. This is the ethanol treated spore preparation. The concentrated slurry is then separated with a density based gradient e.g. a CsCl gradient, sucrose gradient or combination of the two generating a ethanol treated, gradient-purified spore preparation. For example, a CsCl gradient

is performed by loading a 20% volume of spore suspension on top a 80% volume of a stepwise CsCl gradient (w/v) containing the steps of 64%, 50%, 40% CsCl (w/v) and centrifuging for 20 min at 3200 rcf. The spore fraction is then run on a sucrose step gradient with steps of 67%, 50%, 40%, and 30% (w/v). When centrifuged in a swinging bucket rotor for 10 min at 3200 rcf. The spores run roughly in the 30% and 40% sucrose fractions. The lower spore fraction (Figure 2) is then removed and washed to produce a concentrated ethanol treated, gradient-purified spore preparation. Taking advantage of the refractive properties of spores observed by phase contrast microscopy (spores are bright and refractive while germinated spores and vegetative cells are dark) one can see an enrichment of the spore fraction from a fecal bacterial cell suspension (Figure 3, left) compared to an ethanol treated, CsCl gradient purified, spore preparation (Figure 3, center), and to an ethanol treated, CsCl gradient purified, sucrose gradient purified, spore preparation (Figure 3, right).

[0259] Furthermore, growth of spores after treatment with a germinant can also be used to quantify a viable spore population. Briefly, samples were incubated with a germinant (Oxgall, 0.25% for up to 1 hour), diluted and plated anaerobically on BBA (Brucella Blood Agar) or similar media (e.g. see Examples 4 and 5). Individual colonies were picked and DNA isolated for full-length 16S sequencing to identify the species composition (e.g. see Examples 2 and 3). Analysis revealed that 22 species were observed in total (Table 2) with a vast majority present in both the material purified with the gradient and without the gradient, indicating no or inconsequential shift in the ecology as a result of gradient purification. Spore yield calculations demonstrate an efficient recovery of 38% of the spores from the initial fecal material as measured by germination and plating of spores on BBA or measuring DPA count in the sample.

Example 7: Bacterial Compositions Prevent *C. difficile* Infection in a Mouse Model

[0260] To test the therapeutic potential of the bacterial composition such as but not limited to a spore population, a prophylactic mouse model of *C. difficile* infection (model based on Chen, et al., A mouse model of *Clostridium difficile* associated disease, Gastroenterology 135(6):1984-1992) was used. Two cages of five mice each were tested for each arm of the experiment. All mice received an antibiotic cocktail consisting of 10% glucose, kanamycin (0.5 mg/ml), gentamicin (0.044 mg/ml), colistin (1062.5 U/ml), metronidazole (0.269 mg/ml), ciprofloxacin (0.156 mg/ml), ampicillin (0.1 mg/ml) and Vancomycin (0.056 mg/ml) in their drinking water on days -14 through -5 and a dose of 10mg/kg Clindamycin by oral gavage on day -3. On day -1, they received either the test article or vehicle control via oral gavage. On day 0 they were challenged by administration of approximately 4.5 log₁₀ cfu of *C. difficile* (ATCC 43255) via oral gavage. Optionally a positive control group received vancomycin from day -1 through day 3 in addition to the antibiotic protocol and *C. difficile* challenge specified above. Feces were collected from the cages for analysis of bacterial carriage, mortality was assessed every day from day 0 to day 6 and the weight and subsequent weight change of the animal was assessed with weight loss being associated with *C. difficile* infection. Mortality and reduced weight loss of the test article compared to the vehicle were used to assess the success of the test article. Additionally, a *C. difficile* symptom scoring was performed each day from day -1 through day 6. Clinical Score was based on a 0-4 scale by combining scores for Appearance (0-2 pts based on normal, hunched, piloerection, or lethargic), and Clinical Signs (0-2 points based on normal, wet tail, cold-to-the-touch, or isolation from other animals).

[0261] In a naive control arm, animals were challenged with *C. difficile*. In the vancomycin positive control arm animals were dosed with *C. difficile* and treated with vancomycin from day -1 through day 3. The negative control was gavaged with PBS alone and no bacteria. The test arms of the experiment tested 1x, 0.1x, 0.01x dilutions derived from a single donor preparation of ethanol treated spores (e.g. see example AAAF) or the heat treated feces prepared by treating a 20% slurry for 30min at 80C. Dosing for CFU counts was determined from the final ethanol treated spores and dilutions of total spores were administered at 1x, 0.1x, 0.01x of the spore mixture for the ethanol treated fraction and a 1x dose for the heat treated fraction.

[0262] Weight loss and mortality were assessed on day 3. The negative control, treated with *C. difficile* only, exhibits 20% mortality and weight loss on Day 3, while the positive control of 10% human fecal suspension displays no mortality or weight loss on Day 3 (Table 5). Table 5 contains the mortality and weight change of mice treated with a donor fecal suspension and an ethanol and/or heat-treated spore preparation at various dilutions. EtOH-treated feces prevents mortality and weight loss at three dilutions, while the heat-treated fraction was protective at the only dose tested. These data indicate that the spore fraction is efficacious in preventing *C. difficile* infection in the mouse.

Example 8: Prophylactic and Relapse Prevention Hamster Models

[0263] Previous studies with hamsters using toxigenic and nontoxigenic strains of *C. difficile* demonstrated the utility of the hamster model in examining relapse post antibiotic treatment and the effects of prophylaxis treatments with cecal flora in *C. difficile* infection (Wilson et al. 1981, Wilson et al. 1983, Borriello et al. 1985) and more broadly gastrointestinal infectious disease. To demonstrate prophylactic use of a bacterial composition such as but not limited to a spore population, spore

preparation, vegetative cell population, to ameliorate *C. difficile* infection, the following hamster model is used. In a prophylactic model, Clindamycin (10mg/kg s.c.) is given on day -5, the bacterial composition or control is administered on day -3, and *C. difficile* challenge occurs on day 0. In the positive control arm, vancomycin is then administered on day 1-5 (and vehicle control is delivered on day -3). Feces are collected on day -5, -4, -1, 1, 3, 5, 7, 9 and fecal samples are assessed for pathogen carriage and reduction by microbiological methods, 16S sequencing approaches or other methods utilized by one skilled in the art. Mortality is assessed throughout the experiment through 21 days post *C. difficile* challenge. The percentage survival curves show that ethanol treated spores and ethanol treated, gradient-purified spores better protect the hamsters compared to the Vancomycin control, and vehicle control.

[0264] Figure 4 shows a prophylaxis model with the ethanol treated spore preparation and the ethanol treated, gradient-purified spore preparation.

[0265] In the relapse prevention model, hamsters are challenged with toxigenic *C. difficile* strains on day 0, and treated with clindamycin by oral gavage on day 1, and vancomycin dosing day 2-6. Test or control treatment was then administered on day 7, 8, and 9. The groups of hamsters for each arm consist of 8 hamsters per group. Fecal material is collected on day -1, 1, 3, 5, 7, 10 and 13 and hamster mortality is assessed throughout. Survival curves are used to assess the success of the test article e.g. ethanol treated or ethanol treated, gradient purified spores versus the control treatment in preventing hamster death. The survival curves demonstrate maximum efficacy for the ethanol treated, gradient-purified spores followed by the ethanol treated spores. Both treatments improved survival percentage over vancomycin treatment alone.

[0266] Figure 5 shows the relapse prevention model with ethanol treated spores and ethanol treated, gradient purified spores

Example 9: Clinical treatment of recurrent *C. difficile* in patients

[0267] To assess the efficacy of test articles like bacterial composition including but not limited to a ethanol treated spore preparations (e.g. see Example 6) to treat recurrent *C. difficile* in human patients, the following procedure was performed to take feces from a healthy donor, inactivate via the ethanol treated spore preparation protocol described below, and treat recurrent *C. difficile* in patients presenting with this indication. Non-related donors were screened for general health history for absence of chronic medical conditions (including inflammatory bowel disease; irritable bowel syndrome; Celiac disease; or any history of gastrointestinal malignancy or polyposis), absence of risk factors for transmissible infections, antibiotic non-use in the previous 6 months, and negative results in laboratory assays for blood-borne pathogens (HIV, HTLV, HCV, HBV, CMV, HAV and *Treponema pallidum*) and fecal bacterial pathogens (*Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, *E. coli* 0157), ova and parasites, and other infectious agents (*Giardia*, *Cryptosporidium*, *Cyclospora*, *Isospora*) prior to stool donation.

[0268] Donor stool was frozen shortly after donation and sampled for testing. At the time of use, approximately 75 g of donor stool was thawed and resuspended in 500 mL of non-bacteriostatic normal saline and mixed in a single use glass or plastic blender. The resulting slurry was sequentially passed through sterile, disposable mesh screens that remove particles of size 600, 300 and 200 microns. The slurry was then centrifuged briefly (200 rcf for 4 min) to separate fibrous and particulate materials, and the supernatant (containing bacterial cells and spores) was transferred to a fresh container. Ethanol was added to a final concentration of 50%

and the resulting ~1500 ml slurry was incubated at room temperature for 1 hr with continuous mixing to inactivate vegetative bacterial cells. Midway through inactivation the slurry was transferred to a new bottle to ensure complete contact with the ethanol. The solid matter was pelleted in a centrifuge and washed 3 times with normal saline to remove residual ethanol. The final pellet was resuspended in 100% sterile, USP glycerol at a minimum volume, and filled into approximately 30 size 0 delayed release capsules (hypromellose DRcaps, Capsugel, Inc.) at 0.65 mL suspension each. The capsules were immediately capped and placed onto an aluminum freezing block held at -80° C via dry ice to freeze. The frozen capsules were in turn over-capsulated with size 00 DRcaps to enhance capsule stability, labeled, and placed into < -65° C storage immediately. The final product was stored at < -65° C until the day and time of use. Encapsulated product may be stored for indefinitely at < -65° C. On the day of dosing capsules were warmed on wet ice for 1 to 2 hours to improve tolerability, and were then dosed with water ad libitum.

[0269] Patient 1 is a 45-year old woman with a history of *C. difficile* infection and diarrhea for at least 1 year prior to treatment. She has been previously treated with multiple courses of antibiotics followed each time by recurrence of *C. difficile*-associated diarrhea.

[0270] Patient 2 is an 81-year old female who has experienced recurrent *C. difficile* infection for 6 months prior to treatment despite adequate antibiotic therapy following each recurrence.

[0271] 24 hours prior to starting oral treatment, CDAD antibiotic therapy was discontinued. Each patient received a colon preparation procedure intended to reduce the competing microbial burden in the gastrointestinal tract and to facilitate repopulation by the spore forming organisms in the investigational product.

[0272] On the morning of the first treatment day, the patients received a dose of delayed release capsules containing the investigational product with water ad libitum. Patients were requested to avoid food for 1 hour thereafter. The next day, the patient returned to the clinic to receive an additional dose. Patients were asked to avoid food for 4 hours prior to receiving their second dose and for 1 hour following dosing.

[0273] Both patients were followed closely for evidence of relapse or adverse symptoms following treatment. Patients were contacted by phone on Day 2, Day 4, and Weeks 1, 2 and 4 and each was queried about her general status and the condition of her CDAD and related symptoms. Stool samples were collected at baseline and Weeks 1, 2, 4 and 8 post-treatment to assess changes in the gut microbiota via 16S sequencing and spore count with methods explained previously (e.g. see Examples 2 and 3). Through 4 weeks post treatment, each patient has gradually improved with no evidence of *C. difficile* recurrence.

[0274] Six other patients with recurrent *C. difficile*-associated diarrhea were treated in a similar fashion, with no CDI recurrence and no requirement for resumption of antibiotics (total of 8 patients). Additionally, there were no treatment-related serious adverse events.

[0275] The above protocol could be modified to deliver other bacterial compositions e.g. vegetative cells, spore preparations, and combinations thereof.

Example 10: Enrichment and Purification of Bacteria

[0276] To purify individual bacterial strains, dilution plates were selected in which the density enables distinct separation of single colonies. Colonies were picked with a sterile implement (either a sterile loop or toothpick) and re-streaked to BBA or other solid media. Plates were incubated at 37°C for 3-7 days. One or more well-

isolated single colonies of the major morphology type were re-streaked. This process was repeated at least three times until a single, stable colony morphology is observed. The isolated microbe was then cultured anaerobically in liquid media for 24 hours or longer to obtain a pure culture of 10^6 - 10^{10} cfu/ml. Liquid growth medium might include Brain Heart Infusion-based medium (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010) supplemented with yeast extract, hemin, cysteine, and carbohydrates (for example, maltose, cellobiose, soluble starch) or other media described previously (e.g. see example AAAE). The culture was centrifuged at 10,000 x g for 5 min to pellet the bacteria, the spent culture media was removed, and the bacteria were resuspended in sterile PBS. Sterile 75% glycerol was added to a final concentration of 20%. An aliquot of glycerol stock was titered by serial dilution and plating. The remainder of the stock was frozen on dry ice for 10-15 min and then placed at -80C for long term storage.

Example 11: Cell Bank Preparation

[0277] Cell banks (RCBs) of bacterial strains were prepared as follows. Bacterial strains were struck from -80°C frozen glycerol stocks to Brucella blood agar with Hemin or Vitamin K (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010), M2GSC (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010) or other solid growth media and incubated for 24 to 48 h at 37°C in an anaerobic chamber with a gas mixture of H₂:CO₂:N₂ of 10:10:80. Single colonies were then picked and used to inoculate 250 ml to 1 L of Wilkins-Chalgren broth, Brain-Heart Infusion broth, M2GSC broth or other growth media, and grown to mid to late exponential phase or into the stationary phase of growth. Alternatively, the single colonies may be used to inoculate a pilot culture of 10 ml, which were then used to inoculate a large volume culture. The growth media and the growth phase at

harvest were selected to enhance cell titer, sporulation (if desired) and phenotypes that might be associated desired in vitro or in vivo. Optionally, Cultures were grown static or shaking, depending which yielded maximal cell titer. The cultures were then concentrated 10 fold or more by centrifugation at 5000 rpm for 20 min, and resuspended in sterile phosphate buffered saline (PBS) plus 15% glycerol. 1 ml aliquots were transferred into 1.8 ml cryovials which were then frozen on dry ice and stored at -80C. The identity of a given cell bank was confirmed by PCR amplification of the 16S rDNA gene, followed by Sanger direct cycle sequencing, and comparison to a curated rDNA database to determine a taxonomic ID. Each bank was confirmed to yield colonies of a single morphology upon streaking to Brucella blood agar or M2GSC agar. When more than one morphology was observed, colonies were confirmed to be the expected species by PCR and sequencing analysis of the 16S rDNA gene. Variant colony morphologies can be observed within pure cultures, and in a variety of bacteria the mechanisms of varying colony morphologies have been well described (van der Woude, Clinical Microbiology Reviews, 17:518, 2004), including in Clostridium species (Wadsworth-KTL Anaerobic Bacteriology Manual, 6th Ed, Jousimie-Somer, et al 2002). For obligate anaerobes, RCBs were confirmed to lack aerobic colony forming units at a limit of detection of 10 cfu/ml.

Example 12: Titer Determination

[0278] The number of viable cells per ml was determined on the freshly harvested, washed and concentrated culture by plating serial dilutions of the RCB to Brucella blood agar or other solid media, and varied from 10^6 to 10^{10} cfu/ml. The impact of freezing on viability was determined by titering the banks after one or two freeze-thaw cycles on dry ice or at -80°C, followed by thawing in an anaerobic chamber at room temperature. Some strains displayed a 1-3 log drop in viable

cfu/ml after the 1st and/or 2nd freeze thaw, while the viability of others were unaffected.

Example 13: Preparation of bacterial compositions

[0279] Individual strains were typically thawed on ice and combined in an anaerobic chamber to create mixtures, followed by a second freeze at -80°C to preserve the mixed samples. When making combinations of strains for in vitro or in vivo assays, the cfu in the final mixture was estimated based on the second freeze-thaw titer of the individual strains. For experiments in rodents, strains may be combined at equal counts in order to deliver between 1e4 and 1e10 per strain. Additionally, some bacteria may not grow to sufficient titer to yield cell banks that allowed the production of compositions where all bacteria were present at 1e10.

Example 14: Provision of Gut Microbiome Sample Material

[0280] Fresh gut microbiome samples e.g. fecal samples were obtained from healthy human donors who have been screened for general good health and for the absence of infectious diseases, and meet inclusion and exclusion criteria, inclusion criteria include being in good general health, without significant medical history, physical examination findings, or clinical laboratory abnormalities, regular bowel movements with stool appearance typically Type 2, 3, 4, 5 or 6 on the Bristol Stool Scale, and having a BMI $\geq 18 \text{ kg/m}^2$ and $\leq 25 \text{ kg/m}^2$. Exclusion criteria generally included significant chronic or acute medical conditions including renal, hepatic, pulmonary, gastrointestinal, cardiovascular, genitourinary, endocrine, immunologic, metabolic, neurologic or hematological disease, a family history of, inflammatory bowel disease including Crohn's disease and ulcerative colitis, Irritable bowel syndrome, colon, stomach or other gastrointestinal malignancies, or gastrointestinal

polyposis syndromes, or recent use of yogurt or commercial probiotic materials in which an organism(s) is a primary component. Samples were collected directly using a commode specimen collection system, which contains a plastic support placed on the toilet seat and a collection container that rests on the support. Gut microbiome samples e.g. feces were deposited into the container, and the lid was then placed on the container and sealed tightly. The sample was then delivered on ice within 1-4 hours for processing. Samples were mixed with a sterile disposable tool, and 2-4 g aliquots were weighed and placed into tubes and flash frozen in a dry ice/ethanol bath. Aliquots are frozen at -80 degrees Celsius until use.

[0281] Optionally, the microbiome sample was suspended in a solution, and/or fibrous and/or particulate materials were removed. A frozen aliquot containing a known weight of sample was removed from storage at -80 degrees Celsius and allowed to thaw at room temperature. Sterile 1x PBS was added to create a 10% w/v suspension, and vigorous vortexing was performed to suspend the sample until the material appeared homogeneous. The sample was then left to sit for 10 minutes at room temperature to sediment fibrous and particulate matter. The suspension above the sediment was then carefully removed into a new tube and contains a purified spore population. Optionally, the suspension was then centrifuged at a low speed, e.g., 1000 x g, for 5 minutes to pellet particulate matter including fibers. The pellet was discarded and the supernatant, which contained vegetative organisms and spores, was removed into a new tube. The supernatant was then centrifuged at 6000 x g for 10 minutes to pellet the vegetative organisms and spores. The pellet was then resuspended in 1x PBS with vigorous vortexing until the sample material appears homogenous.

Example 15: Bacterial Compositions Populating the Gut in a Mouse Model

[0282] Two bacterial compositions were evaluated in a mouse model to demonstrate the ability to populate the gastrointestinal tract. Bacteria were grown as described in Example 13. Compositions were pre-made under anaerobic conditions and suspended in PBS + 15% glycerol and stored at $\geq -70^{\circ}\text{C}$ prior to use.

[0283] Groups of mice (10 females/group; 5 per cage) were pre-treated on Days -14 to -5 with an antibiotic cocktail consisting of 10% glucose, kanamycin (0.5 mg/ml), gentamicin (0.044 mg/ml), colistin (1062.5 U/ml), metronidazole (0.269 mg/ml), ciprofloxacin (0.156 mg/ml), ampicillin (0.1 mg/ml) and vancomycin (0.056 mg/ml) in their drinking water. On Day -3 they received 10 mg/kg Clindamycin by oral gavage. On Day -1, they were dosed with a microbial compositions by oral gavage in a volume of 0.2 mL (Table 6). Microbial compositions comprised approximately equal numbers of each OTU and were dosed at approximately 1×10^9 , 1×10^8 and 1×10^7 per OTU for each composition (e.g. microbial composition 1, comprising 15 strains, was dosed at approximately 1.5×10^{10} , 1.5×10^9 , and 1.5×10^8 total CFU). Fecal samples were collected from each cage on Day -1 (approximately 1 hour before dosing) and on Days 2, 3 and 4 post-dosing. Feces were stored frozen prior to processing and sequencing. Weight gain of mice treated with either microbial compositions was similar to that of naive, control mice.

[0284] In parallel, groups of animals treated with the same microbial compositions on Day -1 were challenged on Day 0 with approximately $10^{4.5}$ spores of *Clostridium difficile* (ATCC 43255) via oral gavage. Mortality for *C. difficile* challenged animals was assessed every day from Day 0 to Day 6 and the weight and subsequent weight change of the animal was assessed with weight loss being associated with *C. difficile*

infection. Mortality and reduced weight loss of the test article compared to the empty vehicle was used to assess the success of the test article.

[0285] Fecal samples were processed by isolating and sequencing DNA according to Examples 2 and 3. The OTU assignment of fecal samples from Days -1, 2, 3 and 4 was determined by analyzing 16S-V4 sequence reads and assigning OTUs as described in Example 2. Clades were assigned as described in Example 2. Total read counts were determined for each OTU or each clade by summing the results from cages of the same experimental group. Samples with 10 or fewer sequence reads for a given OTU or clade were considered to be below background and were not included in the summation process. Results are shown by OTU (Table 7) and by clade (Table 8).

[0286] Upon examining the OTU data in Table 7, several patterns emerge. First, there are a group of OTUs with no sequence reads on Day -1 that populate large numbers of sequence reads on Days 2, 3, or 4; this group includes *Cl. butyricum*, *Cl. hylemonae*, *Cl. orbiscindens*, *Cl. symbiosum*, and *L. bacterium_5_1_57FAA*. *Cl. disporicum* is comparable to this group as it has sequence reads on Day -1 that are very close to background (10 and 29 in compositions 1 and 2, respectively), which subsequently increase their population by as much as 1000-fold on Days 2, 3 or 4. Second, there are OTUs such as *Co. aerofaciens*, *C. comes*, *R. bromii*, *B. producta*, *Cl. bolteae*, *Cl. mayombeii*, *Cl. innocuum*, *Cl. tertium* and *R. gnavus* which are not detectable at the OTU level in either the Day -1 sample or in subsequent samples. In composition 2, *Co. aerofaciens* is detected transiently on Day 2 in the 1×10^8 and 1×10^7 dose groups; *E. rectale* in the same experimental groups is detected on Day 3, suggesting a possible relationship between transient population by *Co. aerofaciens* followed by *E. rectale* in these groups of mice. A striking observation is that the

observed population of OTU sequence reads is not highly dose dependent. Overall, the data is consistent with a model whereby OTUs populate rapidly following oral administration.

[0287] The clade-based analysis in Table 8 was performed to more thoroughly evaluate the population of the GI tract. Clade-based analysis obscures some of the details afforded by an OTU analysis. For instance, *Cl. tertium* and *Cl. butyricum* are members of the same clade and thus a clade-based analysis cannot distinguish the dynamics of these individual OTUs. However, clade-based analysis has the compensatory benefit that it is sensitive to measuring population changes that can be missed by an OTU-based analysis. The ability of 16S-V4 OTU identification to assign an OTU as a specific species depends in part on the resolving power of the 16S-V4 region for a particular species or group of species. Both the density of available reference 16S sequences for different regions of the tree as well as the inherent variability in the 16S gene between different species will determine the definitiveness of a taxonomic annotation. So in some cases, the population of a species can be followed using clade-based assignments when OTU based-detection is insensitive in a complex population. For instance, the clade-based analysis in Table 2B supports the case that *R. bromii*, *B. producta*, *Cl. innocuum*, and *R. gnavus* were able to populate since each OTU is a sole member of a clade in the microbial compositions and sequence reads went from undetectable on Day -1 to well above background on Days 2, 3 or 4. 16S V4 sequencing and clade-based analysis could not determine whether *Cl. tertium* or *Cl. bolteae* populated due to the fact that other members of their clades (*Cl. butyricum* and *Cl. symbiosum*, respectively) were present and shown to populate at the OTU level in the mice.

[0288] In the mice challenged in parallel with *C. difficile*, animals were significantly protected as shown in Table 9. Mice gavaged with vehicle (phosphate buffered saline) experienced 100% mortality while microbial compositions 1 and 2 protected at all dose levels with between 0 and 10% mortality by Day 6, the last day of the experiment. In addition, weight loss in animals treated with microbial compositions 1 and 2 was minimal compared to animals receiving the vehicle gavage. These data confirm that population of the gastrointestinal tract with microbial compositions confers a clinical benefit by restoring a state of dysbiosis so that animals can resist infection by a pathogen.

Example 16: Quantification of Spore Concentrations Using DPA Assay

[0289] Methods to assess spore concentration in complex mixtures typically require the separation and selection of spores and subsequent growth of individual species to determine the colony forming units. The art does not teach how to quantitatively germinate all the spores in a complex mixture as there are many species for which appropriate germinants have not been identified. Furthermore, sporulation is thought to be a stochastic process as a result of evolutionary selection, meaning that not all spores from a single species germinate with same response to germinant concentration, time and other environmental conditions. Alternatively, a key metabolite of bacterial spores, dipicolinic acid (DPA) has been developed to quantify spores particles in a sample and avoid interference from fecal contaminants. The assay utilizes the fact that DPA chelates Terbium 3+ to form a luminescent complex (Fichtel et al, FEMS Microbiology Ecology, 2007; Kort et al, Applied and Environmental Microbiology, 2005; Shafaat and Ponce, Applied and Environmental Microbiology, 2006; Yang and Ponce, International Journal of Food Microbiology, 2009; Hindle and Hall, Analyst, 1999). A time-resolved fluorescence assay detects

terbium luminescence in the presence of DPA giving a quantitative measurement of DPA concentration in a solution.

[0290] To perform the assay 1mL of the spore standard to be measured was transferred to a 2mL microcentrifuge tube. The samples were centrifuged at 13000 RCF for 10 min and the sample is washed in 1mL sterile deionized H₂O. Wash an additional time by repeating the centrifugation. Transfer the 1mL solution to hungate tubes and autoclave samples on a steam cycle for 30 min at 250C. Add 100uL of 30uM TbCl₃ solution (400mM sodium acetate, pH 5.0, 30μM TbCl₃) to the sample. Make serial dilutions of of the autoclaved material and measure the fluorescence of each sample by exciting with 275nm light and measuring the emission wavelength of 543nm for an integration time of 1.25ms and a 0.1ms delay.

[0291] Purified spores are produced as described previously (e.g. see <http://www.epa.gov/pesticides/methods/MB-28-00.pdf>). Serial dilutions of purified spores from *C. bifermentans*, *C. sporogenes*, and *C. butyricum* cultures were prepared and measured by plating on BBA media and incubating overnight at 37°C to determine CFU/ml. Figure 7 shows the linear correspondence across different spore producing bacteria across several logs demonstrating the DPA assay as means to assess spore content (linear range of DPA assay compared to CFU counts/ml).

[0292] The discrepancy for complex spore populations between spore counts measured by germinable spore CFU and by DPA has important implications for determining the potency of an ethanol treated spore preparation for clinical use. Table 10 below shows spore content data from 3 different ethanol treated spore preparations used to successfully treat 3 patients suffering from recurrent *C. difficile*

infection. The spore content of each spore preparation is characterized using the two described methods.

[0293] What is immediately apparent is that spore content varies greatly per 30 capsules. As measured by germinable SCFU, spore content varies by greater than 10,000-fold. As measured by DPA, spore content varies by greater than 100-fold. In the absence of the DPA assay, it would be difficult to set a minimum dose for administration to a patient. For instance, without data from the DPA assay, one would conclude that a minimum effective dose of spores is 4×10^5 or less using the SCFU assay (e.g. Preparation 1, See Table 10 below). If that SCFU dose was used to normalize dosing in a clinical setting, however, then the actual spore doses given to patients would be much lower for other ethanol treated spore preparations as measured as by the DPA assay (See Table 11 below).

[0294] It becomes immediately obvious from the variability of SCFU and DPA counts across various donations that using SCFU as the measure of potency would lead to significant underdosing in certain cases. For instance, setting a dose specification of 4×10^5 SCFU (the apparent effective dose from donor Preparation 1) for product Preparation 3 would lead to a potential underdosing of more than 100-fold. This can be rectified only by setting potency specifications based on the DPA assay which better reflects total spore counts in an ethanol treated spore preparation. The unexpected finding of this work is that the DPA assay is uniquely suited to set potency and determine dosing for an ethanol treated spore preparation.

Example 17: Demonstration of Enhanced Growth With a Germinant

[0295] To enhance the ethanol treated spores germination capability and demonstrate spore viability, spores from three different donors were germinated by various treatments and plated on various media. Germination with BHIS/oxgall (BHIS

ox), Ca-DPA, rumen fluid (RF), simulated gastric fluid (SGF), mucin medium (Muc), fetal bovine serum (FBS), or thioglycollate (Thi) for 1 hour at 37°C in anaerobic conditions was performed as described previously (e.g. see Examples 4 and 5) with samples derived from two independent donors (Figure 8). The spore-germinal mixture was serially diluted and plated on different plate media including BBA, Sweet B, Sweet B + lysozyme (2 µg/ml), M2GSC and M2GSC + lysozyme (2 µg/ml) as previously described (e.g. see Examples 4 and 5) to determine spore germination. Colony forming units were tallied and titers were determined using standard techniques by one skilled in the art. As Figure 8 shows, maximum colony forming units are derived from BHI-oxgall treatment. This germination treatment also greatly increases the diversity as measured by the number of OTUs identified when samples were submitted for 16S sequencing (e.g. see Examples 2 and 3) compared to plating without a germination step (Figure 9).

[0296] Figure 8 shows different germinal treatments have variable effects on CFU counts from donor A (upper left) and donor B (lower right). The Y-Axes are spore CFU per ml.

[0297] Figure 9 shows germinates greatly increase the diversity of cultured spore forming OTUs.

[0298] To test the effect of heat activation to promote germination, ethanol treated fecal samples were treated for 15 min at room temperature, 55°C, 65°C, 75°C or 85°C from three different donors and germinated subsequently with BHIS+ Oxgall for 1 hr at 37°C then plated on BBA media (Figure 10) as previously described (e.g. see Examples 4 and 5). Pretreatment at room temperature produced equal if not more spores than the elevated temperatures in all three donors. The temperature of germinating was also examined by incubating samples at room temperature or 37°C

for 1 hr in anaerobic conditions before plating on BBA. No difference in the number of CFUs was observed between the two conditions. Lysozyme addition to the plates (2ug/ml) was also tested on a single donor sample by the testing of various activation temperature followed by an incubation in the presence or absence of lysozyme. The addition of lysozyme had a small effect when plated on Sweet B or M2GSC media but less so than treatment with BHIS oxgall without lysozyme for 1hr (Figure 11).

[0299] Figure 10 shows heat activation as a germination treatment with BHIS+oxgall.

[0300] Figure 11 shows the effect of lysozyme slightly enhances germination

[0301] Germination time was also tested by treating a 10% suspension of a single donor ethanol treated feces (e.g. see Example AA AF) incubated in either BHIS, taurocholate, oxgall, or germix for 0, 15, 30, or 60 minutes and subsequently plated on BHIS, EYA, or BBA media (e.g. see Examples AA AD and AA AE). 60 minutes resulted in the most CFU units across all various combinations germinates and plate media tested. Furthermore, this protocol and germination treatment can be applied to mixtures of bacteria and spore forming species to enrich the population of spore-forming bacterial growth. In this manner, spore formers growth can be enhanced in a mixed population by addition of a germinate or germination treatment step as described above.

Example 18: Construction of an *In Vitro* Assay to Screen for Combinations of Microbes Inhibitory to the Growth of *Clostridium difficile*

[0302] The *in vitro* assay is a high-throughput assay designed to screen for combinations of microbes that inhibit the growth of a pathogen or pathobiont. Feces from a human donor is resuspended 10% w/v in pre-reduced PBS under anaerobic

conditions and then is pelleted in an Eppendorf Centrifuge 5810R, Rotor F34-6-38 at 8,500 rpm (9289 rcf) for 5 min to remove cells and particulates. The supernatant is then filter-sterilized using a 0.22 μ m filter to produce the background media for *in vitro* competitions. The 10% sterile-filtered feces media is then added in 178 μ L aliquots to wells of a 96-well plate. 20 μ L of candidate bacterial strains or combinations of strains are added to each well. The *in vitro* assay plate is then incubated anaerobically at 37°C for 3 hr. before inoculation of *Clostridium difficile* ATCC 9689. The *C. difficile* inoculum is prepared by inoculating 5 mL BHI (Brain Heart Infusion Broth) and incubating 37°C for 18 hr. The overnight culture is then diluted 100 fold in PBS before inoculating 2 μ L into each well to produce a final dilution of 10,000 fold from the overnight culture. *In vitro* assay plates are then incubated anaerobically for 24 hr. before assaying for viable counts of *C. difficile* by plating serial dilutions on *C. difficile* selective agar plates with cycloserine cefoxitin mannitol agar (CCMA).

[0303] Colonies of *C. difficile* are confirmed by their characteristic diffuse colony edge morphology as well as fluorescence under UV light.

Example 19: Quantification of Pathogen using Quantitative PCR (qPCR)

Standard Curve Preparation

[0304] The standard curve is generated from wells containing pathogenic *C. difficile* grown in 10% sterile-filtered feces media from Example 2 by selective spot plating. Serial dilutions of duplicate cultures are performed in sterile phosphate-buffered saline. Genomic DNA is then extracted from the standard curve samples along with the other wells.

Genomic DNA Extraction

[0305] Genomic DNA can be extracted from 50 µl of each sample using the Mo Bio Powersoil®-htp 96 Well Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) according to the manufacturer's instructions with two exceptions: the bead beating is performed for 2 x 4:40 minutes using a BioSpec Mini-Beadbeater-96 (BioSpec Products, Bartlesville, OK) and the DNA is eluted in 50 µl of Solution C6. Alternatively the genomic DNA could be isolated using the Mo Bio Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) or the QIAamp DNA Stool Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions.

qPCR Composition and Conditions

[0306] The qPCR contains 1x HotMasterMix (5PRIME, Gaithersburg, MD), 900 nM of Wr-tcdB-F (AGCAGTTGAATATAGTGGTTTAGTTAGAGTTG, IDT, Coralville, IA), 900 nM of Wr-tcdB-R (CATGCTTTTTTAGTTTCTGGATTGAA, IDT, Coralville, IA), 250 nM of We-tcdB-P (6FAM-CATCCAGTCTCAATTGTATATGTTTCTCCA-MGB, Life Technologies, Grand Island, NY), and PCR Water (Mo Bio Laboratories, Carlsbad, CA) to 18 µl (Primers adapted from: Wroblewski, D. et al., Rapid Molecular Characterization of Clostridium difficile and Assessment of Populations of C. difficile in Stool Specimens, Journal of Clinical Microbiology 47:2142–2148 (2009)). This reaction mixture is aliquoted to wells of a MicroAmp® Fast Optical 96-well Reaction Plate with Barcode (0.1mL) (Life Technologies, Grand Island, NY). To this reaction mixture, 2 µl of extracted genomic DNA is added. The qPCR is performed on a BioRad C1000™ Thermal Cycler equipped with a CFX96™ Real-Time System (BioRad, Hercules, CA). The thermocycling conditions are 95°C for 2 minutes followed by 45 cycles of 95°C for 3 seconds, 60°C for 30 seconds, and fluorescent readings of the FAM and ROX channels.

Data Analysis

[0307] The Cq value for each well on the FAM channel is determined by the CFX Manager™ software version 2.1. The $\log_{10}(\text{cfu/ml})$ of each experimental sample is calculated by inputting a given sample's Cq value into linear regression model generated from the standard curve comparing the Cq values of the standard curve wells to the known $\log_{10}(\text{cfu/ml})$ of those samples.

Example 20. Construction of an *In Vitro* Assay to Screen for Combinations of Microbes Inhibitory to the Growth of Pathogenic *E. coli*

[0308] The *in vitro* assay is used to screen for combinations of bacteria inhibitory to the growth of *E. coli* by modifying the media used for growth of the pathogen inoculum. One of several choices of media is used for growth of the pathogen such as Reinforced Clostridial Media (RCM), Brain Heart Infusion Broth (BHI) or Luria Bertani Broth (LB) (also known as Lysogeny Broth). *E. coli* is quantified by using alternative selective media specific for *E. coli* or using qPCR probes specific for the pathogen. For example, aerobic growth on MacConkey lactose medium selects for enteric Gram negatives, including *E. coli*. qPCR is conducted using probes specific for the shiga toxin of pathogenic *E. coli*.

Example 21. Construction of an *In Vitro* Assay to Screen for Combinations of Microbes Inhibitory to the Growth of Vancomycin-Resistant Enterococcus (VRE)

[0309] The *in vitro* assay is used to screen for combinations of bacteria inhibitory to the growth of Vancomycin-Resistant Enterococcus spp. (VRE) by modifying the media used for growth of the pathogen inoculum. Several choices of media are used for growth of the pathogen such as Reinforced Clostridial Media (RCM), Brain Heart Infusion Broth (BHI) or Luria Bertani Broth (LB). VRE is quantified by using alternative selective media specific for VRE or using qPCR probes specific for the pathogen. For example, m-Enterococcus agar containing sodium azide is selective

for *Enterococcus* spp. and a small number of other species. Probes specific to the van genes conferring vancomycin resistance are used in the qPCR.

Example 22: Prophylactic Use and Treatment In a Mouse Model of Vancomycin Resistant *Enterococcus* (VRE) colonization

[0310] The emergence and spread of highly antibiotic-resistant bacteria represent a major clinical challenge (Snitkin et al Science Translational Medicine, 2012). In recent years, the numbers of infections caused by organisms such as methicillin-resistant *Staphylococcus aureus*, carbapenem-resistant *Enterobacteriaceae*, vancomycin-resistant *Enterococcus* (VRE), and *Clostridium difficile* have increased markedly, and many of these strains are acquiring resistance to the few remaining active antibiotics. Most infections produced by highly antibiotic-resistant bacteria are acquired during hospitalizations, and preventing patient- to-patient transmission of these pathogens is one of the major challenges confronting hospitals and clinics. Most highly antibiotic-resistant bacterial strains belong to genera that colonize mucosal surfaces, usually at low densities. The highly complex microbiota that normally colonizes mucosal surfaces inhibits expansion of and domination by bacteria such as *Enterobacteriaceae* and *Enterococcaceae*. Destruction of the normal flora by antibiotic administration, however, disinhibition antibiotic-resistant members of these bacterial families, leading to their expansion to very high densities (Ubeda et al Journal of Clinical Investigation 2010). High-density colonization by these organisms can be calamitous for the susceptible patient, resulting in bacteremia and sepsis (Taur et al, Clinical Infectious Disease, 2012).

[0311] To test prophylactic use and treatment of a bacterial composition test article, a VRE infection mouse model is used as previously described (Ubeda et al, Infectious Immunity 2013, Ubeda et al, Journal of clinical investigation, 2010). Briefly, experiments are done with 7-week-old C57BL/6J female mice purchased

from Jackson Laboratory, housed with irradiated food, and provided with acidified water. Mice are individually housed to avoid contamination between mice due to coprophagia. For experimental infections with VRE, mice are treated with ampicillin (0.5 g/liter) in their drinking water, which is changed every 3 days.

[0312] In the treatment model, on day 1, mice are infected by means of oral gavage with 10^8 CFU of the vancomycin-resistant *Enterococcus faecium* strain purchased from ATCC (ATCC 700221). One day after infection (day 1), antibiotic treatment is stopped and VRE levels are determined at different time points by plating serial dilutions of fecal pellets on Enterococcosel agar plates (Difco) with vancomycin (8 ug/ml; Sigma). VRE colonies are identified by appearance and confirmed by Gram staining or other methods previously described (e.g. see Examples 1-3). In addition, as previously described (Ubeda et al, Journal of Clinical Investigation 2010), PCR of the vanA gene, which confers resistance to vancomycin, confirms the presence of VRE in infected mice. The bacterial composition test article such as but not limited to an ethanol treated, gradient purified spore preparation (as described herein), fecal suspension, or antibiotic treatment is delivered in PBS on days 1-3 while the negative control contains only PBS and is also delivered on days 1-3 by oral gavage. Fresh fecal stool pellets are obtained daily for the duration of the experiment from days -7 to day 10. The samples are immediately frozen and stored at -80°C. DNA was extracted using standard techniques and analyzed with 16S or comparable methods (e.g. see Examples 2 and 3).

[0313] In the colonization model, ampicillin is administered as described above for day -7 to day 1, treatment with the test article or vehicle control is administered on day 0-2 and the VRE resistant bacteria at 10^8 CFU are administered on day 14.

Fecal samples are taken throughout the experiment daily from -7 to day 21 and submitted for 16S sequencing as previously described (e.g. see Examples 2 and 3).

[0314] In both models titers of VRE in feces are used to evaluate the success of the test article versus the negative control. Furthermore, microbiota composition is assessed for the ability of the bacterial composition test article to induce a healthy microbiome.

Example 23: Prophylactic Use and Treatment of a Mouse model of Carbapenem resistant klebsiella (CRKB) Colonization

[0315] The emergence of *Klebsiella pneumoniae* strains with decreased susceptibility to carbapenems is a significant threat to hospitalized patients. Resistance to carbapenems in these organisms is most frequently mediated by *K. pneumoniae* carbapenemase (KPC), a class A beta-lactamase that also confers resistance to broad-spectrum cephalosporins and commercially available beta-lactam/beta-lactamase inhibitor combinations (Queenan et al, Clinical Microbiology Review, 2007). KPC-producing *K. pneumoniae* (KPC-Kp) strains often harbor resistance determinants against several other classes of antimicrobials, including aminoglycosides and fluoroquinolones, resulting in truly multidrug-resistant (MDR) organisms (Hirsch et al, Journal of Antimicrobial Chemotherapy, 2009). Considering the limited antimicrobial options, infections caused by KPC-Kp pose a tremendous therapeutic challenge and are associated with poor clinical outcomes

[0316] A treatment protocol in a mouse model as previously described (e.g. Perez et al, Antimicrobial Agents Chemotherapy, 2011) is used to evaluate the bacterial composition (test article) for treating carbapenem resistant *Klebsiella* and reducing carriage in the GI tract. Female CF1 mice (Harlan Sprague-Dawley, Indianapolis, IN) are used and are individually housed and weighed between 25 and 30 g.

[0317] The thoroughly characterized strain of *K. pneumoniae*, VA-367 (8, 9, 25) is used in this study. This clinical isolate is genetically related to the KPC-Kp strain circulating in the Eastern United States. Characterization of the resistance mechanisms in *K. pneumoniae* VA-367 with PCR and DNA sequence analysis revealed the presence of *bla*_{KPC-3}, *bla*_{TEM-1}, *bla*_{SHV-11}, and *bla*_{SHV-12} as well as *qnrB19* and *aac(6')-Ib*. Additionally, PCR and DNA sequencing revealed disruptions in the coding sequences of the following outer membrane protein genes: *ompK35*, *ompK36*, and *ompK37*. Antibiotic susceptibility testing (AST) was performed with the agar dilution method and interpreted according to current recommendations from the Clinical and Laboratory Standards Institute (CLSI). A modified Hodge test were performed, according to a method described previously (e.g. see Anderson et al, Journal of Clinical Microbiology, 2007) with ertapenem, meropenem, and imipenem. Tigecycline and polymyxin E were evaluated by Etest susceptibility assays (AB bioMérieux, Solna, Sweden). Results for tigecycline were interpreted as suggested by the U.S. Food and Drug Administration (FDA) and according to CLSI recommendations (criteria for *Pseudomonas*) for polymyxin E.

[0318] Mice (10 per group) are assigned to either a bacterial composition (test article), ethanol treated, spore preparation (e.g. see Example 6), antibiotic clindamycin, piperacillin-tazobactam, tigecycline, ertapenem, cefepime, ciprofloxacin, or combination thereof or control group receiving only the vehicle. They are administered the test article daily from day -10 to day 0, On day 0, 10³ CFU of KPC-Kp VA-367 diluted in 0.5 ml phosphate-buffered saline (PBS) was administered by oral gavage using a stainless-steel feeding tube (Perfektum; Popper & Sons, New Hyde Park, NY). Stool samples were collected 1, 4, 6, and 11 days after the administration of KPC-Kp in order to measure the concentration of carbapenem-

resistant *K. pneumoniae*. Stool samples (100 mg diluted in 800 ml of PBS) are plated onto MacConkey agar with and without 0.5 ug/ml of imipenem, and the number of CFU per gram of stool was determined. Alternatively other methods may be used to measure the levels of carbapenem-resistant *K. pneumoniae* e.g. pcr, antigen testing, as one who's skilled in the art could perform.

[0319] Stool samples were collected after 5 days of treatment to assess the effects of the antibiotics on the stool microflora and to measure antibiotic levels in stool. To assess the effects on the microflora, fresh stool samples as previously described (e.g. see Examples 2 and 3). Additional experiments are performed to examine whether the administration the bacterial composition (test article) resulted in the elimination or persistence of colonization with KPC-Kp VA-367.

[0320] Mice are treated with subcutaneous clindamycin to reduce the normal intestinal flora 1 day before receiving 10^4 CFU of KPC-Kp VA-367 by oral gavage, and the mice continued to receive subcutaneous clindamycin every other day for 7 days. Concurrently, for 7 days after oral gavage with KPC-Kp, mice received oral gavage of normal saline (control group), or the bacterial composition as specified. An additional dose of subcutaneous clindamycin was administered 20 days after the administration of KPC-Kp VA-367 to assess whether low levels of carbapenem-resistant *K. pneumoniae* were present that could be augmented by the elimination of the anaerobic microflora. Stool samples were collected at baseline and at 3, 6, 8, 11, 16, and 21 days after KPC-Kp VA-367 was given by gavage. The bacterial composition will be examined by the reduction of CRKB in feces.

Example 24: Identification of Keystone OTUs and Functions

[0321] The human body is an ecosystem in which the microbiota, and the microbiome, play a significant role in the basic healthy function of human systems

(e.g. metabolic, immunological, and neurological). The microbiota and resulting microbiome comprise an ecology of microorganisms that co-exist within single subjects interacting with one another and their host (i.e., the mammalian subject) to form a dynamic unit with inherent biodiversity and functional characteristics. Within these networks of interacting microbes (i.e. ecologies), particular members can contribute more significantly than others; as such these members are also found in many different ecologies, and the loss of these microbes from the ecology can have a significant impact on the functional capabilities of the specific ecology. Robert Paine coined the concept “Keystone Species” in 1969 (see Paine RT. 1969. A note on trophic complexity and community stability. *The American Naturalist* 103: 91–93.) to describe the existence of such lynchpin species that are integral to a given ecosystem regardless of their abundance in the ecological community. Paine originally describe the role of the starfish *Pisaster ochraceus* in marine systems and since the concept has been experimentally validated in numerous ecosystems.

[0322] Keystone OTUs and/or Functions are computationally-derived by analysis of network ecologies elucidated from a defined set of samples that share a specific phenotype. Keystone OTUs and/or Functions are defined as all Nodes within a defined set of networks that meet two or more of the following criteria. Using Criterion 1, the node is frequently observed in networks, and the networks in which the node is observed are found in a large number of individual subjects; the frequency of occurrence of these Nodes in networks and the pervasiveness of the networks in individuals indicates these Nodes perform an important biological function in many individuals. Using Criterion 2, the node is frequently observed in networks, and each the networks in which the node is observed contain a large number of Nodes -- these Nodes are thus “super-connectors”, meaning that they

form a nucleus of a majority of networks and as such have high biological significance with respect to their functional contributions to a given ecology. Using Criterion 3, the node is found in networks containing a large number of Nodes (i.e. they are large networks), and the networks in which the node is found occur in a large number of subjects; these networks are potentially of high interest as it is unlikely that large networks occurring in many individuals would occur by chance alone strongly suggesting biological relevance. Optionally, the required thresholds for the frequency at which a node is observed in network ecologies, the frequency at which a given network is observed across subject samples, and the size of a given network to be considered a Keystone node are defined by the 50th, 70th, 80th, or 90th percentiles of the distribution of these variables. Optionally, the required thresholds are defined by the value for a given variable that is significantly different from the mean or median value for a given variable using standard parametric or non-parametric measures of statistical significance. In another embodiment a Keystone node is defined as one that occurs in a sample phenotype of interest such as but not limited to “health” and simultaneously does not occur in a sample phenotype that is not of interest such as but not limited to “disease.” Optionally, a Keystone Node is defined as one that is shown to be significantly different from what is observed using permuted test datasets to measure significance.

Example 25: Microbial Population (Engraftment and Augmentation) and Reduction of Pathogen Carriage in Patients Treated with Spore Compositions

[0323] Complementary genomic and microbiological methods were used to characterize the composition of the microbiota from Patient 1, 2, 3, 4, and 5, 6, 7, 8, 9, and 10 at pretreatment (pretreatment) and on up to 4 weeks post-treatment.

[0324] To determine the OTUs that engraft from treatment with an ethanol treated spore preparation in the patients and how their microbiome changed in response, the microbiome was characterized by 16S-V4 sequencing prior to treatment (pretreatment) with an ethanol treated spore preparation and up to 25 days after receiving treatment. Alternatively, one might use a bacterial composition in the vegetative state, or a mixture of vegetative bacteria and bacterial spores. As example, the treatment of patient 1 with an ethanol treated spore preparation led to microbial population via the engraftment of OTUs from the spore treatment and augmentation in the microbiome of the patient (Figure 12 and Figure 13). By day 25 following treatment, the total microbial carriage was dominated by species of the following taxonomic groups: Bacteroides, Sutterella, Ruminococcus, Blautia, Eubacterium, Gemmiger/Faecalibacterium, and the non-sporeforming Lactobacillus (see Tables 2 and 12 for specific OTUs). The first two genera represent OTUs that do not form spores while the latter taxonomic groups represent OTUs that are believed to form spores.

[0325] Table 12 shows bacterial OTUs associated with engraftment and ecological augmentation and establishment of a more diverse microbial ecology in patients treated with an ethanol treated spore preparation. OTUs that comprise an augmented ecology are not present in the patient prior to treatment and/or exist at extremely low frequencies such that they do not comprise a significant fraction of the total microbial carriage and are not detectable by genomic and/or microbiological assay methods. OTUs that are members of the engrafting and augmented ecologies were identified by characterizing the OTUs that increase in their relative abundance post treatment and that respectively are: (i) present in the ethanol treated spore preparation and absent in the patient pretreatment (engrafting OTUs), or (ii) absent

in the ethanol treated spore preparation, but increase in their relative abundance through time post treatment with the preparation due to the formation of favorable growth conditions by the treatment (augmenting OTUs). Notably, the latter OTUs can grow from low frequency reservoirs in the patient, or be introduced from exogenous sources such as diet. OTUs that comprise a “core” augmented or engrafted ecology can be defined by the percentage of total patients in which they are observed to engraft and/or augment; the greater this percentage the more likely they are to be part of a core ecology responsible for catalyzing a shift away from a dysbiotic ecology. The dominant OTUs in an ecology can be identified using several methods including but not limited to defining the OTUs that have the greatest relative abundance in either the augmented or engrafted ecologies and defining a total relative abundance threshold. As example, the dominant OTUs in the augmented ecology of Patient-1 were identified by defining the OTUs with the greatest relative abundance, which together comprise 60% of the microbial carriage in this patient’s augmented ecology.

[0326] Patient treatment with the ethanol treated spore preparation leads to the population of a microbial ecology that has greater diversity than prior to treatment (Figure 12). Genomic-based microbiome characterization confirmed engraftment of a range of OTUs that were absent in the patient pretreatment (Table 12). These OTUs comprised both bacterial species that were capable and not capable of forming spores, and OTUs that represent multiple phylogenetic clades. Organisms absent in Patient 1 pre-treatment either engraft directly from the ethanol treated spore fraction or are augmented by the creation of a gut environment favoring a healthy, diverse microbiota. Furthermore, *Bacteroides fragilis* group species were increased by 4 and 6 logs in patients 1 and 2 (Figure 14).

[0327] Figure 12 shows the microbial diversity measured in the ethanol treated spore treatment sample and patient pre- and post-treatment samples. Total microbial diversity is defined using the Chao1 Alpha-Diversity Index and is measured at different genomic sampling depths to confirm adequate sequence coverage to assay the microbiome in the target samples. The patient pretreatment (purple) harbored a microbiome that was significantly reduced in total diversity as compared to the ethanol treated spore treatment (red) and patient post treatment at days 5 (blue), 14 (orange), and 25 (green).

[0328] Figure 13 shows patient microbial ecology is shifted by treatment with an ethanol treated spore treatment from a dysbiotic state to a state of health. Principle Coordinates Analysis based on the total diversity and structure of the microbiome (Bray-Curtis Beta-Diversity) of the patient pre- and post-treatment delineates that the engraftment of OTUs from the spore treatment and the augmentation of the patient microbial ecology leads to a microbial ecology that is distinct from both the pretreatment microbiome and the ecology of the ethanol treated spore treatment (Table 12).

[0329] Figure 14 shows the augmentation of *Bacteroides* species in patients. Comparing the number of *Bacteroides fragilis* groups species per cfu/g of feces pre-treatment and in week 4 post treatment reveals an increase of 4 logs or greater. The ability of 16S-V4 OTU identification to assign an OTU as a specific species depends in part on the resolution of the 16S-V4 region of the 16S gene for a particular species or group of species. Both the density of available reference 16S sequences for different regions of the tree as well as the inherent variability in the 16S gene between different species will determine the definitiveness of a taxonomic annotation to a given sequence read. Given the topological nature of a phylogenetic tree and

that the tree represents hierarchical relationships of OTUs to one another based on their sequence similarity and an underlying evolutionary model, taxonomic annotations of a read can be rolled up to a higher level using a clade-based assignment procedure (Table 1). Using this approach, clades are defined based on the topology of a phylogenetic tree that is constructed from full-length 16S sequences using maximum likelihood or other phylogenetic models familiar to individuals with ordinary skill in the art of phylogenetics. Clades are constructed to ensure that all OTUs in a given clade are: (i) within a specified number of bootstrap supported nodes from one another (generally, 1-5 bootstraps), and (ii) within a 5% genetic similarity. OTUs that are within the same clade can be distinguished as genetically and phylogenetically distinct from OTUs in a different clade based on 16S-V4 sequence data. OTUs falling within the same clade are evolutionarily closely related and may or may not be distinguishable from one another using 16S-V4 sequence data. The power of clade based analysis is that members of the same clade, due to their evolutionary relatedness, play similar functional roles in a microbial ecology such as that found in the human gut. Compositions substituting one species with another from the same clade are likely to have conserved ecological function and therefore are useful in the present invention.

[0330] Stool samples were aliquoted and resuspended 10x vol/wt in either 100% ethanol (for genomic characterization) or PBS containing 15% glycerol (for isolation of microbes) and then stored at -80°C until needed for use. For genomic 16S sequence analysis colonies picked from plate isolates had their full-length 16S sequence characterized as described in Examples 2 and 3, and primary stool samples were prepared targeting the 16S-V4 region using the method for heterogeneous samples described herein.

[0331] Notably, 16S sequences of isolates of a given OTU are phylogenetically placed within their respective clades despite that the actual taxonomic assignment of species and genus may suggest they are taxonomically distinct from other members of the clades in which they fall. Discrepancies between taxonomic names given to an OTU is based on microbiological characteristics versus genetic sequencing are known to exist from the literature. The OTUs footnoted in this table are known to be discrepant between the different methods for assigning a taxonomic name.

[0332] Engraftment of OTUs from the ethanol treated spore preparation treatment into the patient as well as the resulting augmentation of the resident microbiome led to a significant decrease in and elimination of the carriage of pathogenic species other than *C. difficile* in the patient. 16S-V4 sequencing of primary stool samples demonstrated that at pretreatment, 20% of reads were from the genus *Klebsiella* and an additional 19% were assigned to the genus *Fusobacterium*. These striking data are evidence of a profoundly dysbiotic microbiota associated with recurrent *C. difficile* infection and chronic antibiotic use. In healthy individuals, *Klebsiella* is a resident of the human microbiome in only about 2% of subjects based on an analysis of HMP database (www.hmpdacc.org), and the mean relative abundance of *Klebsiella* is only about 0.09% in the stool of these people. Its surprising presence at 20% relative abundance in Patient 1 before treatment is an indicator of a proinflammatory gut environment enabling a “pathobiont” to overgrow and outcompete the commensal organisms normally found in the gut. Similarly, the dramatic overgrowth of *Fusobacterium* indicates a profoundly dysbiotic gut microbiota. One species of *Fusobacterium*, *F. nucleatum* (an OTU phylogenetically indistinguishable from *Fusobacterium* sp. 3_1_33 based on 16S-V4), has been termed “an emerging gut pathogen” based on its association with IBD, Crohn’s

disease, and colorectal cancer in humans and its demonstrated causative role in the development of colorectal cancer in animal models [Allen-Vercoe, Gut Microbes (2011) 2:294-8]. Importantly, neither *Klebsiella* nor *Fusobacterium* was detected in the 16S-V4 reads by Day 25 (Table 13).

[0333] To further characterize the colonization of the gut by *Klebsiella* and other Enterobacteriaceae and to speciate these organisms, pretreatment and Day 25 fecal samples stored at -80C as PBS-glycerol suspensions were plated on a variety of selective media including MacConkey lactose media (selective for gram negative enterobacteria) and Simmons Citrate Inositol media (selective for *Klebsiella* spp) [Van Cregten et al, J. Clin. Microbiol. (1984) 20: 936-41]. Enterobacteria identified in the patient samples included *K. pneumoniae*, *Klebsiella* sp. Co_9935 and *E. coli*. Strikingly, each *Klebsiella* species was reduced by 2-4 logs whereas *E. coli*, a normal commensal organism present in a healthy microbiota, was reduced by less than 1 log (Table 14 below). This decrease in *Klebsiella* spp. carriage is consistent across multiple patients. Four separate patients were evaluated for the presence of *Klebsiella* spp. pre treatment and 4 weeks post treatment. *Klebsiella* spp. were detected by growth on selective Simmons Citrate Inositol media as previously described. Serial dilution and plating, followed by determining cfu/mL titers of morphologically distinct species and 16S full length sequence identification of representatives of those distinct morphological classes, allowed calculation of titers of specific species.

[0334] The genus *Bacteroides* is an important member of the gastrointestinal microbiota; 100% of stool samples from the Human Microbiome Project contain at least one species of *Bacteroides* with total relative abundance in these samples ranging from 0.96% to 93.92% with a median relative abundance of 52.67%

(www.hmpdacc.org reference data set HMSMCP). *Bacteroides* in the gut has been associated with amino acid fermentation and degradation of complex polysaccharides. Its presence in the gut is enhanced by diets rich in animal-derived products as found in the typical western diet [David, L. A. et al, *Nature* (2013) doi:10.1038/nature12820]. Strikingly, prior to treatment, fewer than 0.008% of the 16S-V4 reads from Patient 1 mapped to the genus *Bacteroides* strongly suggesting that *Bacteroides* species were absent or that viable *Bacteroides* were reduced to an extremely minor component of the patient's gut microbiome. Post treatment, $\geq 42\%$ of the 16S-V4 reads could be assigned to the genus *Bacteroides* within 5 days of treatment and by Day 25 post treatment 59.48% of the patients gut microbiome was comprised of *Bacteroides*. These results were confirmed microbiologically by the absence of detectable *Bacteroides* in the pretreatment sample plated on two different *Bacteroides* selective media: *Bacteroides* Bile Esculin (BBE) agar which is selective for *Bacteroides fragilis* group species [Livingston, S.J. et al *J. Clin. Microbiol* (1978). 7: 448-453] and Polyamine Free Arabinose (PFA) agar [Noack et al. *J. Nutr.* (1998) 128: 1385-1391; modified by replacing glucose with arabinose]. The highly selective BBE agar had a limit of detection of $< 2 \times 10^3$ cfu/g, while the limit of detection for *Bacteroides* on PFA agar was approximately 2×10^7 cfu/g due to the growth of multiple non-*Bacteroides* species in the pretreatment sample on that medium. Colony counts of *Bacteroides* species on Day 25 were up to 2×10^{10} cfu/g, consistent with the 16S-V4 sequencing, demonstrating a profound reconstitution of the gut microbiota in Patient 1 (Table 15 below).

[0335] The significant abundance of *Bacteroides* in Patient 1 on Day 25 (and as early as Day 5 as shown by 16S-V4 sequencing) is remarkable. Viable *Bacteroides fragilis* group species were not present in the ethanol treated spore population based

on microbiological plating (limit of detection of 10 cfu/ml). Thus, administration of the ethanol treated spore population to Patient 1 resulted in microbial population of the patient's GI tract, not only due to the engraftment of bacterial species such as but not limited to spore forming species, but also the restoration of high levels of non-spore forming species commonly found in healthy individuals through the creation of a niche that allowed for the repopulation of *Bacteroides* species. These organisms were most likely either present at extremely low abundance in the GI tract of Patient 1, or present in a reservoir in the GI tract from which they could rebound to high titer. Those species may also be reinoculated via oral uptake from food following treatment. We term this healthy repopulation of the gut with OTUs that are not present in the bacterial composition such as but not limited to a spore population or ethanol treated spore population, "Augmentation." Augmentation is an important phenomenon in that it shows the ability to use an ethanol treated spore ecology or other bacterial composition to restore a healthy microbiota by seeding a diverse array of commensal organisms beyond the actual component organisms in the bacterial composition such as but not limited to an ethanol treated spore population itself; specifically the spore composition treatment itself and the engraftment of OTUs from the spore composition create a niche that enables the outgrowth of OTUs required to shift a dysbiotic microbiome to a microbial ecology that is associated with health. The diversity of *Bacteroides* species and their approximate relative abundance in the gut of Patient 1 is shown in Table 16, comprising at least 8 different species.

[0336] Figure 15 shows species engrafting versus species augmenting in patients microbiomes after treatment with a bacterial composition such as but not limited to an ethanol-treated spore population. Relative abundance of species that engrafted

or augmented as described were determined based on the number of 16S sequence reads. Each plot is from a different patient treated with the bacterial composition such as but not limited to an ethanol-treated spore population for recurrent *C. difficile*.

[0337] The impact of the bacterial composition such as but not limited to an ethanol treated spore population treatment on carriage of imipenem resistant Enterobacteriaceae was assessed by plating pretreatment and Day 28 clinical samples from Patients 2, 4 and 5 on MacConkey lactose plus 1 ug/mL of imipenem. Resistant organisms were scored by morphology, enumerated and DNA was submitted for full length 16S rDNA sequencing as described above. Isolates were identified as *Morganella morganii*, *Providencia rettgeri* and *Proteus pennerii*. Each of these are gut commensal organisms; overgrowth can lead to bacteremia and/or urinary tract infections requiring aggressive antibiotic treatment and, in some cases, hospitalization [Kim, B-N, et al *Scan J. Inf Dis* (2003) **35**: 98-103; Lee, I-K and Liu, J-W *J. Microbiol Immunol Infect* (2006) 39: 328-334; O'Hara et al, *Clin Microbiol Rev* (2000) **13**: 534]. The titer of organisms at pretreatment and Day 28 by patient is shown in Table 17. Importantly, administration of the bacterial composition such as but not limited to an ethanol treated spore preparation resulted in greater than 100-fold reduction in 4 of 5 cases of Enterobacteriaceae carriage with multiple imipenem resistant organisms (See Table 17 which shows titers (in cfu/g) of imipenem-resistant *M. morganii*, *P. rettgeri* and *P. pennerii* from Patients 2, 4 & 5).

[0338] In addition to speciation and enumeration, multiple isolates of each organism from Patient 4 were grown overnight in 96-well trays containing a 2-fold dilution series of imipenem in order to quantitatively determine the minimum inhibitory concentration (MIC) of antibiotic. Growth of organisms was detected by

light scattering at 600 nm on a SpectraMax M5e plate reader. In the clinical setting, these species are considered resistant to imipenem if they have an MIC of 1 ug/mL or greater. *M. morganii* isolates from pretreatment samples from Patient D had MICs of 2-4 ug/mL and *P. pennerii* isolates had MICs of 4-8 ug/mL. Thus, the bacterial composition, such as but not limited to, an ethanol treated spores administered to Patient 4 caused the clearance of 2 imipenem resistant organisms (Table 12). While this example specifically uses a spore preparation, the methods herein describe how one skilled in the art would use a more general bacterial composition to achieve the same effects. The specific example should not be viewed as a limitation of the scope of this disclosure.

Example 26: Identifying the Core Ecology From the Bacterial Combination

[0339] Ten different bacterial compositions were made by the ethanol treated spore preparation methods from 6 different donors (as described in Example 6). The spore preparations were used to treat 10 patients, each suffering from recurrent *C. difficile* infection. Patients were identified using the inclusion/exclusion criteria described in Example 9, and donors were identified using the criteria described in Example 10. None of the patients experienced a relapse of *C. difficile* in the 4 weeks of follow up after treatment, whereas the literature would predict that 70-80% of subjects would experience a relapse following cessation of antibiotic [Van Nood, et al, *NEJM* (2013)]. Thus, the ethanol treated spore preparations derived from multiple different donors and donations showed remarkable clinical efficacy. These ethanol treated spore preparations are a subset of the bacterial compositions described herein and the results should not be viewed as a limitation on the scope of the broader set of bacterial compositions.

[0340] To define the Core Ecology underlying the remarkable clinical efficacy of the bacterial compositions e.g. ethanol treated spore preparations, the following analysis was carried out. The OTU composition of the spore preparation was determined by 16S-V4 rDNA sequencing and computational assignment of OTUs per Example 2. A requirement to detect at least ten sequence reads in the ethanol treated spore preparation was set as a conservative threshold to define only OTUs that were highly unlikely to arise from errors during amplification or sequencing. Methods routinely employed by those familiar to the art of genomic-based microbiome characterization use a read relative abundance threshold of 0.005% (see e.g. Bokulich, A. et al. 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature Methods* 10: 57-59), which would equate to ≥ 2 reads given the sequencing depth obtained for the samples analyzed in this example, as cut-off which is substantially lower than the ≥ 10 reads used in this analysis. All taxonomic and clade assignments were made for each OTU as described in Example 3. The resulting list of OTUs, clade assignments, and frequency of detection in the spore preparations are shown in Table 18. Table 18 shows OTUs detected by a minimum of ten 16S-V4 sequence reads in at least a one ethanol treated spore preparation (pan-microbiome). OTUs that engraft in a treated patients and the percentage of patients in which they engraft are denoted, as are the clades, spore forming status, and Keystone OTU status. Starred OTUs occur in $\geq 80\%$ of the ethanol preps and engraft in $\geq 50\%$ of the treated patients.

[0341] Next, it was reasoned that for an OTU to be considered a member of the Core Ecology of the bacterial composition, that OTU must be shown to engraft in a patient. Engraftment is important for two reasons. First, engraftment is a sine qua non of the mechanism to reshape the microbiome and eliminate *C. difficile*

colonization. OTUs that engraft with higher frequency are highly likely to be a component of the Core Ecology of the spore preparation or broadly speaking a set bacterial composition. Second, OTUs detected by sequencing a bacterial composition (as in Table 18 may include non-viable cells or other contaminant DNA molecules not associated with the composition. The requirement that an OTU must be shown to engraft in the patient eliminates OTUs that represent non-viable cells or contaminating sequences. Table 18 also identifies all OTUs detected in the bacterial composition that also were shown to engraft in at least one patient post-treatment. OTUs that are present in a large percentage of the bacterial composition e.g. ethanol spore preparations analyzed and that engraft in a large number of patients represent a subset of the Core Ecology that are highly likely to catalyze the shift from a dysbiotic disease ecology to a healthy microbiome.

[0342] A third lens was applied to further refine insights into the Core Ecology of the bacterial composition e.g. spore preparation. Computational-based, network analysis has enabled the description of microbial ecologies that are present in the microbiota of a broad population of healthy individuals. These network ecologies are comprised of multiple OTUs, some of which are defined as Keystone OTUs.

Keystone OTUs are computationally defined as described in Example 24. Keystone OTUs form a foundation to the microbially ecologies in that they are found and as such are central to the function of network ecologies in healthy subjects. Keystone OTUs associated with microbial ecologies associated with healthy subjects are often missing or exist at reduced levels in subjects with disease. Keystone OTUs may exist in low, moderate, or high abundance in subjects. Table 18 further notes which of the OTUs in the bacterial composition e.g. spore preparation are Keystone OTUs exclusively associated with individuals that are healthy and do not harbor disease.

[0343] There are several important findings from this data. A relatively small number of species, 16 in total, are detected in all of the spore preparations from 6 donors and 10 donations. This is surprising because the HMP database (www.hmpdacc.org) describes the enormous variability of commensal species across healthy individuals. The presence of a small number of consistent OTUs lends support to the concept of a Core Ecology. The engraftment data further supports this conclusion. A regression analysis shows a significant correlation between frequency of detection in a spore preparation and frequency of engraftment in a donor: $R = 0.43$ ($p < 0.001$). While this may seem obvious, there is no a priori requirement that an OTU detected frequently in the bacterial composition e.g. spore preparation will or should engraft. For instance, *Lutispora thermophila*, a spore former found in all ten spore preparations, did not engraft in any of the patients. *Bilophila wadsworthia*, a gram negative anaerobe, is present in 9 of 10 donations, yet it does not engraft in any patient, indicating that it is likely a non-viable contaminant in the ethanol treated spore preparation. Finally, it is worth noting the high preponderance of previously defined Keystone OTUs among the most frequent OTUs in the spore preparations.

[0344] These three factors--prevalence in the bacterial composition such as but not limited to a spore preparation, frequency of engraftment, and designation as a Keystone OTUs--enabled the creation of a "Core Ecology Score" (CES) to rank individual OTUs. CES was defined as follows:

- [0345] ● 40% weighting for presence of OTU in spore preparation
- [0346] ○ multiplier of 1 for presence in 1-3 spore preparations
- [0347] ○ multiplier of 2.5 for presence in 4-8 spore preparations
- [0348] ○ multiplier of 5 for presences in ≥ 9 spore preparations

- [0349] • 40% weighting for engraftment in a patient
 - [0350] ○ multiplier of 1 for engraftment in 1-4 patients
 - [0351] ○ multiplier of 2.5 for engraftment in 5-6 patients
 - [0352] ○ multiplier of 5 for engraftment in ≥ 7 patients
 - [0353] • 20% weighting to Keystone OTUs
 - [0354] ○ multiplier of 1 for a Keystone OTU
 - [0355] ○ multiplier of 0 for a non-Keystone OTU
- [0356] Using this guide, the CES has a maximum possible score of 5 and a minimum possible score of 0.8. As an example, an OTU found in 8 of the 10 bacterial composition such as but not limited to a spore preparations that engrafted in 3 patients and was a Keystone OTU would be assigned the follow CES:
- [0357] $CES = (0.4 \times 2.5) + (0.4 \times 1) + (0.2 \times 1) = 1.6$
- [0358] Table 19 ranks the top 20 OTUs by CES with the further requirement that an OTU must be shown to engraft to be a considered an element of a core ecology.

Example 27: Defining Efficacious Subsets of the Core Ecology

[0359] The number of organisms in the human gastrointestinal tract, as well as the diversity between healthy individuals, is indicative of the functional redundancy of a healthy gut microbiome ecology (see The Human Microbiome Consortia. 2012. Structure, function and diversity of the healthy human microbiome. Nature 486: 207-214). This redundancy makes it highly likely that subsets of the Core Ecology describe therapeutically beneficial components of the bacterial composition such as but not limited to an ethanol treated spore preparation and that such subsets may themselves be useful compositions for populating the GI tract and for the treatment of *C. difficile* infection given the ecologies functional characteristics. Using the CES,

individual OTUs can be prioritized for evaluation as an efficacious subset of the Core Ecology.

[0360] Another aspect of functional redundancy is that evolutionarily related organisms (i.e. those close to one another on the phylogenetic tree, e.g. those grouped into a single clade) will also be effective substitutes in the Core Ecology or a subset thereof for treating *C. difficile*.

[0361] To one skilled in the art, the selection of appropriate OTU subsets for testing in vitro (e.g. see Example 28 below) or in vivo (e.g. see Examples 7 or 8) is straightforward. Subsets may be selected by picking any 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 OTUs from Table 18, with a particular emphasis on those with higher CES, such as the OTUs described Table 19. In addition, using the clade relationships defined in Example 2 and Table 1 above, related OTUs can be selected as substitutes for OTUs with acceptable CES values. These organisms can be cultured anaerobically in vitro using the appropriate media (selected from those described in Example 5 above), and then combined in a desired ratio. A typical experiment in the mouse *C. difficile* model utilizes at least 10^4 and preferably at least 10^5 , 10^6 , 10^7 , 10^8 , 10^9 or more than 10^9 colony forming units of a each microbe in the composition. Variations in the culture yields may sometimes mean that organisms are combined in unequal ratios, e.g. 1:10, 1:100, 1:1,000, 1:10,000, 1:100,000, or greater than 1:100,000. What is important in these compositions is that each strain be provided in a minimum amount so that the strain's contribution to the efficacy of the Core Ecology subset can be measured. Using the principles and instructions described here, it is straightforward for one of skill in the art to make clade-based substitutions to test the efficacy of subsets of the Core Ecology. Table 18 describes

the clades for each OTU detected in a spore preparation and Table 1 describes the OTUs that can be used for substitutions based on clade relationships.

Example 28: Testing Subsets of the Core Ecology in the Mouse Model

[0362] Several subsets of the Core Ecology were tested in the *C. difficile* mouse model. The negative control was phosphate buffered saline and the positive control was a 10% human fecal suspension. The subsets are described in Table 20 (Subsets of the Core Ecology tested in the *C. difficile* mouse model).

[0363] Two cages of five mice each were tested for each arm of the experiment. All mice received an antibiotic cocktail consisting of 10% glucose, kanamycin (0.5 mg/ml), gentamicin (0.044 mg/ml), colistin (1062.5 U/ml), metronidazole (0.269 mg/ml), ciprofloxacin (0.156 mg/ml), ampicillin (0.1 mg/ml) and Vancomycin (0.056 mg/ml) in their drinking water on days -14 through -5 and a dose of 10mg/kg Clindamycin by oral gavage on day -3. On day -1, they received either the test articles or control articles via oral gavage. On day 0, they were challenged by administration of approximately 4.5 log₁₀ cfu of *C. difficile* (ATCC 43255) via oral gavage. Mortality was assessed every day from day 0 to day 6 and the weight and subsequent weight change of the animal was assessed with weight loss being associated with *C. difficile* infection. Mortality and reduced weight loss of the test article compared to the empty vehicle was used to assess the success of the test article. Additionally, a *C. difficile* symptom scoring was performed each day from day -1 through day 6. Symptom scoring was based on Appearance (0-2 pts based on normal, hunched, piloerection, or lethargic), Respiration (0-2 pts based on normal, rapid or shallow, with abdominal breathing), Clinical Signs (0-2 points based on normal, wet tail, cold-to-the-touch, or isolation from other animals).

[0364] In addition to compiling the cumulative mortality for each arm, the average minimum relative weight is calculated as the mean of each mouse's minimum weight relative to Day -1 and the average maximum clinical score is calculated as the mean of each mouse's maximum combined clinical score with a score of 4 assigned in the case of death. The results are reported in Table 21 below (Results of bacterial compositions tested in a *C. difficile* mouse model).

Example 29. Defining Subsets of the Core Ecology in the *in vitro* *C. difficile* Inhibition Assay

[0365] Vials of -80 °C glycerol stock banks were thawed and diluted to 1e8 CFU/mL. Selected strains and their clade assignment are given in Table 22. Each strain was then diluted 10x (to a final concentration of 1e7 CFU/mL of each strain) into 200 uL of PBS + 15% glycerol in the wells of a 96-well plate. Plates were then frozen at -80 °C. When needed for the assay, plates were removed from -80 °C and thawed at room temperature under anaerobic conditions when testing in a *in vitro* *C. difficile* inhibition assay (CivSim).

[0366] An overnight culture of *Clostridium difficile* is grown under anaerobic conditions in SweetB-FosIn or other suitable media for the growth of *C. difficile*. SweetB-FosIn is a complex media composed of brain heart infusion, yeast extract, cysteine, cellobiose, maltose, soluble starch, and fructooligosaccharides/inulin, and hemin, and is buffered with MOPs. After 24 hr of growth the culture is diluted 100,000 fold into a complex media such as SweetB-FosIn which is suitable for the growth of a wide variety of anaerobic bacterial species. The diluted *C. difficile* mixture is then aliquoted to wells of a 96-well plate (180 uL to each well). 20 uL of a subset Core Ecology is then added to each well at a final concentration of 1e6 CFU/mL of each species. Alternatively the assay can be tested each species at different initial concentrations (1e9 CFU/mL, 1e8 CFU/mL, 1e7 CFU/mL, 1e5

CFU/mL, 1e4 CFU/mL, 1e3 CFU/mL, 1e2 CFU/mL). Control wells only inoculated with *C. difficile* are included for a comparison to the growth of *C. difficile* without inhibition. Additional wells are used for controls that either inhibit or do not inhibit the growth of *C. difficile*. One example of a positive control that inhibits growth is a combination of *Blautia producta*, *Clostridium bifermentans* and *Escherichia coli*. One example of a control that shows reduced inhibition of *C. difficile* growth is a combination of *Bacteroides thetaiotaomicron*, *Bacteroides ovatus* and *Bacteroides vulgatus*. Plates are wrapped with parafilm and incubated for 24 hr at 37 °C under anaerobic conditions. After 24 hr the wells containing *C. difficile* alone are serially diluted and plated to determine titer. The 96-well plate is then frozen at -80C before quantifying *C. difficile* by qPCR assay.

[0367] A standard curve is generated from a well on each assay plate containing only pathogenic *C. difficile* grown in SweetB + FosIn media and quantified by selective spot plating. Serial dilutions of the culture are performed in sterile phosphate-buffered saline. Genomic DNA is extracted from the standard curve samples along with the other wells.

[0368] Genomic DNA is extracted from 5 µl of each sample using a dilution, freeze/thaw, and heat lysis protocol. 5 µL of thawed samples is added to 45 µL of UltraPure water (Life Technologies, Carlsbad, CA) and mixed by pipetting. The plates with diluted samples are frozen at -20 °C until use for qPCR which includes a heated lysis step prior to amplification. Alternatively the genomic DNA is isolated using the Mo Bio Powersoil®-htp 96 Well Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA), Mo Bio Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA), or the QIAamp DNA Stool Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions.

[0369] The qPCR reaction mixture contains 1x SsoAdvanced Universal Probes Supermix, 900 nM of Wr-tcdB-F primer (AGCAGTTGAATATAGTGGTTTAGTTAGAGTTG, IDT, Coralville, IA), 900 nM of Wr-tcdB-R primer (CATGCTTTTTTAGTTTCTGGATTGAA, IDT, Coralville, IA), 250 nM of Wr-tcdB-P probe (6FAM-CATCCAGTCTCAATTGTATATGTTTCTCCA-MGB, Life Technologies, Grand Island, NY), and Molecular Biology Grade Water (Mo Bio Laboratories, Carlsbad, CA) to 18 μ l (Primers adapted from: Wroblewski, D. et al., Rapid Molecular Characterization of *Clostridium difficile* and Assessment of Populations of *C. difficile* in Stool Specimens, Journal of Clinical Microbiology 47:2142–2148 (2009)). This reaction mixture is aliquoted to wells of a Hard-shell Low-Profile Thin Wall 96-well Skirted PCR Plate (BioRad, Hercules, CA). To this reaction mixture, 2 μ l of diluted, frozen, and thawed samples are added and the plate sealed with a Microseal 'B' Adhesive Seal (BioRad, Hercules, CA). The qPCR is performed on a BioRad C1000™ Thermal Cycler equipped with a CFX96™ Real-Time System (BioRad, Hercules, CA). The thermocycling conditions are 95°C for 15 minutes followed by 45 cycles of 95°C for 5 seconds, 60°C for 30 seconds, and fluorescent readings of the FAM channel. Alternatively, the qPCR is performed with other standard methods known to those skilled in the art.

[0370] The Cq value for each well on the FAM channel is determined by the CFX Manager™ 3.0 software. The $\log_{10}(\text{cfu/mL})$ of *C. difficile* each experimental sample is calculated by inputting a given sample's Cq value into a linear regression model generated from the standard curve comparing the Cq values of the standard curve wells to the known $\log_{10}(\text{cfu/mL})$ of those samples. The log inhibition is calculated for each sample by subtracting the $\log_{10}(\text{cfu/mL})$ of *C. difficile* in the sample from the $\log_{10}(\text{cfu/mL})$ of *C. difficile* in the sample on each assay plate used for the generation

of the standard curve that has no additional bacteria added. The mean log inhibition is calculated for all replicates for each composition.

[0371] A histogram of the range and standard deviation of each composition is plotted. Ranges or standard deviations of the log inhibitions that are distinct from the overall distribution are examined as possible outliers. If the removal of a single log inhibition datum from one of the binary pairs that is identified in the histograms would bring the range or standard deviation in line with those from the majority of the samples, that datum is removed as an outlier, and the mean log inhibition is recalculated.

[0372] The pooled variance of all samples evaluated in the assay is estimated as the average of the sample variances weighted by the sample's degrees of freedom. The pooled standard error is then calculated as the square root of the pooled variance divided by the square root of the number of samples. Confidence intervals for the null hypothesis are determined by multiplying the pooled standard error to the z score corresponding to a given percentage threshold. Mean log inhibitions outside the confidence interval are considered to be inhibitory if positive or stimulatory if negative with the percent confidence corresponding to the interval used. Ternary combinations with mean log inhibition greater than 0.312 are reported as ++++ ($\geq$ 99% confidence interval (C.I.) of the null hypothesis), those with mean log inhibition between 0.221 and 0.312 as +++ ($95\% < \text{C.I.} < 99\%$), those with mean log inhibition between 0.171 and 0.221 as ++ ($90\% < \text{C.I.} < 95\%$), those with mean log inhibition between 0.113 and 0.171 as + ($80\% < \text{C.I.} < 90\%$), those with mean log inhibition between -0.113 and -0.171 as - ($80\% < \text{C.I.} < 90\%$), those with mean log inhibition between -0.171 and -0.221 as -- ($90\% < \text{C.I.} < 95\%$), those with mean log

inhibition between -0.221 and -0.312 as --- (95% < C.I. < 99%), and those with mean log inhibition less than -0.312 as ---- (99% < C.I.).

[0373] Table 22 below shows OTUs and their clade assignments tested in ternary combinations with results in the in vitro inhibition assay. The CivSim shows that many ternary combinations inhibit *C. difficile*. 39 of 56 combinations show inhibition with a confidence interval >80%; 36 of 56 with a C.I. > 90%; 36 of 56 with a C.I. > 95%; 29 of 56 with a C.I. of >99%. Non-limiting but exemplary ternary combinations include those with mean log reduction greater than 0.171, e.g. any combination shown in Table 22 with a score of +++, such as *Colinsella aerofaciens*, *Coprococcus comes*, and *Blautia producta*. Equally important, the CivSim assay describes ternary combinations that do not effectively inhibit *C. difficile*. 5 of 56 combinations promote growth with >80% confidence; 2 of 56 promote growth with >90% confidence; 1 of 56, *Coprococcus comes*, *Clostridium symbiosum* and *Eubacterium rectale*, promote growth with >95% confidence. 12 of 56 combinations are neutral in the assay, meaning they neither promote nor inhibit *C. difficile* growth to the limit of measurement.

[0374] It is straightforward for one of skill in the art to use the in vitro competition assay described below to determine efficacious subsets of the Core Ecology derived from the bacterial composition shown to be efficacious in treating *C. difficile* in humans.

Use of *in vitro* competition assay to test potential bacterial competitor consortia for functionality

[0375] An *in vitro* assay is performed to test the ability of a chosen species or combination of species to inhibit the growth of a pathogen such as *Clostridium difficile* in media that is otherwise suitable for growth of the pathogen. A liquid media suitable for growth of the pathogen is chosen, such as Brain Heart Infusion Broth

(BHI) for *C. difficile* (see Example 5). The potential competitor species or a combination of competitor species are inoculated into 3 mL of the media and incubated anaerobically for 24 hr at 37° C. After incubation the cells are pelleted in a centrifuge at 10,000 rcf for 5 min. Supernatant is removed and filtered through a 0.22 µm filter to remove all cells. *C. difficile* or another pathogen of interest is then inoculated into the filtered spent supernatant and grown anaerobically at 37° C for 24 hr. A control culture in fresh media is incubated in parallel. After incubation, the titer of *C. difficile* is determined by serially diluting and plating to Brucella Blood Agar (BBA) plates and incubated anaerobically for 24 hr at 37° C. Colonies are counted to determine the final titer of the pathogen after incubation in competitor conditioned media and control media. The percent reduction in final titer is calculated and considered inhibitory if a statistically significant reduction in growth is measured. Alternatively, the inhibition of pathogen growth is monitored by OD₆₀₀ measurement of the test and control cultures.

Example 30: Testing of Bacterial Composition Against Salmonella

[0376] The *in vitro* assay is used to screen for combinations of bacteria inhibitory to the growth of Salmonella spp. by modifying the media used for growth of the pathogen inoculum. Several choices of media are used for growth of the pathogen such as Reinforced Clostridial Media (RCM), Brain Heart Infusion Broth (BHI) or Luria Bertani Broth (LB). Salmonella spp. are quantified by using alternative selective media specific for Salmonella spp. or using qPCR probes specific for the pathogen. For example, MacConkey agar is used to select for Salmonella spp. and the *invA* gene is targeted with qPCR probes; this gene encodes an invasion protein carried by many pathogenic Salmonella spp. and is used in invading eukaryotic cells.

Example 31: Method of Preparing the Bacterial Composition for Administration to a Patient

[0377] Two strains for the bacterial composition are independently cultured and mixed together before administration. Both strains are independently be grown at 37°C, pH 7, in a GMM or other animal-products-free medium, pre-reduced with 1 g/L cysteine·HCl. After each strain reaches a sufficient biomass, it is preserved for banking by adding 15% glycerol and then frozen at -80°C in 1 ml cryotubes.

[0378] Each strain is then be cultivated to a concentration of 10^{10} CFU/mL, then concentrated 20-fold by tangential flow microfiltration; the spent medium is exchanged by diafiltering with a preservative medium consisting of 2% gelatin, 100 mM trehalose, and 10 mM sodium phosphate buffer, or other suitable preservative medium. The suspension is freeze-dried to a powder and titrated.

[0379] After drying, the powder is blended with microcrystalline cellulose and magnesium stearate and formulated into a 250 mg gelatin capsule containing 10 mg of lyophilized powder (10^8 to 10^{11} bacteria), 160 mg microcrystalline cellulose, 77.5 mg gelatin, and 2.5 mg magnesium stearate.

Example 32: Method of Treating a Subject with a Bacterial Composition

[0380] A patient has suffered from recurrent bouts of *C. difficile*. In the most recent acute phase of illness, the patient is treated with an antibiotic sufficient to ameliorate the symptoms of the illness. In order to prevent another relapse of *C. difficile*, the patient is administered one of the present bacterial compositions. Specifically, the patient is administered *Bacillus circulans* and *Roseburia inulinivorans* at a dose of 10^8 bacteria total in a lyophilized form, specifically in a 250 mg gelatin capsule containing 10 mg of lyophilized bacteria, 160 mg microcrystalline cellulose, 77.5 mg gelatin, and 2.5 mg magnesium stearate. The patient takes the capsule by mouth and resumes a normal diet after 4, 8, 12, or 24 hours. In another

embodiment, the patient may take the capsule by mouth before, during, or immediately after a meal.

[0381] Feces is collected before and at 1 day, 3 days, 1 week, and 1 month after administration. The presence of *C. difficile* is found in the feces before administration of the bacterial composition, but feces collections after administration show reducing (such as at least 50% less, 60%, 70%, 80%, 90%, or 95%) to no detectable levels of *C. difficile*, as measured by qPCR, as described above. ELISA for toxin protein or traditional microbiological identification techniques may also be used.

[0382] As another measure of patient success, a positive response may be defined as absence of diarrhea, which itself is defined as 3 or more loose or watery stools per day for at least 2 consecutive days or 8 or more loose or watery stools in 48 hours, or persisting diarrhea (due to other causes) with repeating (three times) negative stool tests for toxins of *C. difficile*.

[0383] Treatment failure is defined as persisting diarrhea with a positive *C. difficile* toxin stool test or no reduction in levels of *C. difficile*, as measured by qPCR sequencing. ELISA or traditional microbiological identification techniques may also be used.

Example 33: Method of Treating a Subject with a Bacterial Composition

[0384] A patient has suffered from recurrent bouts of *C. difficile*. In the most recent acute phase of illness, the patient is treated with an antibiotic sufficient to ameliorate the symptoms of the illness. In order to prevent another relapse of *C. difficile*, the patient is administered one of the present bacterial compositions. Specifically, the patient is administered a bacterial composition containing two bacterial types from Table 1, at a dose of 10^8 bacteria total in a lyophilized form formulated in an enteric coated capsule. Example of the patient or samples derived

from the patient is expected to demonstrate at least one measure of success as described herein (reducing levels of *C. difficile* as measured by qPCR, ELISA, or traditional microbiological identification; absence of diarrhea; persisting diarrhea with repeating (three times) negative stool tests for toxins of *C. difficile*).

[0385] Other embodiments will be apparent to those skilled in the art from consideration of the specification and practice of the embodiments. Consider the specification and examples as exemplary only, with a true scope and spirit being indicated by the following claims.

[0386] Unless otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification, including claims, are to be understood as being modified in all instances by the term “about.” Accordingly, unless otherwise indicated to the contrary, the numerical parameters are approximations and may vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should be construed in light of the number of significant digits and ordinary rounding approaches.

[0387] Unless otherwise indicated, the term “at least” preceding a series of elements is to be understood to refer to every element in the series.

[0388] While the invention has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the invention.

[0389] All references, issued patents and patent applications cited within the body of the instant specification are hereby incorporated by reference in their entirety, for all purposes.

TABLES

Table 1

OTU	SEQ ID Number	Public DB Accession	Clade	Spore Former	Pathogen Status
Eubacterium saburreum	858	AB525414	clade_178	Y	N
Eubacterium sp. oral clone IR009	866	AY349376	clade_178	Y	N
Lachnospiraceae bacterium ICM62	1061	HQ616401	clade_178	Y	N
Lachnospiraceae bacterium MSX33	1062	HQ616384	clade_178	Y	N
Lachnospiraceae bacterium oral taxon 107	1063	ADDS01000069	clade_178	Y	N
Alicyclobacillus acidocaldarius	122	NR_074721	clade_179	Y	N
Clostridium baratii	555	NR_029229	clade_223	Y	N
Clostridium colicanis	576	FJ957863	clade_223	Y	N
Clostridium paraputrificum	611	AB536771	clade_223	Y	N
Clostridium sardiniense	621	NR_041006	clade_223	Y	N
Eubacterium budayi	837	NR_024682	clade_223	Y	N
Eubacterium moniliforme	851	HF558373	clade_223	Y	N
Eubacterium multiforme	852	NR_024683	clade_223	Y	N
Eubacterium nitritogenes	853	NR_024684	clade_223	Y	N
Anoxybacillus flavithermus	173	NR_074667	clade_238	Y	N
Bacillus aerophilus	196	NR_042339	clade_238	Y	N
Bacillus aestuarii	197	GQ980243	clade_238	Y	N
Bacillus amyloliquefaciens	199	NR_075005	clade_238	Y	N
Bacillus anthracis	200	AAEN01000020	clade_238	Y	Category -A
Bacillus atrophaeus	201	NR_075016	clade_238	Y	OP
Bacillus badius	202	NR_036893	clade_238	Y	OP
Bacillus cereus	203	ABDJ01000015	clade_238	Y	OP
Bacillus circulans	204	AB271747	clade_238	Y	OP
Bacillus firmus	207	NR_025842	clade_238	Y	OP
Bacillus flexus	208	NR_024691	clade_238	Y	OP
Bacillus fordii	209	NR_025786	clade_238	Y	OP
Bacillus halmapalus	211	NR_026144	clade_238	Y	OP
Bacillus herbersteinensis	213	NR_042286	clade_238	Y	OP
Bacillus idriensis	215	NR_043268	clade_238	Y	OP
Bacillus lentus	216	NR_040792	clade_238	Y	OP
Bacillus licheniformis	217	NC_006270	clade_238	Y	OP
Bacillus megaterium	218	GU252124	clade_238	Y	OP
Bacillus nealsonii	219	NR_044546	clade_238	Y	OP
Bacillus niabensis	220	NR_043334	clade_238	Y	OP
Bacillus niacini	221	NR_024695	clade_238	Y	OP
Bacillus pocheonensis	222	NR_041377	clade_238	Y	OP
Bacillus pumilus	223	NR_074977	clade_238	Y	OP
Bacillus safensis	224	JQ624766	clade_238	Y	OP
Bacillus simplex	225	NR_042136	clade_238	Y	OP
Bacillus sonorensis	226	NR_025130	clade_238	Y	OP
Bacillus sp. 10403023 MM10403188	227	CAET01000089	clade_238	Y	OP
Bacillus sp. 2_A_57_CT2	230	ACWD01000095	clade_238	Y	OP
Bacillus sp. 2008724126	228	GU252108	clade_238	Y	OP

OTU	SEQ ID Number	Public DB Accession	Clade	Spore Former	Pathogen Status
Bacillus sp. 2008724139	229	GU252111	clade_238	Y	OP
Bacillus sp. 7_16AIA	231	FN397518	clade_238	Y	OP
Bacillus sp. AP8	233	JX101689	clade_238	Y	OP
Bacillus sp. B27(2008)	234	EU362173	clade_238	Y	OP
Bacillus sp. BT1B_CT2	235	ACWC01000034	clade_238	Y	OP
Bacillus sp. GB1.1	236	FJ897765	clade_238	Y	OP
Bacillus sp. GB9	237	FJ897766	clade_238	Y	OP
Bacillus sp. HU19.1	238	FJ897769	clade_238	Y	OP
Bacillus sp. HU29	239	FJ897771	clade_238	Y	OP
Bacillus sp. HU33.1	240	FJ897772	clade_238	Y	OP
Bacillus sp. JC6	241	JF824800	clade_238	Y	OP
Bacillus sp. oral taxon F79	248	HM099654	clade_238	Y	OP
Bacillus sp. SRC_DSF1	243	GU797283	clade_238	Y	OP
Bacillus sp. SRC_DSF10	242	GU797292	clade_238	Y	OP
Bacillus sp. SRC_DSF2	244	GU797284	clade_238	Y	OP
Bacillus sp. SRC_DSF6	245	GU797288	clade_238	Y	OP
Bacillus sp. tc09	249	HQ844242	clade_238	Y	OP
Bacillus sp. zh168	250	FJ851424	clade_238	Y	OP
Bacillus sphaericus	251	DQ286318	clade_238	Y	OP
Bacillus sporothermodurans	252	NR_026010	clade_238	Y	OP
Bacillus subtilis	253	EU627588	clade_238	Y	OP
Bacillus thermoamylovorans	254	NR_029151	clade_238	Y	OP
Bacillus thuringiensis	255	NC_008600	clade_238	Y	OP
Bacillus weihenstephanensis	256	NR_074926	clade_238	Y	OP
Geobacillus kaustophilus	933	NR_074989	clade_238	Y	N
Geobacillus stearothermophilus	936	NR_040794	clade_238	Y	N
Geobacillus thermodenitrificans	938	NR_074976	clade_238	Y	N
Geobacillus thermoglucosidasius	939	NR_043022	clade_238	Y	N
Lysinibacillus sphaericus	1193	NR_074883	clade_238	Y	N
Clostridiales sp. SS3_4	543	AY305316	clade_246	Y	N
Clostridium beijerinckii	557	NR_074434	clade_252	Y	N
Clostridium botulinum	560	NC_010723	clade_252	Y	Category -A
Clostridium butyricum	561	ABDT01000017	clade_252	Y	N
Clostridium chauvoei	568	EU106372	clade_252	Y	N
Clostridium favosporum	582	X76749	clade_252	Y	N
Clostridium histolyticum	592	HF558362	clade_252	Y	N
Clostridium isatidis	597	NR_026347	clade_252	Y	N
Clostridium limosum	602	FR870444	clade_252	Y	N
Clostridium sartagoforme	622	NR_026490	clade_252	Y	N
Clostridium septicum	624	NR_026020	clade_252	Y	N
Clostridium sp. 7_2_43FAA	626	ACDK01000101	clade_252	Y	N
Clostridium sporogenes	645	ABKW02000003	clade_252	Y	N
Clostridium tertium	653	Y18174	clade_252	Y	N
Clostridium carnis	564	NR_044716	clade_253	Y	N
Clostridium celatum	565	X77844	clade_253	Y	N
Clostridium disporicum	579	NR_026491	clade_253	Y	N
Clostridium gasigenes	585	NR_024945	clade_253	Y	N
Clostridium quinii	616	NR_026149	clade_253	Y	N
Clostridium hylemonae	593	AB023973	clade_260	Y	N
Clostridium scindens	623	AF262238	clade_260	Y	N
Lachnospiraceae bacterium 5_1_57FAA	1054	ACTR01000020	clade_260	Y	N
Clostridium glycyrrhizinilyticum	588	AB233029	clade_262	Y	N

OTU	SEQ ID Number	Public DB Accession	Clade	Spore Former	Pathogen Status
Clostridium nexile	607	X73443	clade_262	Y	N
Coprococcus comes	674	ABVR01000038	clade_262	Y	N
Lachnospiraceae bacterium 1_1_57FAA	1048	ACTM01000065	clade_262	Y	N
Lachnospiraceae bacterium 1_4_56FAA	1049	ACTN01000028	clade_262	Y	N
Lachnospiraceae bacterium 8_1_57FAA	1057	ACWQ01000079	clade_262	Y	N
Ruminococcus lactaris	1663	ABOU02000049	clade_262	Y	N
Ruminococcus torques	1670	AAVP02000002	clade_262	Y	N
Paenibacillus lautus	1397	NR_040882	clade_270	Y	N
Paenibacillus polymyxa	1399	NR_037006	clade_270	Y	N
Paenibacillus sp. HGF5	1402	AEXS01000095	clade_270	Y	N
Paenibacillus sp. HGF7	1403	AFDH01000147	clade_270	Y	N
Eubacterium sp. oral clone JI012	868	AY349379	clade_298	Y	N
Alicyclobacillus contaminans	124	NR_041475	clade_301	Y	N
Alicyclobacillus herbarius	126	NR_024753	clade_301	Y	N
Alicyclobacillus pomorum	127	NR_024801	clade_301	Y	N
Blautia coccoides	373	AB571656	clade_309	Y	N
Blautia glucerasea	374	AB588023	clade_309	Y	N
Blautia glucerasei	375	AB439724	clade_309	Y	N
Blautia hansenii	376	ABYU02000037	clade_309	Y	N
Blautia luti	378	AB691576	clade_309	Y	N
Blautia producta	379	AB600998	clade_309	Y	N
Blautia schinkii	380	NR_026312	clade_309	Y	N
Blautia sp. M25	381	HM626178	clade_309	Y	N
Blautia stercoris	382	HM626177	clade_309	Y	N
Blautia wexlerae	383	EF036467	clade_309	Y	N
Bryantella formatexigens	439	ACCL02000018	clade_309	Y	N
Clostridium coccoides	573	EF025906	clade_309	Y	N
Eubacterium cellulosolvens	839	AY178842	clade_309	Y	N
Lachnospiraceae bacterium 6_1_63FAA	1056	ACTV01000014	clade_309	Y	N
Ruminococcus hansenii	1662	M59114	clade_309	Y	N
Ruminococcus obeum	1664	AY169419	clade_309	Y	N
Ruminococcus sp. 5_1_39BFAA	1666	ACII01000172	clade_309	Y	N
Ruminococcus sp. K_1	1669	AB222208	clade_309	Y	N
Syntrophococcus sucromutans	1911	NR_036869	clade_309	Y	N
Bacillus alcalophilus	198	X76436	clade_327	Y	N
Bacillus clausii	205	FN397477	clade_327	Y	OP
Bacillus gelatini	210	NR_025595	clade_327	Y	OP
Bacillus halodurans	212	AY144582	clade_327	Y	OP
Bacillus sp. oral taxon F26	246	HM099642	clade_327	Y	OP
Clostridium innocuum	595	M23732	clade_351	Y	N
Clostridium sp. HGF2	628	AENW01000022	clade_351	Y	N
Clostridium perfringens	612	ABDW01000023	clade_353	Y	Category -B
Sarcina ventriculi	1687	NR_026146	clade_353	Y	N
Clostridium bartlettii	556	ABEZ02000012	clade_354	Y	N
Clostridium bifermentans	558	X73437	clade_354	Y	N
Clostridium ghonii	586	AB542933	clade_354	Y	N
Clostridium glycolicum	587	FJ384385	clade_354	Y	N
Clostridium mayombeii	605	FR733682	clade_354	Y	N
Clostridium sordellii	625	AB448946	clade_354	Y	N

OTU	SEQ ID Number	Public DB Accession	Clade	Spore Former	Pathogen Status
Clostridium sp. MT4 E	635	FJ159523	clade_354	Y	N
Eubacterium tenue	872	M59118	clade_354	Y	N
Clostridium argentinense	553	NR_029232	clade_355	Y	N
Clostridium sp. JC122	630	CAEV01000127	clade_355	Y	N
Clostridium sp. NMBHI_1	636	JN093130	clade_355	Y	N
Clostridium subterminale	650	NR_041795	clade_355	Y	N
Clostridium sulfidigenes	651	NR_044161	clade_355	Y	N
Dorea formicigenerans	773	AAXA02000006	clade_360	Y	N
Dorea longicatena	774	AJ132842	clade_360	Y	N
Lachnospiraceae bacterium 2_1_46FAA	1050	ADLB01000035	clade_360	Y	N
Lachnospiraceae bacterium 2_1_58FAA	1051	ACTO01000052	clade_360	Y	N
Lachnospiraceae bacterium 4_1_37FAA	1053	ADCR01000030	clade_360	Y	N
Lachnospiraceae bacterium 9_1_43BFAA	1058	ACTX01000023	clade_360	Y	N
Ruminococcus gnavus	1661	X94967	clade_360	Y	N
Ruminococcus sp. ID8	1668	AY960564	clade_360	Y	N
Blautia hydrogenotrophica	377	ACBZ01000217	clade_368	Y	N
Lactonifactor longoviformis	1147	DQ100449	clade_368	Y	N
Robinsoniella peoriensis	1633	AF445258	clade_368	Y	N
Eubacterium infirmum	849	U13039	clade_384	Y	N
Eubacterium sp. WAL 14571	864	FJ687606	clade_384	Y	N
Erysipelotrichaceae bacterium 5_2_54FAA	823	ACZW01000054	clade_385	Y	N
Eubacterium bifforme	835	ABYT01000002	clade_385	Y	N
Eubacterium cylindroides	842	FP929041	clade_385	Y	N
Eubacterium dolichum	844	L34682	clade_385	Y	N
Eubacterium sp. 3_1_31	861	ACTL01000045	clade_385	Y	N
Eubacterium tortuosum	873	NR_044648	clade_385	Y	N
Bulleidia extructa	441	ADFR01000011	clade_388	Y	N
Solobacterium moorei	1739	AECQ01000039	clade_388	Y	N
Coprococcus catus	673	EU266552	clade_393	Y	N
Lachnospiraceae bacterium oral taxon F15	1064	HM099641	clade_393	Y	N
Clostridium cochlearium	574	NR_044717	clade_395	Y	N
Clostridium malenominatum	604	FR749893	clade_395	Y	N
Clostridium tetani	654	NC_004557	clade_395	Y	N
Acetivibrio ethanolgignens	6	FR749897	clade_396	Y	N
Anaerosporeobacter mobilis	161	NR_042953	clade_396	Y	N
Bacteroides pectinophilus	288	ABVQ01000036	clade_396	Y	N
Clostridium aminovalericum	551	NR_029245	clade_396	Y	N
Clostridium phytofermentans	613	NR_074652	clade_396	Y	N
Eubacterium hallii	848	L34621	clade_396	Y	N
Eubacterium xylanophilum	875	L34628	clade_396	Y	N
Ruminococcus callidus	1658	NR_029160	clade_406	Y	N
Ruminococcus champanellensis	1659	FP929052	clade_406	Y	N
Ruminococcus sp. 18P13	1665	AJ515913	clade_406	Y	N
Ruminococcus sp. 9SE51	1667	FM954974	clade_406	Y	N
Anaerostipes caccae	162	ABAX03000023	clade_408	Y	N
Anaerostipes sp. 3_2_56FAA	163	ACWB01000002	clade_408	Y	N
Clostridiales bacterium 1_7_47FAA	541	ABQR01000074	clade_408	Y	N

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Clostridiales sp. SM4_1	542	FP929060	clade_408	Y	N
Clostridiales sp. SSC_2	544	FP929061	clade_408	Y	N
Clostridium aerotolerans	546	X76163	clade_408	Y	N
Clostridium aldenense	547	NR_043680	clade_408	Y	N
Clostridium algidixylanolyticum	550	NR_028726	clade_408	Y	N
Clostridium amygdalinum	552	AY353957	clade_408	Y	N
Clostridium asparagiforme	554	ACCJ01000522	clade_408	Y	N
Clostridium bolteae	559	ABCC02000039	clade_408	Y	N
Clostridium celerecrescens	566	JQ246092	clade_408	Y	N
Clostridium citroniae	569	ADLJ01000059	clade_408	Y	N
Clostridium clostridiiformes	571	M59089	clade_408	Y	N
Clostridium clostridioforme	572	NR_044715	clade_408	Y	N
Clostridium hathewayi	590	AY552788	clade_408	Y	N
Clostridium indolis	594	AF028351	clade_408	Y	N
Clostridium lavalense	600	EF564277	clade_408	Y	N
Clostridium saccharolyticum	620	CP002109	clade_408	Y	N
Clostridium sp. M62_1	633	ACFX02000046	clade_408	Y	N
Clostridium sp. SS2_1	638	ABGC03000041	clade_408	Y	N
Clostridium sphenoides	643	X73449	clade_408	Y	N
Clostridium symbiosum	652	ADLQ01000114	clade_408	Y	N
Clostridium xylanolyticum	658	NR_037068	clade_408	Y	N
Eubacterium hadrum	847	FR749933	clade_408	Y	N
Lachnospiraceae bacterium 3_1_57FAA_CT1	1052	ACTP01000124	clade_408	Y	N
Lachnospiraceae bacterium 5_1_63FAA	1055	ACTS01000081	clade_408	Y	N
Lachnospiraceae bacterium A4	1059	DQ789118	clade_408	Y	N
Lachnospiraceae bacterium DJF VP30	1060	EU728771	clade_408	Y	N
Lachnospiraceae genomosp. C1	1065	AY278618	clade_408	Y	N
Clostridium difficile	578	NC_013315	clade_409	Y	OP
Eubacterium sp. AS15b	862	HQ616364	clade_428	Y	N
Eubacterium sp. OBRC9	863	HQ616354	clade_428	Y	N
Eubacterium sp. oral clone OH3A	871	AY947497	clade_428	Y	N
Eubacterium yurii	876	AEES01000073	clade_428	Y	N
Clostridium acetobutylicum	545	NR_074511	clade_430	Y	N
Clostridium algidicarnis	549	NR_041746	clade_430	Y	N
Clostridium cadaveris	562	AB542932	clade_430	Y	N
Clostridium carboxidivorans	563	FR733710	clade_430	Y	N
Clostridium estertheticum	580	NR_042153	clade_430	Y	N
Clostridium fallax	581	NR_044714	clade_430	Y	N
Clostridium felsineum	583	AF270502	clade_430	Y	N
Clostridium frigidicarnis	584	NR_024919	clade_430	Y	N
Clostridium kluyveri	598	NR_074165	clade_430	Y	N
Clostridium magnum	603	X77835	clade_430	Y	N
Clostridium putrefaciens	615	NR_024995	clade_430	Y	N
Clostridium sp. HPB_46	629	AY862516	clade_430	Y	N
Clostridium tyrobutyricum	656	NR_044718	clade_430	Y	N
Sutterella parvirubra	1899	AB300989	clade_432	Y	N
Acetanaerobacterium elongatum	4	NR_042930	clade_439	Y	N
Clostridium cellulosi	567	NR_044624	clade_439	Y	N
Ethanoligenens harbinense	832	AY675965	clade_439	Y	N
Eubacterium rectale	856	FP929042	clade_444	Y	N
Eubacterium sp. oral clone GI038	865	AY349374	clade_444	Y	N

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Lachnobacterium bovis	1045	GU324407	clade_444	Y	N
Roseburia cecicola	1634	GU233441	clade_444	Y	N
Roseburia faecalis	1635	AY804149	clade_444	Y	N
Roseburia faecis	1636	AY305310	clade_444	Y	N
Roseburia hominis	1637	AJ270482	clade_444	Y	N
Roseburia intestinalis	1638	FP929050	clade_444	Y	N
Roseburia inulinivorans	1639	AJ270473	clade_444	Y	N
Brevibacillus brevis	410	NR_041524	clade_448	Y	N
Brevibacillus laterosporus	414	NR_037005	clade_448	Y	N
Bacillus coagulans	206	DQ297928	clade_451	Y	OP
Sporolactobacillus inulinus	1752	NR_040962	clade_451	Y	N
Kocuria palustris	1041	EU333884	clade_453	Y	N
Nocardia farcinica	1353	NC_006361	clade_455	Y	N
Bacillus sp. oral taxon F28	247	HM099650	clade_456	Y	OP
Catenibacterium mitsuokai	495	AB030224	clade_469	Y	N
Clostridium sp. TM_40	640	AB249652	clade_469	Y	N
Coprobaillus cateniformis	670	AB030218	clade_469	Y	N
Coprobaillus sp. 29_1	671	ADKX01000057	clade_469	Y	N
Clostridium rectum	618	NR_029271	clade_470	Y	N
Eubacterium nodatum	854	U13041	clade_476	Y	N
Eubacterium saphenum	859	NR_026031	clade_476	Y	N
Eubacterium sp. oral clone JH012	867	AY349373	clade_476	Y	N
Eubacterium sp. oral clone JS001	870	AY349378	clade_476	Y	N
Faecalibacterium prausnitzii	880	ACOP02000011	clade_478	Y	N
Gemmiger formicilis	932	GU562446	clade_478	Y	N
Subdoligranulum variabile	1896	AJ518869	clade_478	Y	N
Clostridiaceae bacterium JC13	532	JF824807	clade_479	Y	N
Clostridium sp. MLG055	634	AF304435	clade_479	Y	N
Erysipelotrichaceae bacterium 3_1_53	822	ACTJ01000113	clade_479	Y	N
Clostridium cocleatum	575	NR_026495	clade_481	Y	N
Clostridium ramosum	617	M23731	clade_481	Y	N
Clostridium saccharogumia	619	DQ100445	clade_481	Y	N
Clostridium spiroforme	644	X73441	clade_481	Y	N
Coprobaillus sp. D7	672	ACDT01000199	clade_481	Y	N
Clostridiales bacterium SY8519	535	AB477431	clade_482	Y	N
Clostridium sp. SY8519	639	AP012212	clade_482	Y	N
Eubacterium ramulus	855	AJ011522	clade_482	Y	N
Erysipelothrix inopinata	819	NR_025594	clade_485	Y	N
Erysipelothrix rhusiopathiae	820	ACLK01000021	clade_485	Y	N
Erysipelothrix tonsillarum	821	NR_040871	clade_485	Y	N
Holdemania filiformis	1004	Y11466	clade_485	Y	N
Mollicutes bacterium pACH93	1258	AY297808	clade_485	Y	N
Coxiella burnetii	736	CP000890	clade_486	Y	Category -B
Clostridium hiranonis	591	AB023970	clade_487	Y	N
Clostridium irregulare	596	NR_029249	clade_487	Y	N
Clostridium orbiscindens	609	Y18187	clade_494	Y	N
Clostridium sp. NML 04A032	637	EU815224	clade_494	Y	N
Flavonifractor plautii	886	AY724678	clade_494	Y	N
Pseudoflavonifractor capillosus	1591	AY136666	clade_494	Y	N
Ruminococcaceae bacterium D16	1655	ADDX01000083	clade_494	Y	N
Acetivibrio cellulolyticus	5	NR_025917	clade_495	Y	N
Clostridium aldrichii	548	NR_026099	clade_495	Y	N

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<i>Clostridium clariflavum</i>	570	NR_041235	clade_495	Y	N
<i>Clostridium stercorearium</i>	647	NR_025100	clade_495	Y	N
<i>Clostridium straminisolvans</i>	649	NR_024829	clade_495	Y	N
<i>Clostridium thermocellum</i>	655	NR_074629	clade_495	Y	N
<i>Fusobacterium nucleatum</i>	901	ADVK01000034	clade_497	Y	N
<i>Eubacterium barkeri</i>	834	NR_044661	clade_512	Y	N
<i>Eubacterium callanderi</i>	838	NR_026330	clade_512	Y	N
<i>Eubacterium limosum</i>	850	CP002273	clade_512	Y	N
<i>Anaerotruncus colihominis</i>	164	ABGD02000021	clade_516	Y	N
<i>Clostridium methylpentosum</i>	606	ACEC01000059	clade_516	Y	N
<i>Clostridium</i> sp. YIT 12070	642	AB491208	clade_516	Y	N
<i>Hydrogenoanaerobacterium saccharovorans</i>	1005	NR_044425	clade_516	Y	N
<i>Ruminococcus albus</i>	1656	AY445600	clade_516	Y	N
<i>Ruminococcus flavefaciens</i>	1660	NR_025931	clade_516	Y	N
<i>Clostridium haemolyticum</i>	589	NR_024749	clade_517	Y	N
<i>Clostridium novyi</i>	608	NR_074343	clade_517	Y	N
<i>Clostridium</i> sp. LMG 16094	632	X95274	clade_517	Y	N
<i>Eubacterium ventriosum</i>	874	L34421	clade_519	Y	N
<i>Bacteroides galacturonicus</i>	280	DQ497994	clade_522	Y	N
<i>Eubacterium eligens</i>	845	CP001104	clade_522	Y	N
<i>Lachnospira multipara</i>	1046	FR733699	clade_522	Y	N
<i>Lachnospira pectinoschiza</i>	1047	L14675	clade_522	Y	N
<i>Lactobacillus rogosae</i>	1114	GU269544	clade_522	Y	N
<i>Bacillus horti</i>	214	NR_036860	clade_527	Y	OP
<i>Bacillus</i> sp. 9_3AIA	232	FN397519	clade_527	Y	OP
<i>Eubacterium brachy</i>	836	U13038	clade_533	Y	N
<i>Filifactor alocis</i>	881	CP002390	clade_533	Y	N
<i>Filifactor villosus</i>	882	NR_041928	clade_533	Y	N
<i>Clostridium leptum</i>	601	AJ305238	clade_537	Y	N
<i>Clostridium</i> sp. YIT 12069	641	AB491207	clade_537	Y	N
<i>Clostridium sporosphaeroides</i>	646	NR_044835	clade_537	Y	N
<i>Eubacterium coprostanoligenes</i>	841	HM037995	clade_537	Y	N
<i>Ruminococcus bromii</i>	1657	EU266549	clade_537	Y	N
<i>Eubacterium siraeum</i>	860	ABCA03000054	clade_538	Y	N
<i>Clostridium viride</i>	657	NR_026204	clade_540	Y	N
<i>Oscillibacter</i> sp. G2	1386	HM626173	clade_540	Y	N
<i>Oscillibacter valericigenes</i>	1387	NR_074793	clade_540	Y	N
<i>Oscillospira guilliermondii</i>	1388	AB040495	clade_540	Y	N
<i>Butyrivibrio crossotus</i>	455	ABWN01000012	clade_543	Y	N
<i>Clostridium</i> sp. L2_50	631	AAYW02000018	clade_543	Y	N
<i>Coproccoccus eutactus</i>	675	EF031543	clade_543	Y	N
<i>Coproccoccus</i> sp. ART55_1	676	AY350746	clade_543	Y	N
<i>Eubacterium ruminantium</i>	857	NR_024661	clade_543	Y	N
<i>Collinsella aerofaciens</i>	659	AAVN02000007	clade_553	Y	N
<i>Alkaliphilus metalliredigenes</i>	137	AY137848	clade_554	Y	N
<i>Alkaliphilus oremlandii</i>	138	NR_043674	clade_554	Y	N
<i>Clostridium sticklandii</i>	648	L04167	clade_554	Y	N
<i>Turicibacter sanguinis</i>	1965	AF349724	clade_555	Y	N
<i>Fulvimonas</i> sp. NML 060897	892	EF589680	clade_557	Y	N
<i>Desulfotobacterium frappieri</i>	753	AJ276701	clade_560	Y	N
<i>Desulfotobacterium hafniense</i>	754	NR_074996	clade_560	Y	N
<i>Desulfotomaculum nigrificans</i>	756	NR_044832	clade_560	Y	N

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Lutispora thermophila	1191	NR_041236	clade_564	Y	N
Brachyspira pilosicoli	405	NR_075069	clade_565	Y	N
Eggerthella lenta	778	AF292375	clade_566	Y	N
Streptomyces albus	1888	AJ697941	clade_566	Y	N
Chlamydiales bacterium NS11	505	JN606074	clade_567	Y	N
Anaerofustis stercorihominis	159	ABIL02000005	clade_570	Y	N
Butyrivibrio pullicaecorum	453	HH793440	clade_572	Y	N
Eubacterium desmolans	843	NR_044644	clade_572	Y	N
Papillibacter cinnamivorans	1415	NR_025025	clade_572	Y	N
Sporobacter termitidis	1751	NR_044972	clade_572	Y	N
Deferribacteres sp. oral clone JV006	744	AY349371	clade_575	Y	N
Clostridium colinum	577	NR_026151	clade_576	Y	N
Clostridium lactatifermentans	599	NR_025651	clade_576	Y	N
Clostridium piliforme	614	D14639	clade_576	Y	N
Saccharomonospora viridis	1671	X54286	clade_579	Y	N
Thermobifida fusca	1921	NC_007333	clade_579	Y	N
Leptospira licerasiae	1164	EF612284	clade_585	Y	OP
Moorella thermoacetica	1259	NR_075001	clade_590	Y	N
Thermoanaerobacter pseudethanolicus	1920	CP000924	clade_590	Y	N
Flexistipes sinusarabici	888	NR_074881	clade_591	Y	N
Gloeobacter violaceus	942	NR_074282	clade_596	Y	N
Eubacterium sp. oral clone JN088	869	AY349377	clade_90	Y	N
Clostridium oroticum	610	FR749922	clade_96	Y	N
Clostridium sp. D5	627	ADBG01000142	clade_96	Y	N
Eubacterium contortum	840	FR749946	clade_96	Y	N
Eubacterium fissicatena	846	FR749935	clade_96	Y	N
Corynebacterium coyleae	692	X96497	clade_100	N	N
Corynebacterium mucifaciens	711	NR_026396	clade_100	N	N
Corynebacterium ureicelerivorans	733	AM397636	clade_100	N	N
Corynebacterium appendicis	684	NR_028951	clade_102	N	N
Corynebacterium genitalium	698	ACLJ01000031	clade_102	N	N
Corynebacterium glaucum	699	NR_028971	clade_102	N	N
Corynebacterium imitans	703	AF537597	clade_102	N	N
Corynebacterium riegellii	719	EU848548	clade_102	N	N
Corynebacterium sp. L_2012475	723	HE575405	clade_102	N	N
Corynebacterium sp. NML 93_0481	724	GU238409	clade_102	N	N
Corynebacterium sundsvallense	728	Y09655	clade_102	N	N
Corynebacterium tuscaniae	730	AY677186	clade_102	N	N
Prevotella maculosa	1504	AGEK01000035	clade_104	N	N
Prevotella oris	1513	ADDV01000091	clade_104	N	N
Prevotella salivae	1517	AB108826	clade_104	N	N
Prevotella sp. ICM55	1521	HQ616399	clade_104	N	N
Prevotella sp. oral clone AA020	1528	AY005057	clade_104	N	N
Prevotella sp. oral clone GI032	1538	AY349396	clade_104	N	N
Prevotella sp. oral taxon G70	1558	GU432179	clade_104	N	N
Prevotella corporis	1491	L16465	clade_105	N	N
Bacteroides sp. 4_1_36	312	ACTC01000133	clade_110	N	N
Bacteroides sp. AR20	315	AF139524	clade_110	N	N
Bacteroides sp. D20	319	ACPT01000052	clade_110	N	N
Bacteroides sp. F_4	322	AB470322	clade_110	N	N
Bacteroides uniformis	329	AB050110	clade_110	N	N

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Prevotella nanceiensis	1510	JN867228	clade_127	N	N
Prevotella sp. oral taxon 299	1548	ACWZ01000026	clade_127	N	N
Prevotella bergensis	1485	ACKS01000100	clade_128	N	N
Prevotella buccalis	1489	JN867261	clade_129	N	N
Prevotella timonensis	1564	ADEF01000012	clade_129	N	N
Prevotella oralis	1512	AEPE01000021	clade_130	N	N
Prevotella sp. SEQ072	1525	JN867238	clade_130	N	N
Leuconostoc carnosum	1177	NR_040811	clade_135	N	N
Leuconostoc gasicomitatum	1179	FN822744	clade_135	N	N
Leuconostoc inhae	1180	NR_025204	clade_135	N	N
Leuconostoc kimchii	1181	NR_075014	clade_135	N	N
Edwardsiella tarda	777	CP002154	clade_139	N	N
Photorhabdus asymbiotica	1466	Z76752	clade_139	N	N
Psychrobacter arcticus	1607	CP000082	clade_141	N	N
Psychrobacter cibarius	1608	HQ698586	clade_141	N	N
Psychrobacter cryohalolentis	1609	CP000323	clade_141	N	N
Psychrobacter faecalis	1610	HQ698566	clade_141	N	N
Psychrobacter nivimaris	1611	HQ698587	clade_141	N	N
Psychrobacter pulmonis	1612	HQ698582	clade_141	N	N
Pseudomonas aeruginosa	1592	AABQ07000001	clade_154	N	N
Pseudomonas sp. 2_1_26	1600	ACWU01000257	clade_154	N	N
Corynebacterium confusum	691	Y15886	clade_158	N	N
Corynebacterium propinquum	712	NR_037038	clade_158	N	N
Corynebacterium pseudodiphtheriticum	713	X84258	clade_158	N	N
Bartonella bacilliformis	338	NC_008783	clade_159	N	N
Bartonella grahamii	339	CP001562	clade_159	N	N
Bartonella henselae	340	NC_005956	clade_159	N	N
Bartonella quintana	341	BX897700	clade_159	N	N
Bartonella tamiae	342	EF672728	clade_159	N	N
Bartonella washoensis	343	FJ719017	clade_159	N	N
Brucella abortus	430	ACBJ01000075	clade_159	N	Category -B
Brucella canis	431	NR_044652	clade_159	N	Category -B
Brucella ceti	432	ACJD01000006	clade_159	N	Category -B
Brucella melitensis	433	AE009462	clade_159	N	Category -B
Brucella microti	434	NR_042549	clade_159	N	Category -B
Brucella ovis	435	NC_009504	clade_159	N	Category -B
Brucella sp. 83_13	436	ACBQ01000040	clade_159	N	Category -B
Brucella sp. BO1	437	EU053207	clade_159	N	Category -B
Brucella suis	438	ACBK01000034	clade_159	N	Category -B
Ochrobactrum anthropi	1360	NC_009667	clade_159	N	N
Ochrobactrum intermedium	1361	ACQA01000001	clade_159	N	N
Ochrobactrum pseudintermedium	1362	DQ365921	clade_159	N	N
Prevotella genomosp. C2	1496	AY278625	clade_164	N	N
Prevotella multisaccharivorax	1509	AFJE01000016	clade_164	N	N
Prevotella sp. oral clone	1543	AY550997	clade_164	N	N

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IDR_CEC_0055					
Prevotella sp. oral taxon 292	1547	GQ422735	clade_164	N	N
Prevotella sp. oral taxon 300	1549	GU409549	clade_164	N	N
Prevotella marshii	1505	AEEI01000070	clade_166	N	N
Prevotella sp. oral clone IK053	1544	AY349401	clade_166	N	N
Prevotella sp. oral taxon 781	1554	GQ422744	clade_166	N	N
Prevotella stercorea	1562	AB244774	clade_166	N	N
Prevotella brevis	1487	NR_041954	clade_167	N	N
Prevotella ruminicola	1516	CP002006	clade_167	N	N
Prevotella sp. sp24	1560	AB003384	clade_167	N	N
Prevotella sp. sp34	1561	AB003385	clade_167	N	N
Prevotella albensis	1483	NR_025300	clade_168	N	N
Prevotella copri	1490	ACBX02000014	clade_168	N	N
Prevotella oulorum	1514	L16472	clade_168	N	N
Prevotella sp. BI_42	1518	AJ581354	clade_168	N	N
Prevotella sp. oral clone P4PB_83 P2	1546	AY207050	clade_168	N	N
Prevotella sp. oral taxon G60	1557	GU432133	clade_168	N	N
Prevotella amnii	1484	AB547670	clade_169	N	N
Bacteroides caccae	268	EU136686	clade_170	N	N
Bacteroides finegoldii	277	AB222699	clade_170	N	N
Bacteroides intestinalis	283	ABJL02000006	clade_171	N	N
Bacteroides sp. XB44A	326	AM230649	clade_171	N	N
Bifidobacteriaceae genomosp. C1	345	AY278612	clade_172	N	N
Bifidobacterium adolescentis	346	AAXD02000018	clade_172	N	N
Bifidobacterium angulatum	347	ABYS02000004	clade_172	N	N
Bifidobacterium animalis	348	CP001606	clade_172	N	N
Bifidobacterium breve	350	CP002743	clade_172	N	N
Bifidobacterium catenulatum	351	ABXY01000019	clade_172	N	N
Bifidobacterium dentium	352	CP001750	clade_172	N	OP
Bifidobacterium gallicum	353	ABXB03000004	clade_172	N	N
Bifidobacterium infantis	354	AY151398	clade_172	N	N
Bifidobacterium kashiwanohense	355	AB491757	clade_172	N	N
Bifidobacterium longum	356	ABQQ01000041	clade_172	N	N
Bifidobacterium pseudocatenulatum	357	ABXX02000002	clade_172	N	N
Bifidobacterium pseudolongum	358	NR_043442	clade_172	N	N
Bifidobacterium scardovii	359	AJ307005	clade_172	N	N
Bifidobacterium sp. HM2	360	AB425276	clade_172	N	N
Bifidobacterium sp. HMLN12	361	JF519685	clade_172	N	N
Bifidobacterium sp. M45	362	HM626176	clade_172	N	N
Bifidobacterium sp. MSX5B	363	HQ616382	clade_172	N	N
Bifidobacterium sp. TM_7	364	AB218972	clade_172	N	N
Bifidobacterium thermophilum	365	DQ340557	clade_172	N	N
Leuconostoc citreum	1178	AM157444	clade_175	N	N
Leuconostoc lactis	1182	NR_040823	clade_175	N	N
Alicyclobacillus acidoterrestris	123	NR_040844	clade_179	N	N
Alicyclobacillus cycloheptanicus	125	NR_024754	clade_179	N	N
Acinetobacter baumannii	27	ACYQ01000014	clade_181	N	N
Acinetobacter calcoaceticus	28	AM157426	clade_181	N	N
Acinetobacter genomosp. C1	29	AY278636	clade_181	N	N
Acinetobacter haemolyticus	30	ADMT01000017	clade_181	N	N
Acinetobacter johnsonii	31	ACPL01000162	clade_181	N	N
Acinetobacter junii	32	ACPM01000135	clade_181	N	N

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Acinetobacter lwoffii	33	ACPN01000204	clade_181	N	N
Acinetobacter parvus	34	AIEB01000124	clade_181	N	N
Acinetobacter schindleri	36	NR_025412	clade_181	N	N
Acinetobacter sp. 56A1	37	GQ178049	clade_181	N	N
Acinetobacter sp. CIP 101934	38	JQ638573	clade_181	N	N
Acinetobacter sp. CIP 102143	39	JQ638578	clade_181	N	N
Acinetobacter sp. M16_22	41	HM366447	clade_181	N	N
Acinetobacter sp. RUH2624	42	ACQF01000094	clade_181	N	N
Acinetobacter sp. SH024	43	ADCH01000068	clade_181	N	N
Lactobacillus jensenii	1092	ACQD01000066	clade_182	N	N
Alcaligenes faecalis	119	AB680368	clade_183	N	N
Alcaligenes sp. CO14	120	DQ643040	clade_183	N	N
Alcaligenes sp. S3	121	HQ262549	clade_183	N	N
Oligella ureolytica	1366	NR_041998	clade_183	N	N
Oligella urethralis	1367	NR_041753	clade_183	N	N
Eikenella corrodens	784	ACEA01000028	clade_185	N	N
Kingella denitrificans	1019	AEWV01000047	clade_185	N	N
Kingella genomsp. P1 oral clone MB2_C20	1020	DQ003616	clade_185	N	N
Kingella kingae	1021	AFHS01000073	clade_185	N	N
Kingella oralis	1022	ACJW02000005	clade_185	N	N
Kingella sp. oral clone ID059	1023	AY349381	clade_185	N	N
Neisseria elongata	1330	ADBF01000003	clade_185	N	N
Neisseria genomsp. P2 oral clone MB5_P15	1332	DQ003630	clade_185	N	N
Neisseria sp. oral clone JC012	1345	AY349388	clade_185	N	N
Neisseria sp. SMC_A9199	1342	FJ763637	clade_185	N	N
Simonsiella muelleri	1731	ADCY01000105	clade_185	N	N
Corynebacterium glucuronolyticum	700	ABYP01000081	clade_193	N	N
Corynebacterium pyruviciproducens	716	FJ185225	clade_193	N	N
Rothia aerea	1649	DQ673320	clade_194	N	N
Rothia dentocariosa	1650	ADDW01000024	clade_194	N	N
Rothia sp. oral taxon 188	1653	GU470892	clade_194	N	N
Corynebacterium accolens	681	ACGD01000048	clade_195	N	N
Corynebacterium macginleyi	707	AB359393	clade_195	N	N
Corynebacterium pseudogenitalium	714	ABYQ01000237	clade_195	N	N
Corynebacterium tuberculostearicum	729	ACVP01000009	clade_195	N	N
Lactobacillus casei	1074	CP000423	clade_198	N	N
Lactobacillus paracasei	1106	ABQV01000067	clade_198	N	N
Lactobacillus zeae	1143	NR_037122	clade_198	N	N
Prevotella dentalis	1492	AB547678	clade_205	N	N
Prevotella sp. oral clone ASCG10	1529	AY923148	clade_206	N	N
Prevotella sp. oral clone HF050	1541	AY349399	clade_206	N	N
Prevotella sp. oral clone ID019	1542	AY349400	clade_206	N	N
Prevotella sp. oral clone IK062	1545	AY349402	clade_206	N	N
Prevotella genomsp. P9 oral clone MB7_G16	1499	DQ003633	clade_207	N	N
Prevotella sp. oral clone AU069	1531	AY005062	clade_207	N	N
Prevotella sp. oral clone CY006	1532	AY005063	clade_207	N	N
Prevotella sp. oral clone FL019	1534	AY349392	clade_207	N	N
Actinomyces genomsp. C1	56	AY278610	clade_212	N	N

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Actinomyces genomosp. C2	57	AY278611	clade_212	N	N
Actinomyces genomosp. P1 oral clone MB6_C03	58	DQ003632	clade_212	N	N
Actinomyces georgiae	59	GU561319	clade_212	N	N
Actinomyces israelii	60	AF479270	clade_212	N	N
Actinomyces massiliensis	61	AB545934	clade_212	N	N
Actinomyces meyeri	62	GU561321	clade_212	N	N
Actinomyces odontolyticus	66	ACYT01000123	clade_212	N	N
Actinomyces orihominis	68	AJ575186	clade_212	N	N
Actinomyces sp. CCUG 37290	71	AJ234058	clade_212	N	N
Actinomyces sp. ICM34	75	HQ616391	clade_212	N	N
Actinomyces sp. ICM41	76	HQ616392	clade_212	N	N
Actinomyces sp. ICM47	77	HQ616395	clade_212	N	N
Actinomyces sp. ICM54	78	HQ616398	clade_212	N	N
Actinomyces sp. oral clone IP081	87	AY349366	clade_212	N	N
Actinomyces sp. oral taxon 178	91	AEUH01000060	clade_212	N	N
Actinomyces sp. oral taxon 180	92	AEPP01000041	clade_212	N	N
Actinomyces sp. TeJ5	80	GU561315	clade_212	N	N
Haematobacter sp. BC14248	968	GU396991	clade_213	N	N
Paracoccus denitrificans	1424	CP000490	clade_213	N	N
Paracoccus marcusii	1425	NR_044922	clade_213	N	N
Grimontia hollisae	967	ADAQ01000013	clade_216	N	N
Shewanella putrefaciens	1723	CP002457	clade_216	N	N
Afipia genomosp. 4	111	EU117385	clade_217	N	N
Rhodopseudomonas palustris	1626	CP000301	clade_217	N	N
Methylobacterium extorquens	1223	NC_010172	clade_218	N	N
Methylobacterium podarium	1224	AY468363	clade_218	N	N
Methylobacterium radiotolerans	1225	GU294320	clade_218	N	N
Methylobacterium sp. 1sub	1226	AY468371	clade_218	N	N
Methylobacterium sp. MM4	1227	AY468370	clade_218	N	N
Achromobacter denitrificans	18	NR_042021	clade_224	N	N
Achromobacter piechaudii	19	ADMS01000149	clade_224	N	N
Achromobacter xylosoxidans	20	ACRC01000072	clade_224	N	N
Bordetella bronchiseptica	384	NR_025949	clade_224	N	OP
Bordetella holmesii	385	AB683187	clade_224	N	OP
Bordetella parapertussis	386	NR_025950	clade_224	N	OP
Bordetella pertussis	387	BX640418	clade_224	N	OP
Microbacterium chokolatum	1230	NR_037045	clade_225	N	N
Microbacterium flavescens	1231	EU714363	clade_225	N	N
Microbacterium lacticum	1233	EU714351	clade_225	N	N
Microbacterium oleivorans	1234	EU714381	clade_225	N	N
Microbacterium oxydans	1235	EU714348	clade_225	N	N
Microbacterium paraoxydans	1236	AJ491806	clade_225	N	N
Microbacterium phyllosphaerae	1237	EU714359	clade_225	N	N
Microbacterium schleiferi	1238	NR_044936	clade_225	N	N
Microbacterium sp. 768	1239	EU714378	clade_225	N	N
Microbacterium sp. oral strain C24KA	1240	AF287752	clade_225	N	N
Microbacterium testaceum	1241	EU714365	clade_225	N	N
Corynebacterium atypicum	686	NR_025540	clade_229	N	N
Corynebacterium mastitidis	708	AB359395	clade_229	N	N
Corynebacterium sp. NML 97_0186	725	GU238411	clade_229	N	N
Mycobacterium elephantis	1275	AF385898	clade_237	N	OP

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Mycobacterium paraterrae	1288	EU919229	clade_237	N	OP
Mycobacterium phlei	1289	GU142920	clade_237	N	OP
Mycobacterium sp. 1776	1293	EU703152	clade_237	N	N
Mycobacterium sp. 1781	1294	EU703147	clade_237	N	N
Mycobacterium sp. AQ1GA4	1297	HM210417	clade_237	N	N
Mycobacterium sp. GN_10546	1299	FJ497243	clade_237	N	N
Mycobacterium sp. GN_10827	1300	FJ497247	clade_237	N	N
Mycobacterium sp. GN_11124	1301	FJ652846	clade_237	N	N
Mycobacterium sp. GN_9188	1302	FJ497240	clade_237	N	N
Mycobacterium sp. GR_2007_210	1303	FJ555538	clade_237	N	N
Anoxybacillus contaminans	172	NR_029006	clade_238	N	N
Bacillus aeolius	195	NR_025557	clade_238	N	N
Brevibacterium frigoritolerans	422	NR_042639	clade_238	N	N
Geobacillus sp. E263	934	DQ647387	clade_238	N	N
Geobacillus sp. WCH70	935	CP001638	clade_238	N	N
Geobacillus thermocatenulatus	937	NR_043020	clade_238	N	N
Geobacillus thermoleovorans	940	NR_074931	clade_238	N	N
Lysinibacillus fusiformis	1192	FN397522	clade_238	N	N
Planomicrobium koreense	1468	NR_025011	clade_238	N	N
Sporosarcina newyorkensis	1754	AFPZ01000142	clade_238	N	N
Sporosarcina sp. 2681	1755	GU994081	clade_238	N	N
Ureibacillus composti	1968	NR_043746	clade_238	N	N
Ureibacillus suwonensis	1969	NR_043232	clade_238	N	N
Ureibacillus terrenus	1970	NR_025394	clade_238	N	N
Ureibacillus thermophilus	1971	NR_043747	clade_238	N	N
Ureibacillus thermosphaericus	1972	NR_040961	clade_238	N	N
Prevotella micans	1507	AGWK01000061	clade_239	N	N
Prevotella sp. oral clone DA058	1533	AY005065	clade_239	N	N
Prevotella sp. SEQ053	1523	JN867222	clade_239	N	N
Treponema socranskii	1937	NR_024868	clade_240	N	OP
Treponema sp. 6:H:D15A_4	1938	AY005083	clade_240	N	N
Treponema sp. oral taxon 265	1953	GU408850	clade_240	N	N
Treponema sp. oral taxon G85	1958	GU432215	clade_240	N	N
Porphyromonas endodontalis	1472	ACNN01000021	clade_241	N	N
Porphyromonas sp. oral clone BB134	1478	AY005068	clade_241	N	N
Porphyromonas sp. oral clone F016	1479	AY005069	clade_241	N	N
Porphyromonas sp. oral clone P2PB_52 P1	1480	AY207054	clade_241	N	N
Porphyromonas sp. oral clone P4GB_100 P2	1481	AY207057	clade_241	N	N
Acidovorax sp. 98_63833	26	AY258065	clade_245	N	N
Comamonadaceae bacterium NML000135	663	JN585335	clade_245	N	N
Comamonadaceae bacterium NML790751	664	JN585331	clade_245	N	N
Comamonadaceae bacterium NML910035	665	JN585332	clade_245	N	N
Comamonadaceae bacterium NML910036	666	JN585333	clade_245	N	N
Comamonas sp. NSP5	668	AB076850	clade_245	N	N
Delftia acidovorans	748	CP000884	clade_245	N	N
Xenophilus aerolatus	2018	JN585329	clade_245	N	N

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Oribacterium sp. oral taxon 078	1380	ACIQ02000009	clade_246	N	N
Oribacterium sp. oral taxon 102	1381	GQ422713	clade_246	N	N
Weissella cibaria	2007	NR_036924	clade_247	N	N
Weissella confusa	2008	NR_040816	clade_247	N	N
Weissella hellenica	2009	AB680902	clade_247	N	N
Weissella kandleri	2010	NR_044659	clade_247	N	N
Weissella koreensis	2011	NR_075058	clade_247	N	N
Weissella paramesenteroides	2012	ACKU01000017	clade_247	N	N
Weissella sp. K LDS 7.0701	2013	EU600924	clade_247	N	N
Mobiluncus curtisii	1251	AEPZ01000013	clade_249	N	N
Enhydrobacter aerosaccus	785	ACYI01000081	clade_256	N	N
Moraxella osloensis	1262	JN175341	clade_256	N	N
Moraxella sp. GM2	1264	JF837191	clade_256	N	N
Brevibacterium casei	420	JF951998	clade_257	N	N
Brevibacterium epidermidis	421	NR_029262	clade_257	N	N
Brevibacterium sanguinis	426	NR_028016	clade_257	N	N
Brevibacterium sp. H15	427	AB177640	clade_257	N	N
Acinetobacter radioresistens	35	ACVR01000010	clade_261	N	N
Lactobacillus alimentarius	1068	NR_044701	clade_263	N	N
Lactobacillus farciminis	1082	NR_044707	clade_263	N	N
Lactobacillus kimchii	1097	NR_025045	clade_263	N	N
Lactobacillus nodensis	1101	NR_041629	clade_263	N	N
Lactobacillus tuceti	1138	NR_042194	clade_263	N	N
Pseudomonas mendocina	1595	AAUL01000021	clade_265	N	N
Pseudomonas pseudoalcaligenes	1598	NR_037000	clade_265	N	N
Pseudomonas sp. NP522b	1602	EU723211	clade_265	N	N
Pseudomonas stutzeri	1603	AM905854	clade_265	N	N
Paenibacillus barcinonensis	1390	NR_042272	clade_270	N	N
Paenibacillus barengoltzii	1391	NR_042756	clade_270	N	N
Paenibacillus chibensis	1392	NR_040885	clade_270	N	N
Paenibacillus cookii	1393	NR_025372	clade_270	N	N
Paenibacillus durus	1394	NR_037017	clade_270	N	N
Paenibacillus glucanolyticus	1395	D78470	clade_270	N	N
Paenibacillus lactis	1396	NR_025739	clade_270	N	N
Paenibacillus pabuli	1398	NR_040853	clade_270	N	N
Paenibacillus popilliae	1400	NR_040888	clade_270	N	N
Paenibacillus sp. CIP 101062	1401	HM212646	clade_270	N	N
Paenibacillus sp. JC66	1404	JF824808	clade_270	N	N
Paenibacillus sp. R_27413	1405	HE586333	clade_270	N	N
Paenibacillus sp. R_27422	1406	HE586338	clade_270	N	N
Paenibacillus timonensis	1408	NR_042844	clade_270	N	N
Rothia mucilaginosa	1651	ACVO01000020	clade_271	N	N
Rothia nasimurium	1652	NR_025310	clade_271	N	N
Prevotella sp. oral taxon 302	1550	ACZK01000043	clade_280	N	N
Prevotella sp. oral taxon F68	1556	HM099652	clade_280	N	N
Prevotella tanneriae	1563	ACIJ02000018	clade_280	N	N
Prevotellaceae bacterium P4P_62 P1	1566	AY207061	clade_280	N	N
Porphyromonas asaccharolytica	1471	AENO01000048	clade_281	N	N
Porphyromonas gingivalis	1473	AE015924	clade_281	N	N
Porphyromonas macacae	1475	NR_025908	clade_281	N	N
Porphyromonas sp. UQD 301	1477	EU012301	clade_281	N	N
Porphyromonas uenonis	1482	ACLR01000152	clade_281	N	N

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Leptotrichia buccalis	1165	CP001685	clade_282	N	N
Leptotrichia hofstadii	1168	ACVB02000032	clade_282	N	N
Leptotrichia sp. oral clone HE012	1173	AY349386	clade_282	N	N
Leptotrichia sp. oral taxon 223	1176	GU408547	clade_282	N	N
Bacteroides fluxus	278	AFBN01000029	clade_285	N	N
Bacteroides helcogenes	281	CP002352	clade_285	N	N
Parabacteroides johnsonii	1419	ABYH01000014	clade_286	N	N
Parabacteroides merdae	1420	EU136685	clade_286	N	N
Treponema denticola	1926	ADEC01000002	clade_288	N	OP
Treponema genomsp. P5 oral clone MB3_P23	1929	DQ003624	clade_288	N	N
Treponema putidum	1935	AJ543428	clade_288	N	OP
Treponema sp. oral clone P2PB_53 P3	1942	AY207055	clade_288	N	N
Treponema sp. oral taxon 247	1949	GU408748	clade_288	N	N
Treponema sp. oral taxon 250	1950	GU408776	clade_288	N	N
Treponema sp. oral taxon 251	1951	GU408781	clade_288	N	N
Anaerococcus hydrogenalis	144	ABXA01000039	clade_289	N	N
Anaerococcus sp. 8404299	148	HM587318	clade_289	N	N
Anaerococcus sp. gpac215	156	AM176540	clade_289	N	N
Anaerococcus vaginalis	158	ACXU01000016	clade_289	N	N
Propionibacterium acidipropionici	1569	NC_019395	clade_290	N	N
Propionibacterium avidum	1571	AJ003055	clade_290	N	N
Propionibacterium granulosum	1573	FJ785716	clade_290	N	N
Propionibacterium jensenii	1574	NR_042269	clade_290	N	N
Propionibacterium propionicum	1575	NR_025277	clade_290	N	N
Propionibacterium sp. H456	1577	AB177643	clade_290	N	N
Propionibacterium thoenii	1581	NR_042270	clade_290	N	N
Bifidobacterium bifidum	349	ABQP01000027	clade_293	N	N
Leuconostoc mesenteroides	1183	ACKV01000113	clade_295	N	N
Leuconostoc pseudomesenteroides	1184	NR_040814	clade_295	N	N
Johnsonella ignava	1016	X87152	clade_298	N	N
Propionibacterium acnes	1570	ADJM01000010	clade_299	N	N
Propionibacterium sp. 434_HC2	1576	AFIL01000035	clade_299	N	N
Propionibacterium sp. LG	1578	AY354921	clade_299	N	N
Propionibacterium sp. S555a	1579	AB264622	clade_299	N	N
Alicyclobacillus sp. CCUG 53762	128	HE613268	clade_301	N	N
Actinomyces cardiffensis	53	GU470888	clade_303	N	N
Actinomyces funkei	55	HQ906497	clade_303	N	N
Actinomyces sp. HKU31	74	HQ335393	clade_303	N	N
Actinomyces sp. oral taxon C55	94	HM099646	clade_303	N	N
Kerstersia gyiorum	1018	NR_025669	clade_307	N	N
Pigmentiphaga daeguensis	1467	JN585327	clade_307	N	N
Aeromonas allosaccharophila	104	S39232	clade_308	N	N
Aeromonas enteropelogenes	105	X71121	clade_308	N	N
Aeromonas hydrophila	106	NC_008570	clade_308	N	N
Aeromonas jandaei	107	X60413	clade_308	N	N
Aeromonas salmonicida	108	NC_009348	clade_308	N	N
Aeromonas trota	109	X60415	clade_308	N	N
Aeromonas veronii	110	NR_044845	clade_308	N	N
Marvinbryantia formatexigens	1196	AJ505973	clade_309	N	N
Rhodobacter sp. oral taxon C30	1620	HM099648	clade_310	N	N
Rhodobacter sphaeroides	1621	CP000144	clade_310	N	N

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Lactobacillus antri	1071	ACLL01000037	clade_313	N	N
Lactobacillus coleohominis	1076	ACOH01000030	clade_313	N	N
Lactobacillus fermentum	1083	CP002033	clade_313	N	N
Lactobacillus gastricus	1085	AICN01000060	clade_313	N	N
Lactobacillus mucosae	1099	FR693800	clade_313	N	N
Lactobacillus oris	1103	AEKL01000077	clade_313	N	N
Lactobacillus pontis	1111	HM218420	clade_313	N	N
Lactobacillus reuteri	1112	ACGW02000012	clade_313	N	N
Lactobacillus sp. KLDS 1.0707	1127	EU600911	clade_313	N	N
Lactobacillus sp. KLDS 1.0709	1128	EU600913	clade_313	N	N
Lactobacillus sp. KLDS 1.0711	1129	EU600915	clade_313	N	N
Lactobacillus sp. KLDS 1.0713	1131	EU600917	clade_313	N	N
Lactobacillus sp. KLDS 1.0716	1132	EU600921	clade_313	N	N
Lactobacillus sp. KLDS 1.0718	1133	EU600922	clade_313	N	N
Lactobacillus sp. oral taxon 052	1137	GQ422710	clade_313	N	N
Lactobacillus vaginalis	1140	ACGV01000168	clade_313	N	N
Brevibacterium aurantiacum	419	NR_044854	clade_314	N	N
Brevibacterium linens	423	AJ315491	clade_314	N	N
Lactobacillus pentosus	1108	JN813103	clade_315	N	N
Lactobacillus plantarum	1110	ACGZ02000033	clade_315	N	N
Lactobacillus sp. KLDS 1.0702	1123	EU600906	clade_315	N	N
Lactobacillus sp. KLDS 1.0703	1124	EU600907	clade_315	N	N
Lactobacillus sp. KLDS 1.0704	1125	EU600908	clade_315	N	N
Lactobacillus sp. KLDS 1.0705	1126	EU600909	clade_315	N	N
Agrobacterium radiobacter	115	CP000628	clade_316	N	N
Agrobacterium tumefaciens	116	AJ389893	clade_316	N	N
Corynebacterium argenteorotense	685	EF463055	clade_317	N	N
Corynebacterium diphtheriae	693	NC_002935	clade_317	N	OP
Corynebacterium pseudotuberculosis	715	NR_037070	clade_317	N	N
Corynebacterium renale	717	NR_037069	clade_317	N	N
Corynebacterium ulcerans	731	NR_074467	clade_317	N	N
Aurantimonas coralicida	191	AY065627	clade_318	N	N
Aureimonas altamirensis	192	FN658986	clade_318	N	N
Lactobacillus acidipiscis	1066	NR_024718	clade_320	N	N
Lactobacillus salivarius	1117	AEBA01000145	clade_320	N	N
Lactobacillus sp. KLDS 1.0719	1134	EU600923	clade_320	N	N
Lactobacillus buchneri	1073	ACGH01000101	clade_321	N	N
Lactobacillus genomosp. C1	1086	AY278619	clade_321	N	N
Lactobacillus genomosp. C2	1087	AY278620	clade_321	N	N
Lactobacillus hilgardii	1089	ACGP01000200	clade_321	N	N
Lactobacillus kefir	1096	NR_042230	clade_321	N	N
Lactobacillus parabuchneri	1105	NR_041294	clade_321	N	N
Lactobacillus parakefir	1107	NR_029039	clade_321	N	N
Lactobacillus curvatus	1079	NR_042437	clade_322	N	N
Lactobacillus sakei	1116	DQ989236	clade_322	N	N
Aneurinibacillus aneurinilyticus	167	AB101592	clade_323	N	N
Aneurinibacillus danicus	168	NR_028657	clade_323	N	N
Aneurinibacillus migulanus	169	NR_036799	clade_323	N	N
Aneurinibacillus terranovensis	170	NR_042271	clade_323	N	N
Staphylococcus aureus	1757	CP002643	clade_325	N	Category -B
Staphylococcus auricularis	1758	JQ624774	clade_325	N	N
Staphylococcus capitis	1759	ACFR01000029	clade_325	N	N

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Staphylococcus caprae	1760	ACRH01000033	clade_325	N	N
Staphylococcus carnosus	1761	NR_075003	clade_325	N	N
Staphylococcus cohnii	1762	JN175375	clade_325	N	N
Staphylococcus condimentii	1763	NR_029345	clade_325	N	N
Staphylococcus epidermidis	1764	ACHE01000056	clade_325	N	N
Staphylococcus equorum	1765	NR_027520	clade_325	N	N
Staphylococcus haemolyticus	1767	NC_007168	clade_325	N	N
Staphylococcus hominis	1768	AM157418	clade_325	N	N
Staphylococcus lugdunensis	1769	AEQA01000024	clade_325	N	N
Staphylococcus pasteurii	1770	FJ189773	clade_325	N	N
Staphylococcus pseudintermedius	1771	CP002439	clade_325	N	N
Staphylococcus saccharolyticus	1772	NR_029158	clade_325	N	N
Staphylococcus saprophyticus	1773	NC_007350	clade_325	N	N
Staphylococcus sp. clone bottae7	1777	AF467424	clade_325	N	N
Staphylococcus sp. H292	1775	AB177642	clade_325	N	N
Staphylococcus sp. H780	1776	AB177644	clade_325	N	N
Staphylococcus succinus	1778	NR_028667	clade_325	N	N
Staphylococcus warneri	1780	ACPZ01000009	clade_325	N	N
Staphylococcus xylosus	1781	AY395016	clade_325	N	N
Cardiobacterium hominis	490	ACKY01000036	clade_326	N	N
Cardiobacterium valvarum	491	NR_028847	clade_326	N	N
Pseudomonas fluorescens	1593	AY622220	clade_326	N	N
Pseudomonas gessardii	1594	FJ943496	clade_326	N	N
Pseudomonas monteilii	1596	NR_024910	clade_326	N	N
Pseudomonas poae	1597	GU188951	clade_326	N	N
Pseudomonas putida	1599	AF094741	clade_326	N	N
Pseudomonas sp. G1229	1601	DQ910482	clade_326	N	N
Pseudomonas tolaasii	1604	AF320988	clade_326	N	N
Pseudomonas viridiflava	1605	NR_042764	clade_326	N	N
Listeria grayi	1185	ACCR02000003	clade_328	N	OP
Listeria innocua	1186	JF967625	clade_328	N	N
Listeria ivanovii	1187	X56151	clade_328	N	N
Listeria monocytogenes	1188	CP002003	clade_328	N	Category -B
Listeria welshimeri	1189	AM263198	clade_328	N	OP
Capnocytophaga sp. oral clone ASCH05	484	AY923149	clade_333	N	N
Capnocytophaga sputigena	489	ABZV01000054	clade_333	N	N
Leptotrichia genomosp. C1	1166	AY278621	clade_334	N	N
Leptotrichia shahii	1169	AY029806	clade_334	N	N
Leptotrichia sp. neutropenicPatient	1170	AF189244	clade_334	N	N
Leptotrichia sp. oral clone GT018	1171	AY349384	clade_334	N	N
Leptotrichia sp. oral clone GT020	1172	AY349385	clade_334	N	N
Bacteroides sp. 20_3	296	ACRQ01000064	clade_335	N	N
Bacteroides sp. 3_1_19	307	ADCJ01000062	clade_335	N	N
Bacteroides sp. 3_2_5	311	ACIB01000079	clade_335	N	N
Parabacteroides distasonis	1416	CP000140	clade_335	N	N
Parabacteroides goldsteinii	1417	AY974070	clade_335	N	N
Parabacteroides gordonii	1418	AB470344	clade_335	N	N
Parabacteroides sp. D13	1421	ACPW01000017	clade_335	N	N
Capnocytophaga genomosp. C1	477	AY278613	clade_336	N	N
Capnocytophaga ochracea	480	AEOH01000054	clade_336	N	N
Capnocytophaga sp. GEJ8	481	GU561335	clade_336	N	N
Capnocytophaga sp. oral strain	486	AY005077	clade_336	N	N

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A47ROY					
Capnocytophaga sp. S1b	482	U42009	clade_336	N	N
Paraprevotella clara	1426	AFFY01000068	clade_336	N	N
Bacteroides heparinolyticus	282	JN867284	clade_338	N	N
Prevotella heparinolytica	1500	GQ422742	clade_338	N	N
Treponema genomsp. P4 oral clone MB2_G19	1928	DQ003618	clade_339	N	N
Treponema genomsp. P6 oral clone MB4_G11	1930	DQ003625	clade_339	N	N
Treponema sp. oral taxon 254	1952	GU408803	clade_339	N	N
Treponema sp. oral taxon 508	1956	GU413616	clade_339	N	N
Treponema sp. oral taxon 518	1957	GU413640	clade_339	N	N
Chlamydia muridarum	502	AE002160	clade_341	N	OP
Chlamydia trachomatis	504	U68443	clade_341	N	OP
Chlamydia psittaci	503	NR_036864	clade_342	N	Category -B
Chlamydophila pneumoniae	509	NC_002179	clade_342	N	OP
Chlamydophila psittaci	510	D85712	clade_342	N	OP
Anaerococcus octavius	146	NR_026360	clade_343	N	N
Anaerococcus sp. 8405254	149	HM587319	clade_343	N	N
Anaerococcus sp. 9401487	150	HM587322	clade_343	N	N
Anaerococcus sp. 9403502	151	HM587325	clade_343	N	N
Gardnerella vaginalis	923	CP001849	clade_344	N	N
Campylobacter lari	466	CP000932	clade_346	N	OP
Anaerobiospirillum succiniciproducens	142	NR_026075	clade_347	N	N
Anaerobiospirillum thomasi	143	AJ420985	clade_347	N	N
Ruminobacter amylophilus	1654	NR_026450	clade_347	N	N
Succinatimonas hippei	1897	AEVO01000027	clade_347	N	N
Actinomyces europaeus	54	NR_026363	clade_348	N	N
Actinomyces sp. oral clone GU009	82	AY349361	clade_348	N	N
Moraxella catarrhalis	1260	CP002005	clade_349	N	N
Moraxella lincolni	1261	FR822735	clade_349	N	N
Moraxella sp. 16285	1263	JF682466	clade_349	N	N
Psychrobacter sp. 13983	1613	HM212668	clade_349	N	N
Actinobaculum massiliae	49	AF487679	clade_350	N	N
Actinobaculum schaalii	50	AY957507	clade_350	N	N
Actinobaculum sp. BM#101342	51	AY282578	clade_350	N	N
Actinobaculum sp. P2P_19 P1	52	AY207066	clade_350	N	N
Actinomyces sp. oral clone IO076	84	AY349363	clade_350	N	N
Actinomyces sp. oral taxon 848	93	ACUY01000072	clade_350	N	N
Actinomyces neuii	65	X71862	clade_352	N	N
Mobiluncus mulieris	1252	ACKW01000035	clade_352	N	N
Blastomonas natatoria	372	NR_040824	clade_356	N	N
Novosphingobium aromaticivorans	1357	AAAV03000008	clade_356	N	N
Sphingomonas sp. oral clone FI012	1745	AY349411	clade_356	N	N
Sphingopyxis alaskensis	1749	CP000356	clade_356	N	N
Oxalobacter formigenes	1389	ACDQ01000020	clade_357	N	N
Veillonella atypica	1974	AEDS01000059	clade_358	N	N
Veillonella dispar	1975	ACIK02000021	clade_358	N	N
Veillonella genomsp. P1 oral clone MB5_P17	1976	DQ003631	clade_358	N	N
Veillonella parvula	1978	ADFU01000009	clade_358	N	N

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Veillonella sp. 3_1_44	1979	ADCV01000019	clade_358	N	N
Veillonella sp. 6_1_27	1980	ADCW01000016	clade_358	N	N
Veillonella sp. ACP1	1981	HQ616359	clade_358	N	N
Veillonella sp. AS16	1982	HQ616365	clade_358	N	N
Veillonella sp. BS32b	1983	HQ616368	clade_358	N	N
Veillonella sp. ICM51a	1984	HQ616396	clade_358	N	N
Veillonella sp. MSA12	1985	HQ616381	clade_358	N	N
Veillonella sp. NVG 100cf	1986	EF108443	clade_358	N	N
Veillonella sp. OK11	1987	JN695650	clade_358	N	N
Veillonella sp. oral clone ASCG01	1990	AY923144	clade_358	N	N
Veillonella sp. oral clone ASCG02	1991	AY953257	clade_358	N	N
Veillonella sp. oral clone OH1A	1992	AY947495	clade_358	N	N
Veillonella sp. oral taxon 158	1993	AENU01000007	clade_358	N	N
Kocuria marina	1040	GQ260086	clade_365	N	N
Kocuria rhizophila	1042	AY030315	clade_365	N	N
Kocuria rosea	1043	X87756	clade_365	N	N
Kocuria varians	1044	AF542074	clade_365	N	N
Clostridiaceae bacterium END_2	531	EF451053	clade_368	N	N
Micrococcus antarcticus	1242	NR_025285	clade_371	N	N
Micrococcus luteus	1243	NR_075062	clade_371	N	N
Micrococcus lylae	1244	NR_026200	clade_371	N	N
Micrococcus sp. 185	1245	EU714334	clade_371	N	N
Lactobacillus brevis	1072	EU194349	clade_372	N	N
Lactobacillus parabrevis	1104	NR_042456	clade_372	N	N
Pediococcus acidilactici	1436	ACXB01000026	clade_372	N	N
Pediococcus pentosaceus	1437	NR_075052	clade_372	N	N
Lactobacillus dextrinicus	1081	NR_036861	clade_373	N	N
Lactobacillus perolens	1109	NR_029360	clade_373	N	N
Lactobacillus rhamnosus	1113	ABWJ01000068	clade_373	N	N
Lactobacillus saniviri	1118	AB602569	clade_373	N	N
Lactobacillus sp. BT6	1121	HQ616370	clade_373	N	N
Mycobacterium mageritense	1282	FR798914	clade_374	N	OP
Mycobacterium neoaurum	1286	AF268445	clade_374	N	OP
Mycobacterium smegmatis	1291	CP000480	clade_374	N	OP
Mycobacterium sp. HE5	1304	AJ012738	clade_374	N	N
Dysgonomonas gadei	775	ADLV01000001	clade_377	N	N
Dysgonomonas mossii	776	ADLW01000023	clade_377	N	N
Porphyromonas levii	1474	NR_025907	clade_377	N	N
Porphyromonas somerae	1476	AB547667	clade_377	N	N
Bacteroides barnesi	267	NR_041446	clade_378	N	N
Bacteroides coprocola	272	ABIY02000050	clade_378	N	N
Bacteroides coprophilus	273	ACBW01000012	clade_378	N	N
Bacteroides dorei	274	ABWZ01000093	clade_378	N	N
Bacteroides massiliensis	284	AB200226	clade_378	N	N
Bacteroides plebeius	289	AB200218	clade_378	N	N
Bacteroides sp. 3_1_33FAA	309	ACPS01000085	clade_378	N	N
Bacteroides sp. 3_1_40A	310	ACRT01000136	clade_378	N	N
Bacteroides sp. 4_3_47FAA	313	ACDR02000029	clade_378	N	N
Bacteroides sp. 9_1_42FAA	314	ACAA01000096	clade_378	N	N
Bacteroides sp. NB_8	323	AB117565	clade_378	N	N
Bacteroides vulgatus	331	CP000139	clade_378	N	N
Bacteroides ovatus	287	ACWH01000036	clade_38	N	N
Bacteroides sp. 1_1_30	294	ADCL01000128	clade_38	N	N

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Bacteroides sp. 2_1_22	297	ACPQ01000117	clade_38	N	N
Bacteroides sp. 2_2_4	299	ABZZ01000168	clade_38	N	N
Bacteroides sp. 3_1_23	308	ACRS01000081	clade_38	N	N
Bacteroides sp. D1	318	ACAB02000030	clade_38	N	N
Bacteroides sp. D2	321	ACGA01000077	clade_38	N	N
Bacteroides sp. D22	320	ADCK01000151	clade_38	N	N
Bacteroides xylanisolvens	332	ADKP01000087	clade_38	N	N
Treponema lecithinolyticum	1931	NR_026247	clade_380	N	OP
Treponema parvum	1933	AF302937	clade_380	N	OP
Treponema sp. oral clone JU025	1940	AY349417	clade_380	N	N
Treponema sp. oral taxon 270	1954	GQ422733	clade_380	N	N
Parascardovia denticolens	1428	ADEB01000020	clade_381	N	N
Scardovia inopinata	1688	AB029087	clade_381	N	N
Scardovia wiggisiae	1689	AY278626	clade_381	N	N
Clostridiales bacterium 9400853	533	HM587320	clade_384	N	N
Mogibacterium diversum	1254	NR_027191	clade_384	N	N
Mogibacterium neglectum	1255	NR_027203	clade_384	N	N
Mogibacterium pumilum	1256	NR_028608	clade_384	N	N
Mogibacterium timidum	1257	Z36296	clade_384	N	N
Borrelia burgdorferi	389	ABG101000001	clade_386	N	OP
Borrelia garinii	392	ABJV01000001	clade_386	N	OP
Borrelia sp. NE49	397	AJ224142	clade_386	N	OP
Caldimonas manganoxidans	457	NR_040787	clade_387	N	N
Comamonadaceae bacterium oral taxon F47	667	HM099651	clade_387	N	N
Lautropia mirabilis	1149	AEQP01000026	clade_387	N	N
Lautropia sp. oral clone AP009	1150	AY005030	clade_387	N	N
Peptoniphilus asaccharolyticus	1441	D14145	clade_389	N	N
Peptoniphilus duerdenii	1442	EU526290	clade_389	N	N
Peptoniphilus harei	1443	NR_026358	clade_389	N	N
Peptoniphilus indolicus	1444	AY153431	clade_389	N	N
Peptoniphilus lacrimalis	1446	ADDO01000050	clade_389	N	N
Peptoniphilus sp. gpac077	1450	AM176527	clade_389	N	N
Peptoniphilus sp. JC140	1447	JF824803	clade_389	N	N
Peptoniphilus sp. oral taxon 386	1452	ADCS01000031	clade_389	N	N
Peptoniphilus sp. oral taxon 836	1453	AEAA01000090	clade_389	N	N
Peptostreptococcaceae bacterium ph1	1454	JN837495	clade_389	N	N
Dialister pneumosintes	765	HM596297	clade_390	N	N
Dialister sp. oral taxon 502	767	GQ422739	clade_390	N	N
Cupriavidus metallidurans	741	GU230889	clade_391	N	N
Herbaspirillum seropedicae	1001	CP002039	clade_391	N	N
Herbaspirillum sp. JC206	1002	JN657219	clade_391	N	N
Janthinobacterium sp. SY12	1015	EF455530	clade_391	N	N
Massilia sp. CCUG 43427A	1197	FR773700	clade_391	N	N
Ralstonia pickettii	1615	NC_010682	clade_391	N	N
Ralstonia sp. 5_7_47FAA	1616	ACUF01000076	clade_391	N	N
Francisella novicida	889	ABSS01000002	clade_392	N	N
Francisella philomiragia	890	AY928394	clade_392	N	N
Francisella tularensis	891	ABAZ01000082	clade_392	N	Category -A
Ignatzschineria indica	1009	HQ823562	clade_392	N	N
Ignatzschineria sp. NML 95_0260	1010	HQ823559	clade_392	N	N
Streptococcus mutans	1814	AP010655	clade_394	N	N

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<i>Lactobacillus gasseri</i>	1084	ACOZ01000018	clade_398	N	N
<i>Lactobacillus hominis</i>	1090	FR681902	clade_398	N	N
<i>Lactobacillus iners</i>	1091	AEKJ01000002	clade_398	N	N
<i>Lactobacillus johnsonii</i>	1093	AE017198	clade_398	N	N
<i>Lactobacillus senioris</i>	1119	AB602570	clade_398	N	N
<i>Lactobacillus</i> sp. oral clone HT002	1135	AY349382	clade_398	N	N
<i>Weissella beninensis</i>	2006	EU439435	clade_398	N	N
<i>Sphingomonas echinoides</i>	1744	NR_024700	clade_399	N	N
<i>Sphingomonas</i> sp. oral taxon A09	1747	HM099639	clade_399	N	N
<i>Sphingomonas</i> sp. oral taxon F71	1748	HM099645	clade_399	N	N
<i>Zymomonas mobilis</i>	2032	NR_074274	clade_399	N	N
<i>Arcanobacterium haemolyticum</i>	174	NR_025347	clade_400	N	N
<i>Arcanobacterium pyogenes</i>	175	GU585578	clade_400	N	N
<i>Trueperella pyogenes</i>	1962	NR_044858	clade_400	N	N
<i>Lactococcus garvieae</i>	1144	AF061005	clade_401	N	N
<i>Lactococcus lactis</i>	1145	CP002365	clade_401	N	N
<i>Brevibacterium mcbrellneri</i>	424	ADNU01000076	clade_402	N	N
<i>Brevibacterium paucivorans</i>	425	EU086796	clade_402	N	N
<i>Brevibacterium</i> sp. JC43	428	JF824806	clade_402	N	N
<i>Selenomonas artemidis</i>	1692	HM596274	clade_403	N	N
<i>Selenomonas</i> sp. FOBRC9	1704	HQ616378	clade_403	N	N
<i>Selenomonas</i> sp. oral taxon 137	1715	AENV01000007	clade_403	N	N
<i>Desmospora activa</i>	751	AM940019	clade_404	N	N
<i>Desmospora</i> sp. 8437	752	AFHT01000143	clade_404	N	N
<i>Paenibacillus</i> sp. oral taxon F45	1407	HM099647	clade_404	N	N
<i>Corynebacterium ammoniagenes</i>	682	ADNS01000011	clade_405	N	N
<i>Corynebacterium aurimucosum</i>	687	ACLH01000041	clade_405	N	N
<i>Corynebacterium bovis</i>	688	AF537590	clade_405	N	N
<i>Corynebacterium canis</i>	689	GQ871934	clade_405	N	N
<i>Corynebacterium casei</i>	690	NR_025101	clade_405	N	N
<i>Corynebacterium durum</i>	694	Z97069	clade_405	N	N
<i>Corynebacterium efficiens</i>	695	ACLI01000121	clade_405	N	N
<i>Corynebacterium falsenii</i>	696	Y13024	clade_405	N	N
<i>Corynebacterium flavescens</i>	697	NR_037040	clade_405	N	N
<i>Corynebacterium glutamicum</i>	701	BA000036	clade_405	N	N
<i>Corynebacterium jeikeium</i>	704	ACYW01000001	clade_405	N	OP
<i>Corynebacterium kroppenstedtii</i>	705	NR_026380	clade_405	N	N
<i>Corynebacterium lipophiloflavum</i>	706	ACHJ01000075	clade_405	N	N
<i>Corynebacterium matruchotii</i>	709	ACSH02000003	clade_405	N	N
<i>Corynebacterium minutissimum</i>	710	X82064	clade_405	N	N
<i>Corynebacterium resistens</i>	718	ADGN01000058	clade_405	N	N
<i>Corynebacterium simulans</i>	720	AF537604	clade_405	N	N
<i>Corynebacterium singulare</i>	721	NR_026394	clade_405	N	N
<i>Corynebacterium</i> sp. 1 ex sheep	722	Y13427	clade_405	N	N
<i>Corynebacterium</i> sp. NML 99_0018	726	GU238413	clade_405	N	N
<i>Corynebacterium striatum</i>	727	ACGE01000001	clade_405	N	OP
<i>Corynebacterium urealyticum</i>	732	X81913	clade_405	N	OP
<i>Corynebacterium variabile</i>	734	NR_025314	clade_405	N	N
<i>Aerococcus sanguinicola</i>	98	AY837833	clade_407	N	N
<i>Aerococcus urinae</i>	99	CP002512	clade_407	N	N
<i>Aerococcus urinaeequi</i>	100	NR_043443	clade_407	N	N
<i>Aerococcus viridans</i>	101	ADNT01000041	clade_407	N	N

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<i>Fusobacterium naviforme</i>	898	HQ223106	clade_408	N	N
<i>Moryella indoligenes</i>	1268	AF527773	clade_408	N	N
<i>Selenomonas genomosp. P5</i>	1697	AY341820	clade_410	N	N
<i>Selenomonas sp. oral clone IQ048</i>	1710	AY349408	clade_410	N	N
<i>Selenomonas sputigena</i>	1717	ACKP02000033	clade_410	N	N
<i>Hyphomicrobium sulfonivorans</i>	1007	AY468372	clade_411	N	N
<i>Methylocella silvestris</i>	1228	NR_074237	clade_411	N	N
<i>Legionella pneumophila</i>	1153	NC_002942	clade_412	N	OP
<i>Lactobacillus coryniformis</i>	1077	NR_044705	clade_413	N	N
<i>Arthrobacter agilis</i>	178	NR_026198	clade_414	N	N
<i>Arthrobacter arilaitensis</i>	179	NR_074608	clade_414	N	N
<i>Arthrobacter bergerei</i>	180	NR_025612	clade_414	N	N
<i>Arthrobacter globiformis</i>	181	NR_026187	clade_414	N	N
<i>Arthrobacter nicotianae</i>	182	NR_026190	clade_414	N	N
<i>Mycobacterium abscessus</i>	1269	AGQU01000002	clade_418	N	OP
<i>Mycobacterium chelonae</i>	1273	AB548610	clade_418	N	OP
<i>Bacteroides salanitronis</i>	291	CP002530	clade_419	N	N
<i>Paraprevotella xylaniphila</i>	1427	AFBR01000011	clade_419	N	N
<i>Barnesiella intestinihominis</i>	336	AB370251	clade_420	N	N
<i>Barnesiella viscericola</i>	337	NR_041508	clade_420	N	N
<i>Parabacteroides sp. NS31_3</i>	1422	JN029805	clade_420	N	N
<i>Porphyromonadaceae bacterium NML 060648</i>	1470	EF184292	clade_420	N	N
<i>Tannerella forsythia</i>	1913	CP003191	clade_420	N	N
<i>Tannerella sp. 6_1_58FAA_CT1</i>	1914	ACWX01000068	clade_420	N	N
<i>Mycoplasma amphoriforme</i>	1311	AY531656	clade_421	N	N
<i>Mycoplasma genitalium</i>	1317	L43967	clade_421	N	N
<i>Mycoplasma pneumoniae</i>	1322	NC_000912	clade_421	N	N
<i>Mycoplasma penetrans</i>	1321	NC_004432	clade_422	N	N
<i>Ureaplasma parvum</i>	1966	AE002127	clade_422	N	N
<i>Ureaplasma urealyticum</i>	1967	AAYN01000002	clade_422	N	N
<i>Treponema genomosp. P1</i>	1927	AY341822	clade_425	N	N
<i>Treponema sp. oral taxon 228</i>	1943	GU408580	clade_425	N	N
<i>Treponema sp. oral taxon 230</i>	1944	GU408603	clade_425	N	N
<i>Treponema sp. oral taxon 231</i>	1945	GU408631	clade_425	N	N
<i>Treponema sp. oral taxon 232</i>	1946	GU408646	clade_425	N	N
<i>Treponema sp. oral taxon 235</i>	1947	GU408673	clade_425	N	N
<i>Treponema sp. ovine footrot</i>	1959	AJ010951	clade_425	N	N
<i>Treponema vincentii</i>	1960	ACYH01000036	clade_425	N	OP
<i>Burkholderiales bacterium 1_1_47</i>	452	ADCQ01000066	clade_432	N	OP
<i>Parasutterella excrementihominis</i>	1429	AFBP01000029	clade_432	N	N
<i>Parasutterella secunda</i>	1430	AB491209	clade_432	N	N
<i>Sutterella morbirenis</i>	1898	AJ832129	clade_432	N	N
<i>Sutterella sanguinus</i>	1900	AJ748647	clade_432	N	N
<i>Sutterella sp. YIT 12072</i>	1901	AB491210	clade_432	N	N
<i>Sutterella stercoricanis</i>	1902	NR_025600	clade_432	N	N
<i>Sutterella wadsworthensis</i>	1903	ADMF01000048	clade_432	N	N
<i>Propionibacterium freudenreichii</i>	1572	NR_036972	clade_433	N	N
<i>Propionibacterium sp. oral taxon 192</i>	1580	GQ422728	clade_433	N	N
<i>Tessaracoccus sp. oral taxon F04</i>	1917	HM099640	clade_433	N	N
<i>Peptoniphilus ivorii</i>	1445	Y07840	clade_434	N	N
<i>Peptoniphilus sp. gpac007</i>	1448	AM176517	clade_434	N	N
<i>Peptoniphilus sp. gpac018A</i>	1449	AM176519	clade_434	N	N

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Peptoniphilus sp. gpac148	1451	AM176535	clade_434	N	N
Flexispira rappini	887	AY126479	clade_436	N	N
Helicobacter bilis	993	ACDN01000023	clade_436	N	N
Helicobacter cinaedi	995	ABQT01000054	clade_436	N	N
Helicobacter sp. None	998	U44756	clade_436	N	N
Brevundimonas subvibrioides	429	CP002102	clade_438	N	N
Hyphomonas neptunium	1008	NR_074092	clade_438	N	N
Phenylobacterium zucineum	1465	AY628697	clade_438	N	N
Streptococcus downei	1793	AEKN01000002	clade_441	N	N
Streptococcus sp. SHV515	1848	Y07601	clade_441	N	N
Acinetobacter sp. CIP 53.82	40	JQ638584	clade_443	N	N
Halomonas elongata	990	NR_074782	clade_443	N	N
Halomonas johnsoniae	991	FR775979	clade_443	N	N
Butyrivibrio fibrisolvens	456	U41172	clade_444	N	N
Roseburia sp. 11SE37	1640	FM954975	clade_444	N	N
Roseburia sp. 11SE38	1641	FM954976	clade_444	N	N
Shuttleworthia satelles	1728	ACIP02000004	clade_444	N	N
Shuttleworthia sp. MSX8B	1729	HQ616383	clade_444	N	N
Shuttleworthia sp. oral taxon G69	1730	GU432167	clade_444	N	N
Bdellovibrio sp. MPA	344	AY294215	clade_445	N	N
Desulfobulbus sp. oral clone CH031	755	AY005036	clade_445	N	N
Desulfovibrio desulfuricans	757	DQ092636	clade_445	N	N
Desulfovibrio fairfieldensis	758	U42221	clade_445	N	N
Desulfovibrio piger	759	AF192152	clade_445	N	N
Desulfovibrio sp. 3_1_syn3	760	ADDR01000239	clade_445	N	N
Geobacter bemidjensis	941	CP001124	clade_445	N	N
Brachybacterium alimentarium	401	NR_026269	clade_446	N	N
Brachybacterium conglomeratum	402	AB537169	clade_446	N	N
Brachybacterium tyrofermentans	403	NR_026272	clade_446	N	N
Dermabacter hominis	749	FJ263375	clade_446	N	N
Aneurinibacillus thermoaerophilus	171	NR_029303	clade_448	N	N
Brevibacillus agri	409	NR_040983	clade_448	N	N
Brevibacillus centrosporus	411	NR_043414	clade_448	N	N
Brevibacillus choshinensis	412	NR_040980	clade_448	N	N
Brevibacillus invocatus	413	NR_041836	clade_448	N	N
Brevibacillus parabrevis	415	NR_040981	clade_448	N	N
Brevibacillus reuszeri	416	NR_040982	clade_448	N	N
Brevibacillus sp. pH8	417	JN837488	clade_448	N	N
Brevibacillus thermoruber	418	NR_026514	clade_448	N	N
Lactobacillus murinus	1100	NR_042231	clade_449	N	N
Lactobacillus oeni	1102	NR_043095	clade_449	N	N
Lactobacillus ruminis	1115	ACGS02000043	clade_449	N	N
Lactobacillus vini	1141	NR_042196	clade_449	N	N
Gemella haemolysans	924	ACDZ02000012	clade_450	N	N
Gemella morbillorum	925	NR_025904	clade_450	N	N
Gemella morbillorum	926	ACRX01000010	clade_450	N	N
Gemella sanguinis	927	ACRY01000057	clade_450	N	N
Gemella sp. oral clone ASCE02	929	AY923133	clade_450	N	N
Gemella sp. oral clone ASCF04	930	AY923139	clade_450	N	N
Gemella sp. oral clone ASCF12	931	AY923143	clade_450	N	N
Gemella sp. WAL 1945J	928	EU427463	clade_450	N	N
Sporolactobacillus nakayamae	1753	NR_042247	clade_451	N	N

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Gluconacetobacter entanii	945	NR_028909	clade_452	N	N
Gluconacetobacter europaeus	946	NR_026513	clade_452	N	N
Gluconacetobacter hansenii	947	NR_026133	clade_452	N	N
Gluconacetobacter oboediens	949	NR_041295	clade_452	N	N
Gluconacetobacter xylinus	950	NR_074338	clade_452	N	N
Auritibacter ignavus	193	FN554542	clade_453	N	N
Dermacoccus sp. Ellin185	750	AEIQ01000090	clade_453	N	N
Janibacter limosus	1013	NR_026362	clade_453	N	N
Janibacter melonis	1014	EF063716	clade_453	N	N
Acetobacter aceti	7	NR_026121	clade_454	N	N
Acetobacter fabarum	8	NR_042678	clade_454	N	N
Acetobacter lovaniensis	9	NR_040832	clade_454	N	N
Acetobacter malorum	10	NR_025513	clade_454	N	N
Acetobacter orientalis	11	NR_028625	clade_454	N	N
Acetobacter pasteurianus	12	NR_026107	clade_454	N	N
Acetobacter pomorum	13	NR_042112	clade_454	N	N
Acetobacter syzygii	14	NR_040868	clade_454	N	N
Acetobacter tropicalis	15	NR_036881	clade_454	N	N
Gluconacetobacter azotocaptans	943	NR_028767	clade_454	N	N
Gluconacetobacter diazotrophicus	944	NR_074292	clade_454	N	N
Gluconacetobacter johannae	948	NR_024959	clade_454	N	N
Nocardia brasiliensis	1351	AIHV01000038	clade_455	N	N
Nocardia cyriacigeorgica	1352	HQ009486	clade_455	N	N
Nocardia puris	1354	NR_028994	clade_455	N	N
Nocardia sp. 01_Je_025	1355	GU574059	clade_455	N	N
Rhodococcus equi	1623	ADNW01000058	clade_455	N	N
Oceanobacillus caeni	1358	NR_041533	clade_456	N	N
Oceanobacillus sp. Ndiop	1359	CAER01000083	clade_456	N	N
Ornithinibacillus bavariensis	1384	NR_044923	clade_456	N	N
Ornithinibacillus sp. 7_10AIA	1385	FN397526	clade_456	N	N
Virgibacillus proomii	2005	NR_025308	clade_456	N	N
Corynebacterium amycolatum	683	ABZU01000033	clade_457	N	OP
Corynebacterium hansenii	702	AM946639	clade_457	N	N
Corynebacterium xerosis	735	FN179330	clade_457	N	OP
Staphylococcaceae bacterium NML 92_0017	1756	AY841362	clade_458	N	N
Staphylococcus fleurettii	1766	NR_041326	clade_458	N	N
Staphylococcus sciuri	1774	NR_025520	clade_458	N	N
Staphylococcus vitulinus	1779	NR_024670	clade_458	N	N
Stenotrophomonas maltophilia	1782	AAVZ01000005	clade_459	N	N
Stenotrophomonas sp. FG_6	1783	EF017810	clade_459	N	N
Mycobacterium africanum	1270	AF480605	clade_46	N	OP
Mycobacterium alsiensis	1271	AJ938169	clade_46	N	OP
Mycobacterium avium	1272	CP000479	clade_46	N	OP
Mycobacterium colombiense	1274	AM062764	clade_46	N	OP
Mycobacterium gordonae	1276	GU142930	clade_46	N	OP
Mycobacterium intracellulare	1277	GQ153276	clade_46	N	OP
Mycobacterium kansasii	1278	AF480601	clade_46	N	OP
Mycobacterium lacus	1279	NR_025175	clade_46	N	OP
Mycobacterium leprae	1280	FM211192	clade_46	N	OP
Mycobacterium lepromatosis	1281	EU203590	clade_46	N	OP
Mycobacterium mantenii	1283	FJ042897	clade_46	N	OP
Mycobacterium marinum	1284	NC_010612	clade_46	N	OP

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Mycobacterium microti	1285	NR_025234	clade_46	N	OP
Mycobacterium parascrofulaceum	1287	ADNV01000350	clade_46	N	OP
Mycobacterium seoulense	1290	DQ536403	clade_46	N	OP
Mycobacterium sp. 1761	1292	EU703150	clade_46	N	N
Mycobacterium sp. 1791	1295	EU703148	clade_46	N	N
Mycobacterium sp. 1797	1296	EU703149	clade_46	N	N
Mycobacterium sp. B10_07.09.0206	1298	HQ174245	clade_46	N	N
Mycobacterium sp. NLA001000736	1305	HM627011	clade_46	N	N
Mycobacterium sp. W	1306	DQ437715	clade_46	N	N
Mycobacterium tuberculosis	1307	CP001658	clade_46	N	Category -C
Mycobacterium ulcerans	1308	AB548725	clade_46	N	OP
Mycobacterium vulneris	1309	EU834055	clade_46	N	OP
Xanthomonas campestris	2016	EF101975	clade_461	N	N
Xanthomonas sp. kmd_489	2017	EU723184	clade_461	N	N
Dietzia natronolimnaea	769	GQ870426	clade_462	N	N
Dietzia sp. BBDP51	770	DQ337512	clade_462	N	N
Dietzia sp. CA149	771	GQ870422	clade_462	N	N
Dietzia timorensis	772	GQ870424	clade_462	N	N
Gordonia bronchialis	951	NR_027594	clade_463	N	N
Gordonia polyisoprenivorans	952	DQ385609	clade_463	N	N
Gordonia sp. KTR9	953	DQ068383	clade_463	N	N
Gordonia sputi	954	FJ536304	clade_463	N	N
Gordonia terrae	955	GQ848239	clade_463	N	N
Leptotrichia goodfellowii	1167	ADAD01000110	clade_465	N	N
Leptotrichia sp. oral clone IK040	1174	AY349387	clade_465	N	N
Leptotrichia sp. oral clone P2PB_51 P1	1175	AY207053	clade_465	N	N
Bacteroidales genomosp. P7 oral clone MB3_P19	264	DQ003623	clade_466	N	N
Butyrivibrio virosa	454	AB443949	clade_466	N	N
Odoribacter laneus	1363	AB490805	clade_466	N	N
Odoribacter splanchnicus	1364	CP002544	clade_466	N	N
Capnocytophaga gingivalis	478	ACLQ01000011	clade_467	N	N
Capnocytophaga granulosa	479	X97248	clade_467	N	N
Capnocytophaga sp. oral clone AH015	483	AY005074	clade_467	N	N
Capnocytophaga sp. oral strain S3	487	AY005073	clade_467	N	N
Capnocytophaga sp. oral taxon 338	488	AEXX01000050	clade_467	N	N
Capnocytophaga canimorsus	476	CP002113	clade_468	N	N
Capnocytophaga sp. oral clone ID062	485	AY349368	clade_468	N	N
Lactobacillus cateniformis	1075	M23729	clade_469	N	N
Lactobacillus vitulinus	1142	NR_041305	clade_469	N	N
Cetobacterium somerae	501	AJ438155	clade_470	N	N
Fusobacterium gonidiaformans	896	ACET01000043	clade_470	N	N
Fusobacterium mortiferum	897	ACDB02000034	clade_470	N	N
Fusobacterium necrogenes	899	X55408	clade_470	N	N
Fusobacterium necrophorum	900	AM905356	clade_470	N	N
Fusobacterium sp. 12_1B	905	AGWJ01000070	clade_470	N	N
Fusobacterium sp. 3_1_5R	911	ACDD01000078	clade_470	N	N
Fusobacterium sp. D12	918	ACDG02000036	clade_470	N	N

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<i>Fusobacterium ulcerans</i>	921	ACDH01000090	clade_470	N	N
<i>Fusobacterium varium</i>	922	ACIE01000009	clade_470	N	N
<i>Mycoplasma arthritidis</i>	1312	NC_011025	clade_473	N	N
<i>Mycoplasma faucium</i>	1314	NR_024983	clade_473	N	N
<i>Mycoplasma hominis</i>	1318	AF443616	clade_473	N	N
<i>Mycoplasma orale</i>	1319	AY796060	clade_473	N	N
<i>Mycoplasma salivarium</i>	1324	M24661	clade_473	N	N
<i>Mitsuokella jalaludinii</i>	1247	NR_028840	clade_474	N	N
<i>Mitsuokella multacida</i>	1248	ABWK02000005	clade_474	N	N
<i>Mitsuokella</i> sp. oral taxon 521	1249	GU413658	clade_474	N	N
<i>Mitsuokella</i> sp. oral taxon G68	1250	GU432166	clade_474	N	N
<i>Selenomonas genomsp.</i> C1	1695	AY278627	clade_474	N	N
<i>Selenomonas genomsp.</i> P8 oral clone MB5_P06	1700	DQ003628	clade_474	N	N
<i>Selenomonas ruminantium</i>	1703	NR_075026	clade_474	N	N
Veillonellaceae bacterium oral taxon 131	1994	GU402916	clade_474	N	N
<i>Alloscardovia omnicolens</i>	139	NR_042583	clade_475	N	N
<i>Alloscardovia</i> sp. OB7196	140	AB425070	clade_475	N	N
<i>Bifidobacterium urinalis</i>	366	AJ278695	clade_475	N	N
<i>Prevotella loeschei</i>	1503	JN867231	clade_48	N	N
<i>Prevotella</i> sp. oral clone ASCG12	1530	DQ272511	clade_48	N	N
<i>Prevotella</i> sp. oral clone GU027	1540	AY349398	clade_48	N	N
<i>Prevotella</i> sp. oral taxon 472	1553	ACZS01000106	clade_48	N	N
<i>Selenomonas diana</i>	1693	GQ422719	clade_480	N	N
<i>Selenomonas flueggei</i>	1694	AF287803	clade_480	N	N
<i>Selenomonas genomsp.</i> C2	1696	AY278628	clade_480	N	N
<i>Selenomonas genomsp.</i> P6 oral clone MB3_C41	1698	DQ003636	clade_480	N	N
<i>Selenomonas genomsp.</i> P7 oral clone MB5_C08	1699	DQ003627	clade_480	N	N
<i>Selenomonas infelix</i>	1701	AF287802	clade_480	N	N
<i>Selenomonas noxia</i>	1702	GU470909	clade_480	N	N
<i>Selenomonas</i> sp. oral clone FT050	1705	AY349403	clade_480	N	N
<i>Selenomonas</i> sp. oral clone GI064	1706	AY349404	clade_480	N	N
<i>Selenomonas</i> sp. oral clone GT010	1707	AY349405	clade_480	N	N
<i>Selenomonas</i> sp. oral clone HU051	1708	AY349406	clade_480	N	N
<i>Selenomonas</i> sp. oral clone IK004	1709	AY349407	clade_480	N	N
<i>Selenomonas</i> sp. oral clone JI021	1711	AY349409	clade_480	N	N
<i>Selenomonas</i> sp. oral clone JS031	1712	AY349410	clade_480	N	N
<i>Selenomonas</i> sp. oral clone OH4A	1713	AY947498	clade_480	N	N
<i>Selenomonas</i> sp. oral clone P2PA_80 P4	1714	AY207052	clade_480	N	N
<i>Selenomonas</i> sp. oral taxon 149	1716	AEEJ01000007	clade_480	N	N
Veillonellaceae bacterium oral taxon 155	1995	GU470897	clade_480	N	N
<i>Agrococcus jenensis</i>	117	NR_026275	clade_484	N	N
<i>Microbacterium gubbeenense</i>	1232	NR_025098	clade_484	N	N
<i>Pseudoclavibacter</i> sp. Timone	1590	FJ375951	clade_484	N	N
<i>Tropheryma whipplei</i>	1961	BX251412	clade_484	N	N
<i>Zimmermannella bifida</i>	2031	AB012592	clade_484	N	N
<i>Legionella hackeliae</i>	1151	M36028	clade_486	N	OP

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Legionella longbeachae	1152	M36029	clade_486	N	OP
Legionella sp. D3923	1154	JN380999	clade_486	N	OP
Legionella sp. D4088	1155	JN381012	clade_486	N	OP
Legionella sp. H63	1156	JF831047	clade_486	N	OP
Legionella sp. NML 93L054	1157	GU062706	clade_486	N	OP
Legionella steelei	1158	HQ398202	clade_486	N	OP
Tatlockia micdadei	1915	M36032	clade_486	N	N
Helicobacter pullorum	996	ABQU01000097	clade_489	N	N
Acetobacteraceae bacterium AT_5844	16	AGEZ01000040	clade_490	N	N
Roseomonas cervicalis	1643	ADVL01000363	clade_490	N	N
Roseomonas mucosa	1644	NR_028857	clade_490	N	N
Roseomonas sp. NML94_0193	1645	AF533357	clade_490	N	N
Roseomonas sp. NML97_0121	1646	AF533359	clade_490	N	N
Roseomonas sp. NML98_0009	1647	AF533358	clade_490	N	N
Roseomonas sp. NML98_0157	1648	AF533360	clade_490	N	N
Rickettsia akari	1627	CP000847	clade_492	N	OP
Rickettsia conorii	1628	AE008647	clade_492	N	OP
Rickettsia prowazekii	1629	M21789	clade_492	N	Category -B
Rickettsia rickettsii	1630	NC_010263	clade_492	N	OP
Rickettsia slovaca	1631	L36224	clade_492	N	OP
Rickettsia typhi	1632	AE017197	clade_492	N	OP
Anaeroglobus geminatus	160	AGCJ01000054	clade_493	N	N
Megasphaera genomsp. C1	1201	AY278622	clade_493	N	N
Megasphaera micronuciformis	1203	AECs01000020	clade_493	N	N
Clostridiales genomsp. BVAB3	540	CP001850	clade_495	N	N
Tsukamurella paurometabola	1963	X80628	clade_496	N	N
Tsukamurella tyrosinosolvens	1964	AB478958	clade_496	N	N
Abiotrophia para_adiacens	2	AB022027	clade_497	N	N
Carnobacterium divergens	492	NR_044706	clade_497	N	N
Carnobacterium maltaromaticum	493	NC_019425	clade_497	N	N
Enterococcus avium	800	AF133535	clade_497	N	N
Enterococcus caccae	801	AY943820	clade_497	N	N
Enterococcus casseliflavus	802	AEWT01000047	clade_497	N	N
Enterococcus durans	803	AJ276354	clade_497	N	N
Enterococcus faecalis	804	AE016830	clade_497	N	N
Enterococcus faecium	805	AM157434	clade_497	N	N
Enterococcus gallinarum	806	AB269767	clade_497	N	N
Enterococcus gilvus	807	AY033814	clade_497	N	N
Enterococcus hawaiiensis	808	AY321377	clade_497	N	N
Enterococcus hirae	809	AF061011	clade_497	N	N
Enterococcus italicus	810	AEPV01000109	clade_497	N	N
Enterococcus mundtii	811	NR_024906	clade_497	N	N
Enterococcus raffinosus	812	FN600541	clade_497	N	N
Enterococcus sp. BV2CASA2	813	JN809766	clade_497	N	N
Enterococcus sp. CCRI_16620	814	GU457263	clade_497	N	N
Enterococcus sp. F95	815	FJ463817	clade_497	N	N
Enterococcus sp. RfL6	816	AJ133478	clade_497	N	N
Enterococcus thailandicus	817	AY321376	clade_497	N	N
Fusobacterium canifelinum	893	AY162222	clade_497	N	N
Fusobacterium genomsp. C1	894	AY278616	clade_497	N	N
Fusobacterium genomsp. C2	895	AY278617	clade_497	N	N
Fusobacterium periodonticum	902	ACJY01000002	clade_497	N	N

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Fusobacterium sp. 1_1_41FAA	906	ADGG01000053	clade_497	N	N
Fusobacterium sp. 11_3_2	904	ACUO01000052	clade_497	N	N
Fusobacterium sp. 2_1_31	907	ACDC02000018	clade_497	N	N
Fusobacterium sp. 3_1_27	908	ADGF01000045	clade_497	N	N
Fusobacterium sp. 3_1_33	909	ACQE01000178	clade_497	N	N
Fusobacterium sp. 3_1_36A2	910	ACPU01000044	clade_497	N	N
Fusobacterium sp. AC18	912	HQ616357	clade_497	N	N
Fusobacterium sp. ACB2	913	HQ616358	clade_497	N	N
Fusobacterium sp. AS2	914	HQ616361	clade_497	N	N
Fusobacterium sp. CM1	915	HQ616371	clade_497	N	N
Fusobacterium sp. CM21	916	HQ616375	clade_497	N	N
Fusobacterium sp. CM22	917	HQ616376	clade_497	N	N
Fusobacterium sp. oral clone ASCF06	919	AY923141	clade_497	N	N
Fusobacterium sp. oral clone ASCF11	920	AY953256	clade_497	N	N
Granulicatella adiacens	959	ACKZ01000002	clade_497	N	N
Granulicatella elegans	960	AB252689	clade_497	N	N
Granulicatella paradiacens	961	AY879298	clade_497	N	N
Granulicatella sp. oral clone ASC02	963	AY923126	clade_497	N	N
Granulicatella sp. oral clone ASCA05	964	DQ341469	clade_497	N	N
Granulicatella sp. oral clone ASCB09	965	AY953251	clade_497	N	N
Granulicatella sp. oral clone ASCG05	966	AY923146	clade_497	N	N
Tetragenococcus halophilus	1918	NR_075020	clade_497	N	N
Tetragenococcus koreensis	1919	NR_043113	clade_497	N	N
Vagococcus fluvialis	1973	NR_026489	clade_497	N	N
Chryseobacterium anthropi	514	AM982793	clade_498	N	N
Chryseobacterium gleum	515	ACKQ02000003	clade_498	N	N
Chryseobacterium hominis	516	NR_042517	clade_498	N	N
Treponema refringens	1936	AF426101	clade_499	N	OP
Treponema sp. oral clone JU031	1941	AY349416	clade_499	N	N
Treponema sp. oral taxon 239	1948	GU408738	clade_499	N	N
Treponema sp. oral taxon 271	1955	GU408871	clade_499	N	N
Alistipes finegoldii	129	NR_043064	clade_500	N	N
Alistipes onderdonkii	131	NR_043318	clade_500	N	N
Alistipes putredinis	132	ABFK02000017	clade_500	N	N
Alistipes shahii	133	FP929032	clade_500	N	N
Alistipes sp. HGB5	134	AENZ01000082	clade_500	N	N
Alistipes sp. JC50	135	JF824804	clade_500	N	N
Alistipes sp. RMA 9912	136	GQ140629	clade_500	N	N
Mycoplasma agalactiae	1310	AF010477	clade_501	N	N
Mycoplasma bovoculi	1313	NR_025987	clade_501	N	N
Mycoplasma fermentans	1315	CP002458	clade_501	N	N
Mycoplasma flocculare	1316	X62699	clade_501	N	N
Mycoplasma ovipneumoniae	1320	NR_025989	clade_501	N	N
Arcobacter butzleri	176	AEPT01000071	clade_502	N	N
Arcobacter cryaerophilus	177	NR_025905	clade_502	N	N
Campylobacter curvus	461	NC_009715	clade_502	N	OP
Campylobacter rectus	467	ACFU01000050	clade_502	N	OP
Campylobacter showae	468	ACVQ01000030	clade_502	N	OP

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Campylobacter sp. FOBRC14	469	HQ616379	clade_502	N	OP
Campylobacter sp. FOBRC15	470	HQ616380	clade_502	N	OP
Campylobacter sp. oral clone BB120	471	AY005038	clade_502	N	OP
Campylobacter sputorum	472	NR_044839	clade_502	N	OP
Bacteroides ureolyticus	330	GQ167666	clade_504	N	N
Campylobacter gracilis	463	ACYG01000026	clade_504	N	OP
Campylobacter hominis	464	NC_009714	clade_504	N	OP
Dialister invisus	762	ACIM02000001	clade_506	N	N
Dialister microaerophilus	763	AFBB01000028	clade_506	N	N
Dialister microaerophilus	764	AENT01000008	clade_506	N	N
Dialister propionificiens	766	NR_043231	clade_506	N	N
Dialister succinatiphilus	768	AB370249	clade_506	N	N
Megasphaera elsdenii	1200	AY038996	clade_506	N	N
Megasphaera genomsp. type_1	1202	ADGP01000010	clade_506	N	N
Megasphaera sp. BLPYG_07	1204	HM990964	clade_506	N	N
Megasphaera sp. UPII 199_6	1205	AFIJ01000040	clade_506	N	N
Chromobacterium violaceum	513	NC_005085	clade_507	N	N
Laribacter hongkongensis	1148	CP001154	clade_507	N	N
Methylophilus sp. ECd5	1229	AY436794	clade_507	N	N
Finegoldia magna	883	ACHM02000001	clade_509	N	N
Parvimonas micra	1431	AB729072	clade_509	N	N
Parvimonas sp. oral taxon 110	1432	AFII01000002	clade_509	N	N
Peptostreptococcus micros	1456	AM176538	clade_509	N	N
Peptostreptococcus sp. oral clone FJ023	1460	AY349390	clade_509	N	N
Peptostreptococcus sp. P4P_31 P3	1458	AY207059	clade_509	N	N
Helicobacter pylori	997	CP000012	clade_510	N	OP
Anaplasma marginale	165	ABOR01000019	clade_511	N	N
Anaplasma phagocytophilum	166	NC_007797	clade_511	N	N
Ehrlichia chaffeensis	783	AAIF01000035	clade_511	N	OP
Neorickettsia risticii	1349	CP001431	clade_511	N	N
Neorickettsia sennetsu	1350	NC_007798	clade_511	N	N
Pseudoramibacter alactolyticus	1606	AB036759	clade_512	N	N
Veillonella montpellierensis	1977	AF473836	clade_513	N	N
Veillonella sp. oral clone ASCA08	1988	AY923118	clade_513	N	N
Veillonella sp. oral clone ASCB03	1989	AY923122	clade_513	N	N
Inquilinus limosus	1012	NR_029046	clade_514	N	N
Sphingomonas sp. oral clone FZ016	1746	AY349412	clade_514	N	N
Anaerococcus lactolyticus	145	ABYO01000217	clade_515	N	N
Anaerococcus prevotii	147	CP001708	clade_515	N	N
Anaerococcus sp. gpac104	152	AM176528	clade_515	N	N
Anaerococcus sp. gpac126	153	AM176530	clade_515	N	N
Anaerococcus sp. gpac155	154	AM176536	clade_515	N	N
Anaerococcus sp. gpac199	155	AM176539	clade_515	N	N
Anaerococcus tetradius	157	ACGC01000107	clade_515	N	N
Bacteroides coagulans	271	AB547639	clade_515	N	N
Clostridiales bacterium 9403326	534	HM587324	clade_515	N	N
Clostridiales bacterium ph2	539	JN837487	clade_515	N	N
Peptostreptococcus sp. 9succ1	1457	X90471	clade_515	N	N
Peptostreptococcus sp. oral clone AP24	1459	AB175072	clade_515	N	N

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Tissierella praeacuta	1924	NR_044860	clade_515	N	N
Helicobacter canadensis	994	ABQS01000108	clade_518	N	N
Peptostreptococcus anaerobius	1455	AY326462	clade_520	N	N
Peptostreptococcus stomatis	1461	ADGQ01000048	clade_520	N	N
Bilophila wadsworthia	367	ADCP01000166	clade_521	N	N
Desulfovibrio vulgaris	761	NR_074897	clade_521	N	N
Actinomyces nasicola	64	AJ508455	clade_523	N	N
Cellulosimicrobium funkei	500	AY501364	clade_523	N	N
Lactococcus raffinolactis	1146	NR_044359	clade_524	N	N
Bacteroidales genomsp. P1	258	AY341819	clade_529	N	N
Bacteroidales genomsp. P2 oral clone MB1_G13	259	DQ003613	clade_529	N	N
Bacteroidales genomsp. P3 oral clone MB1_G34	260	DQ003615	clade_529	N	N
Bacteroidales genomsp. P4 oral clone MB2_G17	261	DQ003617	clade_529	N	N
Bacteroidales genomsp. P5 oral clone MB2_P04	262	DQ003619	clade_529	N	N
Bacteroidales genomsp. P6 oral clone MB3_C19	263	DQ003634	clade_529	N	N
Bacteroidales genomsp. P8 oral clone MB4_G15	265	DQ003626	clade_529	N	N
Bacteroidetes bacterium oral taxon D27	333	HM099638	clade_530	N	N
Bacteroidetes bacterium oral taxon F31	334	HM099643	clade_530	N	N
Bacteroidetes bacterium oral taxon F44	335	HM099649	clade_530	N	N
Flavobacterium sp. NF2_1	885	FJ195988	clade_530	N	N
Myroides odoratimimus	1326	NR_042354	clade_530	N	N
Myroides sp. MY15	1327	GU253339	clade_530	N	N
Chlamydiales bacterium NS16	507	JN606076	clade_531	N	N
Chlamydophila pecorum	508	D88317	clade_531	N	OP
Parachlamydia sp. UWE25	1423	BX908798	clade_531	N	N
Fusobacterium russii	903	NR_044687	clade_532	N	N
Streptobacillus moniliformis	1784	NR_027615	clade_532	N	N
Eubacteriaceae bacterium P4P_50 P4	833	AY207060	clade_533	N	N
Abiotrophia defectiva	1	ACIN02000016	clade_534	N	N
Abiotrophia sp. oral clone P4PA_155 P1	3	AY207063	clade_534	N	N
Catonella genomsp. P1 oral clone MB5_P12	496	DQ003629	clade_534	N	N
Catonella morbi	497	ACIL02000016	clade_534	N	N
Catonella sp. oral clone FL037	498	AY349369	clade_534	N	N
Eremococcus coleocola	818	AENN01000008	clade_534	N	N
Facklamia hominis	879	Y10772	clade_534	N	N
Granulicatella sp. M658_99_3	962	AJ271861	clade_534	N	N
Campylobacter coli	459	AAFL01000004	clade_535	N	OP
Campylobacter concisus	460	CP000792	clade_535	N	OP
Campylobacter fetus	462	ACLG01001177	clade_535	N	OP
Campylobacter jejuni	465	AL139074	clade_535	N	Category -B
Campylobacter upsaliensis	473	AEP01000040	clade_535	N	OP
Atopobium minutum	183	HM007583	clade_539	N	N

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Atopobium parvulum	184	CP001721	clade_539	N	N
Atopobium rimae	185	ACFE01000007	clade_539	N	N
Atopobium sp. BS2	186	HQ616367	clade_539	N	N
Atopobium sp. F0209	187	EU592966	clade_539	N	N
Atopobium sp. ICM42b10	188	HQ616393	clade_539	N	N
Atopobium sp. ICM57	189	HQ616400	clade_539	N	N
Atopobium vaginae	190	AEDQ01000024	clade_539	N	N
Coriobacteriaceae bacterium BV3Ac1	677	JN809768	clade_539	N	N
Actinomyces naeslundii	63	X81062	clade_54	N	N
Actinomyces oricola	67	NR_025559	clade_54	N	N
Actinomyces oris	69	BABV01000070	clade_54	N	N
Actinomyces sp. 7400942	70	EU484334	clade_54	N	N
Actinomyces sp. ChDC B197	72	AF543275	clade_54	N	N
Actinomyces sp. GEJ15	73	GU561313	clade_54	N	N
Actinomyces sp. M2231_94_1	79	AJ234063	clade_54	N	N
Actinomyces sp. oral clone GU067	83	AY349362	clade_54	N	N
Actinomyces sp. oral clone IO077	85	AY349364	clade_54	N	N
Actinomyces sp. oral clone IP073	86	AY349365	clade_54	N	N
Actinomyces sp. oral clone JA063	88	AY349367	clade_54	N	N
Actinomyces sp. oral taxon 170	89	AFBL01000010	clade_54	N	N
Actinomyces sp. oral taxon 171	90	AECW01000034	clade_54	N	N
Actinomyces urogenitalis	95	ACFH01000038	clade_54	N	N
Actinomyces viscosus	96	ACRE01000096	clade_54	N	N
Orientia tsutsugamushi	1383	AP008981	clade_541	N	OP
Megamonas funiformis	1198	AB300988	clade_542	N	N
Megamonas hypermegale	1199	AJ420107	clade_542	N	N
Aeromicrobium marinum	102	NR_025681	clade_544	N	N
Aeromicrobium sp. JC14	103	JF824798	clade_544	N	N
Luteococcus sanguinis	1190	NR_025507	clade_544	N	N
Propionibacteriaceae bacterium NML 02_0265	1568	EF599122	clade_544	N	N
Rhodococcus corynebacterioides	1622	X80615	clade_546	N	N
Rhodococcus erythropolis	1624	ACNO01000030	clade_546	N	N
Rhodococcus fascians	1625	NR_037021	clade_546	N	N
Segniliparus rotundus	1690	CP001958	clade_546	N	N
Segniliparus rugosus	1691	ACZI01000025	clade_546	N	N
Exiguobacterium acetylicum	878	FJ970034	clade_547	N	N
Macroccoccus caseolyticus	1194	NR_074941	clade_547	N	N
Streptomyces sp. 1 AIP_2009	1890	FJ176782	clade_548	N	N
Streptomyces sp. SD 524	1892	EU544234	clade_548	N	N
Streptomyces sp. SD 528	1893	EU544233	clade_548	N	N
Streptomyces thermoviolaceus	1895	NR_027616	clade_548	N	N
Borrelia afzelii	388	ABCU01000001	clade_549	N	OP
Borrelia crocidurae	390	DQ057990	clade_549	N	OP
Borrelia duttonii	391	NC_011229	clade_549	N	OP
Borrelia hermsii	393	AY597657	clade_549	N	OP
Borrelia hispanica	394	DQ057988	clade_549	N	OP
Borrelia persica	395	HM161645	clade_549	N	OP
Borrelia recurrentis	396	AF107367	clade_549	N	OP
Borrelia spielmanii	398	ABKB01000002	clade_549	N	OP
Borrelia turicatae	399	NC_008710	clade_549	N	OP
Borrelia valaisiana	400	ABCY01000002	clade_549	N	OP
Providencia alcalifaciens	1586	ABXW01000071	clade_55	N	N

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Providencia rettgeri	1587	AM040492	clade_55	N	N
Providencia rustigianii	1588	AM040489	clade_55	N	N
Providencia stuartii	1589	AF008581	clade_55	N	N
Treponema pallidum	1932	CP001752	clade_550	N	OP
Treponema phagedenis	1934	AEFH01000172	clade_550	N	N
Treponema sp. clone DDKL_4	1939	Y08894	clade_550	N	N
Acholeplasma laidlawii	17	NR_074448	clade_551	N	N
Mycoplasma putrefaciens	1323	U26055	clade_551	N	N
Mycoplasmataceae genomosp. P1 oral clone MB1_G23	1325	DQ003614	clade_551	N	N
Spiroplasma insolitum	1750	NR_025705	clade_551	N	N
Collinsella intestinalis	660	ABXH02000037	clade_553	N	N
Collinsella stercoris	661	ABXJ01000150	clade_553	N	N
Collinsella tanakaei	662	AB490807	clade_553	N	N
Caminicella sporogenes	458	NR_025485	clade_554	N	N
Acidaminococcus fermentans	21	CP001859	clade_556	N	N
Acidaminococcus intestini	22	CP003058	clade_556	N	N
Acidaminococcus sp. D21	23	ACGB01000071	clade_556	N	N
Phascolarctobacterium faecium	1462	NR_026111	clade_556	N	N
Phascolarctobacterium sp. YIT 12068	1463	AB490812	clade_556	N	N
Phascolarctobacterium succinatutens	1464	AB490811	clade_556	N	N
Acidithiobacillus ferrivorans	25	NR_074660	clade_557	N	N
Xanthomonadaceae bacterium NML 03_0222	2015	EU313791	clade_557	N	N
Catabacter hongkongensis	494	AB671763	clade_558	N	N
Christensenella minuta	512	AB490809	clade_558	N	N
Clostridiales bacterium oral clone P4PA_66 P1	536	AY207065	clade_558	N	N
Clostridiales bacterium oral taxon 093	537	GQ422712	clade_558	N	N
Heliobacterium modesticaldum	1000	NR_074517	clade_560	N	N
Alistipes indistinctus	130	AB490804	clade_561	N	N
Bacteroidales bacterium ph8	257	JN837494	clade_561	N	N
Candidatus Sulcia muelleri	475	CP002163	clade_561	N	N
Cytophaga xylanolytica	742	FR733683	clade_561	N	N
Flavobacteriaceae genomosp. C1	884	AY278614	clade_561	N	N
Gramella forsetii	958	NR_074707	clade_561	N	N
Sphingobacterium faecium	1740	NR_025537	clade_562	N	N
Sphingobacterium mizutaii	1741	JF708889	clade_562	N	N
Sphingobacterium multivorum	1742	NR_040953	clade_562	N	N
Sphingobacterium spiritivorum	1743	ACHA02000013	clade_562	N	N
Jonquetella anthropi	1017	ACOO02000004	clade_563	N	N
Pyramidobacter pisciolens	1614	AY207056	clade_563	N	N
Synergistes genomosp. C1	1904	AY278615	clade_563	N	N
Synergistes sp. RMA 14551	1905	DQ412722	clade_563	N	N
Synergistetes bacterium ADV897	1906	GQ258968	clade_563	N	N
Candidatus Arthromitus sp. SFB_mouse_Yit	474	NR_074460	clade_564	N	N
Gracilibacter thermotolerans	957	NR_043559	clade_564	N	N
Brachyspira aalborgi	404	FM178386	clade_565	N	N
Brachyspira sp. HIS3	406	FM178387	clade_565	N	N
Brachyspira sp. HIS4	407	FM178388	clade_565	N	N

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Brachyspira sp. HIS5	408	FM178389	clade_565	N	N
Adlercreutzia equolifaciens	97	AB306661	clade_566	N	N
Coriobacteriaceae bacterium JC110	678	CAEM01000062	clade_566	N	N
Coriobacteriaceae bacterium phl	679	JN837493	clade_566	N	N
Cryptobacterium curtum	740	GQ422741	clade_566	N	N
Eggerthella sinensis	779	AY321958	clade_566	N	N
Eggerthella sp. 1_3_56FAA	780	ACWN01000099	clade_566	N	N
Eggerthella sp. HGA1	781	AEXR01000021	clade_566	N	N
Eggerthella sp. YY7918	782	AP012211	clade_566	N	N
Gordonibacter pamelaeeae	680	AM886059	clade_566	N	N
Gordonibacter pamelaeeae	956	FP929047	clade_566	N	N
Slackia equolifaciens	1732	EU377663	clade_566	N	N
Slackia exigua	1733	ACUX01000029	clade_566	N	N
Slackia faecicanis	1734	NR_042220	clade_566	N	N
Slackia heliotrinireducens	1735	NR_074439	clade_566	N	N
Slackia isoflavoniconvertens	1736	AB566418	clade_566	N	N
Slackia piriformis	1737	AB490806	clade_566	N	N
Slackia sp. NATTS	1738	AB505075	clade_566	N	N
Chlamydiales bacterium NS13	506	JN606075	clade_567	N	N
Victivallaceae bacterium NML 080035	2003	FJ394915	clade_567	N	N
Victivallis vadensis	2004	ABDE02000010	clade_567	N	N
Streptomyces griseus	1889	NR_074787	clade_573	N	N
Streptomyces sp. SD 511	1891	EU544231	clade_573	N	N
Streptomyces sp. SD 534	1894	EU544232	clade_573	N	N
Cloacibacillus evryensis	530	GQ258966	clade_575	N	N
Deferribacteres sp. oral clone JV001	743	AY349370	clade_575	N	N
Deferribacteres sp. oral clone JV023	745	AY349372	clade_575	N	N
Synergistetes bacterium LBVCM1157	1907	GQ258969	clade_575	N	N
Synergistetes bacterium oral taxon 362	1909	GU410752	clade_575	N	N
Synergistetes bacterium oral taxon D48	1910	GU430992	clade_575	N	N
Peptococcus sp. oral clone JM048	1439	AY349389	clade_576	N	N
Helicobacter winthamensis	999	ACDO01000013	clade_577	N	N
Wolinella succinogenes	2014	BX571657	clade_577	N	N
Olsenella genomsp. C1	1368	AY278623	clade_578	N	N
Olsenella profusa	1369	FN178466	clade_578	N	N
Olsenella sp. F0004	1370	EU592964	clade_578	N	N
Olsenella sp. oral taxon 809	1371	ACVE01000002	clade_578	N	N
Olsenella uli	1372	CP002106	clade_578	N	N
Nocardiopsis dassonvillei	1356	CP002041	clade_579	N	N
Peptococcus niger	1438	NR_029221	clade_580	N	N
Peptococcus sp. oral taxon 167	1440	GQ422727	clade_580	N	N
Akkermansia muciniphila	118	CP001071	clade_583	N	N
Opitutus terrae	1373	NR_074978	clade_583	N	N
Clostridiales bacterium oral taxon F32	538	HM099644	clade_584	N	N
Leptospira borgpetersenii	1161	NC_008508	clade_585	N	OP
Leptospira broomii	1162	NR_043200	clade_585	N	OP

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Leptospira interrogans	1163	NC_005823	clade_585	N	OP
Methanobrevibacter gottschalkii	1213	NR_044789	clade_587	N	N
Methanobrevibacter millerae	1214	NR_042785	clade_587	N	N
Methanobrevibacter oralis	1216	HE654003	clade_587	N	N
Methanobrevibacter thaueri	1219	NR_044787	clade_587	N	N
Methanobrevibacter smithii	1218	ABYV02000002	clade_588	N	N
Deinococcus radiodurans	746	AE000513	clade_589	N	N
Deinococcus sp. R_43890	747	FR682752	clade_589	N	N
Thermus aquaticus	1923	NR_025900	clade_589	N	N
Actinomyces sp. c109	81	AB167239	clade_590	N	N
Syntrophomonadaceae genomsp. P1	1912	AY341821	clade_590	N	N
Anaerobaculum hydrogeniformans	141	ACJX02000009	clade_591	N	N
Microcystis aeruginosa	1246	NC_010296	clade_592	N	N
Prochlorococcus marinus	1567	CP000551	clade_592	N	N
Methanobrevibacter acididurans	1208	NR_028779	clade_593	N	N
Methanobrevibacter arboriphilus	1209	NR_042783	clade_593	N	N
Methanobrevibacter curvatus	1210	NR_044796	clade_593	N	N
Methanobrevibacter cuticularis	1211	NR_044776	clade_593	N	N
Methanobrevibacter filiformis	1212	NR_044801	clade_593	N	N
Methanobrevibacter woesei	1220	NR_044788	clade_593	N	N
Roseiflexus castenholzii	1642	CP000804	clade_594	N	N
Methanobrevibacter olleyae	1215	NR_043024	clade_595	N	N
Methanobrevibacter ruminantium	1217	NR_042784	clade_595	N	N
Methanobrevibacter wolinii	1221	NR_044790	clade_595	N	N
Methanosphaera stadtmanae	1222	AY196684	clade_595	N	N
Chloroflexi genomsp. P1	511	AY331414	clade_596	N	N
Halorubrum lipolyticum	992	AB477978	clade_597	N	N
Methanobacterium formicicum	1207	NR_025028	clade_597	N	N
Acidilobus saccharovorans	24	AY350586	clade_598	N	N
Hyperthermus butylicus	1006	CP000493	clade_598	N	N
Ignicoccus islandicus	1011	X99562	clade_598	N	N
Metallosphaera sedula	1206	D26491	clade_598	N	N
Thermofilum pendens	1922	X14835	clade_598	N	N
Prevotella melaninogenica	1506	CP002122	clade_6	N	N
Prevotella sp. ICM1	1520	HQ616385	clade_6	N	N
Prevotella sp. oral clone FU048	1535	AY349393	clade_6	N	N
Prevotella sp. oral clone GI030	1537	AY349395	clade_6	N	N
Prevotella sp. SEQ116	1526	JN867246	clade_6	N	N
Streptococcus anginosus	1787	AECT01000011	clade_60	N	N
Streptococcus milleri	1812	X81023	clade_60	N	N
Streptococcus sp. 16362	1829	JN590019	clade_60	N	N
Streptococcus sp. 69130	1832	X78825	clade_60	N	N
Streptococcus sp. AC15	1833	HQ616356	clade_60	N	N
Streptococcus sp. CM7	1839	HQ616373	clade_60	N	N
Streptococcus sp. OBRC6	1847	HQ616352	clade_60	N	N
Burkholderia ambifaria	442	AAUZ01000009	clade_61	N	OP
Burkholderia cenocepacia	443	AAHI01000060	clade_61	N	OP
Burkholderia cepacia	444	NR_041719	clade_61	N	OP
Burkholderia mallei	445	CP000547	clade_61	N	Category -B
Burkholderia multivorans	446	NC_010086	clade_61	N	OP
Burkholderia oklahomensis	447	DQ108388	clade_61	N	OP
Burkholderia pseudomallei	448	CP001408	clade_61	N	Category

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					-B
Burkholderia rhizoxinica	449	HQ005410	clade_61	N	OP
Burkholderia sp. 383	450	CP000151	clade_61	N	OP
Burkholderia xenovorans	451	U86373	clade_61	N	OP
Prevotella buccae	1488	ACRB01000001	clade_62	N	N
Prevotella genomsp. P8 oral clone MB3_P13	1498	DQ003622	clade_62	N	N
Prevotella sp. oral clone FW035	1536	AY349394	clade_62	N	N
Prevotella bivia	1486	ADFO01000096	clade_63	N	N
Prevotella disiens	1494	AEDO01000026	clade_64	N	N
Bacteroides faecis	276	GQ496624	clade_65	N	N
Bacteroides fragilis	279	AP006841	clade_65	N	N
Bacteroides nordii	285	NR_043017	clade_65	N	N
Bacteroides salyersiae	292	EU136690	clade_65	N	N
Bacteroides sp. 1_1_14	293	ACRP01000155	clade_65	N	N
Bacteroides sp. 1_1_6	295	ACIC01000215	clade_65	N	N
Bacteroides sp. 2_1_56FAA	298	ACWI01000065	clade_65	N	N
Bacteroides sp. AR29	316	AF139525	clade_65	N	N
Bacteroides sp. B2	317	EU722733	clade_65	N	N
Bacteroides thetaiotaomicron	328	NR_074277	clade_65	N	N
Actinobacillus minor	45	ACFT01000025	clade_69	N	N
Haemophilus parasuis	978	GU226366	clade_69	N	N
Vibrio cholerae	1996	AAUR01000095	clade_71	N	Category -B
Vibrio fluvialis	1997	X76335	clade_71	N	Category -B
Vibrio furnissii	1998	CP002377	clade_71	N	Category -B
Vibrio mimicus	1999	ADAF01000001	clade_71	N	Category -B
Vibrio parahaemolyticus	2000	AAWQ01000116	clade_71	N	Category -B
Vibrio sp. RC341	2001	ACZT01000024	clade_71	N	Category -B
Vibrio vulnificus	2002	AE016796	clade_71	N	Category -B
Lactobacillus acidophilus	1067	CP000033	clade_72	N	N
Lactobacillus amylolyticus	1069	ADNY01000006	clade_72	N	N
Lactobacillus amylovorus	1070	CP002338	clade_72	N	N
Lactobacillus crispatus	1078	ACOG01000151	clade_72	N	N
Lactobacillus delbrueckii	1080	CP002341	clade_72	N	N
Lactobacillus helveticus	1088	ACLM01000202	clade_72	N	N
Lactobacillus kalixensis	1094	NR_029083	clade_72	N	N
Lactobacillus kefiranofaciens	1095	NR_042440	clade_72	N	N
Lactobacillus leichmannii	1098	JX986966	clade_72	N	N
Lactobacillus sp. 66c	1120	FR681900	clade_72	N	N
Lactobacillus sp. KLDS 1.0701	1122	EU600905	clade_72	N	N
Lactobacillus sp. KLDS 1.0712	1130	EU600916	clade_72	N	N
Lactobacillus sp. oral clone HT070	1136	AY349383	clade_72	N	N
Lactobacillus ultunensis	1139	ACGU01000081	clade_72	N	N
Prevotella intermedia	1502	AF414829	clade_81	N	N
Prevotella nigrescens	1511	AFPX01000069	clade_81	N	N
Prevotella pallens	1515	AFPY01000135	clade_81	N	N
Prevotella sp. oral taxon 310	1551	GQ422737	clade_81	N	N

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Prevotella genomosp. C1	1495	AY278624	clade_82	N	N
Prevotella sp. CM38	1519	HQ610181	clade_82	N	N
Prevotella sp. oral taxon 317	1552	ACQH01000158	clade_82	N	N
Prevotella sp. SG12	1527	GU561343	clade_82	N	N
Prevotella denticola	1493	CP002589	clade_83	N	N
Prevotella genomosp. P7 oral clone MB2_P31	1497	DQ003620	clade_83	N	N
Prevotella histicola	1501	JN867315	clade_83	N	N
Prevotella multiformis	1508	AEWX01000054	clade_83	N	N
Prevotella sp. JCM 6330	1522	AB547699	clade_83	N	N
Prevotella sp. oral clone GI059	1539	AY349397	clade_83	N	N
Prevotella sp. oral taxon 782	1555	GQ422745	clade_83	N	N
Prevotella sp. oral taxon G71	1559	GU432180	clade_83	N	N
Prevotella sp. SEQ065	1524	JN867234	clade_83	N	N
Prevotella veroralis	1565	ACVA01000027	clade_83	N	N
Bacteroides acidifaciens	266	NR_028607	clade_85	N	N
Bacteroides cellulosilyticus	269	ACCH01000108	clade_85	N	N
Bacteroides clarus	270	AFBM01000011	clade_85	N	N
Bacteroides eggerthii	275	ACWG01000065	clade_85	N	N
Bacteroides oleiciplenus	286	AB547644	clade_85	N	N
Bacteroides pyogenes	290	NR_041280	clade_85	N	N
Bacteroides sp. 315_5	300	FJ848547	clade_85	N	N
Bacteroides sp. 31SF15	301	AJ583248	clade_85	N	N
Bacteroides sp. 31SF18	302	AJ583249	clade_85	N	N
Bacteroides sp. 35AE31	303	AJ583244	clade_85	N	N
Bacteroides sp. 35AE37	304	AJ583245	clade_85	N	N
Bacteroides sp. 35BE34	305	AJ583246	clade_85	N	N
Bacteroides sp. 35BE35	306	AJ583247	clade_85	N	N
Bacteroides sp. WH2	324	AY895180	clade_85	N	N
Bacteroides sp. XB12B	325	AM230648	clade_85	N	N
Bacteroides stercoris	327	ABFZ02000022	clade_85	N	N
Actinobacillus pleuropneumoniae	46	NR_074857	clade_88	N	N
Actinobacillus ureae	48	AEVG01000167	clade_88	N	N
Haemophilus aegyptius	969	AFBC01000053	clade_88	N	N
Haemophilus ducreyi	970	AE017143	clade_88	N	OP
Haemophilus haemolyticus	973	JN175335	clade_88	N	N
Haemophilus influenzae	974	AADP01000001	clade_88	N	OP
Haemophilus parahaemolyticus	975	GU561425	clade_88	N	N
Haemophilus parainfluenzae	976	AEWU01000024	clade_88	N	N
Haemophilus paraphrophaemolyticus	977	M75076	clade_88	N	N
Haemophilus somnus	979	NC_008309	clade_88	N	N
Haemophilus sp. 70334	980	HQ680854	clade_88	N	N
Haemophilus sp. HK445	981	FJ685624	clade_88	N	N
Haemophilus sp. oral clone ASCA07	982	AY923117	clade_88	N	N
Haemophilus sp. oral clone ASCG06	983	AY923147	clade_88	N	N
Haemophilus sp. oral clone BJ021	984	AY005034	clade_88	N	N
Haemophilus sp. oral clone BJ095	985	AY005033	clade_88	N	N
Haemophilus sp. oral taxon 851	987	AGRK01000004	clade_88	N	N
Haemophilus sputorum	988	AFNK01000005	clade_88	N	N
Histophilus somni	1003	AF549387	clade_88	N	N
Mannheimia haemolytica	1195	ACZX01000102	clade_88	N	N

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Pasteurella bettyae	1433	L06088	clade_88	N	N
Moellerella wisconsensis	1253	JN175344	clade_89	N	N
Morganella morganii	1265	AJ301681	clade_89	N	N
Morganella sp. JB_T16	1266	AJ781005	clade_89	N	N
Proteus mirabilis	1582	ACLE01000013	clade_89	N	N
Proteus penneri	1583	ABVP01000020	clade_89	N	N
Proteus sp. HS7514	1584	DQ512963	clade_89	N	N
Proteus vulgaris	1585	AJ233425	clade_89	N	N
Oribacterium sinus	1374	ACKX01000142	clade_90	N	N
Oribacterium sp. ACB1	1375	HM120210	clade_90	N	N
Oribacterium sp. ACB7	1376	HM120211	clade_90	N	N
Oribacterium sp. CM12	1377	HQ616374	clade_90	N	N
Oribacterium sp. ICM51	1378	HQ616397	clade_90	N	N
Oribacterium sp. OBRC12	1379	HQ616355	clade_90	N	N
Oribacterium sp. oral taxon 108	1382	AFIH01000001	clade_90	N	N
Actinobacillus actinomycetemcomitans	44	AY362885	clade_92	N	N
Actinobacillus succinogenes	47	CP000746	clade_92	N	N
Aggregatibacter actinomycetemcomitans	112	CP001733	clade_92	N	N
Aggregatibacter aphrophilus	113	CP001607	clade_92	N	N
Aggregatibacter segnis	114	AEPS01000017	clade_92	N	N
Averyella dalhousiensis	194	DQ481464	clade_92	N	N
Bisgaard Taxon	368	AY683487	clade_92	N	N
Bisgaard Taxon	369	AY683489	clade_92	N	N
Bisgaard Taxon	370	AY683491	clade_92	N	N
Bisgaard Taxon	371	AY683492	clade_92	N	N
Buchnera aphidicola	440	NR_074609	clade_92	N	N
Cedecea davisae	499	AF493976	clade_92	N	N
Citrobacter amalonaticus	517	FR870441	clade_92	N	N
Citrobacter braakii	518	NR_028687	clade_92	N	N
Citrobacter farmeri	519	AF025371	clade_92	N	N
Citrobacter freundii	520	NR_028894	clade_92	N	N
Citrobacter gillenii	521	AF025367	clade_92	N	N
Citrobacter koseri	522	NC_009792	clade_92	N	N
Citrobacter murlinae	523	AF025369	clade_92	N	N
Citrobacter rodentium	524	NR_074903	clade_92	N	N
Citrobacter sedlakii	525	AF025364	clade_92	N	N
Citrobacter sp. 30_2	526	ACDJ01000053	clade_92	N	N
Citrobacter sp. KMSI_3	527	GQ468398	clade_92	N	N
Citrobacter werkmanii	528	AF025373	clade_92	N	N
Citrobacter youngae	529	ABWL02000011	clade_92	N	N
Cronobacter malonaticus	737	GU122174	clade_92	N	N
Cronobacter sakazakii	738	NC_009778	clade_92	N	N
Cronobacter turicensis	739	FN543093	clade_92	N	N
Enterobacter aerogenes	786	AJ251468	clade_92	N	N
Enterobacter asburiae	787	NR_024640	clade_92	N	N
Enterobacter cancerogenus	788	Z96078	clade_92	N	N
Enterobacter cloacae	789	FP929040	clade_92	N	N
Enterobacter cowanii	790	NR_025566	clade_92	N	N
Enterobacter hormaechei	791	AFHR01000079	clade_92	N	N
Enterobacter sp. 247BMC	792	HQ122932	clade_92	N	N
Enterobacter sp. 638	793	NR_074777	clade_92	N	N
Enterobacter sp. JC163	794	JN657217	clade_92	N	N

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Enterobacter sp. SCSS	795	HM007811	clade_92	N	N
Enterobacter sp. TSE38	796	HM156134	clade_92	N	N
Enterobacteriaceae bacterium 9_2_54FAA	797	ADCU01000033	clade_92	N	N
Enterobacteriaceae bacterium CF01Ent_1	798	AJ489826	clade_92	N	N
Enterobacteriaceae bacterium Smarlab 3302238	799	AY538694	clade_92	N	N
Escherichia albertii	824	ABKX01000012	clade_92	N	N
Escherichia coli	825	NC_008563	clade_92	N	Category -B
Escherichia fergusonii	826	CU928158	clade_92	N	N
Escherichia hermannii	827	HQ407266	clade_92	N	N
Escherichia sp. 1_1_43	828	ACID01000033	clade_92	N	N
Escherichia sp. 4_1_40B	829	ACDM02000056	clade_92	N	N
Escherichia sp. B4	830	EU722735	clade_92	N	N
Escherichia vulneris	831	NR_041927	clade_92	N	N
Ewingella americana	877	JN175329	clade_92	N	N
Haemophilus genomosp. P2 oral clone MB3_C24	971	DQ003621	clade_92	N	N
Haemophilus genomosp. P3 oral clone MB3_C38	972	DQ003635	clade_92	N	N
Haemophilus sp. oral clone JM053	986	AY349380	clade_92	N	N
Hafnia alvei	989	DQ412565	clade_92	N	N
Klebsiella oxytoca	1024	AY292871	clade_92	N	OP
Klebsiella pneumoniae	1025	CP000647	clade_92	N	OP
Klebsiella sp. AS10	1026	HQ616362	clade_92	N	N
Klebsiella sp. Co9935	1027	DQ068764	clade_92	N	N
Klebsiella sp. enrichment culture clone SRC_DSD25	1036	HM195210	clade_92	N	N
Klebsiella sp. OBRC7	1028	HQ616353	clade_92	N	N
Klebsiella sp. SP_BA	1029	FJ999767	clade_92	N	N
Klebsiella sp. SRC_DSD1	1033	GU797254	clade_92	N	N
Klebsiella sp. SRC_DSD11	1030	GU797263	clade_92	N	N
Klebsiella sp. SRC_DSD12	1031	GU797264	clade_92	N	N
Klebsiella sp. SRC_DSD15	1032	GU797267	clade_92	N	N
Klebsiella sp. SRC_DSD2	1034	GU797253	clade_92	N	N
Klebsiella sp. SRC_DSD6	1035	GU797258	clade_92	N	N
Klebsiella variicola	1037	CP001891	clade_92	N	N
Kluyvera ascorbata	1038	NR_028677	clade_92	N	N
Kluyvera cryocrescens	1039	NR_028803	clade_92	N	N
Leminorella grimonii	1159	AJ233421	clade_92	N	N
Leminorella richardii	1160	HF558368	clade_92	N	N
Pantoea agglomerans	1409	AY335552	clade_92	N	N
Pantoea ananatis	1410	CP001875	clade_92	N	N
Pantoea brenneri	1411	EU216735	clade_92	N	N
Pantoea citrea	1412	EF688008	clade_92	N	N
Pantoea conspicua	1413	EU216737	clade_92	N	N
Pantoea septica	1414	EU216734	clade_92	N	N
Pasteurella dagmatis	1434	ACZR01000003	clade_92	N	N
Pasteurella multocida	1435	NC_002663	clade_92	N	N
Plesiomonas shigelloides	1469	X60418	clade_92	N	N
Raoultella ornithinolytica	1617	AB364958	clade_92	N	N
Raoultella planticola	1618	AF129443	clade_92	N	N

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Raoultella terrigena	1619	NR_037085	clade_92	N	N
Salmonella bongori	1683	NR_041699	clade_92	N	Category -B
Salmonella enterica	1672	NC_011149	clade_92	N	Category -B
Salmonella enterica	1673	NC_011205	clade_92	N	Category -B
Salmonella enterica	1674	DQ344532	clade_92	N	Category -B
Salmonella enterica	1675	ABEH02000004	clade_92	N	Category -B
Salmonella enterica	1676	ABAK02000001	clade_92	N	Category -B
Salmonella enterica	1677	NC_011080	clade_92	N	Category -B
Salmonella enterica	1678	EU118094	clade_92	N	Category -B
Salmonella enterica	1679	NC_011094	clade_92	N	Category -B
Salmonella enterica	1680	AE014613	clade_92	N	Category -B
Salmonella enterica	1682	ABFH02000001	clade_92	N	Category -B
Salmonella enterica	1684	ABEM01000001	clade_92	N	Category -B
Salmonella enterica	1685	ABAM02000001	clade_92	N	Category -B
Salmonella typhimurium	1681	DQ344533	clade_92	N	Category -B
Salmonella typhimurium	1686	AF170176	clade_92	N	Category -B
Serratia fonticola	1718	NR_025339	clade_92	N	N
Serratia liquefaciens	1719	NR_042062	clade_92	N	N
Serratia marcescens	1720	GU826157	clade_92	N	N
Serratia odorifera	1721	ADBY01000001	clade_92	N	N
Serratia proteamaculans	1722	AAUN01000015	clade_92	N	N
Shigella boydii	1724	AAKA01000007	clade_92	N	Category -B
Shigella dysenteriae	1725	NC_007606	clade_92	N	Category -B
Shigella flexneri	1726	AE005674	clade_92	N	Category -B
Shigella sonnei	1727	NC_007384	clade_92	N	Category -B
Tatumella ptyseos	1916	NR_025342	clade_92	N	N
Trabulsiella guamensis	1925	AY373830	clade_92	N	N
Yersinia aldovae	2019	AJ871363	clade_92	N	OP
Yersinia aleksiciae	2020	AJ627597	clade_92	N	OP
Yersinia bercovieri	2021	AF366377	clade_92	N	OP
Yersinia enterocolitica	2022	FR729477	clade_92	N	Category -B
Yersinia frederiksenii	2023	AF366379	clade_92	N	OP
Yersinia intermedia	2024	AF366380	clade_92	N	OP
Yersinia kristensenii	2025	ACCA01000078	clade_92	N	OP
Yersinia mollaretii	2026	NR_027546	clade_92	N	OP
Yersinia pestis	2027	AE013632	clade_92	N	Category

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					-A
<i>Yersinia pseudotuberculosis</i>	2028	NC_009708	clade_92	N	OP
<i>Yersinia rohdei</i>	2029	ACCD01000071	clade_92	N	OP
<i>Yokenella regensburgei</i>	2030	AB273739	clade_92	N	N
<i>Conchiformibius kuhniae</i>	669	NR_041821	clade_94	N	N
<i>Morococcus cerebrosus</i>	1267	JN175352	clade_94	N	N
<i>Neisseria bacilliformis</i>	1328	AFAY01000058	clade_94	N	N
<i>Neisseria cinerea</i>	1329	ACDY01000037	clade_94	N	N
<i>Neisseria flavescens</i>	1331	ACQV01000025	clade_94	N	N
<i>Neisseria gonorrhoeae</i>	1333	CP002440	clade_94	N	OP
<i>Neisseria lactamica</i>	1334	ACEQ01000095	clade_94	N	N
<i>Neisseria macacae</i>	1335	AFQE01000146	clade_94	N	N
<i>Neisseria meningitidis</i>	1336	NC_003112	clade_94	N	OP
<i>Neisseria mucosa</i>	1337	ACDX01000110	clade_94	N	N
<i>Neisseria pharyngis</i>	1338	AJ239281	clade_94	N	N
<i>Neisseria polysaccharea</i>	1339	ADBE01000137	clade_94	N	N
<i>Neisseria sicca</i>	1340	ACKO02000016	clade_94	N	N
<i>Neisseria</i> sp. KEM232	1341	GQ203291	clade_94	N	N
<i>Neisseria</i> sp. oral clone AP132	1344	AY005027	clade_94	N	N
<i>Neisseria</i> sp. oral strain B33KA	1346	AY005028	clade_94	N	N
<i>Neisseria</i> sp. oral taxon 014	1347	ADEA01000039	clade_94	N	N
<i>Neisseria</i> sp. TM10_1	1343	DQ279352	clade_94	N	N
<i>Neisseria subflava</i>	1348	ACEO01000067	clade_94	N	N
<i>Okadaella gastrococcus</i>	1365	HQ699465	clade_98	N	N
<i>Streptococcus agalactiae</i>	1785	AAJO01000130	clade_98	N	N
<i>Streptococcus alactolyticus</i>	1786	NR_041781	clade_98	N	N
<i>Streptococcus australis</i>	1788	AEQR01000024	clade_98	N	N
<i>Streptococcus bovis</i>	1789	AEEL01000030	clade_98	N	N
<i>Streptococcus canis</i>	1790	AJ413203	clade_98	N	N
<i>Streptococcus constellatus</i>	1791	AY277942	clade_98	N	N
<i>Streptococcus cristatus</i>	1792	AEVC01000028	clade_98	N	N
<i>Streptococcus dysgalactiae</i>	1794	AP010935	clade_98	N	N
<i>Streptococcus equi</i>	1795	CP001129	clade_98	N	N
<i>Streptococcus equinus</i>	1796	AEVB01000043	clade_98	N	N
<i>Streptococcus gallolyticus</i>	1797	FR824043	clade_98	N	N
<i>Streptococcus genomosp.</i> C1	1798	AY278629	clade_98	N	N
<i>Streptococcus genomosp.</i> C2	1799	AY278630	clade_98	N	N
<i>Streptococcus genomosp.</i> C3	1800	AY278631	clade_98	N	N
<i>Streptococcus genomosp.</i> C4	1801	AY278632	clade_98	N	N
<i>Streptococcus genomosp.</i> C5	1802	AY278633	clade_98	N	N
<i>Streptococcus genomosp.</i> C6	1803	AY278634	clade_98	N	N
<i>Streptococcus genomosp.</i> C7	1804	AY278635	clade_98	N	N
<i>Streptococcus genomosp.</i> C8	1805	AY278609	clade_98	N	N
<i>Streptococcus gordonii</i>	1806	NC_009785	clade_98	N	N
<i>Streptococcus infantarius</i>	1807	ABJK02000017	clade_98	N	N
<i>Streptococcus infantis</i>	1808	AFNN01000024	clade_98	N	N
<i>Streptococcus intermedius</i>	1809	NR_028736	clade_98	N	N
<i>Streptococcus lutetiensis</i>	1810	NR_037096	clade_98	N	N
<i>Streptococcus massiliensis</i>	1811	AY769997	clade_98	N	N
<i>Streptococcus mitis</i>	1813	AM157420	clade_98	N	N
<i>Streptococcus oligofermentans</i>	1815	AY099095	clade_98	N	N
<i>Streptococcus oralis</i>	1816	ADMV01000001	clade_98	N	N
<i>Streptococcus parasanguinis</i>	1817	AEKM01000012	clade_98	N	N

OTU	SEQ ID Number	Public DB Accession	Clade	Spore Former	Pathogen Status
Streptococcus pasteurianus	1818	AP012054	clade_98	N	N
Streptococcus peroris	1819	AEVF01000016	clade_98	N	N
Streptococcus pneumoniae	1820	AE008537	clade_98	N	N
Streptococcus porcinus	1821	EF121439	clade_98	N	N
Streptococcus pseudopneumoniae	1822	FJ827123	clade_98	N	N
Streptococcus pseudoporcinus	1823	AENS01000003	clade_98	N	N
Streptococcus pyogenes	1824	AE006496	clade_98	N	OP
Streptococcus ratti	1825	X58304	clade_98	N	N
Streptococcus sanguinis	1827	NR_074974	clade_98	N	N
Streptococcus sinensis	1828	AF432857	clade_98	N	N
Streptococcus sp. 2_1_36FAA	1831	ACOI01000028	clade_98	N	N
Streptococcus sp. 2285_97	1830	AJ131965	clade_98	N	N
Streptococcus sp. ACS2	1834	HQ616360	clade_98	N	N
Streptococcus sp. AS20	1835	HQ616366	clade_98	N	N
Streptococcus sp. BS35a	1836	HQ616369	clade_98	N	N
Streptococcus sp. C150	1837	ACRI01000045	clade_98	N	N
Streptococcus sp. CM6	1838	HQ616372	clade_98	N	N
Streptococcus sp. ICM10	1840	HQ616389	clade_98	N	N
Streptococcus sp. ICM12	1841	HQ616390	clade_98	N	N
Streptococcus sp. ICM2	1842	HQ616386	clade_98	N	N
Streptococcus sp. ICM4	1844	HQ616387	clade_98	N	N
Streptococcus sp. ICM45	1843	HQ616394	clade_98	N	N
Streptococcus sp. M143	1845	ACRK01000025	clade_98	N	N
Streptococcus sp. M334	1846	ACRL01000052	clade_98	N	N
Streptococcus sp. oral clone ASB02	1849	AY923121	clade_98	N	N
Streptococcus sp. oral clone ASCA03	1850	DQ272504	clade_98	N	N
Streptococcus sp. oral clone ASCA04	1851	AY923116	clade_98	N	N
Streptococcus sp. oral clone ASCA09	1852	AY923119	clade_98	N	N
Streptococcus sp. oral clone ASCB04	1853	AY923123	clade_98	N	N
Streptococcus sp. oral clone ASCB06	1854	AY923124	clade_98	N	N
Streptococcus sp. oral clone ASCC04	1855	AY923127	clade_98	N	N
Streptococcus sp. oral clone ASCC05	1856	AY923128	clade_98	N	N
Streptococcus sp. oral clone ASCC12	1857	DQ272507	clade_98	N	N
Streptococcus sp. oral clone ASCD01	1858	AY923129	clade_98	N	N
Streptococcus sp. oral clone ASCD09	1859	AY923130	clade_98	N	N
Streptococcus sp. oral clone ASCD10	1860	DQ272509	clade_98	N	N
Streptococcus sp. oral clone ASCE03	1861	AY923134	clade_98	N	N
Streptococcus sp. oral clone ASCE04	1862	AY953253	clade_98	N	N
Streptococcus sp. oral clone ASCE05	1863	DQ272510	clade_98	N	N
Streptococcus sp. oral clone	1864	AY923135	clade_98	N	N

OTU	SEQ ID Number	Public DB Accession	Clade	Spore Former	Pathogen Status
ASCE06					
Streptococcus sp. oral clone ASCE09	1865	AY923136	clade_98	N	N
Streptococcus sp. oral clone ASCE10	1866	AY923137	clade_98	N	N
Streptococcus sp. oral clone ASCE12	1867	AY923138	clade_98	N	N
Streptococcus sp. oral clone ASCF05	1868	AY923140	clade_98	N	N
Streptococcus sp. oral clone ASCF07	1869	AY953255	clade_98	N	N
Streptococcus sp. oral clone ASCF09	1870	AY923142	clade_98	N	N
Streptococcus sp. oral clone ASCG04	1871	AY923145	clade_98	N	N
Streptococcus sp. oral clone BW009	1872	AY005042	clade_98	N	N
Streptococcus sp. oral clone CH016	1873	AY005044	clade_98	N	N
Streptococcus sp. oral clone GK051	1874	AY349413	clade_98	N	N
Streptococcus sp. oral clone GM006	1875	AY349414	clade_98	N	N
Streptococcus sp. oral clone P2PA_41 P2	1876	AY207051	clade_98	N	N
Streptococcus sp. oral clone P4PA_30 P4	1877	AY207064	clade_98	N	N
Streptococcus sp. oral taxon 071	1878	AEEP01000019	clade_98	N	N
Streptococcus sp. oral taxon G59	1879	GU432132	clade_98	N	N
Streptococcus sp. oral taxon G62	1880	GU432146	clade_98	N	N
Streptococcus sp. oral taxon G63	1881	GU432150	clade_98	N	N
Streptococcus suis	1882	FM252032	clade_98	N	N
Streptococcus thermophilus	1883	CP000419	clade_98	N	N
Streptococcus salivarius	1826	AGBV01000001	clade_98	N	N
Streptococcus uberis	1884	HQ391900	clade_98	N	N
Streptococcus urinalis	1885	DQ303194	clade_98	N	N
Streptococcus vestibularis	1886	AEKO01000008	clade_98	N	N
Streptococcus viridans	1887	AF076036	clade_98	N	N
Synergistetes bacterium oral clone 03 5 D05	1908	GU227192	clade_98	N	N

Table 2

Species isolated from ethanol treated spore preparation preparation before (left) and after (right) CsCl gradient step

Isolates	ethanol treated spore preparation	ethanol treated, gradient purified spore preparation
<i>Bacillus coagulans</i>	7	2
<i>Blautia luti</i>	1	1
<i>Blautia</i> sp	14	13
<i>Blautia wexlerae</i>	3	1
<i>Ruminococcus obeum</i>	4	2
<i>Clostridiales</i> sp	1	2
<i>Clostridium aerotolerans</i>	1	2
<i>Clostridium disporicum</i>	0	1
<i>Clostridium</i> sp	1	1
<i>Clostridium symbiosum</i>	0	1
<i>Dorea longicatena</i>	8	6
<i>Eubacterium cellulosolvens</i>	1	0
<i>Eubacterium ventriosum</i>	2	2
<i>Gemmiger formicilis</i>	0	1
<i>Robinsoniella peoriensis</i>	0	1
<i>Roseburia hominis</i>	3	6
<i>Roseburia intestinalis</i>	9	7
<i>Ruminococcus</i> sp	5	2
<i>Syntrophococcus sucromutans</i>	1	1
<i>Turicibacter sanguinis</i>	3	4
<i>Clostridiales</i> sp	7	9
<i>Clostridium bartlettii</i>	8	11
<i>Clostridium irregulare</i>	0	1
<i>Clostridium sordellii</i>	4	6
<i>Lachnospiraceae</i> sp	1	0

Table 3

Representative diseases, disorders and conditions potentially associated with a dysbiosis, which are suitable for treatment with the compositions and methods of the invention.

Abdominal abscess
Abdominal cavity inflammation
Abscess
Absidia infection
Achromatopsia
Acidemia
Acinetobacter baumannii infection
Acinetobacter infection
Acinetobacter lwoffii infection
Acne
Acne vulgaris
Acoustic neuroma
Acromegaly
Actinomyces israelii infection
Acute bronchitis
Acute chest syndrome
Acute coronary syndrome
Acute decompensated heart failure
Acute external otitis
Acute lung injury
Acute lymphoblastic leukemia
Acute myelogenous leukemia
Acute promyelocytic leukemia
Acute sinusitis
Addison's disease
Adenocarcinoma
Adenoid hypertrophy
Adenoma
Adenomyosis
Adenosine deaminase deficiency
Adenovirus infection
Adhesive capsulitis
Adrenal cortical carcinoma
Adrenal gland hypofunction
Adrenomyeloneuropathy
Adult respiratory distress syndrome
Adult T-cell lymphoma
Adult varicella zoster virus infection
Advanced solid tumor
Agammaglobulinemia
Age related macular degeneration
Aggression
Aggressive fibromatosis

Aging
Agitation
AL amyloidosis
Albuminuria
Alcoholism (and effects)
Alkaptonuria
Allergic conjunctivitis
Allergic rhinitis
Allergy
Alopecia
Alopecia areata
Alpha mannosidosis
Alpha-1 antitrypsin deficiency
ALS
Alzheimers disease
Amenorrhea
Amnesia
Amoeba infection
Amoebic liver abscess
Amphetamine dependence
Amyloidosis
Anal cancer
Anal fissure
Anal fistula
Anal tumor
Anaplastic astrocytoma
Anaplastic large cell lymphoma
Anemia
Anesthesia
Angina
Angioedema
Angiomyolipoma
Angiosarcoma
Angle closure glaucoma
Ankylosing spondylitis
Anorexia nervosa
Anovulation
Antibiotic treatment
Antiphospholipid syndrome
Antibiotic associated diarrhea
Anus pain
Anxiety disorder
Aortic aneurysm

Aortic stenosis
Aplastic anemia
Apnea
Appendicitis
Appetite loss
Arterial occlusive disease
Arterial thrombosis
Arteriosclerosis
Arteriovenous malformation
Artery disease
Arthralgia
Arthritis
Ascites
Asperger syndrome
Aspergillus fumigatus infection
Aspergillus infection
Asthma
Astrocytoma
Ataxia telangiectasia
Atherosclerosis
Atopic dermatitis
Atopy / Allergic Sensitivity
Atrial fibrillation
Atrial flutter
Atrophic vaginitis
Atrophy
Attention deficit hyperactivity disorder
Autism
Autoimmune disease
Autoimmune hemolytic anemia
Autosomal dominant polycystic kidney disease
B-cell acute lymphoblastic leukemia
B-cell lymphoma
Bacillus anthracis infection
Bacillus infection
Back pain
Bacteremia
Bacterial endocarditis
Bacterial eye infection
Bacterial infection
Bacterial meningitis
Bacterial pneumonia
Bacterial respiratory tract infection

Bacterial skin infection
Bacterial susceptibility
Bacterial urinary tract infection
Bacterial vaginosis
Bacteroides caccae infection
Bacteroides fragilis infection
Bacteroides infection
Bacteroides thetaiotaomicron infection
Bacteroides uniformis infection
Bacteroides vulgatus infection
Balanitis
Bariatric surgery
Bartholinitis
Bartonella bacilliformis infection
Bartonella infection
Basal cell carcinoma
Becker muscular dystrophy
Behcets disease
Beta thalassemia
Bifidobacterium infection
Biliary cancer
Biliary cirrhosis
Biliary tract disease
Biliary tract infection
Biliary tumor
Binge eating disorder
Bipolar disorder
Bipolar I disorder
Birth complication
BK virus infection
Bladder cancer
Bladder tumor
Blastomyces infection
Bleeding
Blepharitis
Blepharospasm
Blood clotting disorder
Blood clotting factor deficiency
Blood transfusion
Bone and joint infection
Bone disease
Bone infection
Bone injury

Bone marrow disease
Bone marrow failure
Bone marrow transplantation
Bone metastases
Bone resorption
Bone tumor
Bordetella pertussis infection
Borrelia burgdorferi infection
Borrelia recurrentis infection
Brain disease
Brain hemorrhage
Brain infarction
Brain injury
Brain ischemia
Brain tumor
Breast cancer
Breast disease
Breast tumor
Bronchiectasis
Bronchiolitis
Bronchitis
Bronchospasm
Brucella infection
Budd Chiari syndrome
Bulimia nervosa
Burkholderia infection
Burkitts lymphoma
Bursitis
Cachexia
Campylobacter fetus infection
Campylobacter infection
Campylobacter jejuni infection
Cancer
Cancer pain
Candida albicans infection
Candida infection
Candida krusei infection
Capillary leak syndrome
Carbamoyl phosphate synthase I deficiency
Carcinoid syndrome
Carcinoid tumor
Carcinoma
Cardiac edema

Cardiac failure
Cardiac hypertrophy
Cardiac reperfusion injury
Cardiotoxicity drug-induced
Cardiovascular disease
Carnitine palmitoyltransferase deficiency
Cartilage disease
Castlemans disease
Cataplexy
Cataract
Celiac Disease
Cellulitis
Central nervous system disease
Central nervous system injury
Central nervous system tumor
Cerebral edema
Cerebral hemorrhage
Cerebral hypoxia
Cerebral infarction
Cerebral palsy
Cerebrotendinous xanthomatosis
Cerebrovascular disease
Cerebrovascular ischemia
Cervical dysplasia
Cervical dystonia
Cervicitis
Cervix disease
Cervix infection
Cervix injury
Chemotherapy induced nausea and vomiting
Chemotherapy-induced diarrhea
Chemotherapy-induced emesis
Chlamydia infection
Chlamydia pneumoniae infection
Chlamydia trachomatis infection
Chlamydiae infection
Cholangiocarcinoma
Cholangitis
Cholecystitis
Cholelithiasis
Cholesteryl ester storage disease
Chondrosarcoma
Chorioretinitis

Choroidal neovascularization
Choroiditis
Chromoblastomycosis
Chronic bronchitis
Chronic fatigue syndrome
Chronic granulomatous disease
Chronic infection
Chronic inflammatory demyelinating polyneuropathy
Chronic lymphocytic leukemia
Chronic myelocytic leukemia
Chronic obstructive airway disease
Chronic obstructive pulmonary disease
Chronic Polio Shedders
Chronic thromboembolic pulmonary hypertension
Churg-Strauss syndrome
CINCA syndrome
Circadian rhythm sleep disorder
Cirrhosis
Citrobacter infection
Citrullinuria
Cladophialophora infection
Clostridiaceae infection
Clostridium botulinum infection
Clostridium difficile infection
Clostridium infection
Clostridium tetani infection
Cluster headache
CMV retinitis
Coats disease
Cocaine addiction
Coccidioides infection
Cognitive disorder
Cold agglutinin disease
Colitis
Colon cancer
Colon polyps
Colon tumor
Colorectal cancer
Colorectal tumor
Common cold
Compensated liver cirrhosis
Complement diseases

Complex partial seizure
Complex regional pain syndrome
Complicated skin and skin structure infection
Complicated urinary tract infection
Compulsive gambling
Condyloma
Congenital adrenal hyperplasia
Congenital heart defect
Congenital hemophilia
Congestive heart failure
Conjunctiva disease
Conjunctival neoplasm
Conjunctivitis
Connective tissue disease
Constipation
Constipation predominant irritable bowel syndrome
Contact dermatitis
Contraception
Copper metabolism disorder
Corneal disease
Corneal injury
Corneal transplant rejection
Corneal transplantation
Corneal ulcer
Coronary artery disease
Coronary thrombosis
Corynebacterium diphtheriae infection
Corynebacterium infection
Cough
Coxiella infection
CRE infection
Crohns disease
Cryopyrin associated periodic syndrome
Cryptococcus infection
Cryptococcus neoformans infection
Cryptococcus neoformans meningitis
Cryptosporidium infection
Cushings disease
Cutaneous lupus erythematosus
Cutaneous T-cell lymphoma
Cystic fibrosis
Cystic hygroma

Cystinosis
Cystinuria
Cystitis
Cytomegalovirus infection
Dacryocystitis
Deafness
Decubitus ulcer
Deep vein thrombosis
Degenerative disc disease
Dementia
Dengue virus infection
Depression
Dermatitis
Dermatofibrosarcoma
Dermatological disease
Dermatomycosis
Dermatomyositis
Dermatophytosis
Diabetes insipidus
Diabetes mellitus
Diabetic complication
Diabetic foot ulcer
Diabetic ketoacidosis
Diabetic macular edema
Diabetic nephropathy
Diabetic neuropathy
Diabetic retinopathy
Diarrhea
Diarrhea predominant irritable bowel syndrome
Diastolic heart failure
Diffuse large B-cell lymphoma
Digoxin overdose
Discoid lupus erythematosus
Disseminated intravascular coagulation
Diverticulitis
Diverticulosis
DNA virus infection
Drug withdrawal syndrome
Dry age related macular degeneration
Duchenne dystrophy
Dumping syndrome
Duodenal ulcer
Dupuytren contracture

Dysgeusia
Dysmenorrhea
Dyspareunia
Dyspepsia
Dystonia
Dysuria
Ebola virus infection
Eczema
Edema
Eisenmenger syndrome
Embolism
Emesis
Emotional lability
Emphysema
Empyema
End stage renal disease
Endocarditis
Endocrine disease
Endocrine tumor
Endometrioid carcinoma
Endometriosis
Endophthalmitis
Entamoeba histolytica infection
Enteritis
Enterobacter aerogenes infection
Enterobacter cloacae infection
Enterobacter infection
Enterobacteriaceae infection
Enterococcus faecalis infection
Enterococcus faecium infection
Enterococcus infection
Enterocolitis
Enterotoxigenic Escherichia coli (ETEC)
Enterovirus 71 infection
Enuresis
Eosinophilic esophagitis
Ependymoma
Epidermolysis bullosa
Epidermophyton infection
Epididymitis
Epididymo-orchitis
Epilepsy
Epstein Barr virus infection

Erectile dysfunction
Erysipelas
Erythema
Erythema multiforme
Erythema nodosum leprae
Erythropoietic protoporphyria
ESBL (Extended Spectrum Beta Lactamase) Producing Bacterial Infection
Escherichia coli infection
Esophageal cancer
Esophageal varices
Esophagitis
Esophagus tumor
Essential hypertension
Estrogen deficiency
Ewing sarcoma
Exocrine pancreatic insufficiency
Exophiala infection
Extrinsic allergic alveolitis
Fabry disease
Factor I deficiency
Factor II deficiency
Factor IX deficiency
Factor VII deficiency
Factor VIII deficiency
Factor XIII deficiency
Fallopian tube cancer
Fallopian tube tumor
Familial adenomatous polyposis
Familial amyloid neuropathy
Familial cold autoinflammatory syndrome
Familial hypercholesterolemia
Familial mediterranean fever
Fasciola hepatica infection
Fatigue
Febrile neutropenia
Fecal incontinence
Female contraception
Female genital tract infection
Female genital tract tumor
Female infertility
Female sexual dysfunction
Fever

Fibromyalgia
Fibrosis
Fistula
Flavivirus infection
Folic acid deficiency
Follicle center lymphoma
Folliculitis
Food Allergy
Food poisoning
Fragile X syndrome
Francisella tularensis infection
Friedreich ataxia
Frontotemporal dementia
Functional bowel disorder
Fungal infection
Fungal meningitis
Fungal respiratory tract infection
Fungal urinary tract infection
Fusarium infection
Fusobacterium infection
Galactorrhea
Gallbladder tumor
GALT
Gastric injury
Gastric motility disorder
Gastric ulcers
Gastritis
Gastroesophageal reflux
Gastrointestinal disease
Gastrointestinal infection
Gastrointestinal pain
Gastrointestinal stromal tumor
Gastrointestinal tumor
Gastrointestinal ulcer
Gastroparesis
Gaucher disease
Gauchers disease type I
Gauchers disease type III
General anesthesia
Generalized anxiety disorder
Genetic disorder
Genital herpes
Genital system disease

Genital tract infection
Genital ulcer
Genitourinary disease
Genitourinary tract tumor
Germ cell and embryonic cancer
Gestational diabetes
Giant bone cell tumor
Giardia lamblia infection
Gigantism
Gingivitis
Glaucoma
Glaucoma surgical procedure
Glioblastoma
Glioma
Gliosarcoma
Glomerulonephritis
Glossitis
Glucose intolerance
Goiter
Gout
Graft versus host disease
Gram negative bacterium infection
Gram positive bacterium infection
Granulomatosis
Growth disorder
Growth hormone deficiency
Guillain Barre syndrome
Gynecological disorder
Gynecomastia
Haemophilus aegyptus infection
Haemophilus ducreyi infection
Haemophilus infection
Haemophilus influenzae infection
Haemophilus parainfluenzae infection
Hairy cell leukemia
Hallervorden-Spatz syndrome
Hantavirus infection
Head and neck tumor
Headache
Hearing loss
Heart arrhythmia
Heart disease
Heart transplant rejection

Helicobacter pylori infection
Helminth infection
Hemangioma
Hematological disease
Hematological neoplasm
Hematoma
Hematopoietic stem cell transplantation
Hemiplegia
Hemochromatosis
Hemoglobinopathy
Hemolytic anemia
Hemolytic uremic syndrome
Hemophilia
Hemorrhagic shock
Hemorrhoids
Heparin induced thrombocytopenia
Hepatic edema
Hepatic encephalopathy
Hepatic porphyria
Hepatitis
Hepatitis A virus infection
Hepatitis B virus infection
Hepatitis C virus infection
Hepatitis D virus infection
Hepatitis E virus infection
Hepatitis virus infection
Hepatobiliary disease
Hepatobiliary system tumor
Hepatocellular carcinoma
Hepatorenal syndrome
Hereditary angioedema
Herpes simplex virus infection
Herpesvirus infection
Herpetic keratitis
Hidradenitis
Hidradenitis suppurativa
Hippel Lindau syndrome
Histoplasma infection
HIV infection
HIV-1 infection
HIV-2 infection
HIV-associated lipodystrophy
Hodgkins disease

Homocystinuria
Hormone deficiency
Hormone dependent prostate cancer
Hormone refractory prostate cancer
Hot flashes
HSV-1 infection
HSV-2 infection
Human T cell leukemia virus 1 infection
Hunter syndrome
Huntingtons chorea
Hydrocephalus
Hyperactivity
Hyperammonemia
Hyperandrogenism
Hyperbilirubinemia
Hypercalcemia
Hypercholesterolemia
Hypereosinophilic syndrome
Hyperglycemia
Hyperhidrosis
Hyperkalemia
Hyperlipidemia
Hyperlipoproteinemia type I
Hypernatremia
Hyperoxaluria
Hyperparathyroidism
Hyperphosphatemia
Hyperpigmentation
Hyperprolactinemia
Hypersensitivity
Hypersomnia
Hypertension
Hyperthyroidism
Hypertriglyceridemia
Hypertrophic cardiomyopathy
Hyperuricemia
Hypoalbuminemia
Hypocalcemia
Hypogammaglobulinemia
Hypoglycemia
Hypogonadism
Hyponatremia
Hypoparathyroidism

Hypophosphatasia
Hypophosphatemia
Hypotension
Hypothyroidism
Hypovolemia
Ichthyosis vulgaris
Idiopathic pulmonary fibrosis
IgA nephropathy
Ileus
Immediate type hypersensitivity
Immune complex disease
Immune deficiency
Immune disorder
Immune thrombocytopenic purpura
Immunocompromised
Immunoglobulin G deficiency
Impetigo
Inappropriate ADH syndrome
Inborn error of metabolism
Inclusion body myositis
Indigestion
Infantile spasms
Infectious arthritis
Infectious disease
Infectious endocarditis
Infertility
Inflammatory bowel disease
Inflammatory disease
Influenza virus A infection
Influenza virus B infection
Influenza virus infection
Injury
Insomnia
Insulin dependent diabetes
Intermittent claudication
Interstitial cystitis
Interstitial lung disease
Intervertebral disc disease
Intestine disease
Intestine infection
Intoxication
Iritis
Iron deficiency anemia

Iron metabolism disorder
Iron overload
Irritable bowel syndrome
Ischemia
Ischemic heart disease
Ischemic stroke
Islet cell transplant rejection
Japanese encephalitis virus infection
Jaundice
Jaw disease
Joint disease
Joint infection
Juvenile myoclonic epilepsy
Juvenile rheumatoid arthritis
Kaposi sarcoma
Kawasaki disease
Keratitis
Keratoconjunctivitis
Keratosi
Kidney dialysis
Kidney transplant rejection
Klebsiella granulomatis infection
Klebsiella infection
Klebsiella pneumoniae infection
Labor complication
Lacrimal gland disease
Lactation disorder
Lambert-Eaton syndrome
Large intestine tumor
Laron syndrome
Laryngitis
Laryngopharyngitis
Lebers hereditary optic atrophy
Left heart disease pulmonary hypertension
Leg ulcer
Legionella infection
Legionella pneumophila infection
Leigh disease
Leishmania braziliensis infection
Leishmania donovani infection
Leishmania infection
Leishmania tropica infection
Lennox Gastaut syndrome

Leprosy
Leptospiraceae infection
Lesch Nyhan syndrome
Leukemia
Leukopenia
Leukopenia drug induced
Leukoplakia
Lewy body dementia
Lichen
Lipid metabolism disorder
Lipodystrophy
Lipoma
Lipoprotein lipase deficiency
Liposarcoma
Listeria monocytogenes infection
Listeriosis
Liver abscess
Liver cancer
Liver cirrhosis
Liver disease
Liver fibrosis
Liver injury
Liver transplant rejection
Liver tumor
Lower back pain
Lower limb fracture
Lower respiratory tract infection
Lung abscess
Lung cancer
Lung disease
Lung disease pulmonary hypertension
Lung embolism
Lung infection
Lung inflammation
Lung malformation
Lung transplant rejection
Lung tumor
Lupus erythematosus panniculitis
Lupus nephritis
Lyme disease
Lymphadenitis
Lymphadenopathy
Lymphangiomyomatosis

Lymphangitis
Lymphatic system disease
Lymphedema
Lymphoblastic leukemia
Lymphoid leukemia
Lymphoma
Lysosomal acid lipase deficiency
Macroglobulinemia
Macular disease
Macular edema
Macular telangiectasia 2
Major depressive disorder
Male contraception
Male hypogonadism
Male infertility
Male osteoporosis
Male sexual dysfunction
Mania
Mantle cell lymphoma
Marburg virus infection
Marginal zone B-cell lymphoma
Maroteaux-Lamy syndrome
Mastalgia
Mastitis
Mastocytosis
Mastoiditis
Measles virus infection
Meconium aspiration syndrome
Medullary thyroid cancer
Medulloblastoma
Melanoma
Membranoproliferative glomerulonephritis
Membranous glomerulonephritis
MEN1
MEN2
Meningioma
Meningitis
Menopause
Menorrhagia
Menstruation disorder
Mental deficiency
Merkel cell carcinoma
Mesothelioma

Metabolic bone disease
Metabolic disorder
Metabolic Syndrome
Metabolic syndrome X
Metal intoxication
Metastasis
Metastatic brain cancer
Metastatic breast cancer
Metastatic colon cancer
Metastatic colorectal cancer
Metastatic esophageal cancer
Metastatic gastrointestinal cancer
Metastatic head and neck cancer
Metastatic liver cancer
Metastatic lung cancer
Metastatic non small cell lung cancer
Metastatic ovary cancer
Metastatic pancreas cancer
Metastatic prostate cancer
Metastatic renal cancer
Metastatic stomach cancer
Methylmalonic acidemia
Metrorrhagia
Micrococcaceae infection
Micrococcus infection
Microsporidial infection
Microsporum infection
Migraine
Mild cognitive impairment
Mitochondrial disease
Molluscum contagiosum infection
Monkeypox virus infection
Moraxella catarrhalis infection
Moraxella infection
Morganella infection
Morganella morganii infection
Morning sickness
Morquio syndrome
Motion sickness
Motor neurone disease
Mouth tumor
Mouth ulcer
Movement disorder

MRSA infection
Muckle Wells syndrome
Mucopolysaccharidosis type I
Mucor infection
Mucositis
Multidrug resistant infection
Multiple myeloma
Multiple sclerosis
Mumps virus infection
Muscle hypertonia
Muscle injury
Muscle spasm
Muscle weakness
Muscular dystrophy
Musculoskeletal disease
Musculoskeletal pain
Musculoskeletal system inflammation
Myalgia
Myasthenia gravis
Mycobacterium infection
Mycobacterium leprae infection
Mycobacterium tuberculosis infection
Mycoplasma infection
Mycoplasma pneumoniae infection
Mycosis fungoides
Mydriasis
Myelitis
Myelodysplastic syndrome
Myelofibrosis
Myeloid leukemia
Myeloproliferative disorder
Myocardial disease
Myocardial infarction
N-acetylglutamate synthase deficiency
Nail disease
Narcolepsy
Nasal polyps
Nasopharyngeal carcinoma
Nausea
Nausea drug-induced
Necrotizing enterocolitis
Necrotizing Pancreatitis
Neisseria gonorrhoeae infection

Neisseria infection
Neisseria meningitidis infection
Neisseria meningitidis meningitis
Nelson syndrome
Nematode infection
Neonatal respiratory distress syndrome
Neoplasm
Neoplastic meningitis
Nephritis
Nephroblastoma
Nephropathic cystinosis
Nephrotic syndrome
Nerve injury
Nerve tumor
Neuroblastoma
Neurodegenerative disease
Neuroendocrine tumor
Neurofibromatosis
Neurofibromatosis type I
Neurological disease
Neuromuscular blockade
Neuromuscular disease
Neuromyelitis optica
Neuropathic pain
Neuropathy
Neutropenia
Nicotine dependence
Niemann Pick disease type C
Nocturia
Non alcoholic fatty liver disease
Non segmental vitiligo
Non small cell lung cancer
Non-alcoholic steatohepatitis
Non-Hodgkin lymphoma
Non-insulin dependent diabetes
Non-small-cell lung cancer
Obesity
Obsessive compulsive disorder
Obstructive sleep apnea
Occlusive coronary artery disease
Ocular cystinosis
Ocular disease
Ocular hypertension

Ocular infection
Ocular inflammation
Ocular melanoma
Ocular pain
Ocular surgical procedure
Onychomycosis
Open angle glaucoma
Opiate dependence
Optic neuritis
Oral mucositis
Orbital inflammatory disease
Organ transplant rejection
Organ transplantation
Ornithine transcarbamylase deficiency
Orthostatic hypotension
Ostealgia
Osteoarthritis
Osteogenesis imperfecta
Osteolysis
Osteomalacia
Osteomyelitis
Osteonecrosis
Osteopenia
Osteopetrosis
Osteoporosis
Osteosarcoma
Otitis
Otitis externa
Otitis media
Otorhinolaryngological disease
Otorhinolaryngological infection
Ovarian hyperstimulation syndrome
Ovary cancer
Ovary tumor
Overactive bladder
Overgrowth syndrome
Pagets bone disease
Pain
Pancreas disease
Pancreas transplant rejection
Pancreas tumor
Pancreatic ductal adenocarcinoma
Pancreatic endocrine tumor

Pancreatic ulcers
Pancreatitis
Panic disorder
Panophthalmitis
Papillary thyroid tumor
Papillomavirus infection
Papular skin disease
Papulosis
Paracetamol overdose
Paralysis
Paraproteinemia
Parasitic infection
Parkinsons disease
Paroxysmal nocturnal hemoglobinuria
Partial seizure
Parturition
Patent ductus arteriosus
Pediatric varicella zoster virus infection
Pediculosis capitis infestation
Pelvic inflammatory disease
Pemphigoid
Pemphigus
Penile induration
Penis tumor
Peptic ulcer
Peptostreptococcus infection
Perennial allergic rhinitis
Perianal fistula
Periarthritis
Pericoronitis
Periodontal disease
Periodontitis
Peripheral arterial occlusive disease
Peripheral neuropathy
Peripheral T-cell lymphoma
Peripheral vascular disease
Peritoneal tumor
Peritonitis
Peritonsillitis
Pervasive child developmental disorder
Pharyngitis
Phenylketonuria
Phlebothrombosis

Pink eye infection
Pituitary tumor
Pityriasis
Planned abortion
Plasmodium falciparum infection
Plasmodium infection
Plasmodium malariae infection
Plasmodium vivax infection
Pleural disease
Pneumocystis carinii infection
Pneumonia
POEMS syndrome
Poison intoxication
Poliovirus infection
Polyarteritis nodosa
Polychondritis
Polycystic kidney disease
Polycystic ovary syndrome
Polycythemia
Polycythemia vera
Polymorphus light eruption
Polymyalgia rheumatica
Polymyositis
Polyomavirus infection
Pompes disease
Port wine stain
Portal hypertension
Post traumatic stress disorder
Post-surgical bacterial leakage
Postherpetic neuralgia
Postmenopausal osteoporosis
Postnatal depression
Pouchitis
Pre-eclampsia
Precocious puberty
Premature ejaculation
Premature labor
Premature ovarian failure
Premenstrual syndrome
Prevotella infection
Priapism
Primary biliary cirrhosis
Primary sclerosing cholangitis

Proctitis
Progestogen deficiency
Progressive supranuclear palsy
Proliferative diabetic retinopathy
Prolymphocytic leukemia
Prophylaxis
Propionibacterium acnes infection
Propionibacterium infection
Prostate cancer
Prostate hyperplasia
Prostate tumor
Prostatitis
Protein C deficiency
Proteinuria
Proteus infection
Proteus mirabilis infection
Protozoal infection
Providencia infection
Prurigo
Pruritus
Pseudomembranous colitis
Pseudomonas aeruginosa infection
Pseudomonas infection
Psoriasis
Psoriatic arthritis
Psychiatric disorder
Psychotic disorder
Pterygium
Pulmonary artery hypertension
Pulmonary fibrosis
Pulmonary heart disease
Pulmonary hypertension
Purpura
Pyelonephritis
Pyoderma gangrenosum
Rabies virus infection
Radiation sickness
Radiotherapy induced emesis
Raynauds disease
Rectal cancer
Rectal tumor
Rectosigmoiditis
Renal cell carcinoma

Renal disease
Renal failure
Renal injury
Renal osteodystrophy
Renal tubule disease
Renal tumor
Renovascular hypertension
Reperfusion injury
Reproductive disorder
Respiratory congestion
Respiratory disease
Respiratory disorder
Respiratory distress syndrome
Respiratory insufficiency
Respiratory syncytial virus infection
Respiratory tract infection
Respiratory tract inflammation
Restenosis
Restless legs syndrome
Retinal dystrophy
Retinal venous occlusion
Retinitis pigmentosa
Retinoblastoma
Retinopathy
Retinopathy of prematurity
Rett syndrome
Rheumatoid arthritis
Rhinitis
Rhinopharyngitis
Rhizomucor infection
Rhizopus infection
Rickets
Rickettsia infection
Rocky mountain spotted fever
Rosacea
Ross River virus infection
Rotavirus infection
Rotavirus Vaccine Adjunct
Rubella virus infection
Salmonella infection
Salmonella typhi infection
Salpingitis
Sandhoff disease

Sarcoidosis
Sarcoma
Sarcopenia
SARS coronavirus infection
Scabies infection
Scar tissue
Scedosporium infection
Schistosomiasis
Schizoaffective disorder
Schizophrenia
Scleritis
Scleroderma
Seasonal affective disorder
Seasonal allergic rhinitis
Sebaceous gland disease
Seborrheic dermatitis
Secondary hyperparathyroidism
Seizure disorder
Seminoma
Sepsis
Septic shock
Serratia infection
Serratia marcescens infection
Serum sickness
Severe combined immunodeficiency syndrome
Sexual deviation
Sexual dysfunction
Sezary syndrome
Shigella boydii infection
Shigella dysenteriae infection
Shigella flexneri infection
Shigella infection
Shigella sonnei infection
Shock
Short bowel syndrome
Sialadenitis
Sialorrhea
Sickle beta 0 thalassemia
Sickle cell anemia
Sickle cell crisis
Sinusitis
Sitosterolemia
Sjogrens syndrome

Skin allergy
Skin burns
Skin cancer
Skin infection
Skin Inflammatory disease
Skin lymphoma
Skin tumor
Skin ulcer
Sleep apnea
Sleep disorder
Small-cell lung cancer
Social phobia
Soft tissue sarcoma
Solid tumor
Spinal cord disease
Spinal cord injury
Spinal cord tumor
Spinal muscular atrophy
Spinal stenosis
Spinocerebellar ataxia
Spinocerebellar degenerations
Splenomegaly
Spondylarthritis
Spondylosynthesis
Spontaneous abortion
Squamous cell carcinoma
Stage III melanoma
Stage IV melanoma
Staphylococcus aureus infection
Staphylococcus epidermidis infection
Staphylococcus infection
Staphylococcus saprophyticus infection
Stem cell transplantation
Stenotrophomonas maltophilia infection
Stevens Johnson syndrome
Stomach cancer
Stomach disease
Stomach infection
Stomach tumor
Stomach ulcer
Stomatitis
Strabismus
Streptococcus agalactiae infection

Streptococcus constellatus infection
Streptococcus infection
Streptococcus intermedius infection
Streptococcus mitis infection
Streptococcus oralis infection
Streptococcus pneumoniae infection
Streptococcus pyogenes infection
Stress urinary incontinence
Stroke
Stutter
Subcortical vascular dementia
Sucrase isomaltase deficiency
Sunburn
Surgery
Surgical procedure
Synovitis
Systemic lupus erythematosus
Systemic mastocytosis
Systolic heart failure
T-cell acute lymphoblastic leukemia
T-cell lymphoma
Tachycardia
Tardive dyskinesia
Tay Sachs disease
Temporal arteritis
Tendon injury
Tenosynovitis
Testis tumor
Testosterone deficiency
Thalassemia major
Thrombasthenia
Thromboangiitis obliterans
Thrombocyte disorder
Thrombocythemia
Thrombocytopenia
Thrombocytopenic purpura
Thrombocytosis
Thromboembolism
Thrombosis
Thymoma
Thyroid associated ophthalmopathy
Thyroid tumor
Tinea

Tinea capitis
Tinea corporis
Tinea cruris
Tinea pedis
Tinnitus
Tonic clonic epilepsy
Tonsillitis
Tooth disease
Topical anesthesia
Torticollis
Tourette syndrome
Toxic epidermal necrolysis
Toxicity
Transitional cell carcinoma
Transplant rejection
Transplantation
Traumatic brain injury
Traveler's diarrhea
Trench mouth
Treponema infection
Treponema pallidum infection
Trichomonas infection
Trichophyton infection
Trigeminal neuralgia
Trypanosoma brucei infection
Trypanosoma cruzi infection
Trypanosomiasis
Tuberous sclerosis
Tumor lysis syndrome
Turners syndrome
Type 1 Diabetes
Type 2 Diabetes
Type I tyrosinemia
Ulcer
Ulcerative colitis
Ulcerative proctitis
Umbilical cord stem cell transplantation
Unidentified indication
Unstable angina
Upper respiratory tract infection
Ureaplasma urealyticum infection
Uremia
Urethritis

Urinary dysfunction
Urinary incontinence
Urinary retention
Urinary tract disease
Urinary tract infection
Urinary tract tumor
Urogenital tract infection
Urolithiasis
Urticaria
Uterine cervix tumor
Uterine fibroids
Uterus infection
Uterus tumor
Uveal melanoma
Uveitis
Vaccination
Vaccinia virus infection
Vaginal infection
Vaginitis
Variant angina pectoris
Varicella zoster virus infection
Varices
Varicosis ulcer
Variola virus infection
Vascular dementia
Vascular disease
Vascular neoplasm
Vascular occlusive disease
Vasculitis
Venous occlusive disease
Ventricular arrhythmia
Ventricular fibrillation
Ventricular tachycardia
Verruca vulgaris
Vertigo
Vibrio cholerae infection
Viral encephalitis
Viral eye infection
Viral hemorrhagic fever
Viral infection
Viral respiratory tract infection
Viral susceptibility
Viridans group Streptococcus infection

Vitamin B12 deficiency
Vitamin D deficiency
Vitamin E deficiency
Vitamin K deficiency
Vitiligo
Vitreous hemorrhage
Von Willebrands disease
Vancomycin-Resistant Enterococcus Infection
Vulva disease
Vulvovaginitis
Wasting disease
Wasting Syndrome
Wegener granulomatosis
Weight loss
West Nile virus infection

Wet age related macular degeneration
Whipple's disease
Wilson disease
Wound healing
X linked dominant hypophosphatemic rickets
Xenobiotic metabolism
Xerophthalmia
Xerostomia
Yellow fever virus infection
Yersinia pestis infection
Zollinger-Ellison syndrome
Flatulence
Gastrointestinal Disorder
General Inflammation

Table 4

Representative diseases, disorders and conditions potentially associated with a dysbiosis, which are suitable for treatment with the compositions and methods of the invention

Abdominal cavity inflammation
Absidia infection
Acinetobacter baumannii infection
Acinetobacter infection
Acinetobacter Iwoffii infection
Acne
Actinomyces israelii infection
Adenovirus infection
Adult varicella zoster virus infection
Aging
Alcoholism (and effects)
Allergic conjunctivitis
Allergic rhinitis
Allergy
ALS
Alzheimers disease
Amoeba infection
Anal cancer
Antibiotic treatment
Antitbiotic associated diarrhea
Arteriosclerosis
Arthritis
Aspergillus fumigatus infection
Aspergillus infection
Asthma
Atherosclerosis
Atopic dermatitis
Atopy / Allergic Sensitivity
Autism
Autoimmune disease
Bacillus anthracis infection
Bacillus infection
Bacterial endocarditis
Bacterial eye infection

Bacterial infection
Bacterial meningitis
Bacterial pneumonia
Bacterial respiratory tract infection
Bacterial skin infection
Bacterial susceptibility
Bacterial urinary tract infection
Bacterial vaginosis
Bacteroides caccae infection
Bacteroides fragilis infection
Bacteroides infection
Bacteroides thetaiotaomicron infection
Bacteroides uniformis infection
Bacteroides vulgatus infection
Bartonella bacilliformis infection
Bartonella infection
Bifidobacterium infection
Biliary cancer
Biliary cirrhosis
Biliary tract disease
Biliary tract infection
Biliary tumor
BK virus infection
Blastomyces infection
Bone and joint infection
Bone infection
Bordetella pertussis infection
Borrelia burgdorferi infection
Borrelia recurrentis infection
Brucella infection
Burkholderia infection
Cachexia
Campylobacter fetus infection
Campylobacter infection
Campylobacter jejuni infection
Cancer
Candida albicans infection
Candida infection
Candida krusei infection
Celiac Disease
Cervix infection
Chemotherapy-induced diarrhea
Chlamydia infection

Chlamydia pneumoniae infection
Chlamydia trachomatis infection
Chlamydiae infection
Chronic fatigue syndrome
Chronic infection
Chronic inflammatory demyelinating polyneuropathy
Chronic Polio Shedders
Circadian rhythm sleep disorder
Cirrhosis
Citrobacter infection
Cladophialophora infection
Clostridiaceae infection
Clostridium botulinum infection
Clostridium difficile infection
Clostridium infection
Clostridium tetani infection
Coccidioides infection
Colitis
Colon cancer
Colorectal cancer
Common cold
Compensated liver cirrhosis
Complicated skin and skin structure infection
Complicated urinary tract infection
Constipation
Constipation predominant irritable bowel syndrome
Corynebacterium diphtheriae infection
Corynebacterium infection
Coxiella infection
CRE infection
Crohns disease
Cryptococcus infection
Cryptococcus neoformans infection
Cryptosporidium infection
Cutaneous lupus erythematosus
Cystic fibrosis
Cystitis
Cytomegalovirus infection
Dementia
Dengue virus infection
Depression
Dermatitis

Diabetes mellitus
Diabetic complication
Diabetic foot ulcer
Diarrhea
Diarrhea predominant irritable bowel syndrome
Discoid lupus erythematosus
Diverticulitis
DNA virus infection
Duodenal ulcer
Ebola virus infection
Entamoeba histolytica infection
Enterobacter aerogenes infection
Enterobacter cloacae infection
Enterobacter infection
Enterobacteriaceae infection
Enterococcus faecalis infection
Enterococcus faecium infection
Enterococcus infection
Enterocolitis
Enterovirus 71 infection
Epidermophyton infection
Epstein Barr virus infection
ESBL (Extended Spectrum Beta Lactamase) Producing Bacterial Infection
Escherichia coli infection
Esophageal cancer
Exophiala infection
Familial cold autoinflammatory syndrome
Fasciola hepatica infection
Female genital tract infection
Female genital tract tumor
Female infertility
Fibrosis
Flavivirus infection
Food Allergy
Francisella tularensis infection
Functional bowel disorder
Fungal infection
Fungal respiratory tract infection
Fungal urinary tract infection
Fusarium infection
Fusobacterium infection
Gastric ulcers

Gastrointestinal infection
Gastrointestinal pain
Gastrointestinal ulcer
Genital tract infection
Genitourinary disease
Genitourinary tract tumor
Gestational diabetes
Giardia lamblia infection
Gingivitis
Gram negative bacterium infection
Gram positive bacterium infection
Haemophilus aegyptus infection
Haemophilus ducreyi infection
Haemophilus infection
Haemophilus influenzae infection
Haemophilus parainfluenzae infection
Hantavirus infection
Helicobacter pylori infection
Helminth infection
Hepatitis A virus infection
Hepatitis B virus infection
Hepatitis C virus infection
Hepatitis D virus infection
Hepatitis E virus infection
Hepatitis virus infection
Herpes simplex virus infection
Herpesvirus infection
Histoplasma infection
HIV infection
HIV-1 infection
HIV-2 infection
HSV-1 infection
HSV-2 infection
Human T cell leukemia virus 1 infection
Hypercholesterolemia
Hyperoxaluria
Hypertension
Infectious arthritis
Infectious disease
Infectious endocarditis
Infertility
Inflammatory bowel disease
Inflammatory disease

Influenza virus A infection
Influenza virus B infection
Influenza virus infection
Insomnia
Insulin dependent diabetes
Intestine infection
Irritable bowel syndrome
Japanese encephalitis virus infection
Joint infection
Juvenile rheumatoid arthritis
Klebsiella granulomatis infection
Klebsiella infection
Klebsiella pneumoniae infection
Legionella infection
Legionella pneumophila infection
Leishmania braziliensis infection
Leishmania donovani infection
Leishmania infection
Leishmania tropica infection
Leptospiraceae infection
Listeria monocytogenes infection
Listeriosis
Liver cirrhosis
Liver fibrosis
Lower respiratory tract infection
Lung infection
Lung inflammation
Lupus erythematosus panniculitis
Lupus nephritis
Lyme disease
Male infertility
Marburg virus infection
Measles virus infection
Metabolic disorder
Metabolic Syndrome
Metastatic colon cancer
Metastatic colorectal cancer
Metastatic esophageal cancer
Metastatic gastrointestinal cancer
Metastatic stomach cancer
Micrococcaceae infection
Micrococcus infection
Microsporidial infection

Microsporum infection
Molluscum contagiosum infection
Monkeypox virus infection
Moraxella catarrhalis infection
Moraxella infection
Morganella infection
Morganella morganii infection
MRSA infection
Mucor infection
Multidrug resistant infection
Multiple sclerosis
Mumps virus infection
Musculoskeletal system inflammation
Mycobacterium infection
Mycobacterium leprae infection
Mycobacterium tuberculosis infection
Mycoplasma infection
Mycoplasma pneumoniae infection
Necrotizing enterocolitis
Necrotizing Pancreatitis
Neisseria gonorrhoeae infection
Neisseria infection
Neisseria meningitidis infection
Nematode infection
Non alcoholic fatty liver disease
Non-alcoholic steatohepatitis
Non-insulin dependent diabetes
Obesity
Ocular infection
Ocular inflammation
Orbital inflammatory disease
Osteoarthritis
Otorhinolaryngological infection
Pain
Papillomavirus infection
Parasitic infection
Parkinsons disease
Pediatric varicella zoster virus infection
Pelvic inflammatory disease
Peptostreptococcus infection
Perennial allergic rhinitis
Periarthritis
Pink eye infection

Plasmodium falciparum infection
Plasmodium infection
Plasmodium malariae infection
Plasmodium vivax infection
Pneumocystis carinii infection
Poliovirus infection
Polyomavirus infection
Post-surgical bacterial leakage
Pouchitis
Prevotella infection
Primary biliary cirrhosis
Primary sclerosing cholangitis
Propionibacterium acnes infection
Propionibacterium infection
Prostate cancer
Proteus infection
Proteus mirabilis infection
Protozoal infection
Providencia infection
Pseudomonas aeruginosa infection
Pseudomonas infection
Psoriasis
Psoriatic arthritis
Pulmonary fibrosis
Rabies virus infection
Rectal cancer
Respiratory syncytial virus infection
Respiratory tract infection
Respiratory tract inflammation
Rheumatoid arthritis
Rhinitis
Rhizomucor infection
Rhizopus infection
Rickettsia infection
Ross River virus infection
Rotavirus infection
Rubella virus infection
Salmonella infection
Salmonella typhi infection
Sarcopenia
SARS coronavirus infection
Scabies infection
Scedosporium infection

Scleroderma
Seasonal allergic rhinitis
Serratia infection
Serratia marcescens infection
Shigella boydii infection
Shigella dysenteriae infection
Shigella flexneri infection
Shigella infection
Shigella sonnei infection
Short bowel syndrome
Skin allergy
Skin cancer
Skin infection
Skin Inflammatory disease
Sleep disorder
Spondylarthritis
Staphylococcus aureus infection
Staphylococcus epidermidis infection
Staphylococcus infection
Staphylococcus saprophyticus infection
Stenotrophomonas maltophilia infection
Stomach cancer
Stomach infection
Stomach ulcer
Streptococcus agalactiae infection
Streptococcus constellatus infection
Streptococcus infection
Streptococcus intermedius infection
Streptococcus mitis infection
Streptococcus oralis infection
Streptococcus pneumoniae infection
Streptococcus pyogenes infection
Systemic lupus erythematosus
Traveler's diarrhea
Trench mouth
Treponema infection
Treponema pallidum infection
Trichomonas infection
Trichophyton infection
Trypanosoma brucei infection
Trypanosoma cruzi infection
Type 1 Diabetes
Type 2 Diabetes

Ulcerative colitis
Upper respiratory tract infection
Ureaplasma urealyticum infection
Urinary tract disease
Urinary tract infection
Urinary tract tumor
Urogenital tract infection
Uterus infection
Vaccinia virus infection
Vaginal infection
Varicella zoster virus infection
Variola virus infection
Vibrio cholerae infection
Viral eye infection
Viral infection
Viral respiratory tract infection
Viridans group Streptococcus infection
Vancomycin-Resistant Enterococcus Infection
Wasting Syndrome
Weight loss
West Nile virus infection
Whipple's disease
Xenobiotic metabolism
Yellow fever virus infection
Yersinia pestis infection
Flatulence
Gastrointestinal Disorder
General Inflammation

Table 5

Mortality and weight change in mice challenged with *C. difficile* with or without ethanol treated, spore preparation treatment.

Test article	mortality (n=10)	% weight change on Day 3
vehicle (negative control)	20%	-10.5%
Donor feces (positive control)	0	-0.1%
EtOH-treated spore preparation 1x	0	2.3%
EtOH-treated spore preparation 0.1x	0	2.4%
EtOH-treated spore preparation 0.01x	0	-3%
heat-treated spore preparation	0	0.1%

Table 6

Microbial compositions administered via oral gavage on Day -1

	OTU	Clade
Microbial Composition 1	Clostridium_butyricum	clade_252
	Clostridium_disporicum	clade_253
	Clostridium_hylemonae	clade_260
	Clostridium_orbiscindens	clade_494
	Clostridium_symbiosum	clade_408
	Collinsella_aerofaciens	clade_553
	Coprococcus_comes	clade_262
	Lachnospiraceae_bacterium_5_1_57FAA	clade_260
	Ruminococcus_bromii	clade_537
	Blautia_producta	clade_309
	Clostridium_bolteae	clade_408
	Clostridium_innocuum	clade_351
	Clostridium_mayombeii	clade_354
	Clostridium_tertium	clade_252
	Ruminococcus_gnavus	clade_360
Microbial Composition 2	Clostridium_disporicum	clade_253
	Clostridium_orbiscindens	clade_494
	Clostridium_symbiosum	clade_408
	Collinsella_aerofaciens	clade_553
	Eubacterium_rectale	clade_444
	Lachnospiraceae_bacterium_5_1_57FAA	clade_260
	Blautia_producta	clade_309
	Clostridium_innocuum	clade_351
	Clostridium_mayombeii	clade_354

Table 7

Population of OTUs on Days 2, 3 and 4 following dosing with Microbial Compositions

	1x10 ⁹ per OTU					1x10 ⁸ per OTU					1x10 ⁷ per OTU			
Microbial comp 1	-1	2	3	4		-1	2	3	4		-1	2	3	4
Cl_butyricum	0	106	51	32		0	10	0	34		195	0	0	0
Cl_disporicum	10	1746	1190	887		0	1746	769	1011		201	1117 5	1531	1152
Cl_hylemonae	0	258	258	84		0	203	164	77		0	265	214	90
Cl_orbiscindens	0	188	192	471		0	188	138	276		0	221	174	341
Cl_symbiosum	0	485	482	486		0	444	379	447		0	562	427	775
Co_aerofaciens	0	0	0	0		0	0	0	0		0	0	0	0
C_comes	0	0	0	0		0	0	0	0		0	0	0	0
L_bacterium_5_1_57 FAA	0	341	336	354		0	351	182	356		0	256	240	300
R_bromii	0	0	0	0		0	0	0	0		0	0	0	0
B_producta	0	0	0	0		0	0	0	0		0	0	0	0
Cl_bolteae	0	0	0	0		0	0	0	0		0	0	0	0
Cl_innocuum	0	0	0	0		0	0	0	0		0	0	0	0
Cl_mayombeii	0	0	0	0		0	0	0	0		0	0	0	0
Cl_tertium	0	0	0	0		0	0	0	0		0	0	0	0
R_gnavus	0	0	0	0		0	0	0	0		0	0	0	0
Microbial comp 2	-1	2	3	4		-1	2	3	4		-1	2	3	4
Cl_disporicum	29	1181 0	1094 8	1467 2		0	1134 9	1397 8	3942		0	1199 5	7005	6268
Cl_orbiscindens	0	510	408	764		0	332	545	544		0	310	319	432
Cl_symbiosum	0	559	508	375		0	665	494	450		0	396	639	650
Co_aerofaciens	0	0	0	0		0	0	1172	0		0	0	247	0
E_rectale	0	0	0	0		0	0	0	12		0	0	0	261

L_bacterium_5_1_57 FAA	0	972	801	596		0	860	962	844		0	636	1901	1269
B_producta	0	0	0	0		0	0	0	0		0	0	0	0
Cl_innocuum	0	0	0	0		0	0	0	0		0	0	0	0
Cl_mayombeii	0	0	0	0		0	0	0	0		0	0	0	0

Table 8

Population of Clades on Days 2, 3 and 4 following dosing with Microbial Compositions

	1x10 ⁹ per OTU					1x10 ⁸ per OTU					1x10 ⁷ per OTU			
Microbial comp 1	-1	2	3	4		-1	2	3	4		-1	2	3	4
clade_252	0	444	252	87		0	198	122	125		209	394	231	88
clade_253	10	1746	1190	887		0	1746	769	1011		201	11175	1531	1152
clade_260	0	599	594	438		0	554	346	433		0	521	454	390
clade_262	0	14	151	51		0	0	0	0		0	12	21	57
clade_309	0	11093	9750	4023		0	9991	5208	5145		19	9311	6369	4951
clade_351	0	9064	10647	7751		0	6528	7259	8213		0	8903	10049	8701
clade_354	0	0	0	0		0	0	0	31		173	0	0	0
clade_360	0	14300	10220	11036		0	12553	12989	6889		0	9308	13483	9292
clade_408	13	8892	12985	12101		23	3952	7260	10652		43	4079	8581	14929
clade_494	0	226	227	565		0	188	184	411		0	221	200	351
clade_537	0	0	68	225		0	0	0	0		0	0	0	55
clade_553	0	0	0	0		0	0	0	0		0	0	0	0
Microbial comp 2	-1	2	3	4		-1	2	3	4		-1	2	3	4
clade_253	29	11810	10948	14672		0	11349	13978	3942		0	11995	7005	6268
clade_260	0	1125	1312	854		0	1049	1295	1250		0	792	2121	1637
clade_309	54	12513	13731	7849		0	11610	12004	12672		0	7407	14111	10858
clade_351	0	7651	9939	5936		0	8495	9724	9207		0	6005	9833	7655
clade_354	149	0	127	429		0	0	0	39		12	0	0	0
clade_408	18	2242	4989	10480		12	1688	5580	3789		0	1068	1561	6281
clade_444	41	0	49	202		0	18	0	12		0	14	82	1578
clade_494	0	510	465	1054		0	332	565	596		0	310	319	476
clade_553	0	0	0	0		0	0	1172	0		0	0	247	0

Table 9

Mortality by experimental group in mice challenged with $10^{4.5}$ *C. difficile* spores on Day 0

Group	Dose (CFU per OTU)	Deaths (% mortality)
Vehicle control	N/A	10 (100%)
Microbial composition 1	10^9	1 (10%)
	10^8	1 (10%)
	10^7	0 (0%)
Microbial composition 2	10^9	0 (0%)
	10^8	1 (10%)
	10^7	1 (10%)

Table 10

Spore quantitation for ethanol treated spore preparations using spore CFU (SCFU) assay and DPA assay

Preparation	SCFU / 30 capsules	DPA SEq / 30 capsules	Ratio SCFU / DPA
Preparation 1	4.0×10^5	6.8×10^7	5.9×10^{-3}
Preparation 2	2.1×10^7	9.2×10^8	0.023
Preparation 3	6.9×10^9	9.6×10^9	0.72

Table 11

DPA doses in Table AC when normalized to 4×10^5 SCFU per dose

Preparation	SCFU / 30 capsules	DPA SEq / 30 capsules	Fraction of Preparation 1 Dose
Preparation 1	4.0×10^5	6.8×10^6	1.0
Preparation 2	4.0×10^5	1.8×10^7	0.26
Preparation 3	4.0×10^5	5.6×10^5	0.0082

Table 12

Bacterial OTUs associated with engraftment and ecological augmentation and establishment of a more diverse microbial ecology in patients treated with an ethanol treated spore preparation. OTUs that comprise an augmented ecology are not present in the patient prior to treatment and/or exist at extremely low frequencies such that they do not comprise a significant fraction of the total microbial carriage and are not detectable by genomic and/or microbiological assay methods. OTUs that are members of the engrafting and augmented ecologies were identified by characterizing the OTUs that increase in their relative abundance post treatment and that respectively are: (i) present in the ethanol treated spore preparation and absent in the patient pretreatment, or (ii) absent in the ethanol treated spore preparation, but increase in their relative abundance through time post treatment with the preparation due to the formation of favorable growth conditions by the treatment. Notably, the latter OTUs can grow from low frequency reservoirs in the patient, or be introduced from exogenous sources such as diet. OTUs that comprise a “core” augmented or engrafted ecology can be defined by the percentage of total patients in which they are observed to engraft and/or augment; the greater this percentage the more likely they are to be part of a core ecology responsible for catalyzing a shift away from a dysbiotic ecology. The dominant OTUs in an ecology can be identified using several methods including but not limited to defining the OTUs that have the greatest relative abundance in either the augmented or engrafted ecologies and defining a total relative abundance threshold. As example, the dominant OTUs in the augmented ecology of Patient-1 were identified by defining the OTUs with the greatest relative abundance, which together comprise 60% of the microbial carriage in this patient’s augmented ecology.

OTU	Phylogenetic Clade	Spore Forming OTU	Dominant OTU in Augmented Ecology
Bacteroides sp. 2_1_22	clade38	N	Y
Streptococcus anginosus	clade60	N	
Prevotella intermedia	clade81	N	
Prevotella nigrescens	clade81	N	
Oribacterium sp. ACB7	clade90	N	
Prevotella salivae	clade104	N	
Bacteroides intestinalis	clade171	N	Y

OTU	Phylogenetic Clade	Spore Forming OTU	Dominant OTU in Augmented Ecology
<i>Bifidobacterium dentium</i>	clade172	N	
<i>Alcaligenes faecalis</i>	clade183	N	
<i>Rothia dentocariosa</i>	clade194	N	
<i>Peptoniphilus lacrimalis</i>	clade291	N	
<i>Anaerococcus</i> sp. gpac155	clade294	N	
<i>Sutterella stercoricanis</i>	clade302	N	Y
<i>Bacteroides</i> sp. 3_1_19	clade335	N	Y
<i>Parabacteroides goldsteinii</i>	clade335	N	
<i>Bacteroides dorei</i>	clade378	N	Y
<i>Bacteroides massiliensis</i>	clade378	N	
<i>Lactobacillus iners</i>	clade398	N	
<i>Granulicatella adiacens</i>	clade460	N	
<i>Eggerthella</i> sp. 1_3_56FAA	clade477	N	
<i>Gordonibacter pamelaee</i>	clade477	N	
<i>Finegoldia magna</i>	clade509	N	
<i>Actinomyces nasicola</i>	clade523	N	
<i>Streptobacillus moniliformis</i>	clade532	N	
<i>Oscillospira guilliermondii</i>	clade540	N	
<i>Orientia tsutsugamushi</i>	clade541	N	
<i>Christensenella minuta</i>	clade558	N	
<i>Clostridium oroticum</i>	clade96	Y	
<i>Clostridium</i> sp. D5	clade96	Y	
<i>Clostridium glycyrrhizinilyticum</i>	clade147	Y	
<i>Coprococcus comes</i>	clade147	Y	

OTU	Phylogenetic Clade	Spore Forming OTU	Dominant OTU in Augmented Ecology
Ruminococcus lactaris	clade147	Y	
Ruminococcus torques	clade147	Y	Y
Clostridiales sp. SS3/4	clade246	Y	
Clostridium hylemonae	clade260	Y	
Clostridium aerotolerans	clade269	Y	
Clostridium asparagiforme	clade300	Y	Y
Clostridium sp. M62/1	clade300	Y	
Clostridium symbiosum	clade300	Y	
Lachnospiraceae genomosp. C1	clade300	Y	
Blautia sp. M25	clade304	Y	Y
Blautia stercoris	clade304	Y	
Ruminococcus hansenii	clade304	Y	
Ruminococcus obeum	clade304	Y	
Ruminococcus sp. 5_1_39BFAA	clade304	Y	
Bryantella formatexigens	clade309	Y	
Eubacterium cellulosolvens	clade309	Y	
Clostridium sp. HGF2	clade351	Y	
Clostridium bartlettii	clade354	Y	
Clostridium bifermentans	clade354	Y	
Clostridium glycolicum	clade354	Y	
Eubacterium tenue	clade354	Y	
Dorea formicigenerans	clade360	Y	
Dorea longicatena	clade360	Y	
Lachnospiraceae bacterium 2_1_46FAA	clade360	Y	

OTU	Phylogenetic Clade	Spore Forming OTU	Dominant OTU in Augmented Ecology
Lachnospiraceae bacterium 9_1_43BFAA	clade360	Y	Y
Ruminococcus gnavus	clade360	Y	
Clostridium hathewayi	clade362	Y	
Blautia hydrogenotrophica	clade368	Y	
Clostridiaceae bacterium END-2	clade368	Y	
Roseburia faecis	clade369	Y	
Roseburia hominis	clade370	Y	
Roseburia intestinalis	clade370	Y	
Eubacterium sp. WAL 14571	clade384	Y	
Erysipelotrichaceae bacterium 5_2_54FAA	clade385	Y	
Eubacterium bifforme	clade385	Y	
Eubacterium dolichum	clade385	Y	
Coprococcus catus	clade393	Y	
Acetivibrio ethanolgignens	clade396	Y	
Anaerosporebacter mobilis	clade396	Y	
Bacteroides pectinophilus	clade396	Y	
Eubacterium hallii	clade396	Y	
Eubacterium xylanophilum	clade396	Y	
Anaerostipes caccae	clade408	Y	
Clostridiales bacterium 1_7_47FAA	clade408	Y	
Clostridium aldenense	clade408	Y	
Clostridium citroniae	clade408	Y	
Eubacterium hadrum	clade408	Y	Y
Acetanaerobacterium elongatum	clade439	Y	

OTU	Phylogenetic Clade	Spore Forming OTU	Dominant OTU in Augmented Ecology
Faecalibacterium prausnitzii	clade478	Y	
Gemmiger formicilis	clade478	Y	Y
Eubacterium ramulus	clade482	Y	
Lachnospiraceae bacterium 3_1_57FAA_CT1	clade483	Y	
Lachnospiraceae bacterium A4	clade483	Y	Y
Lachnospiraceae bacterium DJF VP30	clade483	Y	
Holdemania filiformis	clade485	Y	
Clostridium orbiscindens	clade494	Y	
Pseudoflavonifractor capillosus	clade494	Y	
Ruminococcaceae bacterium D16	clade494	Y	
Acetivibrio cellulolyticus	clade495	Y	
Eubacterium limosum	clade512	Y	
Anaerotruncus colihominis	clade516	Y	
Clostridium methylpentosum	clade516	Y	
Clostridium sp. YIT 12070	clade516	Y	
Hydrogenoanaerobacterium saccharovorans	clade516	Y	
Eubacterium ventriosum	clade519	Y	
Eubacterium eligens	clade522	Y	
Lachnospira pectinoschiza	clade522	Y	
Lactobacillus rogosae	clade522	Y	Y
Clostridium leptum	clade537	Y	
Eubacterium coprostanoligenes	clade537	Y	
Ruminococcus bromii	clade537	Y	

OTU	Phylogenetic Clade	Spore Forming OTU	Dominant OTU in Augmented Ecology
<i>Clostridium viride</i>	clade540	Y	
<i>Butyrivibrio crossotus</i>	clade543	Y	
<i>Coprococcus eutactus</i>	clade543	Y	
<i>Eubacterium ruminantium</i>	clade543	Y	
<i>Eubacterium rectale</i>	clade568	Y	Y
<i>Roseburia inulinivorans</i>	clade568	Y	
<i>Butyricicoccus pullicaecorum</i>	clade572	Y	
<i>Eubacterium desmolans</i>	clade572	Y	
<i>Papillibacter cinnamivorans</i>	clade572	Y	
<i>Sporobacter termitidis</i>	clade572	Y	
<i>Clostridium lactatifermentans</i>	clade576	Y	

Table 13

Reduction in the opportunistic pathogen or pathobiont load by ethanol treated spores.

	Pretreatment	Day 5	Day 14	Day 25
Klebsiella (% of total reads)	20.27%	1.32%	7.62%	0.00%
Fusobacterium (% total of reads)	19.14%	3.01%	0.01%	0.00%

Table 14

Changes in Enterobacteria as a function of treatment measured on Simmons Citrate Agar

Patient	Organism	Pretreatment titer (cfu/g)	Day 25 titer (cfu/g)
1	<i>Klebsiella pneumoniae</i>	9×10^6	1×10^3
1	<i>Klebsiella sp. Co9935</i>	4×10^6	1×10^3
1	<i>Escherichia coli</i>	7×10^6	1×10^6
2	<i>Klebsiella sp. Co9935</i>	4×10^6	1×10^3
4	<i>Klebsiella pneumoniae</i>	3×10^8	$< 1 \times 10^4$
4	<i>Klebsiella sp. Co9935</i>	6×10^7	$< 1 \times 10^4$
5	<i>Klebsiella pneumoniae</i>	1×10^6	$< 1 \times 10^4$

Table 15

Augmentation of Bacteroides as a function of bacterial composition treatment of Patient 1.

Media	Bacteroides species	Pretreatment titer (cfu/g)	Day 25 titer (cfu/g)
BBE	<i>B. fragilis</i> group	$<2 \times 10^4$	3×10^8
PFA	All Bacteroides	$<2 \times 10^7$	2×10^{10}

Table 16

Bacteroides spp. in Patient 1 post-treatment with the ethanol treated spore preparation based full-length 16S rDNA sequences of isolated strains.

Species	% of total Bacteroides cfu (1.58×10^{10} cfu/g)
Bacteroides sp. 4_1_36	63%
Bacteroides cellulosilyticus	14%
Bacteroides sp. 1_1_30	14%
Bacteroides uniformis	4.8%
Bacteroides ovatus	1.7%
Bacteroides dorei	0.91%
Bacteroides xylanisolvens	0.83%
Bacteroides sp. 3_1_19	0.23%

Table 17

Titers (in cfu/g) of imipenem-resistant *M. morganii*, *P. rettgeri* and *P. pennerii* from Patients 2, 4 & 5

Patient	Organism	Pretreatment titer	Day 28 titer *
Patient 2	<i>M. morganii</i>	1×10^4	6×10^2
Patient 2	<i>P. rettgeri</i>	9×10^3	$< 5 \times 10^1$
Patient 4	<i>M. morganii</i>	2×10^4	$< 5 \times 10^1$
Patient 4	<i>P. pennerii</i>	2×10^4	$< 5 \times 10^1$
Patient 5	<i>M. morganii</i>	5×10^3	$< 5 \times 10^1$

* Limit of detection based on plating 200 μ L of 10% wt/vol suspension is 5×10^1

Table 18

OTUs detected by a minimum of ten 16S-V4 sequence reads in at least a one ethanol treated spore preparation (pan-microbiome). OTUs that engraft in a treated patients and the percentage of patients in which they engraft are denoted, as are the clades, spore forming status, and Keystone OTU status. Starred OTUs occur in $\geq 80\%$ of the ethanol preps and engraft in $\geq 50\%$ of the treated patients.

OTU	Clade	% of Spore Preps with OTU	% of Patients OTU Engrafts	Spore Former	Keystone OTU
Prevotella_maculosa	clade_104	10%	0%	N	N
Prevotella_copri	clade_168	20%	0%	N	N
Bacteroides_caccae	clade_170	30%	0%	N	Y
Bifidobacterium_sp_TM_7*	clade_172	90%	60%	N	N
Bifidobacterium_gallicum	clade_172	70%	20%	N	N
Bifidobacterium_dentium	clade_172	50%	0%	N	N
Lactobacillus_casei	clade_198	20%	10%	N	N
Actinomyces_odontolyticus	clade_212	20%	30%	N	N
Clostridium_colicanis	clade_223	10%	10%	Y	N
Clostridiales_sp_SS3_4*	clade_246	100%	70%	Y	N
Clostridium_sporogenes	clade_252	40%	40%	Y	N
Clostridium_butyricum	clade_252	20%	20%	Y	N

OTU	Clade	% of Spore Preps with OTU	% of Patients OTU Engrafts	Spore Former	Keystone OTU
Clostridium_disporicum	clade_253	40%	30%	Y	N
Clostridium_hylemonae*	clade_260	100%	50%	Y	N
Clostridium_scindens	clade_260	10%	60%	Y	N
Coprococcus_comes*	clade_262	90%	80%	Y	Y
Lachnospiraceae_bacterium_1_4_56FAA*	clade_262	90%	80%	Y	Y
Ruminococcus_torques	clade_262	30%	70%	Y	Y
Parabacteroides_merdae	clade_286	30%	20%	N	Y
Bifidobacterium_bifidum	clade_293	10%	0%	N	N
Johnsonella_ignava	clade_298	10%	10%	N	N
Blautia_glucerasea*	clade_309	100%	80%	Y	N
Blautia_sp_M25*	clade_309	100%	70%	Y	Y
Lachnospiraceae_bacterium_6_1_63FAA*	clade_309	100%	60%	Y	N
Eubacterium_cellulosolvens	clade_309	10%	30%	Y	Y
Lactobacillus_fermentum	clade_313	10%	0%	N	N
Sarcina_ventriculi	clade_353	10%	10%	Y	N
Clostridium_bartlettii*	clade_354	90%	70%	Y	N
Clostridium_bifermentans	clade_354	70%	70%	Y	N
Clostridium_mayombeii	clade_354	50%	50%	Y	N

OTU	Clade	% of Spore Preps with OTU	% of Patients OTU Engrafts	Spore Former	Keystone OTU
Dorea_longicatena*	clade_360	100%	60%	Y	Y
Lachnospiraceae_bacterium_9_1_43BFAA	clade_360	100%	30%	Y	N
Lachnospiraceae_bacterium_2_1_58FAA*	clade_360	80%	80%	Y	N
Lachnospiraceae_bacterium_2_1_46FAA	clade_360	50%	50%	Y	N
Lactobacillus_perolens	clade_373	10%	0%	N	N
Bacteroides_dorei	clade_378	60%	50%	N	Y
Eubacterium_biforme	clade_385	10%	0%	Y	N
Peptoniphilus_sp_gpac077	clade_389	10%	20%	N	N
Coprococcus_catus*	clade_393	100%	70%	Y	Y
Eubacterium_hallii*	clade_396	90%	60%	Y	Y
Anaerosporobacter_mobilis	clade_396	40%	60%	Y	N
Bacteroides_pectinophilus	clade_396	10%	60%	Y	N
Lactobacillus_hominis	clade_398	10%	0%	N	N
Lactococcus_lactis	clade_401	40%	40%	N	N
Ruminococcus_champanelle nsis*	clade_406	80%	50%	Y	N
Ruminococcus_callidus	clade_406	10%	10%	Y	N
Clostridium_clostridioforme*	clade_408	100%	60%	Y	Y

OTU	Clade	% of Spore Preps with OTU	% of Patients OTU Engrafts	Spore Former	Keystone OTU
Eubacterium_hadrum*	clade_408	100%	90%	Y	Y
Clostridium_symbiosum	clade_408	30%	50%	Y	Y
Anaerostipes_caccae	clade_408	10%	50%	Y	N
Parasutterella_excrementihominis	clade_432	10%	0%	N	N
Sutterella_stercoricanis	clade_432	10%	0%	N	N
Eubacterium_rectale*	clade_444	100%	80%	Y	Y
Lachnobacterium_bovis*	clade_444	100%	80%	Y	N
Desulfovibrio_desulfuricans	clade_445	10%	0%	N	Y
Eubacterium_sp_oral_clone_JS001*	clade_476	80%	70%	Y	N
Faecalibacterium_prausnitzii*	clade_478	100%	60%	Y	Y
Subdoligranulum_variabile*	clade_478	100%	80%	Y	Y
Coprobacillus_sp_D7*	clade_481	90%	60%	Y	N
Clostridium_cocleatum	clade_481	60%	20%	Y	N
Clostridium_spiroforme	clade_481	40%	50%	Y	N
Eubacterium_ramulus*	clade_482	80%	60%	Y	N
Flavonifractor_plautii	clade_494	70%	60%	Y	Y
Pseudoflavonifractor_capillosus	clade_494	60%	60%	Y	Y
Ruminococcaceae_bacterium_	clade_494	30%	50%	Y	Y

OTU	Clade	% of Spore Preps with OTU	% of Patients OTU Engrafts	Spore Former	Keystone OTU
D16					
Acetivibrio_cellulolyticus*	clade_495	70%	80%	Y	N
Clostridium_stercorarium	clade_495	40%	50%	Y	N
Enterococcus_durans	clade_497	10%	10%	N	N
Enterococcus_faecium	clade_497	10%	10%	N	N
Dialister_invisus	clade_506	50%	10%	N	N
Eubacterium_limosum	clade_512	20%	0%	Y	N
Ruminococcus_flavefaciens	clade_516	60%	60%	Y	N
Eubacterium_ventriosum	clade_519	30%	60%	Y	Y
Bilophila_wadsworthia	clade_521	90%	0%	N	Y
Lachnospira_pectinoschiza	clade_522	40%	60%	Y	N
Eubacterium_eligens	clade_522	30%	50%	Y	Y
Catonella_morbi	clade_534	20%	0%	N	N
Clostridium_sporosphaeroide s*	clade_537	100%	80%	Y	N
Ruminococcus_bromii	clade_537	60%	30%	Y	Y
Clostridium_leptum	clade_537	40%	70%	Y	Y
Clostridium_sp_YIT_12069	clade_537	40%	60%	Y	N
Clostridium_viride	clade_540	10%	10%	Y	N

OTU	Clade	% of Spore Preps with OTU	% of Patients OTU Engrafts	Spore Former	Keystone OTU
Megamonas_funiformis	clade_542	50%	0%	N	N
Eubacterium_ruminantium*	clade_543	80%	90%	Y	N
Coprococcus_eutactus	clade_543	20%	20%	Y	N
Collinsella_aerofaciens	clade_553	50%	10%	Y	Y
Alkaliphilus_metalliredigenes	clade_554	40%	10%	Y	N
Turicibacter_sanguinis	clade_555	80%	40%	Y	N
Phascolarctobacterium_faecium	clade_556	20%	0%	N	N
Clostridiales_bacterium_oral_clone_P4PA*	clade_558	80%	50%	N	N
Lutispora_thermophila	clade_564	100%	0%	Y	N
Coriobacteriaceae_bacterium_JC110	clade_566	70%	0%	N	N
Eggerthella_sp_1_3_56FAA	clade_566	70%	30%	N	N
Adlercreutzia_equolifaciens	clade_566	40%	0%	N	N
Gordonibacter_pamelaeae	clade_566	30%	0%	N	Y
Slackia_isoflavoniconvertens	clade_566	10%	0%	N	N
Eubacterium_desmolans*	clade_572	90%	70%	Y	N
Papillibacter_cinnamivorans*	clade_572	90%	80%	Y	N
Clostridium_colinum	clade_576	30%	30%	Y	N
Akkermansia_muciniphila	clade_583	60%	10%	N	Y

OTU	Clade	% of Spore Preps with OTU	% of Patients OTU Engrafts	Spore Former	Keystone OTU
Clostridiales_bacterium_oral_taxon_F32	clade_584	60%	30%	N	N
Prochlorococcus_marinus	clade_592	30%	0%	N	N
Methanobrevibacter_wolinii	clade_595	30%	0%	N	N
Bacteroides_fragilis	clade_65	20%	30%	N	Y
Lactobacillus_delbrueckii	clade_72	10%	0%	N	N
Escherichia_coli	clade_92	50%	0%	N	Y
Clostridium_sp_D5	clade_96	80%	60%	Y	N
Streptococcus_thermophilus	clade_98	90%	20%	N	Y
Streptococcus_sp_CM6	clade_98	20%	10%	N	N
Streptococcus_sp_oral_clone_ASCE05	clade_98	10%	0%	N	N

Table 19

Top 20 OTUs ranked by CES

OTU	Clade	CES	Spore Former	Keystone OTU
Eubacterium_hadrum	clade_408	4.2	Y	Y
Eubacterium_rectale	clade_444	4.2	Y	Y
Subdoligranulum_variabile	clade_478	4.2	Y	Y
Blautia_sp_M25	clade_309	4.2	Y	Y
Coprococcus_catus	clade_393	4.2	Y	Y
Lachnospiraceae_bacterium_1_4_56F AA	clade_262	4.2	Y	Y
Coprococcus_comes	clade_262	4.2	Y	Y
Blautia_glucerasea	clade_309	4.0	Y	N
Lachnobacterium_bovis	clade_444	4.0	Y	N
Clostridium_sporosphaeroides	clade_537	4.0	Y	N
Clostridiales_sp_SS3_4	clade_246	4.0	Y	N
Papillibacter_cinnamivorans	clade_572	4.0	Y	N
Clostridium_bartlettii	clade_354	4.0	Y	N
Eubacterium_desmolans	clade_572	4.0	Y	N
Clostridium_clostridioforme	clade_408	3.2	Y	Y
Dorea_longicatena	clade_360	3.2	Y	Y
Faecalibacterium_prausnitzii	clade_478	3.2	Y	Y
Eubacterium_hallii	clade_396	3.2	Y	Y
Clostridium_leptum	clade_537	3.2	Y	Y
Lachnospiraceae_bacterium_6_1_63F AA	clade_309	3.0	Y	N

Table 20Subsets of the Core Ecology tested in the *C. difficile* mouse model

Subset	OTU	Substitute For OTU in Table 1 (Clade)
Subset 1	<i>Collinsella aerofaciens</i>	none (Clade_553)
	<i>Clostridium tertium</i>	<i>C. sporogenes</i> (Clade_252)
	<i>Clostridium disporicum</i>	none (Clade_253)
	<i>Clostridium innocuum</i>	<i>Clostridium_sp_HGF2</i> (Clade_351)
	<i>Clostridium mayombeii</i>	none (Clade_354)
	<i>Clostridium butyricum</i>	none (Clade_252)
	<i>Coprococcus comes</i>	none (Clade_262)
	<i>Clostridium hylemonae</i>	none (Clade_260)
	<i>Clostridium bolteae</i>	<i>E. hadrum</i> (Clade_408)
	<i>Clostridium symbiosum</i>	<i>C. clostridioforme</i> (Clade_408)
	<i>Clostridium orbiscindens</i>	<i>R._bacterium_D16</i> (Clade_494)
	<i>Lachnospiraceae</i> <i>bacterium_5_1_57FAA</i>	<i>C. scindens</i> (Clade_260)
	<i>Blautia producta</i>	<i>Blautia_sp_M25</i> (Clade_309)
	<i>Ruminococcus gnavus</i>	<i>D. longicatena</i> (Clade_360)
	<i>Ruminococcus bromii</i>	none (Clade_537)

Subset	OTU	Substitute For OTU in Table 1 (Clade)
Subset 2	Collinsella aerofaciens	none (Clade_553)
	Clostridium butyricum	none (Clade_252)
	Clostridium hylemonae	none (Clade_260)
	Blautia producta	Blautia_sp_M25 (Clade_309)
Subset 3	Collinsella aerofaciens	none (Clade_553)
	Clostridium innocuum	Clostridium_sp_HGF2 (Clade_351)
	Coprococcus comes	none (Clade_262)
	Ruminococcus bromii	none (Clade_537)
Subset 4	Clostridium butyricum	none (Clade_252)
	Clostridium hylemonae	none (Clade_260)
	Blautia producta	Blautia_sp_M25 (Clade_309)
Subset 5	Clostridium butyricum	none (Clade_252)
	Clostridium hylemonae	none (Clade_260)
Subset 6	Blautia producta	Blautia_sp_M25 (Clade_309)
	Clostridium butyricum	none (Clade_252)
Subset 7	Clostridium orbiscindens	R._bacterium_D16 (Clade_494)
	Lachnospiraceae bacterium_5_1_57FAA	C. scindens (Clade_260)

Subset	OTU	Substitute For OTU in Table 1 (Clade)
	Eubacterium rectale	none (Clade_444)

Table 21Results of bacterial compositions tested in a *C. difficile* mouse model

Group	Dose	Cumulative Mortality (%)	Avg. Minimum Relative Weight	Avg. Maximum Clinical Score (Death = 4)
Vehicle Control	-	40	0.87	2.8
Feces Control	5.8e8 cfu total	0	0.99	0
Subset 1	1e8 cfu/OTU	0	0.98	0
Subset 2	1e8 cfu/OTU	10	0.84	2.1
Subset 3	1e8 cfu/OTU	10	0.84	2.2
Subset 4	1e8 cfu/OTU	0	0.87	2
Subset 5	1e8 cfu/OTU	20	0.91	1.7
Subset 6	1e8 cfu/OTU	40	0.82	2.8
Subset 7	1e8 cfu/OTU	0	0.90	1

Table 22

OTUs and their clade assignments tested in ternary combinations with results in the in vitro inhibition assay

OTU1	Clade1	OTU2	Clade2	OTU3	Clade3	Results
Clostridium_bolteae	clade_408	Blautia_producta	clade_309	Eubacterium_rectale	clade_444	++++
Clostridium_bolteae	clade_408	Clostridium_symbiosum	clade_408	Blautia_producta	clade_309	++++
Clostridium_bolteae	clade_408	Clostridium_symbiosum	clade_408	Eubacterium_rectale	clade_444	-
Clostridium_bolteae	clade_408	Clostridium_symbiosum	clade_408	Faecalibacterium_prausnitzii	clade_478	-
Clostridium_bolteae	clade_408	Clostridium_symbiosum	clade_408	Lachnospiraceae_bacterium_5_1_57FAA	clade_260	
Clostridium_bolteae	clade_408	Faecalibacterium_prausnitzii	clade_478	Blautia_producta	clade_309	++++
Clostridium_bolteae	clade_408	Faecalibacterium_prausnitzii	clade_478	Eubacterium_rectale	clade_444	
Clostridium_bolteae	clade_408	Faecalibacterium_prausnitzii	clade_478	Lachnospiraceae_bacterium_5_1_57FAA	clade_260	++++
Clostridium_bolteae	clade_408	Lachnospiraceae_bacterium_5_1_57FAA	clade_260	Blautia_producta	clade_309	++++
Clostridium_bolteae	clade_408	Lachnospiraceae_bacterium_5_1_57FAA	clade_260	Eubacterium_rectale	clade_444	+
Clostridium_symbiosum	clade_408	Blautia_producta	clade_309	Eubacterium_rectale	clade_444	++++
Clostridium_symbiosum	clade_408	Faecalibacterium_prausnitzii	clade_478	Blautia_producta	clade_309	++++
Clostridium_symbiosum	clade_408	Faecalibacterium_prausnitzii	clade_478	Eubacterium_rectale	clade_444	
Clostridium_symbiosum	clade_408	Faecalibacterium_prausnitzii	clade_478	Lachnospiraceae_bacterium_5_1_57FAA	clade_260	+
Clostridium_symbiosum	clade_408	Lachnospiraceae_bacterium_5_1_57FAA	clade_260	Blautia_producta	clade_309	++++

OTU1	Clade1	OTU2	Clade2	OTU3	Clade3	Results
m		um_5_1_57FAA				
Clostridium_symbiosu m	clade_408	Lachnospiraceae_bacteri um_5_1_57FAA	clade_260	Eubacterium_rectale	clade_444	
Collinsella_aerofaciens	clade_553	Blautia_producta	clade_309	Eubacterium_rectale	clade_444	++++
Collinsella_aerofaciens	clade_553	Clostridium_bolteae	clade_408	Blautia_producta	clade_309	++++
Collinsella_aerofaciens	clade_553	Clostridium_bolteae	clade_408	Clostridium_symbiosum	clade_408	++++
Collinsella_aerofaciens	clade_553	Clostridium_bolteae	clade_408	Eubacterium_rectale	clade_444	++++
Collinsella_aerofaciens	clade_553	Clostridium_bolteae	clade_408	Faecalibacterium_prausnit zii	clade_478	++++
Collinsella_aerofaciens	clade_553	Clostridium_bolteae	clade_408	Lachnospiraceae_bacteriu m_5_1_57FAA	clade_260	++++
Collinsella_aerofaciens	clade_553	Clostridium_symbiosum	clade_408	Blautia_producta	clade_309	++++
Collinsella_aerofaciens	clade_553	Clostridium_symbiosum	clade_408	Eubacterium_rectale	clade_444	
Collinsella_aerofaciens	clade_553	Clostridium_symbiosum	clade_408	Faecalibacterium_prausnit zii	clade_478	
Collinsella_aerofaciens	clade_553	Clostridium_symbiosum	clade_408	Lachnospiraceae_bacteriu m_5_1_57FAA	clade_260	+
Collinsella_aerofaciens	clade_553	Coprococcus_comes	clade_262	Blautia_producta	clade_309	++++
Collinsella_aerofaciens	clade_553	Coprococcus_comes	clade_262	Clostridium_bolteae	clade_408	++++
Collinsella_aerofaciens	clade_553	Coprococcus_comes	clade_262	Clostridium_symbiosum	clade_408	+++
Collinsella_aerofaciens	clade_553	Coprococcus_comes	clade_262	Eubacterium_rectale	clade_444	+++
Collinsella_aerofaciens	clade_553	Coprococcus_comes	clade_262	Faecalibacterium_prausnit zii	clade_478	++++
Collinsella_aerofaciens	clade_553	Coprococcus_comes	clade_262	Lachnospiraceae_bacteriu m_5_1_57FAA	clade_260	+++
Collinsella_aerofaciens	clade_553	Faecalibacterium_prausn itzi	clade_478	Blautia_producta	clade_309	++++

OTU1	Clade1	OTU2	Clade2	OTU3	Clade3	Results
Collinsella_aerofaciens	clade_553	Faecalibacterium_prausnitzii	clade_478	Eubacterium_rectale	clade_444	+++
Collinsella_aerofaciens	clade_553	Faecalibacterium_prausnitzii	clade_478	Lachnospiraceae_bacterium_5_1_57FAA	clade_260	+++
Collinsella_aerofaciens	clade_553	Lachnospiraceae_bacterium_5_1_57FAA	clade_260	Blautia_producta	clade_309	++++
Collinsella_aerofaciens	clade_553	Lachnospiraceae_bacterium_5_1_57FAA	clade_260	Eubacterium_rectale	clade_444	++++
Coprococcus_comes	clade_262	Blautia_producta	clade_309	Eubacterium_rectale	clade_444	++++
Coprococcus_comes	clade_262	Clostridium_bolteae	clade_408	Blautia_producta	clade_309	++++
Coprococcus_comes	clade_262	Clostridium_bolteae	clade_408	Clostridium_symbiosum	clade_408	
Coprococcus_comes	clade_262	Clostridium_bolteae	clade_408	Eubacterium_rectale	clade_444	--
Coprococcus_comes	clade_262	Clostridium_bolteae	clade_408	Faecalibacterium_prausnitzii	clade_478	+++
Coprococcus_comes	clade_262	Clostridium_bolteae	clade_408	Lachnospiraceae_bacterium_5_1_57FAA	clade_260	+++
Coprococcus_comes	clade_262	Clostridium_symbiosum	clade_408	Blautia_producta	clade_309	++++
Coprococcus_comes	clade_262	Clostridium_symbiosum	clade_408	Eubacterium_rectale	clade_444	---
Coprococcus_comes	clade_262	Clostridium_symbiosum	clade_408	Faecalibacterium_prausnitzii	clade_478	
Coprococcus_comes	clade_262	Clostridium_symbiosum	clade_408	Lachnospiraceae_bacterium_5_1_57FAA	clade_260	
Coprococcus_comes	clade_262	Faecalibacterium_prausnitzii	clade_478	Blautia_producta	clade_309	++++
Coprococcus_comes	clade_262	Faecalibacterium_prausnitzii	clade_478	Eubacterium_rectale	clade_444	-
Coprococcus_comes	clade_262	Faecalibacterium_prausnitzii	clade_478	Lachnospiraceae_bacterium_5_1_57FAA	clade_260	
Coprococcus_comes	clade_262	Lachnospiraceae_bacterium_5_1_57FAA	clade_260	Blautia_producta	clade_309	++++

OTU1	Clade1	OTU2	Clade2	OTU3	Clade3	Results
		um_5_1_57FAA				
Coprococcus_comes	clade_262	Lachnospiraceae_bacterium_5_1_57FAA	clade_260	Eubacterium_rectale	clade_444	
Faecalibacterium_prausnitzii	clade_478	Blautia_producta	clade_309	Eubacterium_rectale	clade_444	++++
Faecalibacterium_prausnitzii	clade_478	Lachnospiraceae_bacterium_5_1_57FAA	clade_260	Blautia_producta	clade_309	++++
Faecalibacterium_prausnitzii	clade_478	Lachnospiraceae_bacterium_5_1_57FAA	clade_260	Eubacterium_rectale	clade_444	
Lachnospiraceae_bacterium_5_1_57FAA	clade_260	Blautia_producta	clade_309	Eubacterium_rectale	clade_444	++++

CLAIMS

What is claimed is:

1. A method of populating the gastrointestinal tract of a mammalian subject, comprising the step of orally administering to the mammalian subject an effective amount of a therapeutic composition comprising a purified population of spore-forming bacteria produced by the steps of a) providing a fecal material and b) subjecting the material to a treatment step resulting in purification of spore-forming bacteria, wherein the purified population is present in an amount effective to engraft and/or augment in the gastrointestinal tract in order to treat or prevent a dysbiosis in the mammalian subject.
2. A method of populating the gastrointestinal tract of a mammalian subject, comprising the step of orally administering to the subject an effective amount of a therapeutic composition comprising a purified population of spore-forming bacteria, in an amount effective to engraft and/or augment in the gastrointestinal tract in order to treat or prevent a dysbiosis in a mammalian subject.
3. The method of claim 1 or 2, wherein the population is effective to treat or prevent a gastrointestinal dysbiosis.
4. The method of claim 1 or 2, wherein the population is effective to reduce the severity of at least one symptom of a gastrointestinal dysbiosis.
5. The method of claim 1 or 2, wherein the population is effective to modulate the microbiota diversity present in the mammalian recipient.
6. The method of claim 1 or 2, wherein the population comprises a bacterial spore population.
7. The method of claim 1 or 2, wherein the population comprises a bacterial spore preparation.
8. The method of claim 1, wherein the fecal material is obtained from a healthy mammalian donor subject or a plurality of mammalian donor subjects.
9. The method of claim 1, wherein the treatment step comprises heating the material above 25 degrees Celsius for at least 30 seconds.

10. The method of claim 1, wherein the treatment step comprises contacting the material with a solvent.
11. The method of claim 1, wherein the treatment step comprises a physical manipulation of the material.
12. The method of claim 1, comprising removing at least a portion of an acellular component of the fecal material, thereby enriching and/or separating spore-forming bacteria from acellular material.
13. The method of claim 1 or 2, wherein the population comprises a single bacterial spore preparation or a combination of bacterial spore preparations, wherein each bacterial spore preparation is purified from a fecal material obtained from a single mammalian donor subject.
14. The method of claim 1 or 2, wherein the population comprises a single bacterial spore preparation or a combination of bacterial spore preparations wherein each bacterial spore preparation is independently purified from a fecal material obtained from a mammalian donor subject.
15. The method of claim 1 or 2, wherein the dysbiosis comprises i) a gastrointestinal disease, disorder or condition selected from the group consisting of *Clostridium difficile*-induced diarrhea, irritable bowel syndrome (IBS), colonization with a pathogen or pathobiont, infection with a drug-resistant pathogen or pathobiont, colitis, and Crohn's Disease, or ii) a non-gastrointestinal disease, disorder or condition.
16. The method of claim 1, comprising wherein the treatment step comprises depleting or inactivating a pathogenic material.
17. The method of claim 16, wherein the purified population is substantially depleted of a detectable level of a first pathogenic material.
18. The method of claim 17, wherein the first pathogenic material is selected from the group consisting of virus, bacterium, eukaryotic parasite, helminth, phage, mycoplasma, toxoplasma, and fungus.
19. The method of claim 1, wherein the purified population is substantially depleted of a residual habitat product.
20. The method of claim 19, wherein the residual habitat product comprises acellular material.

21. The method of claim 20, wherein the acellular material comprises residual fiber, DNA, viral coat material, or non-viable material.
22. The method of claim 19, wherein the residual habitat product comprises a eukaryotic cell from the mammalian donor subject.
23. A therapeutic composition comprising a purified population of spore-forming bacteria or bacteria having a substantial activity of spore-forming bacteria, in an amount effective to populate the gastrointestinal tract of a mammalian subject to whom the therapeutic composition is administered.
24. The therapeutic composition of claim 23, wherein the population is effective to i) treat a gastrointestinal dysbiosis, and/or ii) engraft at least one type of bacteria present in the therapeutic composition but not present in a mammalian subject prior to treatment; and/or iii) augment at least one type of bacteria not present in the therapeutic composition in a mammalian subject to whom the therapeutic composition is administered.
25. The therapeutic composition of claim 23, wherein the populating of the gastrointestinal tract is effective to reduce the severity of at least one symptom of a gastrointestinal dysbiosis.
26. The therapeutic composition of claim 23, wherein the populating of the gastrointestinal tract is effective to either: i) engraft at least one type of bacteria present in the therapeutic composition but not present in a mammalian subject, or ii) augment at least one type of bacteria not present in the therapeutic composition in the mammalian recipient.
27. The therapeutic composition of claim 23, wherein the population comprises a population of bacterial spores.
28. The therapeutic composition of claim 23, wherein the population comprises a bacterial spore preparation.
29. The therapeutic composition of claim 23, wherein the population is purified from a fecal material obtained from a mammalian donor subject.
30. The therapeutic composition of claim 23, wherein the population is purified from a fecal material obtained from a mammalian donor subject or a plurality of mammalian donor subjects.

31. The therapeutic composition of claim 30, wherein the plurality of mammalian donor subjects comprises at least 2 healthy human subjects individually having a body mass index (BMI) below about 25, 26, 27, 28, 29, or 30.
32. The therapeutic composition of claim 23, wherein the population comprises a bacterial spore preparation or a combination of bacterial spore preparations, wherein each bacterial spore preparation is independently purified from a fecal material obtained from a mammalian donor subject.
33. The therapeutic composition of claim 32, wherein the mammalian donor subject is validated as not having a detectable level of a pathogen or a pathobiont prior to production of the fecal material.
34. The therapeutic composition of claim 33, wherein the mammalian donor subject is validated at least 1 hour prior to production of the fecal material.
35. The therapeutic composition of claim 33, wherein the mammalian donor subject is validated as not having a detectable level of a pathogen or pathobiont after production of the fecal material.
36. The therapeutic composition of claim 35, wherein the mammalian donor subject is validated at least 1 hour subsequent to production of the fecal material.
37. The therapeutic composition of claim 23, wherein the dysbiosis comprises a gastrointestinal disease, disorder or condition selected from the group consisting of *Clostridium difficile*-induced diarrhea, irritable bowel syndrome (IBS), colonization with a pathogen or pathobiont, infection by a drug-resistant pathogen or pathobiont, colitis, and Crohn's Disease.
38. The therapeutic composition of claim 23, wherein the purified population is substantially reduced in a detectable level of a first pathogenic material.
39. The therapeutic composition of claim 38, wherein the first pathogenic material is selected from the group consisting of virus, bacterium, eukaryotic parasite, helminth, phage, mycoplasma, toxoplasma, and fungus.
40. The therapeutic composition of claim 23, wherein the purified population is substantially reduced in a residual habitat product.
41. The therapeutic composition of claim 40, wherein the residual habitat product comprises acellular material.

42. The therapeutic composition of claim 41, wherein the acellular material comprises DNA, viral coat material, or non-viable bacterial material.
43. The therapeutic composition of claim 41, wherein the residual habitat product comprises a eukaryotic cell from the mammalian donor subject.
44. The therapeutic composition of claim 40, wherein the residual habitat product is reduced at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or greater than 99% from the fecal material obtained from the mammalian donor subject.
45. The therapeutic composition of claim 23, wherein the purified population is substantially reduced in a detectable level of a pathogenic activity of a first pathogenic material.
46. The therapeutic composition of claim 45, wherein the first pathogenic material is selected from the group consisting of virus, bacterium, eukaryotic parasite, helminth, phage, mycoplasma, toxoplasma, and fungus, and wherein the pathogenic activity is selected from the group consisting of a viable titer of said pathogenic agent, an infection of the mammalian recipient subject, an immunomodulatory activity, an autoimmune response, and an inflammatory response.
47. The therapeutic composition of claim 23, wherein the purified population has a substantially reduced level of a pathogenic activity of a first pathogenic material.
48. The therapeutic composition of claim 23, comprising a purified population of bacterial spores present at a concentration at least 10% greater than the concentration of the bacterial spores present in the fecal material.
49. The therapeutic composition of claim 23, wherein the purified population is obtainable from a miscible or partially miscible solvent treatment of the fecal material or a fraction or derivative thereof.
50. The therapeutic composition of claim 49, wherein the miscible solvent is an alcohol.
51. The therapeutic composition of claim 50, wherein the alcohol is methanol, ethanol, propanol, isopropanol, or butanol.
52. The therapeutic composition of claim 50, wherein the partially miscible solvent is an ether, dimethoxyethane, or tetrahydrofuran.

53. A therapeutic composition comprising a purified population of spore-forming bacteria, in an amount effective to populate the gastrointestinal tract of a mammalian subject to whom the therapeutic composition is administered, wherein the purified population is obtained by the steps of a) providing a fecal material comprising spore-forming bacteria and b) subjecting the spore-forming bacteria to a treatment step resulting in purification of the spore-forming bacteria.

54. The therapeutic composition of claim 53, wherein the purified population is present in an amount effective to i) engraft at least one type of bacteria present in the therapeutic composition but not present in a mammalian recipient and/or ii) augment at least one type of bacteria not present in the therapeutic composition in the mammalian recipient to treat or prevent a dysbiosis.

55. The therapeutic composition of claim 53, wherein the purified population is obtained from a heat treatment, mechanical treatment, or solvent treatment of the spore-forming bacteria.

56. The therapeutic composition of claim 53, wherein the composition optionally comprises a germinant.

57. The therapeutic composition of claim 56, wherein the germinant is selected from BHIS oxgall, CaDPA, one or more amino acids, a sugar, a nucleoside, a bile salt, a metal cation, a fatty acid, and a long-chain alkyl amine, or a combination thereof.

58. The therapeutic composition of claim 53, wherein the purified population comprises i) a substantial reduction in the amount and/or viability of one or more pathogens and/or one or more pathobionts present in the fecal material, or ii) a substantial reduction of pathogen activity and/or pathobiont activity present in the fecal material, or a combination thereof.

59. The therapeutic composition of claim 55, wherein the solvent treatment comprises contacting fecal material and/or spore-forming bacteria with a solution comprising between about 5% and about 95% alcohol for at least one minute at a temperature of at least about 20 degrees Celsius.

60. The therapeutic composition of claim 55, wherein the solvent treatment comprises contacting fecal material and/or spore-forming bacteria with a solution

comprising alcohol under conditions suitable to substantially reduce a pathogen activity and/or a pathobiont activity present in the fecal material.

61. The therapeutic composition of claim 53, wherein the mechanical treatment comprises a centrifugation or filtration.

62. The therapeutic composition of claim 61, wherein the centrifugation comprises velocity gradient centrifugation or equilibrium density sedimentation.

63. The therapeutic composition of claim 61 wherein the purified population is at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or greater than 99% purified from acellular material present in the fecal material.

64. The therapeutic composition of claim 23, wherein the purified population is obtainable from elution of spores adsorbed to a solid medium.

65. The therapeutic composition of claim 64, wherein the solid medium comprises a hydrophobic interaction chromatographic (HIC) medium or an affinity chromatographic medium.

66. The therapeutic composition of claim 64, wherein the purified population is obtainable by contacting the fecal material with a solid medium that differentially adsorbs a residual habitat product and a spore population.

67. The therapeutic composition of claim 53, wherein the purified population is obtainable by subjecting the fecal material to a mechanical disrupting treatment.

68. The therapeutic composition of claim 67, wherein the mechanical disrupting treatment substantially disrupts a non-spore material and does not substantially disrupt a spore.

69. The therapeutic composition of claim 53, wherein the purified population is obtainable by subjecting the fecal material to a thermal disrupting treatment.

70. The therapeutic composition of claim 69, wherein the thermal disrupting treatment comprises subjecting the fecal material or a liquid comprising the fecal material to a heated environment of at least about 30 degrees Celsius for at least about ten minutes.

71. A method for production of a composition comprising a population of bacterial spores suitable for therapeutic administration to a mammalian subject in need thereof, comprising the steps of: (a) providing a fecal material obtained from a mammalian donor

subject; and (b) subjecting the fecal material to at least one purification step under conditions such that a purified population of spore-forming bacteria is produced from the fecal material.

72. The method of claim 71, wherein the mammalian donor subject is a healthy human subject.

73. The method of claim 71, comprising contacting the fecal material or a fraction or derivative thereof with a miscible solvent.

74. The method of claim 71, comprising a miscible solvent treatment, an immiscible solvent extraction, an elution from a solid medium, a thermal disrupting treatment, a radiation treatment, a filtration treatment, a chromatographic separation treatment, a centrifugation treatment, an aqueous extraction treatment, a solvent-aqueous extraction treatment, mechanical disrupting treatment, or a combination thereof.

75. A method of treating or preventing a dysbiosis in a human subject, comprising administering to the human subject the therapeutic composition of claim 23 or claim 53.

76. The method of claim 75, wherein the therapeutic administration comprises oral administration of a composition comprising at least about 1×10^4 colony forming units of bacterial spores per dose of the composition.

77. The method of claim 76, wherein the bacterial spores comprise bacteria from the genera provided in Table 1.

78. The method of claim 76, wherein the composition comprises at least about 0.1%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50% or above 50% spores on a mass basis.

79. The method of claim 76, wherein the composition comprises at least about 1×10^4 spores per gram or dose.

80. The method of claim 76, wherein the composition comprises at least about 1×10^3 , 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 , 1×10^9 , 1×10^{10} , or greater than 1×10^{10} spores per gram or dose.

81. A therapeutic composition comprising a purified bacterial spore population comprising at least about 1×10^3 , 1×10^4 , 1×10^5 , or 1×10^6 spores, wherein the composition does not exceed about 1 gram in weight, formulated for oral administration to populate the gastrointestinal tract in a mammalian subject in need thereof, in order to

i) engraft in the mammalian recipient and/or ii) augment a bacterial population in the mammalian recipient and thereby treat or prevent a dysbiosis.

82. The therapeutic composition of claim 81, formulated to treat or prevent gastrointestinal disease, disorder or condition in a mammalian recipient subject in need thereof.

83. The therapeutic composition of claim 81, wherein the bacterial spore population is purified from fecal material obtained from a mammalian donor subject.

84. The therapeutic composition of claim 81, in an amount effective to treat or prevent a metabolic disorder in a mammalian recipient subject suffering from or at risk of developing such disorder to whom the therapeutic composition is administered.

85. The therapeutic composition of claim 81, wherein the bacterial spore population is purified from a fecal material obtained from at least one mammalian donor subject, wherein the at least one mammalian donor subject has no clinical history of a metabolic disorder.

86. A kit comprising in one or more containers a fecal material collection apparatus and a solvent solution, and instructions for use thereof for generating purified populations of spore-forming bacteria.

87. The kit of claim 86, wherein the solvent solution comprises a detergent.

88. The kit of claim 87, wherein the detergent is Triton X-100, Tween 20, Tween 80, Nonidet P40, a pluronic, or a polyol.

89. A method of populating the gastrointestinal tract of a human subject, comprising the step of administering to the human subject a therapeutic composition comprising a purified population of spore-forming bacteria, under conditions such that a microbial population present in the gastrointestinal tract and/or a microbial population outside the gastrointestinal tract is modulated.

90. The method of claim 89, wherein the modulation comprises reduction or elimination of at least one pathogen and/or pathobiont present in the gastrointestinal tract when the therapeutic composition is administered.

91. The method of claim 89, wherein the modulation comprises engraftment of at least one type of spore-forming bacteria present in the therapeutic composition.

92. The method of claim 89, wherein the modulation comprises augmentation by at least one type of bacteria not present in the therapeutic composition.
93. The method of claim 89, wherein the at least one type of spore-forming bacteria are not detectably present in gastrointestinal tract when the therapeutic composition is administered.
94. The method of claim 89, wherein the modulation comprises augmentation by at least one type of spore-forming bacteria or non-spore forming bacteria not present in the therapeutic composition.
95. The method of claim 89, wherein the at least one type of spore-forming or non-spore forming bacteria are not detectably present in gastrointestinal tract when the therapeutic composition is administered.
96. The method of claim 89, wherein the modulation comprises at least two of: i) reduction or elimination of at least one pathogen and/or pathobiont present in the gastrointestinal tract when the therapeutic composition is administered; ii) engraftment of at least one type of spore-forming bacteria present in the therapeutic composition; and iii) augmentation by at least one type of spore-forming bacteria and/or non-spore forming bacteria not present in the therapeutic composition.
97. The method of claim 89, wherein the modulation comprises reduction or elimination of at least one pathogen and/or pathobiont present in the gastrointestinal tract when the therapeutic composition is administered and at least one of: i) engraftment of at least one type of spore-forming bacteria present in the therapeutic composition; and ii) augmentation by at least one type of bacteria not present in the therapeutic composition.
98. The method of claim 97, wherein the at least one pathogen and/or pathobiont is present at pathogenic amounts in the gastrointestinal tract when the composition is administered.
99. The method of claim 89, wherein the mammalian subject suffers from or is at risk of developing bacterial overgrowth syndrome (BOS).
100. The method of claim 99, wherein the modulation comprises reduction or elimination of at least one pathogen and/or pathobiont associated with the BOS.

101. The method of claim 85, wherein the modulation comprises reduction or elimination of at least one multi-drug resistant pathogen and/or pathobiont.

102. A method of populating a bacterial population in the gastrointestinal tract of a human subject, comprising the step of administering to the human subject a therapeutic composition comprising a purified population of spore-forming bacteria, under conditions such that at least i) a subset of the spore-forming bacteria sustainably engraft within the gastrointestinal tract, or ii) at least one type of bacteria not present in the therapeutic composition is augmented within the gastrointestinal tract.

103. A method of populating the gastrointestinal tract of a mammalian subject, comprising the step of orally administering to the subject an effective amount of a bacterial composition comprising at least a first type of isolated bacteria and a second type of isolated bacteria, wherein the first type and the second type are not identical, under conditions such that the first type of isolated bacteria and the second type of isolated bacteria functionally populate the gastrointestinal tract of the subject.

104. The method of claim 103, wherein the bacterial composition comprises at least about 9 types of isolated bacteria.

105. The method of claim 103, wherein the bacterial composition consists essentially of between two and about twenty types of isolated bacteria.

106. The method of claim 103, wherein the first type of isolated bacteria and the second type of isolated bacteria are selected from Table 1.

107. The method of claim 103, wherein the first type of isolated bacteria and the second type of isolated bacteria comprise an operational taxonomic unit (OTU) distinction.

108. The method of claim 107, wherein OTU distinction comprises 16S rRNA sequence similarity below about 97% identity.

109. The method of claim 107, wherein OTU distinction comprises a gene or plurality of genes with a sequence similarity below about 97% identity.

110. The method of claim 107, wherein the OTU distinction comprises a whole genome sequence similarity below about 95% identity.

111. The method of claim 107, wherein the OTU distinction comprises one or more sequence clusters derived from whole genome shotgun metagenomic sequence and sequence similarity within a cluster is below about 95%.

112. The method of claim 107, wherein the OTU distinction comprises a plurality of nucleotide sequences with a similarity of 80%, wherein the plurality of nucleotide sequences i) comprise a monophyletic clade in a phylogenetic tree and/or ii) comprise distinct monophyletic clades in a phylogenetic tree.

113. The method of claim 103, wherein a functional population comprises detectable amounts of the first type of isolated bacteria and the second type of isolated bacteria present in the gastrointestinal tract and/or the feces of the subject three days after the administration of the bacterial composition.

114. The method of claim 103, wherein the bacterial composition further comprises a pharmaceutically acceptable excipient suitable for oral administration.

115. A method of populating the gastrointestinal tract of a mammalian subject, comprising the step of orally administering to the subject an effective amount of a therapeutic composition comprising a purified population of spore-forming bacteria produced by the steps of a) providing a fecal material and b) subjecting the material to a treatment step resulting in purification of spore-forming bacteria, wherein the purified population is present in an amount effective to treat or prevent a dysbiosis in a mammalian recipient subject to whom the therapeutic composition is administered.

116. A method of populating the gastrointestinal tract of a mammalian subject, comprising the step of orally administering to the subject an effective amount of a therapeutic composition comprising a purified population of spore-forming bacteria, in an amount effective to treat or prevent a dysbiosis in a mammalian recipient subject to whom the therapeutic composition is administered.

117. The method of claim 116, wherein the mammalian subject is suffering from or at risk of developing a disease, disorder or condition other than a gastrointestinal disease, disorder or condition.

118. The method of claim 116, further comprising the step of detecting one or more of the administered spore-forming bacteria in the mammalian subject subsequent to administration.

119. The method of claim 116, further comprising the step of detecting a reduction in one or more of bacterial pathogens and/or pathobionts in the mammalian subject subsequent to administration.

120. The method of claim 118 or 119, wherein the detecting step comprises detecting a 16S nucleic acid sequence.

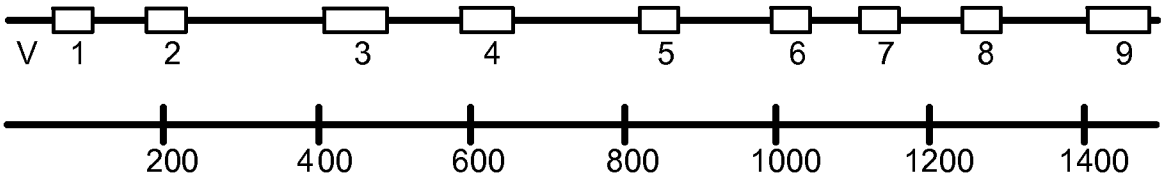


FIG. 1A

1 AAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTA
51 ACACATGCAAGTCGAACGGTAACAGGAAGAAGCTTGCTCTTTGCTGACGA
101 GTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATA
151 ACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGG
201 GGGACCTTCGGGCTCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAG
251 TAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAG
301 GATGACCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGG
351 CAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
401 GCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTACAGCGGGGAGGA
451 AGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGC
501 ACCGGCTAACTCGTGCCCGAGGCATGCGCAGGAATACGGAGGTGCAAGCGT
551 TAATCGGAATTACTGGGCGTAAAGCGCACGCGCAGGCGGTTTGTAAAGTCAG
601 ATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGC
651 TTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGT
701 AGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACT
751 CACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGT
801 AGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTG
851 GCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAA
901 GGTAAAACTCAAATGAATTGACGGGGGGCCCGCACAAGCGGTGGAGCATG
951 TGGTTTAATTTCGATGCAACGCGAAGAACCCTTACCTGGTCTTGACATCCAC
1001 GGAAGTTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGGTGC
1051 TGCATGGCTGTCGTCAGCTCGTGTGTGTGAAATGTTGGGTAAAGTCCCGCA
1101 ACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACCTCAA
1151 AGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCA
1201 TCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAA
1251 AGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCCTCGTAGT
1301 CCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAAT
1351 CGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCCTGTACACACCG
1401 CCCGMCAACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTT
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1501 AAGGTAACCGTAGGGGAACCTGCGGTTGGATCACCTCCTTA

FIG. 1B

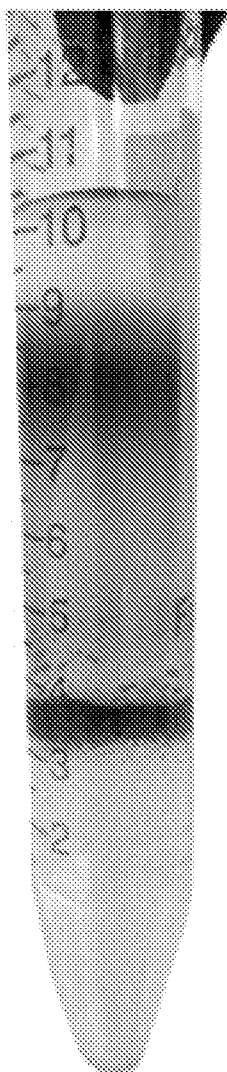


FIG. 2

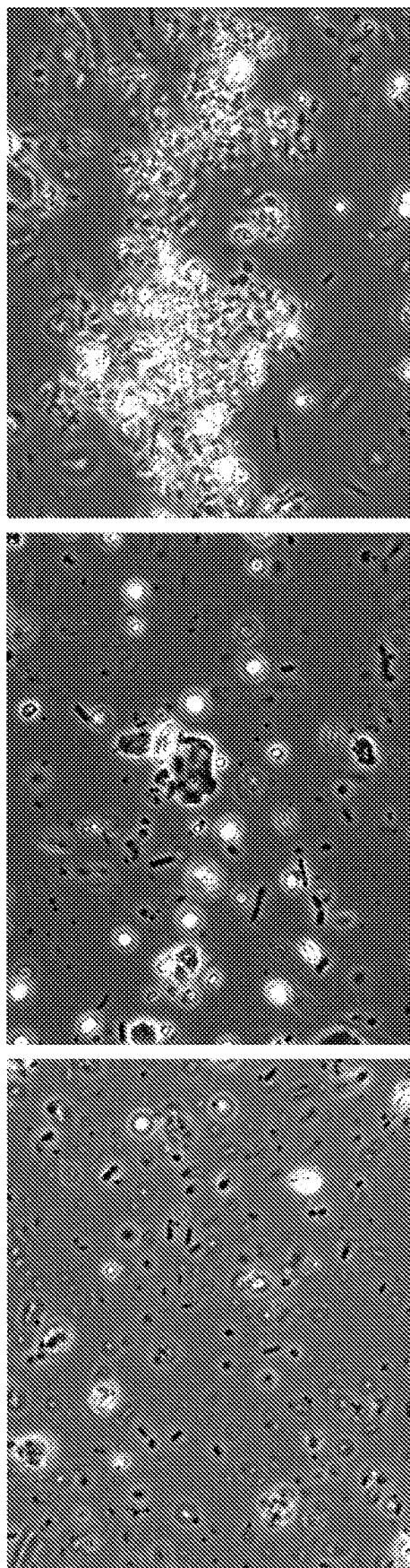


FIG. 3

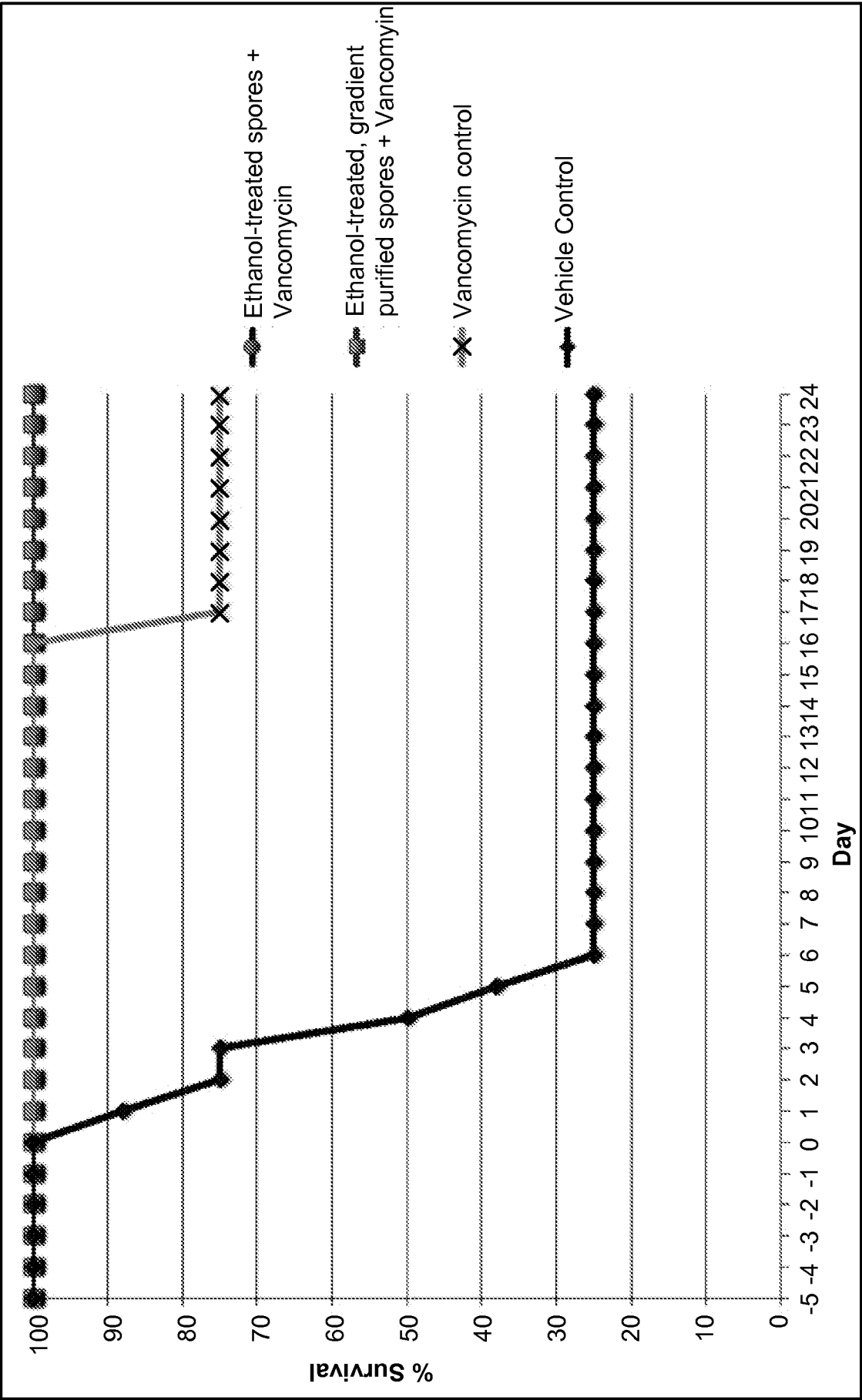


FIG. 4

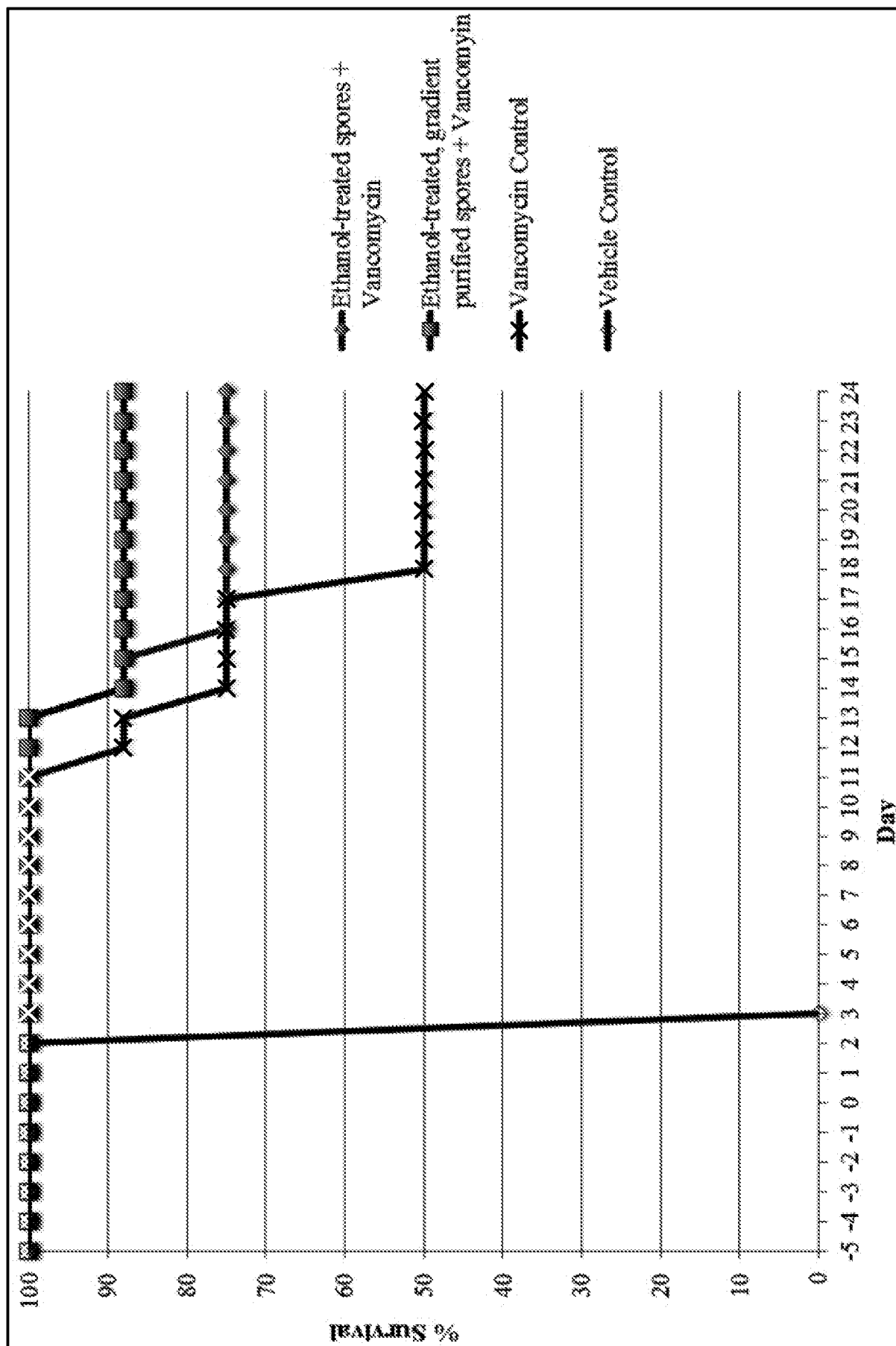


FIG. 5

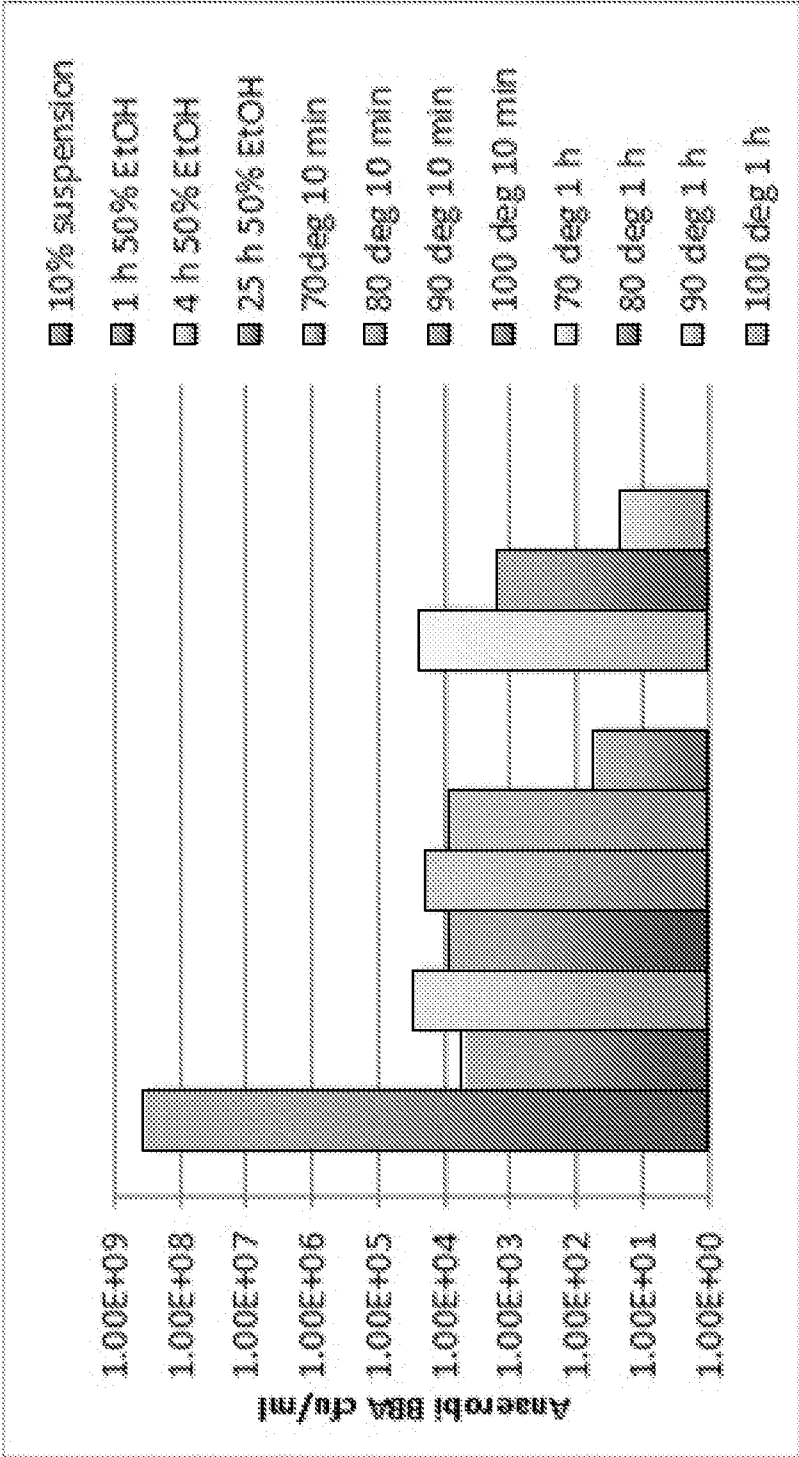


FIG. 6

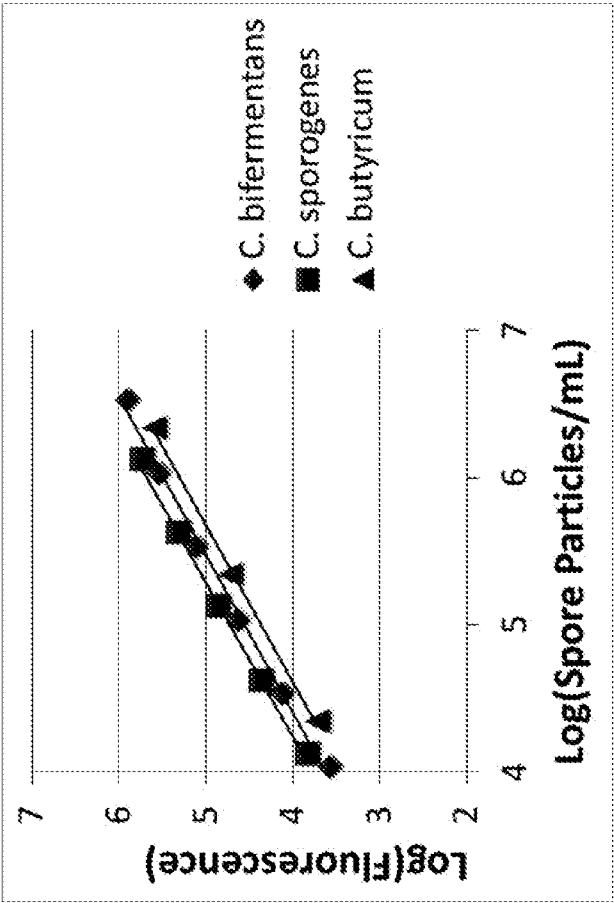


FIG. 7

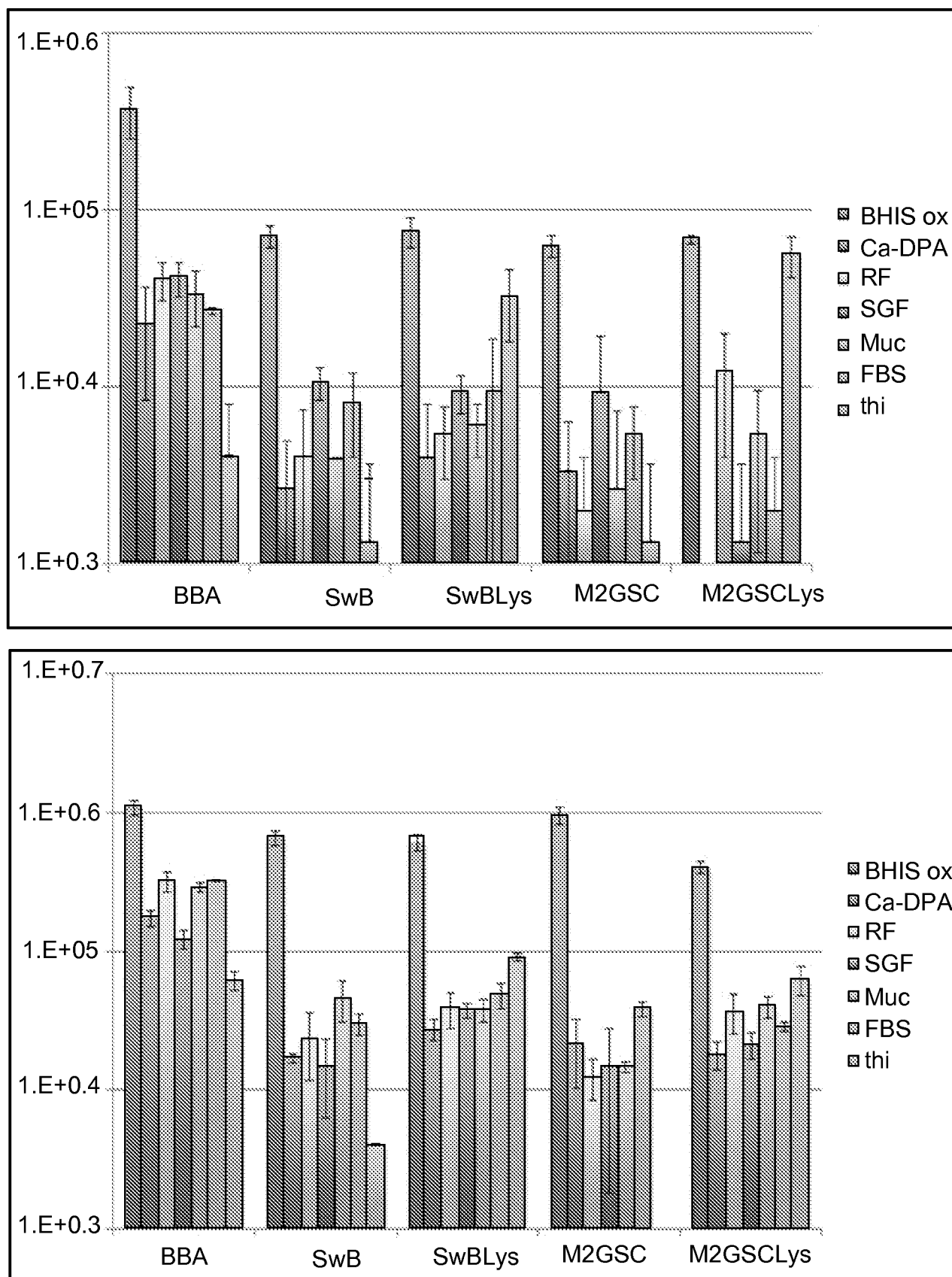


FIG. 8

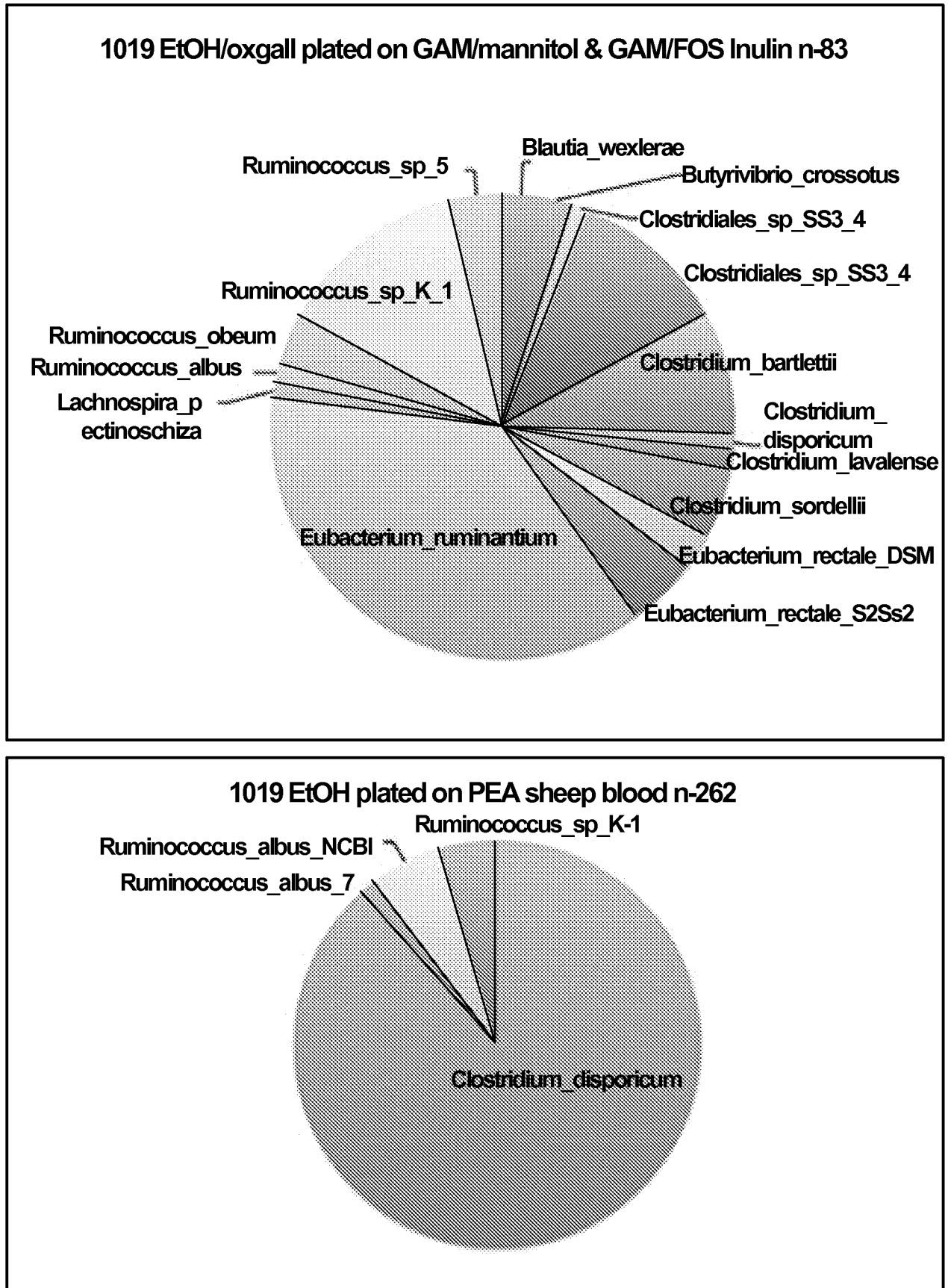


FIG. 9

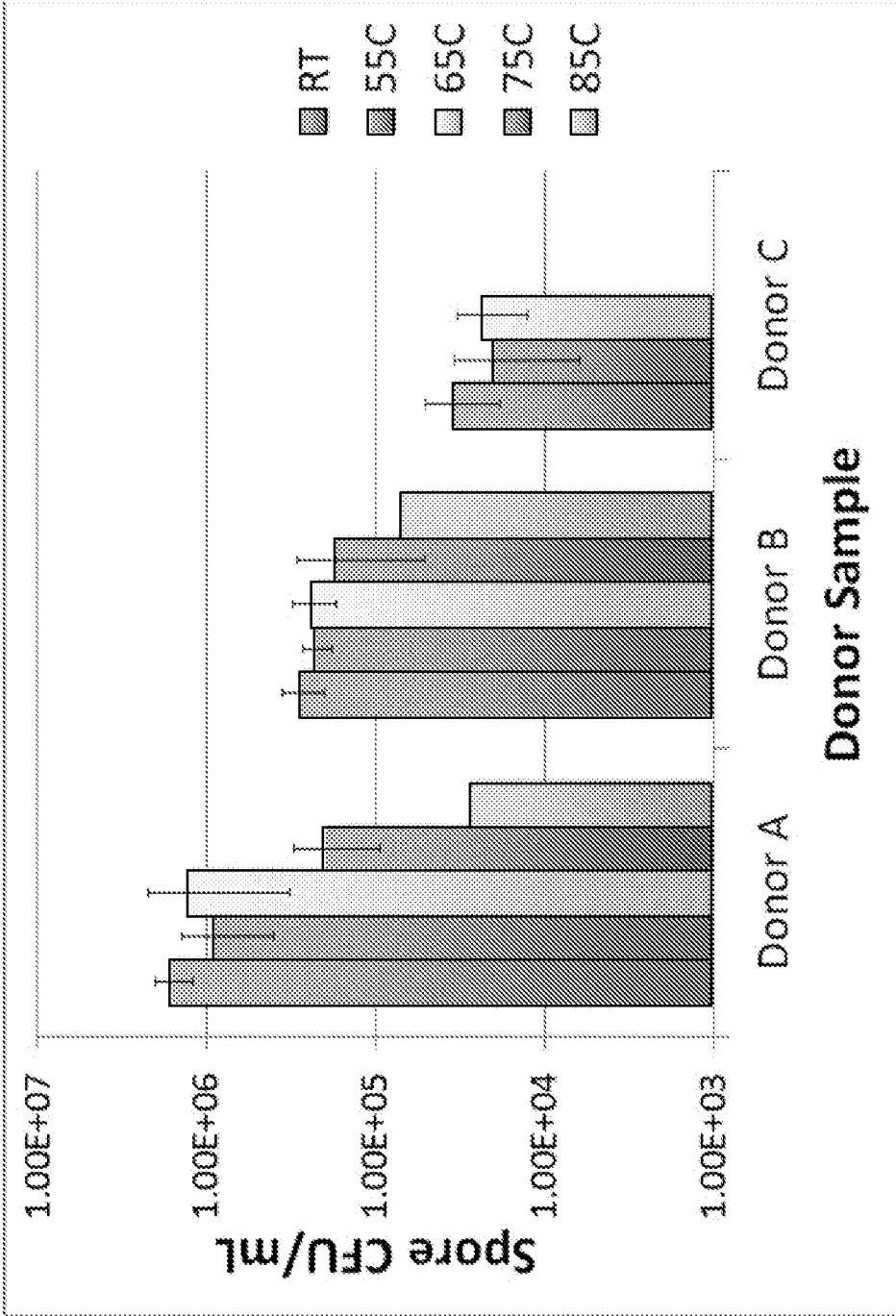


FIG. 10

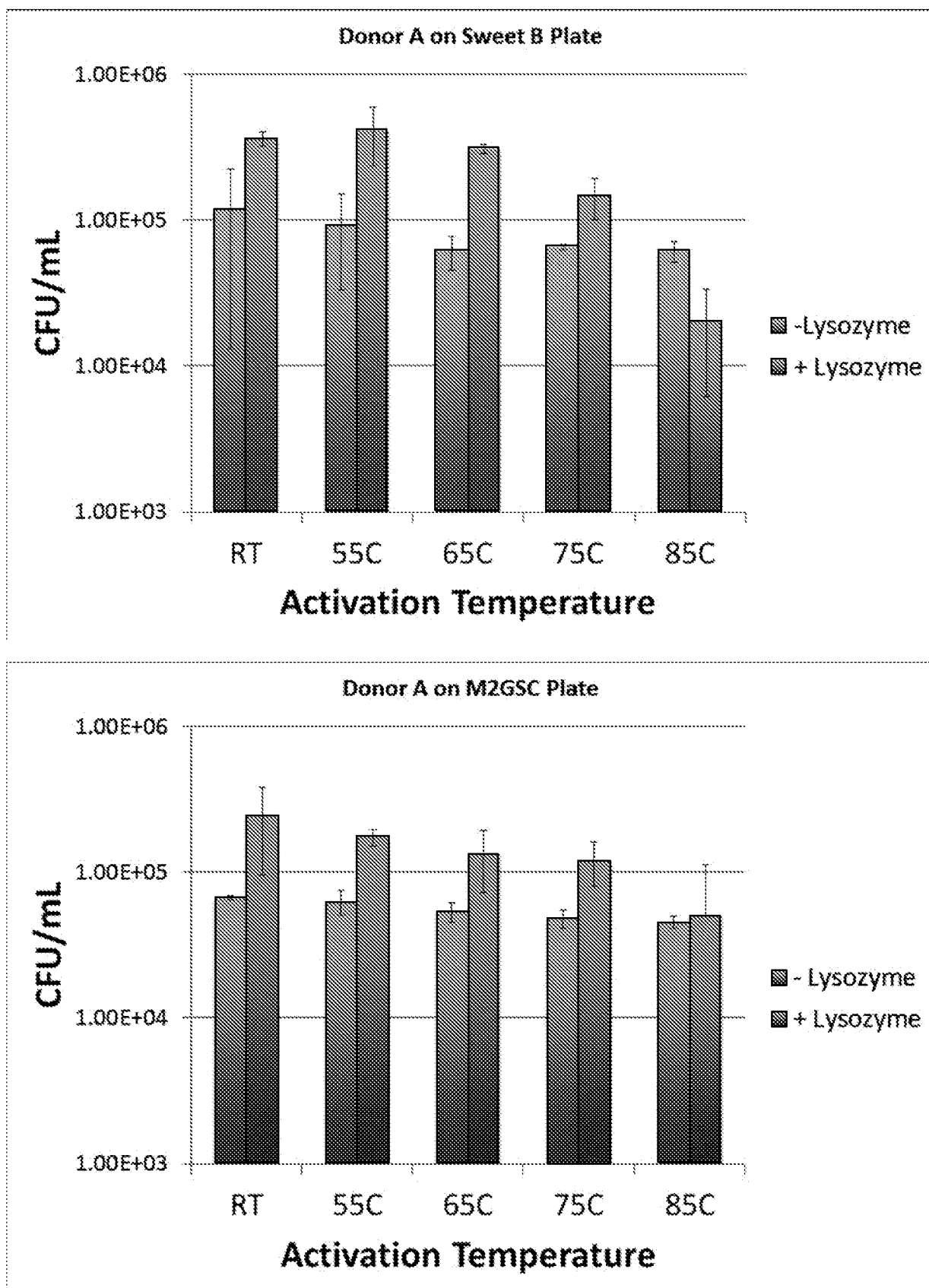


FIG. 11

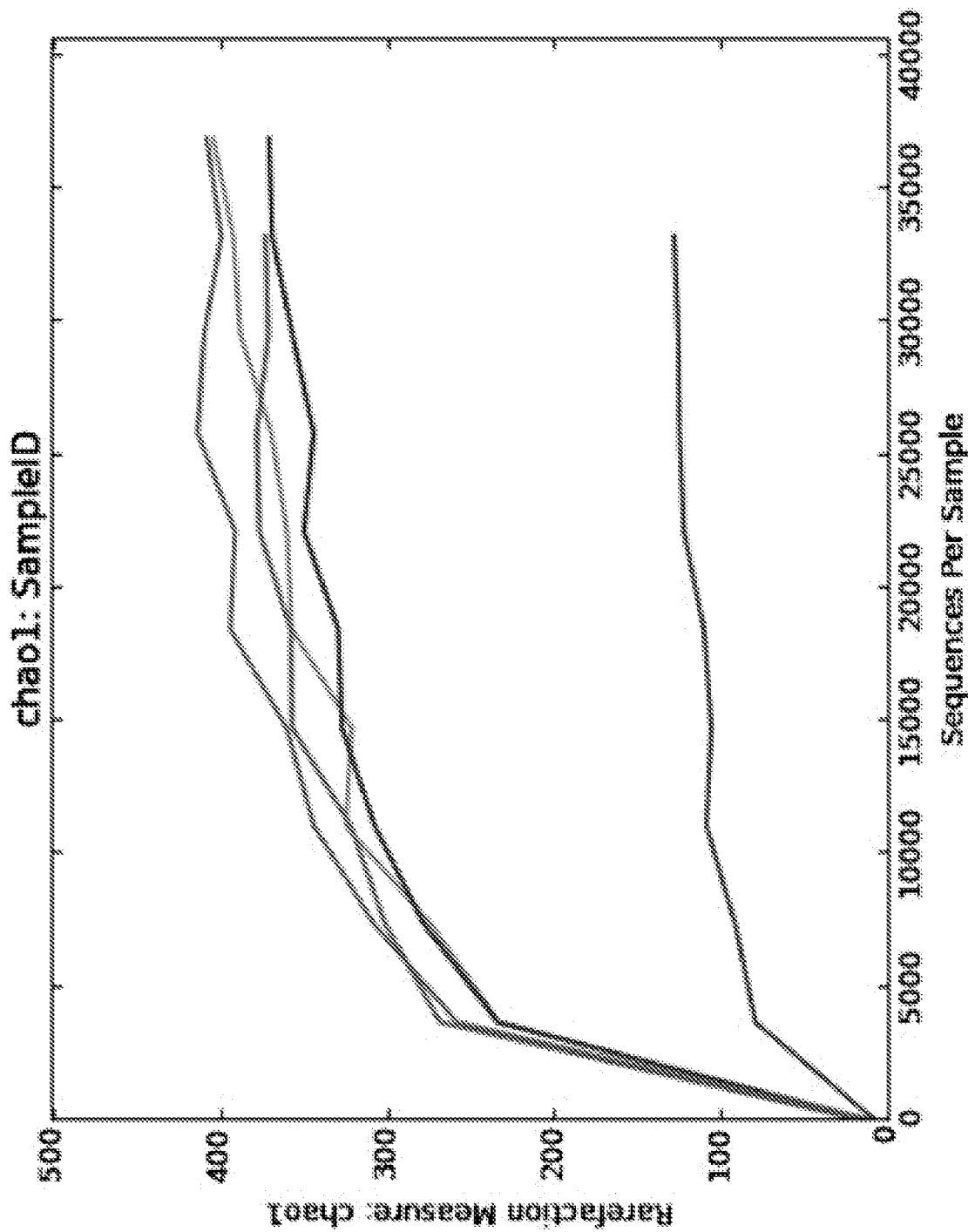


FIG. 12

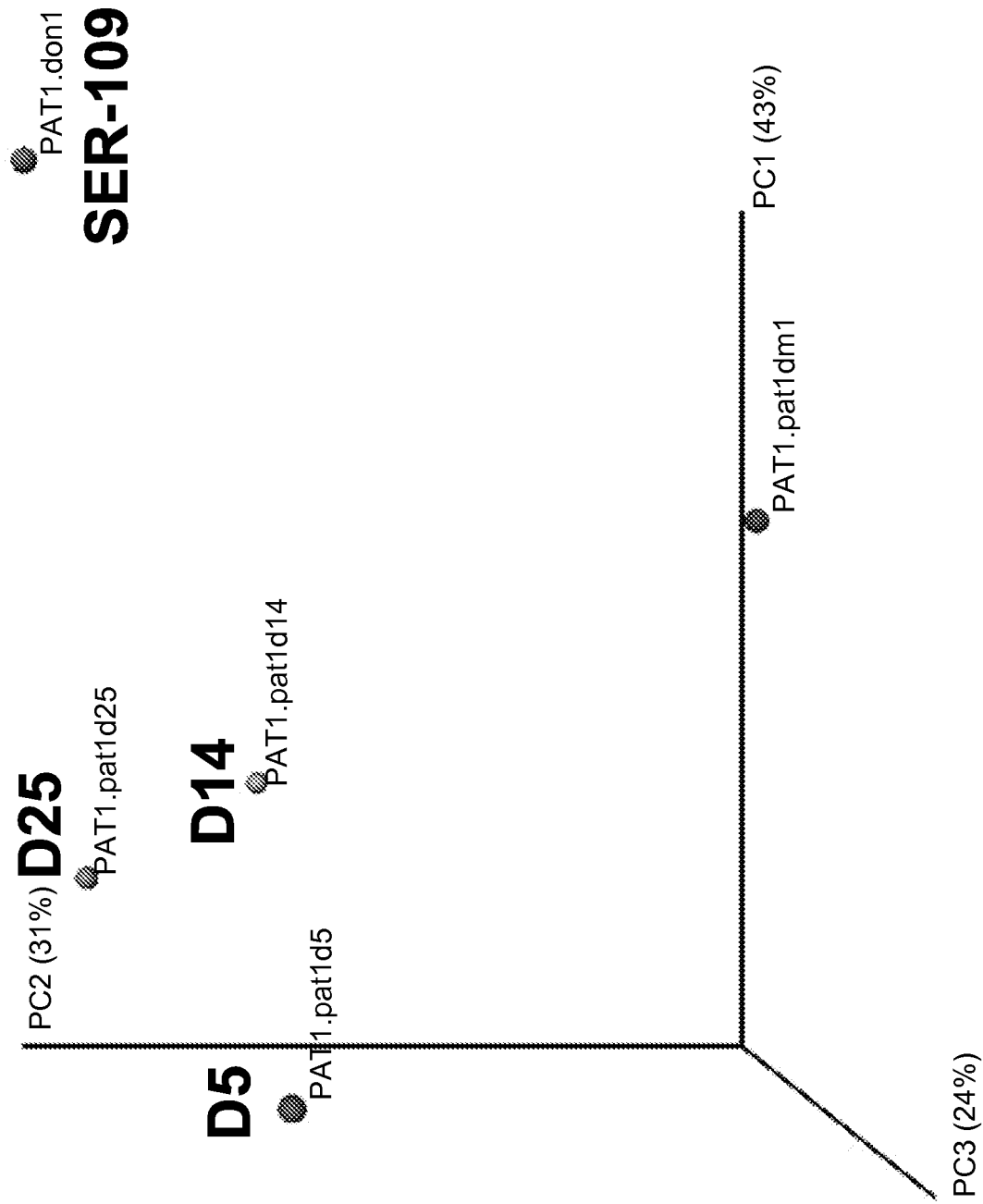


FIG. 13

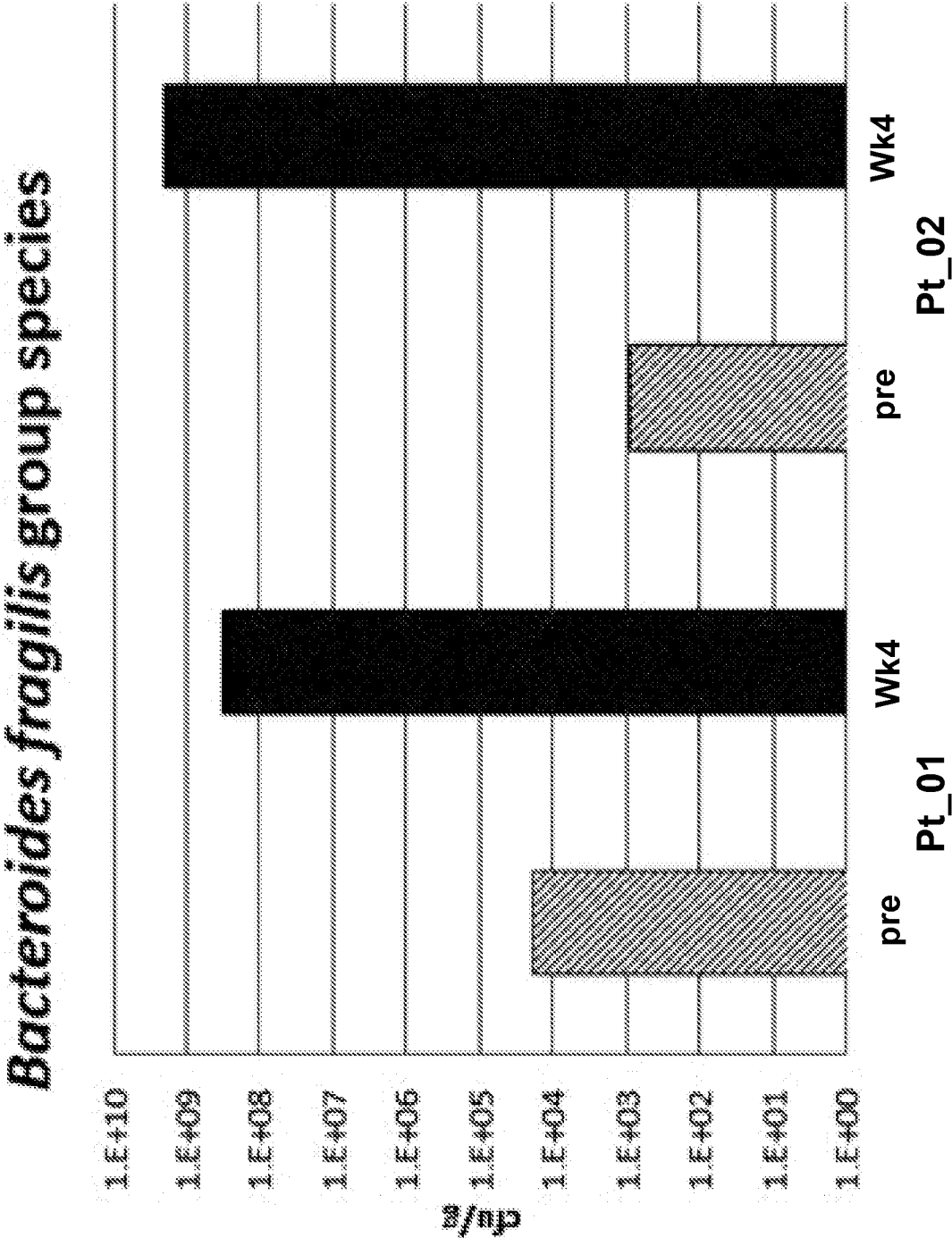


FIG. 14

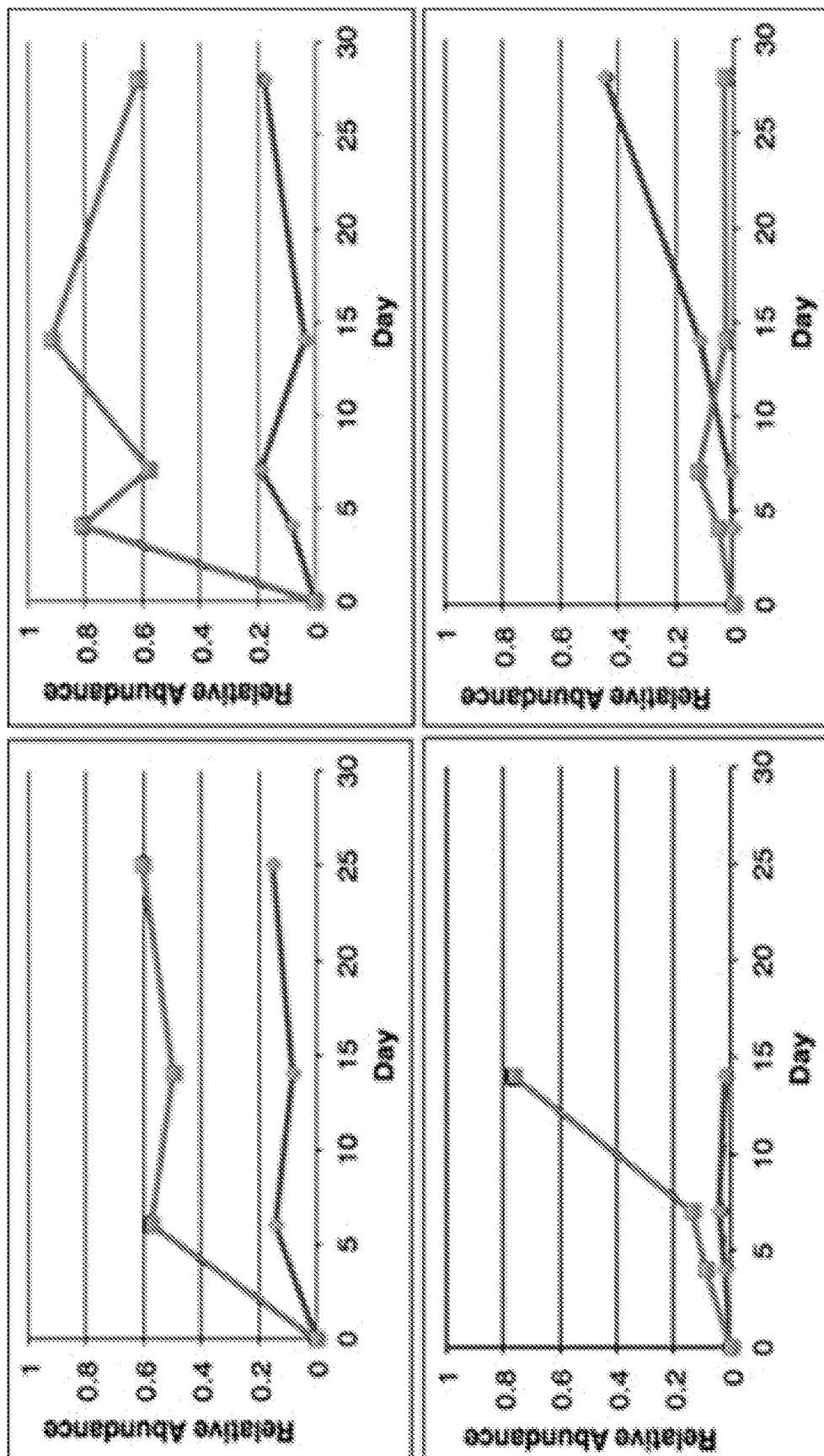


FIG. 15