

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



WIPO | PCT



(10) International Publication Number
WO 2014/121301 A1

(43) International Publication Date
7 August 2014 (07.08.2014)

(51) International Patent Classification:

C12N 1/20 (2006.01) A61K 35/66 (2006.01)
A61K 35/74 (2006.01)

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(21) International Application Number:

PCT/US2014/014744

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(22) International Filing Date:

4 February 2014 (04.02.2014)

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

kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/760,574	4 February 2013 (04.02.2013)	US
61/760,585	4 February 2013 (04.02.2013)	US
61/760,584	4 February 2013 (04.02.2013)	US
61/760,606	4 February 2013 (04.02.2013)	US
61/926,918	13 January 2014 (13.01.2014)	US

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

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

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Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: COMPOSITIONS AND METHODS FOR INHIBITION OF PATHOGENIC BACTERIAL GROWTH

(57) Abstract: Compositions for methods of preventing or reducing pathogenic bacterial growth, proliferation, and/or colonization are described containing one or more types of non-pathogenic bacteria to be introduced into the gastrointestinal tract and effectively compete with pathogenic bacteria for monomeric or polymeric carbohydrate nutrients, and/or amino acid nutrients, and/or vitamin nutrients.



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TITLE

[001] Compositions and Methods for Inhibition of Pathogenic Bacterial Growth.

RELATED APPLICATIONS

[002] This application claims priority to U.S. Provisional Application No. 61/760,584, filed February 4, 2013 and U.S. Provisional Application No. 61/760,585, filed February 4, 2013 and U.S. Provisional Application No. 61/760,574, filed February 4, 2013 and U.S. Provisional Application No. 61/760,606, filed February 4, 2013 and U.S. Provisional Application No. 61/926,918, filed January 13, 2014. These applications are all incorporated by reference in their entirety for all purposes.

REFERENCE TO A SEQUENCE LISTING

[003] This application includes a Sequence Listing submitted electronically as a text file named 25969PCT_sequencelisting.txt, created on February 2, 2014, with a size of 4,100,000 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 nor correct dysbioses of the GI tract in many situations. Additionally, such probiotics are insufficient to prevent, reduce or treat a pathogenic bacterial infection of the gastrointestinal tract, such as by *Clostridium difficile*, by reducing the population of the gastrointestinal tract by the pathogenic bacteria.

[007] Thus practitioners have a need for methods and compositions for preventing or treating pathogenic bacterial infections. We have designed bacterial compositions of isolated bacterial strains with a plurality of beneficial properties, including the ability to metabolize nutrients at rates above and at concentrations below those of pathogenic bacteria, based on our understanding of those bacterial strains and our analysis of the properties that enhance the utility and commercialization of a microbial composition.

[008] Therefore, in response to the need for durable, efficient, and effective compositions and methods for prevention, diagnosis and/or treatment of gastrointestinal diseases by way of restoring or enhancing microbiota functions, we address these and

other shortcomings of the prior art by providing compositions and methods for treating subjects.

SUMMARY OF THE INVENTION

[009] The present invention demonstrates effective compositions for and methods of preventing or reducing pathogenic bacterial growth, proliferation, and/or colonization, whereby bacterial compositions that contain one or more types of non-pathogenic bacteria are introduced into the gastrointestinal tract and effectively compete with pathogenic bacteria for monomeric or polymeric carbohydrate nutrients, and/or amino acid nutrients, and/or vitamin nutrients. Alternatively, organisms may prevent disease by metabolizing available germinants utilized by the pathogenic bacteria and prevent pathogenic spore germination. Alternatively, competition for overlapping nutrients among two or more types of non-pathogenic bacteria that are introduced into the gastrointestinal tract may lead to secretion of molecules inhibitory to a pathogen and/or pathobiont by one or multiple of the types of introduced non-pathogenic bacteria. In certain instances, the inhibitory compound may be a toxin, antibiotic, or a bacterocin (see Chiuchiolo et al. Growth-phase-dependent expression of the cyclopeptide antibiotic microcin J25 *J Bacteriol.* 2001 Mar;183(5):1755-64).

BRIEF DESCRIPTION OF THE DRAWINGS

[010] Figure 1 illustrates variable regions mapped onto the 16s sequence (Figure 1A) and annotated 16s sequence (Figure 1B) with bolded variable regions according to an embodiment of the invention.

[011] Figure 2 is a photograph of a CsCl gradient demonstrating spore separation from other material.

[012] Figure 3 illustrates population of fecal bacterial cell suspension (left) after CsCl separation (center) and after a CsCl and sucrose gradient (right) demonstrates enrichment of spores.

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

[014] Figure 5 illustrates a relapse prevention model with ethanol treated spores and ethanol treated, gradient purified spores.

[015] Figure 6 illustrates a linear range of DPA assay compared to CFU counts/ml.

[016] Figure 7 illustrates bacterial diversity measured in the ethanol treated spore treatment sample and patient pre- and post-treatment samples.

[017] Figure 8 illustrates patient bacterial ecology is shifted by treatment with an ethanol treated spore treatment from a dysbiotic state to a state of health.

[018] Figure 9 illustrates Augmentation of Bacteroides species in patients.

[019] Figure 10 illustrates species Engrafting versus Species Augmenting in patient's microbiomes after treatment with an ethanol-treated spore preparation.

[020] Figure 11 illustrates the results of a nutrient utilization assay with Clostridium difficile and potential competitors of the pathogen. Plus (+) indicates that it is a nutrient for the isolate tested. Minus (-) indicates that it is not a nutrient for the isolate tested.

[021] 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

[022] "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).

[023] "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.

[024] “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.

[025] “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 (as 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.

[026] 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.

[027] “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.

[028] “Ecological Niche” or simply “Niche” refers to the ecological space in which a 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).

[029] “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.

[030] 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.

[031] 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.

[032] “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.

[033] 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.

[034] “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 $1 \times 10^{-2}\%$, $1 \times 10^{-3}\%$, $1 \times 10^{-4}\%$, $1 \times 10^{-5}\%$, $1 \times 10^{-6}\%$, $1 \times 10^{-7}\%$, 1×10^{-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.

[035] “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.

[036] 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.

[037] 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.

[038] 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.

[039] 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.

[040] 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/).

[041] 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.

[042] 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.

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

[044] 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.

[045] 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.

[046] 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.

[047] 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.

[048] 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.

[049] "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.

[050] 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.

[051] 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.

[052] 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).

[053] 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.

[054] 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.

[055] 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.

[056] 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.

[057] 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.

[058] 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.

[059] 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.

[060] "Fermentation products" are compounds that are produced by bacteria or bacterial populations through carbohydrate metabolic processes. These metabolites include but are not limited to lactate, butyrate, acetate, ethanol, succinate, formate.

[061] A substance that organisms use to live and grow. In this context, it includes but is not limited to vitamins, minerals, antioxidant, fermentation products, cofactors, proteins, amino acids, nucleotides, nucleic acids, lipids, carbohydrates and metabolic products of these molecules.

COMPOSITIONS OF THE INVENTION

Bacterial Compositions

[062] 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, germination, 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). 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).

[063] 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.

[064] The present invention demonstrates effective methods of preventing or reducing pathogenic bacterial growth, proliferation, and/or colonization, whereby bacterial compositions that contain one or more types of non-pathogenic bacteria are introduced into the gastrointestinal tract and effectively compete with pathogenic bacteria for monomeric or polymeric carbohydrate nutrients, and/or amino acid nutrients, and/or vitamin nutrients. Alternatively, organisms may prevent disease by metabolizing available germinants utilized by the pathogenic bacteria and prevent pathogenic spore germination. Alternatively, competition for overlapping nutrients among two or more types of non-pathogenic bacteria that are introduced into the gastrointestinal tract may lead to secretion of molecules inhibitory to a pathogen and/or pathobiont by one or multiple of the types of introduced non-pathogenic bacteria. In certain instances, the inhibitory compound may be a toxin, antibiotic, or a bacterocin (see Chiuchiolo et al. Growth-phase-dependent expression of the cyclopeptide antibiotic microcin J25 *J Bacteriol.* 2001 Mar;183(5):1755-64).

[065] In one instance, the enteric bacterium *Clostridium difficile* (*C. difficile*) is an opportunistic pathogen whose infection frequently follows antibiotic treatment. Numerous investigations in animal models as well as clinical observations have demonstrated that the normal gut microbiota is capable of exerting ecological control

over *C. difficile* that prevents it from colonizing the gut, and that this control is often perturbed following antibiotic treatment. One notable effect of antibiotic treatment is to change in the composition and availability nutrients in the gastrointestinal tract as a result of altering the gut microbiota. For instance, treatment of SPF mice with streptomycin leads to a transient 40-fold increase in sialic acid one day after antibiotic exposure, which declines by day three, a timeframe consistent with susceptibility to *C. difficile* infection [Ng KM, et al *Nature* (2013) **502**: 96-9]. *C. difficile* is known to metabolize sialic acids such as N-acetyl-neuraminic acid, which is enzymatically released from mucin by commensal microbes along the intestinal epithelium. One experimental means of re-establishing control in animals has been to transplant the normal fecal flora of a healthy animal via an enteral route [for example, Wilson, K.H., J Silva, and FR Fekety (1981) Suppression of *Clostridium difficile* by normal hamster cecal flora and prevention of antibiotic-associated colitis. *Infect. Immun.* 34 (2): 626-8.] In humans, fecal microbiota transplantation (FMT) has shown similar beneficial results [for example, Shahinas, D, et al (2012) Toward an understanding of changes in diversity associated with fecal microbiome transplantation based on 16s rRNA gene deep sequencing. *mBio* 3].

[066] In order to maintain steady-state concentrations in the gastrointestinal tract of a mammalian subject (where continuous dilution occurs as a result of the peristaltic wave), *C. difficile* is dependent upon the availability of a small number of nutrients, particularly carbohydrates such as glucose, mannitol, fructose, N-acetylglucosamine (NAG) and N-acetylneuraminic acid (NAN), amino acids, and vitamins such as biotin, pantothenate and pyridoxine. Free monosaccharide levels of NAG and NAN are low in the mammalian gastrointestinal tract; instead, NAG and NAN are present in higher order carbohydrate polymers such as mucins and inulin.

[067] In one embodiment of the present invention, the bacterial compositions containing one or more types of non-pathogenic bacteria are introduced into the gastrointestinal tract to prevent or treat infection by utilizing the nutrients and/or metabolizing the germinants utilized by *C. difficile*. In some embodiments, the latter will be achieved when the introduced bacterial composition depletes one or more of the nutrients (e.g., carbohydrate or vitamin) utilized by *C. difficile* to a concentration that

inhibits further growth of *C. difficile* or alternatively, reduces the rate of growth such that the introduced bacteria and/or other resident bacteria outcompete *C. difficile*.

[068] In a first aspect, provided are compositions comprising an effective amount of a first type of bacteria and a second type of bacteria that overlap with *C. difficile* nutrition utilization, and/or are independently capable of proliferating in a nutrient medium having a threshold concentration of a nutrient below that concentration required for *Clostridium difficile* proliferation. In certain embodiments the first and second type of bacteria are isolated, such as from the gastrointestinal tract of a healthy mammal, and are not identical to each other.

[069] Exemplary nutrients include carbohydrates, amino acids, vitamins, minerals, and cofactors such as germinants. Exemplary minerals include iron, phosphate, copper, nickel, and magnesium. Additional nutrients include nitrogen sources, such as ammonia or urea. Table XXX5 contains representative nitrogen sources.

[070] Exemplary fermentation products include lactate, butyrate, acetate, ethanol, succinate, and formate. Exemplary carbohydrate nutrients include glucose, mannitol, fructose, N-acetylglucosamine (NAG) and N-acetylneuraminic acid (NAN). The bacterial compositions contain bacteria that overlap with *C. difficile* nutrition utilization and/or are independently capable of proliferating in a nutrient medium having a threshold concentration of at least one of the following: glucose, mannitol, fructose, NAG and NAN below that concentration required for *Clostridium difficile* proliferation. In certain embodiments, the bacterial compositions include bacteria that are independently capable of proliferating in a nutrient medium having a threshold concentration of each of glucose, mannitol, fructose, NAG and NAN below the concentrations required for *Clostridium difficile* proliferation. The threshold concentration may be about 90%, about 80%, about 70%, about 60%, about 50%, about 40%, about 30%, about 20%, about 10% or less than about 10% of the concentration of that nutrient required for *Clostridium difficile* proliferation. In other embodiments, the bacterial compositions contain bacteria that can proliferate faster than *C. difficile* at a given nutrient concentration, effectively out-competing the *C. difficile* for a limited resource. For example, in a composition having two or more types of bacteria, these two types are independently capable of proliferating at a rate at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%,

100%, 200%, 300%, or more than 300% greater than the proliferation rate of *Clostridium difficile* in a nutrient medium having a concentration of a nutrient such as glucose, mannitol, fructose, NAG and NAN, or a combination thereof. Alternatively, a composition is provided having two or more types of bacteria that are capable of growing to a maximal bacterial concentration in excess of the maximal bacterial concentration capable by *C. difficile*, as determined, e.g., by turbidity, colony counts, total biomass, or other means described herein or otherwise known in the art. In yet another embodiment, the bacterial composition is delivered at an efficacious dose such that the population of bacteria in the composition deplete glucose, mannitol, fructose, NAG and NAN and other carbohydrates necessary for *C. difficile* growth.

[071] Exemplary vitamin nutrients include biotin, pantothenate, folic acid and pyridoxine. Table XXX1 contains representative vitamins, minerals, and cofactors. Without being limited to any particular mechanism, the bacteria present in the therapeutic composition are better able to transport one or more vitamins from the medium into the bacterial cell, or they are more efficient at utilizing the vitamin(s) once it is inside the cell.

[072] The bacterial compositions comprise bacteria that overlap with *C. difficile* nutrition utilization requirements and/or are independently capable of proliferating in a nutrient medium having a threshold concentration of at least one of biotin, pantothenate and pyridoxine below that concentration required for *Clostridium difficile* proliferation. In certain embodiments, the bacterial compositions contain bacteria that are independently capable of proliferating in a nutrient medium having a threshold concentration of each of biotin, pantothenate and pyridoxine below the concentrations required for *Clostridium difficile* proliferation. The threshold concentration may be about 90%, about 80%, about 70%, about 60%, about 50%, about 40%, about 30%, about 20%, about 10% or less than about 10% of the concentration of that nutrient required for *Clostridium difficile* proliferation. In other embodiments, the bacterial compositions contain bacteria that can proliferate faster than *C. difficile* at a given nutrient concentration, effectively out-competing the *C. difficile* for a limited resource. For example, in a composition comprising two or more types of bacteria, these two types are independently capable of proliferating at a rate at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, or more than 300% greater than the

proliferation rate of *Clostridium difficile* in a nutrient medium having a concentration of a nutrient such as biotin, pantothenate, pyridoxine, or a combination thereof.

Alternatively, a composition is provided comprising two or more types of bacteria that are capable of growing to a maximal bacterial concentration in excess of the maximal bacterial concentration capable by *C. difficile*, as determined, e.g., by turbidity, colony counts, total biomass, or other means described herein or otherwise known in the art. In yet another embodiment, the bacterial composition is delivered at an efficacious dose such that the population of bacteria in the composition deplete biotin, pantothenate and pyridoxine and other vitamins necessary for *C. difficile* growth.

[073] In another aspect, provided are compositions comprising effective amounts of one or more types of bacteria that compete with *C. difficile* for nutrients, e.g., polymeric carbohydrates, glycosylated proteins and/or vitamins. In a further aspect, provided are compositions comprising effective amounts of one or more types of bacteria that have rates of saccharification and consumption of the resulting products that exceed other bacterial species in the gastrointestinal tract. These compositions effectively eliminate polymeric carbohydrates from the local gastrointestinal environment, which would otherwise be hydrolyzed by resident bacterial species resulting in free glucose, NAG and/or NAN. Thus provided are bacterial compositions comprising first and second types of bacteria that are independently capable of saccharification at a rate at least 10% greater than *Clostridium difficile*. Also provided are bacterial compositions comprising first and second types of bacteria that are independently capable of saccharification at a rate at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, or more than 300% greater than any other bacterial species in the local environment that digests polymeric carbohydrates and releases the hydrolysis products into the media for consumption by, e.g., *Clostridium difficile*. Suitable carbohydrate polymers to target for selective digestion include a fructan, an arabinoxylan, a lactulose, and a galactooligosaccharide or a glycosylated protein such as a mucin.

[074] Also provided are bacterial compositions comprising bacterial types capable of efficient saccharification of complex carbohydrates and metabolism of simple sugars. For example, provided is a composition comprising an effective amount of a first type of bacteria and a second type of bacteria formulated for oral administration to a

mammalian subject, wherein the first type of bacteria is: (i) isolated, (ii) not identical to the second type of bacteria, and (iii) independently capable of saccharification at a rate at least 10% greater than *Clostridium difficile*, and wherein the second type of bacteria is: (i) isolated, (ii) not identical to the first type of bacteria, and (iii) independently capable of glycolysis of a nutrient selected from the group consisting of glucose, mannitol, fructose, NAG and NAN, at a rate at least 10% greater than *Clostridium difficile* or at a concentration at least 10% less than *Clostridium difficile*, or the combination thereof. In addition, provided is a composition comprising an effective amount of a first type of bacteria and a second type of bacteria formulated for oral administration to a mammalian subject, wherein the first type of bacteria is: (i) isolated, (ii) not identical to the second type of bacteria, and (iii) independently capable of saccharification of a necessary *C. difficile* nutrient, and wherein the second type of bacteria is: (i) isolated, (ii) not identical to the first type of bacteria, and (iii) independently capable of glycolysis of a nutrient selected from the group consisting of glucose, mannitol, fructose, NAG and NAN; and wherein the delivered bacteria effectively metabolize for nutrients to levels below that which is required for *C. difficile* growth.

[075] Also provided are methods for the treatment of *Clostridium difficile* infection in a mammalian subject, by orally administering to the subject a composition comprising an effective amount of a first type of bacteria and a second type of bacteria formulated for oral administration to a mammalian subject, where the first type of bacteria is: (i) isolated, (ii) not identical to the second type of bacteria, and (iii) independently capable of saccharification at a rate at least 10% greater than *Clostridium difficile*, and where the second type of bacteria is: (i) isolated, (ii) not identical to the first type of bacteria, and (iii) independently capable of glycolysis of a nutrient selected from the group consisting of glucose, mannitol, fructose, N-acetylglucosamine (NAG) and N-acetylneuramic acid (NAN), at a rate at least 10% greater than *Clostridium difficile* or at a concentration at least 10% less than *Clostridium difficile*, or the combination thereof, under conditions such that the first type of bacteria and the second type of bacteria functionally populate the gastrointestinal tract of the subject and prevent the population of the gastrointestinal tract by *Clostridium difficile*.

[076] Microbial, e.g., 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.

[077] 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.

Bacterial Compositions Described by Species

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

[079] In one embodiment, the microbial, e.g., 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.

[080] 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.

[081] 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.

[082] 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.

[083] 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*

pilliforme, *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.

[084] 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.

[085] 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.

[086] 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.

[087] 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.

[088] 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.

[089] 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.

[090] 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)

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

[092] 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

[093] 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*).

[094] 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*.

[095] 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*.

[096] 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*.

[0097] 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.

[0098] 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*).

[0099] 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*.

[0100] 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*.

[0101] 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*.

[0102] 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*.

[0103] 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 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.

Inhibition of Bacterial Pathogens

[0104] In some embodiments, the bacterial composition provides a protective or therapeutic effect against infection by one or more GI pathogens of interest.

[0105] A list of exemplary bacterial pathogens is provided in **Table 1** as indicated by pathogen status.

[0106] In some embodiments, 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).

[0107] 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 pneumoniae*, *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*.

[0108] 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

[0109] 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. In some embodiments, yeast spores and other fungal spores are also purified and selected for therapeutic use.

[0110] Disclosed herein are therapeutic compositions containing non-pathogenic, germination-competent bacterial spores, spore forming organisms and non-spore forming organisms 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.

[0111] Provided herein are therapeutic compositions containing a purified population of bacterial spores, spore forming organisms and non-spore forming organisms. 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 or bacteria, 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 or bacteria 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., liquid culture) 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.

[0112] In certain embodiments, the purified populations 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.

[0113] 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%.

[0114] 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.

[0115] 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.

[0116] 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.

[0117] 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.

[0118] 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.

[0119] Preferred bacterial species are provided at **Table X4**. Where specific strains of a species are provided, one of skill in the art will recognize that other strains of the species can be substituted for the named strain.

[0120] 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).

[0121] 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.

[0122] In some embodiments, all or essentially all of the bacterial species present in a purified population are isolated or originally isolated 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 bacteria are generated in culture and combined to form a purified population. In other alternative embodiments, one or more of these culture-generated populations are combined with a fecal material-derived population to generate a hybrid 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.

[0123] 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.

[0124] 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.

[0125] 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.

[0126] 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

Methods for Determining 16S Sequences

[0127] 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.

[0128] 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.

[0129] 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

[0130] 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.

[0131] 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.

[0132] 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.

[0133] 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.

[0134] 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.

[0135] 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.

[0136] 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

[0137] 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.

[0138] 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.

[0139] 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).

[0140] 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).

[0141] 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

[0142] 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.

[0143] 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.

[0144] 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.

[0145] 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

[0146] 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.

[0147] 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.

[0148] 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).

[0149] 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.

[0150] 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

[0151] 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.

[0152] 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.

[0153] 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).

[0154] 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

[0155] 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

[0156] 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.

[0157] 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.

[0158] 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).

[0159] 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, Ibacitabine, Amantadine, Oseltamivir, Rimantidine, Tipranavir, Zalcitabine, Zanamivir and Zidovudine.

[0160] 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.

[0161] 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.

[0162] 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

[0163] 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.

[0164] 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 (cefactor, defadroxil, cefazolin, cefixime, cefixitin, 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.

[0165] 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} .

[0166] 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.

[0167] 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

[0168] 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).

[0169] 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

[0170] 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.

[0171] 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 C_q 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

[0172] 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

[0173] 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

[0174] 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

[0175] 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

[0176] 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

[0177] 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%, 89%, 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

[0178] 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

[0179] 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 to 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

[0180] 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

[0181] 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 cm² will not generally destroy desired spores.

Centrifugation and Density Separation Treatments

[0182] 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.

[0183] 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**

[0184] 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.

[0185] 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.

[0186] 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.

[0187] 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.

[0188] 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.

[0189] 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.

[0190] 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.

[0191] 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.

[0192] 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.

[0193] 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.

[0194] 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.

[0195] 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.

[0196] 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.

[0197] 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, silytol, 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.

[0198] 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.

[0199] 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.

[0200] 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.

[0201] 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.

[0202] 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.

[0203] 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.

[0204] 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.

[0205] 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.

[0206] 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

[0207] 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.

[0208] 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

[0209] 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 -80C. 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). 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 & 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 & 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

[0210] OTUs may 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.

[0211] 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.

[0212] In addition to the 16S rRNA gene, one may 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

[0213] Genomic DNA is extracted from pure microbial cultures using a hot alkaline lysis method. 1 μ l of microbial culture is added to 9 μ l 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 μ l 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

[0214] To amplify bacterial 16S rDNA (Figure 1A), 2 μ l of extracted gDNA is added to a 20 μ l 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.

[0215] The PCR is 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.

[0216] 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

[0217] 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 (AATGATACGGCGACCACCGAGATCTACACTATGGTAATTGTGTGCCAGCMGCCGCGGTAA, 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.

[0218] 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

[0219] 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

[0220] *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.

[0221] *Nucleic Acid Detection.* The prepared library is sequenced on Illumina HiSeq or MiSeq sequencers (Illumina, San Diego, CA) with cluster generation, template hybridization, iso-thermal amplification, linearization, blocking and denaturation 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

[0222] Nucleic acid sequences are analyzed and annotations are 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

[0223] 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.

[0224] 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

[0225] 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 Oxgall, can be used to induce germination. Oxgall 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/oxgall 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 Oxgall provides additional bile acid germinants.

[0226] 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.

[0227] 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 CaCl₂, 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 KH₂PO₄, 0.53 g Na₂HPO₄, 0.3 g NH₄Cl, 0.3 g NaCl, 0.1 g MgCl₂ x 6H₂O, 0.11 g CaCl₂, 1 ml alkaline trace element solution, 1 ml acid trace element solution, 1 ml vitamin solution, 0.5 mg resazurin, 4 g NaHCO₃, 0.25 g Na₂S x 9 H₂O. 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.

[0228] 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: The purification and isolation of a spore forming fraction from feces

[0229] 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).

[0230] 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 6: Bacterial compositions prevent *C. difficile* infection in a mouse model

[0231] 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).

[0232] 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 5) 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.

[0233] 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 3). 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 7: The prophylactic and relapse prevention hamster models

[0234] 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. The results are shown in Figure 4, illustrating the prophylaxis model with the ethanol treated spore preparation and the ethanol treated, gradient-purified spore preparation.

[0235] 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.

[0236] The results are shown in Figure 5 illustrating the relapse prevention model with ethanol treated spores and ethanol treated, gradient purified spores

Example 8: Clinical treatment of recurrent *C. difficile* in patients

[0237] To assess the efficacy of test articles like bacterial composition including but not limited to a ethanol treated spore preparations (e.g. see Example 5) 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.

[0238] 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.

[0239] 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.

[0240] 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.

[0241] 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.

[0242] 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.

[0243] 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.

[0244] 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.

[0245] The above protocol could be modified to deliver other bacterial compositions e.g. vegetative cells, spore preparations, combinations thereof.

Example 9. Enrichment and Purification of bacteria.

[0246] 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 5). 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 10. Cell Bank Preparation.

[0247] 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 -80°C. 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 11. Titer Determination.

[0248] 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 12. Preparation of bacterial compositions.

[0249] 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 13. Provision of gut microbiome sample material.

[0250] 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.

[0251] 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 14: Bacterial compositions populating the gut in a mouse model.

[0252] 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 12. Compositions were pre-made under anaerobic conditions and suspended in PBS + 15% glycerol and stored at $\geq -70^{\circ}\text{C}$ prior to use.

[0253] 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 composition by oral gavage in a volume of 0.2 mL (Table ZA). 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 composition was similar to that of naive, control mice.

[0254] 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.

Table ZA. Microbial compositions administered via oral gavage on Day -1

	OTU	Clade
Microbial Composition 1	<i>Clostridium_butyricum</i>	clade_252
	<i>Clostridium_disporicum</i>	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

[0255] Fecal samples were processed by isolating and sequencing DNA according to ***Example 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 TAB) and by clade (Table TAC).

Table TAB. 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	10 6	51	32	0	10	0	34	195	0	0	0
Cl_disporicum	10	17 46	11 90	88 7	0	17 46	76 9	10 11	201	11 17 5	15 31	11 52
Cl_hylemonae	0	25 8	25 8	84	0	20 3	16 4	77	0	26 5	21 4	90
Cl_orbiscindens	0	18 8	19 2	47 1	0	18 8	13 8	27 6	0	22 1	17 4	34 1
Cl_symbiosum	0	48 5	48 2	48 6	0	44 4	37 9	44 7	0	56 2	42 7	77 5
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_57FAA	0	34 1	33 6	35 4	0	35 1	18 2	35 6	0	25 6	24 0	30 0
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	0	8	2	0	9	8	42	0	5	05	68
Cl_orbiscindens	0	0	8	4	0	2	5	4	0	0	9	2
Cl_symbiosum	0	9	8	5	0	5	4	0	0	6	9	0
Co_aerofaciens	0	0	0	0	0	0	72	0	0	0	7	0
E_rectale	0	0	0	0	0	0	0	12	0	0	0	1
L_bacterium_5_1_57FAA	0	2	1	6	0	0	2	4	0	6	01	69
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 TAC. Population of Clades on Days 2, 3 and 4 following dosing with Microbial Compositions

	1x10 ⁷ per OTU				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	-1	2	3	4
clade_2 52	0	444	252	87	0	198	122	125	209	394	231	88				
clade_2 53	10	174	119	887	0	6	769	1	201	75	1	2				
clade_2 60	0	599	594	438	0	554	346	433	0	521	454	390				

clade_2 62	0	14	151	51	0	0	0	0	0	12	21	57
clade_3 09	110 0	975 93	402 0	3	999 0	520 1	514 8	5	19	931 1	636 9	495 1
clade_3 51	906 0	106 4	775 47	1	652 0	725 8	821 9	3	890 0	100 3	870 49	1
clade_3 54	0	0	0	0	0	0	0	31	173	0	0	0
clade_3 60	143 0	102 00	110 20	36	125 0	129 53	688 89	9	930 0	134 8	929 83	2
clade_4 08	889 13	129 2	121 85	01	395 23	726 2	106 0	52	407 43	858 9	149 1	29
clade_4 94	0	226	227	565	0	188	184	411	0	221	200	351
clade_5 37	0	0	68	225	0	0	0	0	0	0	0	55
clade_5 53	0	0	0	0	0	0	0	0	0	0	0	0
Microbi al comp 2	-1	2	3	4	-1	2	3	4	-1	2	3	4
clade_25 3	118 29	109 10	146 48	72	113 0	139 49	394 78	2	119 0	700 95	626 5	8
clade_26 0	112 0	131 5	854 2		104 0	129 9	125 5	0	212 0	163 792	7	
clade_30 9	125 54	137 13	784 31	9	116 0	120 10	126 04	72	740 0	141 7	108 11	58
clade_35 1	765 0	993 1	593 9	6	849 0	972 5	920 4	7	600 0	983 5	765 3	5
clade_35 4	149	0	127	429	0	0	0	39	12	0	0	0
clade_40 8	224 18	498 2	104 9	80	168 12	558 8	378 0	9	106 0	156 8	628 1	1
clade_44 4	41	0	49	202	0	18	0	12	0	14	82	157 8

[illegible]

[0256] Upon examining the OTU data in Table TAB, several patterns emerge. First, there are a group of OTUs with no sequence reads on Day -1 that show subsequent and 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 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. boltae*, *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 number of OTU sequence reads is not highly dose dependent. Overall, the data is consistent with a model whereby OTUs populate rapidly following oral administration.

[0257] The clade-based analysis in Table TAC 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 1 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.

[0258] In the mice challenged in parallel with *C. difficile*, animals were significantly protected as shown in Table TAD. 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.

Table TAD. 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%)

Example 15 Use of Biolog assay to determine bacterial strain nutrient utilization

[0259] A screen was performed to test the ability of *Clostridium difficile* and potential competitor species to utilize a panel of 190 different carbon sources. The screen was carried out using PM1 and PM2 MicroPlates (Biolog #12111, #12112), IF-0a base media (Biolog #72268) and Biolog Redox Dye Mix D (Biolog #74224). For each strain, a 1 μ L aliquot from -80° C glycerol stock was streaked out for single colonies to solid Brucella Blood Agar plates (BBA) (Anaerobe Systems #AS-111) and incubated anaerobically at 37° C for 24 hr. A single colony was then re-streaked to a BBA plate and incubated anaerobically at 37° C for 24 hr. The MicroPlates were pre-reduced by incubating for at least 24 hr in a hydrogen free anaerobic environment before use. All liquid media and supplements used were pre-reduced by placing them in an anaerobic chamber with loose lids for at least 24 hr before use. Alternatively, combinations of bacteria can also be tested.

[0260] The base media for inoculation was prepared by adding 0.029 mL of 1M potassium ferricyanide to 0.240 mL of Dye Mix D followed by addition of 19.7 mL of IF-0a, 4 mL sterile water and 0.024 mL 0.5 mM menadione. For some species, the concentrations of potassium ferricyanide and menadione were adjusted to achieve the optimal redox balance or to test multiple redox conditions. Potassium ferricyanide was tested at a final concentration of 0.38, 0.12, 0.038 and 0.06 mM. Menadione was tested at a final concentration of 0.5, 0.16 and 0.05 μ M. In total, this yields 9 redox conditions for testing. Reduction of the tetrazolium dye that forms the basis for the endpoint measurement was sensitive to the redox state of each bacterial culture, and thus to the ratio of menadione to potassium ferricyanide. It was therefore important to test various ratios for each bacterial isolate and was also important in some cases to test a species at multiple menadione / potassium ferricyanide ratios in order to detect all conditions in which a possible nutrient utilization was detectable. Some species were tested beyond the 20 hr time point to detect all conditions resulting in a positive result. In these cases plates were read at 20, 44 or 96 hr.

[0261] Using a sterile, 1 μ L microbiological loop, a loopful of biomass was scraped from the BBA plate and resuspended in the base media by vortexing. The OD was adjusted to 0.1 at 600 nm using a SpectraMax M5 plate reader. The bacterial

suspension was then aliquoted into each well of the PM1 and PM2 plates (100 μ L per well). The plates were incubated at 37°C for 20 hr in a rectangular anaerobic jar (Mitsubishi) with 3 anaerobic, hydrogen-free gas packs (Mitsubishi AnaeroPack). After 20 hr, OD at 550 nm was read using a SpectraMax M5 plate reader. Wells were scored as a weak hit if the value was 1.5x above the negative control well, and a strong hit if the value was 2x above the negative control well. The results are shown in the Table in Figure 11.

[0262] The following list of nutrient sources were tested: L-Arabinose, N-Acetyl-D-Glucosamine, D-Saccharic Acid, Succinic Acid, D-Galactose, L-Aspartic Acid, L-Proline, D-Alanine, D-Trehalose, D-Mannose, Dulcitol, D-Serine, D-Sorbitol, Glycerol, L-Fucose, D-Glucuronic Acid, D-Gluconic Acid, D, L-alpha-Glycerol-Phosphate, D-Xylose, L-Lactic Acid, Formic Acid, D-Mannitol, L-Glutamic Acid, D-Glucose-6-Phosphate, D-Galactonic Acid-gamma-Lactone, D,L-Malic Acid, D-Ribose, Tween 20, L-Rhamnose, D-Fructose, Acetic Acid, alpha-D-Glucose, Maltose, D-Melibiose, Thymidine, L-Asparagine, D-Aspartic Acid, D-Glucosaminic Acid, 1,2-Propanediol, Tween 40, alpha-Keto-Glutaric Acid, alpha-Keto-Butyric Acid, alpha-Methyl-D-Galactoside, alpha-D-Lactose, Lactulose, Sucrose, Uridine, L-Glutamine, M-Tartaric Acid, D-Glucose-1-Phosphate, D-Fructose-6-Phosphate, Tween 80, alpha-Hydroxy-Glutaric-gamma-lactone, alpha-Hydroxy Butyric Acid, beta-Methyl-D-Glucoside, Adonitol, Maltotriose, 2-Deoxy Adenosine, Adenosine, Glycyl-L-Aspartic Acid, Citric Acid, M-Inositol, D-Threonine, Fumaric Acid, Bromo Succinic Acid, Propionic Acid, Mucic Acid, Glycolic Acid, Glyoxylic Acid, D-Cellobiose, Inosine, Glycyl-L-Glutamic Acid, Tricarballic Acid, L-Serine, L-Threonine, L-Alanine, L-Alanyl-Glycine, Acetoacetic Acid, N-Acetyl-beta-D-Mannosamine, Mono Methyl Succinate, Methyl Pyruvate, D-Malic Acid, L-Malic Acid, Glycyl-L-Proline, p-Hydroxy Phenyl Acetic Acid, m-Hydroxy Phenyl Acetic Acid, Tyramine, D-Psicose, L-Lyxose, Glucuronamide, Pyruvic Acid, L-Galactonic Acid-gamma-Lactone, D-Galacturonic Acid, Pheylethyl-amine, 2-aminoethanol, Chondroitin Sulfate C, alpha-Cyclodextrin, beta-Cyclodextrin, gamma-Cyclodextrin, Dextrin, Gelatin, Glycogen, Inulin, Laminarin, Mannan, Pectin, N-Acetyl-D-Galactosamine, N-Acetyl-Neuramic Acid, beta-D-Allose, Amygdalin, D-Arabinose, D-Arabitol, L-Arabitol, Arbutin, 2-Deoxy-D-Ribose, I-Erythritol, D-Fucose, 3-O-beta-D-Galacto-pyranosyl-D-Arabinose, Gentibiose, L-Glucose, Lactitol, D-Melezitose, Maltitol, alpha-Methyl-D-Glucoside, beta-Methyl-D-Galactoside, 3-Methyl Glucose, beta-Methyl-D-Glucuronic Acid, alpha-Methyl-

D-Mannoside, beta-Methyl-D-Xyloside, Palatinose, D-Raffinose, Salicin, Sedoheptulosan, L-Sorbose, Stachyose, D-Tagatose, Turanose, Xylitol, N-Acetyl-D-Glucosaminitol, gamma-Amino Butyric Acid, delta-Amino Valeric Acid, Butyric Acid, Capric Acid, Caproic Acid, Citraconic Acid, Citramalic Acid, D-Glucosamine, 2-Hydroxy Benzoic Acid, 4-Hydroxy Benzoic Acid, beta-Hydroxy Butyric Acid, gamma-Hydroxy Butyric Acid, alpha-Keto Valeric Acid, Itaconic Acid, 5-Keto-D-Gluconic Acid, D-Lactic Acid Methyl Ester, Malonic Acid, Melibionc Acid, Oxalic Acid, Oxalomalic Acid, Quinic Acid, D-Ribino-1,4-Lacton, Sebacic Acid, Sorbic Acid, Succinamic Acid, D-Tartaric Acid, L-Tartaric Acid, Acetamide, L-Alaninamide, N-Acetyl-L-Glutamic Acid, L-Arginine, Glycine, L-Histidine, L-Homserine, Hydroxy-L-Proline, L-Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Ornithine, L-Phenylalanine, L-Pyroglutamic Acid, L-Valine, D,L-Carnithine, Sec-Butylamine, D,L-Octopamine, Putrescine, Dihydroxy Acetone, 2,3-Butanediol, 2,3-Butanone, 3-Hydroxy 2-Butanone.

[0263] Additionally, one of skill in the art could design nutrient utilization assays for an even broader set of nutrients using the using methods described above.

[0264] A similar screen can be performed to test the utilization of vitamins, amino acids, or cofactors. In these instances, Biolog MicroPlates for screening of vitamins, amino acids or cofactors that are of interest would be used in place of the PM1 and PM2 plates, for example PM5. Table XXX1 contains a list of representative vitamins, minerals, and cofactors. For each strain tested, a universal carbon source such as glucose will be used as a positive control to demonstrate reduction of the tetrazolium dye under the specific conditions of the assay.

Example 16: Use of transcriptomics to determine bacterial strain nutrient utilization

[0265] Alternatively, the ability of a strain to utilize a particular carbon source can be determined by a transcriptomics based approach.

[0266] In this instance, a pure culture of *Clostridium difficile* is streaked to a Brucella Blood Agar plate (BBA) (Anaerobe Systems #AS-111) and incubated anaerobically at 37° C for 24 hr. A single colony is then inoculated into a minimal defined base medium (as described in Karasawa, T, et al., *Microbiol* (1995) **141**: 371-5) substituting with a single carbon source such as fructose or mannitol. Several cultures can be tested in parallel altering the carbon source being tested. A control culture is also

inoculated which lacks a carbon source. RNA is extracted from all cultures at 8, 14 and 38 hr. Extraction of total RNA is performed immediately using a FastPrep instrument and an RNA ProBlue kit (QBiogene). RNA quality is assessed by analysis on an Agilent Bioanalyser.

[0267] The *C. difficile* microarray based on the genome sequence of strain 630 can be found in ArrayExpress (accession number A-BUG- 20). The microarray contains 3,679 coding sequences (CDSs). A Genisphere 3DNA Array 900 MPX microarray kit is used for cDNA synthesis, labeling, and hybridization. Ten micrograms of starting RNA is used for the cDNA synthesis reactions. The microarray slides are hybridized competitively with each cDNA.

[0268] The microarray slides are scanned using a ScanArray 4000 instrument (Packard Instrument Co.), and fluorescence intensities are quantified using ImaGene (BioDiscovery) software. Raw expression data from the arrays are analyzed with R and Limma (linear model for microarray data) software from the Bioconductor project. Further data processing and statistical analysis is performed by correcting background with the Normexp method, resulting in strictly positive values and reducing variability in the log ratios for genes with low hybridization signals. Each strain is then normalized by using the Loess method. In order to identify differentially expressed genes, Bayesian adjusted *t* statistics is performed. A gene is considered differentially expressed when the *P* value is < 0.05. The 8 hr values are chosen as a reference for comparing values from later time points to identify genes and pathways that are upregulated during the time course of the experiment. Alternatively, the values can be compared relative to a no carbon source control, or a glucose control, as glucose represses expression of genes involved in carbon utilization in many bacteria via catabolite repression.

[0269] Potential competitors of *Clostridium difficile* are tested in the same manner, singly or in combination, using custom microarray slides for each strain or species. Alternatively, an RNA-seq based approach can be used to identify and quantify the relative abundance of mRNA in each sample [can we insert a reference for how to perform an RNA-seq experiment?].

Example 17A: Use of *in vivo* transcriptomics to determine bacterial strain nutrient utilization

[0270] Alternatively, carbon source utilization of *Clostridium difficile* and potential competitors can be determined by a transcriptomics approach that detects the presence of mRNA levels in an *in vivo* mouse model. For example, in Ng et al (Nature 2013, 502:7469), *C. difficile* genes for catabolism of sialic acid and fucose were found to have increased levels of expression *in vivo* relative to when grown *in vitro* in growth medium, and in Janoir et al (I&I 2013 81:3757), expression of genes required for catabolism of glucose, sorbitol and fructose are induced *in vivo* relative to *in vitro*, suggesting their use during infection.

[0271] A group of mice are inoculated with *Clostridium difficile*, and subgroups are sacrificed after 8, 12 or 24 hr, at which time cecal contents are collected. Total nucleic acid is extracted from 50 µl aliquots of the cecal contents using a MasterPure RNA purification kit (Epicentre Biotechnologies, Madison, WI). DNA is removed using a Turbo DNA-free kit (Ambion, Austin, TX), using the rigorous protocol. Total RNA is quantified with a NanoDrop ND-1000 (Thermo Scientific, Wilmington, DE), and integrity is analyzed using an RNA FlashGel (Lonza, Basel, Switzerland). Total RNA is enriched for mRNA using MicroExpress (Ambion, Austin, TX). Enriched RNA is quantified using RiboGreen (Invitrogen, Carlsbad, CA). One hundred nanograms of mRNA-enriched RNA is used for library preparation using SuperScript II (Invitrogen, Carlsbad, CA) and an mRNA-seq kit (Illumina, San Diego, CA). The cDNA library is sequenced using an Illumina HiSeq 2500. Reads are then mapped against the appropriate *C. difficile* reference genome. The 8 hr time point can be used as a reference to identify genes and pathways that are upregulated *in vivo*, or alternatively a control sample grown *in vitro* in a standard growth medium can be used as a reference. The pattern of upregulated catabolic genes defines a map of nutrients for which an effective anti-*C. difficile* consortium of organisms would compete.

[0272] The same process is repeated or performed in parallel for bacterial strains that are potential competitors of *C. difficile*. In an alternative version, combinations of bacterial strains can be inoculated into a mouse and analysed simultaneously from the same cecal sample using publicly available reference genomes from the human microbiome for read mapping. Species with potential ability to compete

with *C. difficile* are selected based on their pattern of overlap for carbon sources analyzed.

Example 17B: Use of literature search to determine bacterial strain nutrient utilization

[0273] The nutrient utilization capabilities of a pathogen are also determined by means of a literature search. Compounds that can support growth of *C. difficile* were determined from scientific publications describing nutrients that are utilized by the organism (for example, Nakamura et al, 1982, Microbiol Immunol 26:107), from scientific papers describing a defined media that allows growth of the organism (for example, George et al, J. Clin. Microbiol. 1979, 9:214), reference manuals that compile data from the literature such as Bergey's Manual of Systematic Bacteriology (Bergey's Manual of Systematic Bacteriology: Vol. 3: The Firmicutes By Paul Vos, George Garrity, Dorothy Jones, Noel R. Krieg, Wolfgang Ludwig, Fred A. Rainey, Karl-Heinz Schleifer, William B. Whitman, 2009 Ed) or The Prokaryotes (The Prokaryotes: Vol. 4: Bacteria: Firmicutes, Cyanobacteria, 2006 Ed, edited by Martin Dworkin, Stanley Falkow). For example, Bergey's Manual indicates carbon sources typically used by *C. difficile*: glucose, cellobiose, fructose, mannitol, mannose, melezitose, salicin, sorbitol, sucrose, trehalose, xylose, proline, aspartic acid, serine, leucine, alanine, threonine, valine, phenylalanine, methionine, isoleucine. Note, not all strains use all of these carbon sources, making the testing of several individual strains useful.

[0274] Genomics studies describing analysis of reference genomes can suggest nutrients that are potentially used by a pathogen, based on the presence of metabolic pathways and transport proteins for given substrates. For example, Sebaihia et al., Nature Genetics, 2006 which presents the genome for the virulent *Clostridium difficile* strain 630, describes a 19 gene cluster for ethanolamine degradation suggesting ethanolamine is likely a carbon source used by *C. difficile*.

[0275] Transcriptomics studies, in which mRNA transcript levels are determined by microarray or RNA-seq methods, can reveal metabolic pathways that are upregulated in one condition relative to another. For example, for *Clostridium difficile* in Ng et al. (Nature 2013, 502:7469), genes for catabolism of sialic acid and fucose were found to have increased levels of expression *in vivo* relative to when grown *in vitro* in growth medium, and in Janoir et al. (I&I 2013 81:3757), expression of genes required for

catabolism of glucose, sorbitol and fructose are induced *in vivo* relative to *in vitro*, suggesting their use during infection.

[0276] Metabolomic studies in mice infected with *C. difficile*, indicate that sorbitol, mannitol, arabitol, xylitol, gluconate, sucrose and lactate levels were elevated when the mice were made susceptible to *C. difficile* infection by treatment with antibiotics, implicating those as important nutrients *in vivo* (Theriot et al., 2013 (*Nature Communications*)).

Example 18: Quantification of Spore Concentrations using DPA assay

[0277] 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.

[0278] 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.

[0279] 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 37C to determine CFU/ml. Figure 6 shows the linear correspondence across different spore producing bacteria across several logs demonstrating the DPA assay as means to assess spore content.

[0280] 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 AC 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.

Table AC. 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

[0281] 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, Table AC). 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 (Table AD).

Table AD. 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	6.8×10^7	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

[0282] 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 19: Engraftment, Augmentation and reduction of Pathogen Carriage in Patients Treated with Spore Compositions

[0283] 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.

[0284] 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 the

engraftment of OTUs from the spore treatment and augmentation in the microbiome of the patient (Figure 7 and Figure 8). 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 Table 16 and Table 1 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.

[0285] Patient treatment with the ethanol treated spore preparation leads to the establishment of a microbial ecology that has greater diversity than prior to treatment (Figure 7). Genomic-based microbiome characterization confirmed engraftment of a range of OTUs that were absent in the patient pretreatment (Table 16). 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 9).

[0286] The results are shown in Figure 7: 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).

[0287] The results are shown in Figure 8. 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 16).

[0288] Results are shown in Figure 9 illustrating 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.

[0289] 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 in Example 10.

[0290] 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.

[0291] 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. It's 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 18).

[0292] 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 19). 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.

[0293] 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 20).

[0294] 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 not only in 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 21, comprising at least 8 different species.

[0295] The results are shown in Figure 10; the figure illustrates 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*.

[0296] 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 22. 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 (Table 22).

[0297] 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 16). 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 20. Identifying the Core Ecology from the bacterial combination.

[0298] Ten different bacterial compositions were made by the ethanol treated spore preparation methods from 6 different donors (as described herein). 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 herein, and donors were identified using the criteria described herein. 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.

[0299] 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 12. 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 herein. The resulting list of OTUs, clade assignments, and frequency of detection in the spore preparations are shown in **Table GB**. 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. Bolded OTUs occur in $\geq 80\%$ of the ethanol preps and engraft in $\geq 50\%$ of the treated patients.

Table GB. OTUs detected by a minimum of ten 16S-V4 sequence reads in at least a one ethanol treated spore preparation (pan-microbiome).

OTU	Clade	% of Spore Preparation with OTU	% of Patient's 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
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_56 FAA	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_63 FAA	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
Dorea_longicatena	clade_360	100 %	60%	Y	Y
Lachnospiraceae_bacterium_9_1_43	clade_360	100	30%	Y	N

BFAA		%			
Lachnospiraceae_bacterium_2_1_58 FAA	clade_360	80%	80%	Y	N
Lachnospiraceae_bacterium_2_1_46 FAA	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
Anaerosporeobacter_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_champanellensis	clade_406	80%	50%	Y	N
Ruminococcus_callidus	clade_406	10%	10%	Y	N
Clostridium_clostridioforme	clade_408	100 %	60%	Y	Y
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_D16	clade_494	30%	50%	Y	Y
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_sporosphaeroides	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
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_pamelaee	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
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

[0300] 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 GB) 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 GB 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.

[0301] 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 herein. 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 GB 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.

[0302] 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.

[0303] 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:

- 40% weighting for presence of OTU in spore preparation
 - multiplier of 1 for presence in 1-3 spore preparations
 - multiplier of 2.5 for presence in 4-8 spore preparations
 - multiplier of 5 for presences in ≥ 9 spore preparations
- 40% weighting for engraftment in a patient
 - multiplier of 1 for engraftment in 1-4 patients
 - multiplier of 2.5 for engraftment in 5-6 patients
 - multiplier of 5 for engraftment in ≥ 7 patients
- 20% weighting to Keystone OTUs
 - multiplier of 1 for a Keystone OTU
 - multiplier of 0 for a non-Keystone OTU

[0304] 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:

$$\text{CES} = (0.4 \times 2.5) + (0.4 \times 1) + (0.2 \times 1) = 1.6$$

[0305] **Table GC** 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.

Table GC. 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_56FA A	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_63FA A	clade_309	3.0	Y	N

Example 21. Defining Efficacious Subsets of the Core Ecology

[0306] 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 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.

[0307] 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*.

[0308] To one skilled in the art, the selection of appropriate OTU subsets for testing in vitro or in vivo (e.g. see Examples 6 or 7) 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 GB, with a particular emphasis on those with higher CES, such as the OTUs described in Table GC. 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 GB 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 22: Prophylactic use and treatment in a Mouse model of vancomycin resistant enterococcus (VRE) colonization.

[0309] 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).

[0310] 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.

[0311] 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 example 1, 2 and 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 example 2 and 3).

[0312] 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).

[0313] 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

[0314] 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

[0315] 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.

[0316] 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 blaKPC-3, blaTEM-1, blaSHV-11, and blaSHV-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.

[0317] Mice (10 per group) are assigned to either a bacterial composition (test article), ethanol treated, spore preparation (e.g. as described herein), 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.

[0318] 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.

[0319] Mice are treated with subcutaneous clindamycin to reduce the normal intestinal flora 1 day before receiving 10⁴ 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

[0320] 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.

[0321] 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: Method of Preparing the Bacterial Composition for Administration to a Patient

[0322] 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.

[0323] 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.

[0324] 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 26: Use of microbiological assay to determine bacterial strain germinant utilization

[0325] In the case of pathogenic sporulating bacteria, typically bacterial spores must germinate and initiate vegetative cell growth in order to trigger disease

symptoms. An example is *C. difficile*, which is highly infectious when in the dormant, spore phase, but must germinate and grow in order to cause the symptoms of *Clostridium difficile*-associated disease (CDAD) (Burns et al., Research in Microbiology, Volume 161, Issue 9, November 2010, Pages 730–734). Preventing pathogenic spore germination by using sets of strains to metabolize available germinants is therefore a viable strategy for preventing disease caused by sporulating bacteria.

[0326] One method to isolate and identify single strains or sets of strains that utilize similar nutrients and germinants to a given pathogen involves selecting for such microbes from complex communities of bacteria. Plating a complex community of bacteria on plates that are selective for the pathogenic vegetative or spore of interest can lead to the isolation of species with similar nutrient and germinant utilization profiles.

[0327] Fecal suspensions from samples that tested negative for the presence of *C. difficile* were left untreated or treated with 50% ethanol for 1 hour to select for bacterial spores and eliminate vegetative bacteria. These samples were then plated on commercially available plates for the selective isolation of *C. difficile* (chromID® *C. difficile* plates from Biomerieux, or *Clostridium difficile* selective agar (CDSA) plates from BD). These plates contain mixtures of germinants which support *C. difficile* germination and nutrients which support *C. difficile* vegetative growth. Individual colonies of strains that grew on these plates were picked for identification via 16S ribosomal sequencing. These strains utilize essential nutrients and germinants necessary for *C. difficile* germination and growth, and thus represent candidates for use in multi-strain bacterial compositions to treat and prevent CDAD.

[0328] , Treatment 3 consists of 15 organisms that comprise *C. disporicum*, *C. mayombeii*, *C. tertium*, *C. innocuum*, and *Collinsella aerofaciens* each of which was shown to utilized similar nutrients and germinants as *C. difficile* in the present example (Table X). This treatment prevented *C. difficile*-associated mortality, weight loss, and clinical symptoms upon challenge with the pathogen.

Table X: Species isolated from a fecal suspension after ethanol treatment and plating on media agars that are selective for *C. difficile*

Plate Type	Treatment	OTUs Isolated from Fecal Suspension on Designated Plate Type
ChromID® <i>C. difficile</i> Agar	Ethanol Treated	Clostridium_celatum Clostridium_citroniae Clostridium_disporicum Clostridium_hathewayi Clostridium_mayombeii Clostridium_tertium
CDSA	Ethanol Treated	Clostridium_sp_HGF2 Clostridium_innocuum
CDSA	Untreated	Bacteroides_sp_3_2_5 Clostridium_sp_HGF2 Collinsella_aerofaciens Enterobacter_cloacae Enterobacter_sp_247BMC Enterococcus_faecium Enterococcus_faecium Enterococcus_faecium Lactobacillus_rhamnosus

Example 27: Use of Biolog assay to determine bacterial strain germinant utilization

[0329] Bacterial species that inhibit the germination of spores of a pathogen can be determined by finding a competitor species or combination of competitor species that overlap for germination requirements. As an example, the method described in Example 15 can be used to find a list of germination requirements for a spore forming pathogen. Potential competitors can be tested in parallel and chosen based on the level of overlap for germination requirements when the bacteria are grown from spores. For this assay, a culture consisting of pure spores is used as the inoculating material. A pure spore sample is prepared by treating a culture of a spore forming species with 50% ethanol for 1 hr with mixing. After incubation in ethanol the suspension is pelleted in a centrifuge at 12,000 rpm for 5 min. The spores are then resuspended in PBS. Germination from spores are tested in parallel with and without added germinants. After incubation for 1 hr to allow germination, each sample is serially diluted and plated for titer. Nutrients are scored as germinants if titer is higher in the presence of the nutrient. A combination of competitors which overlap sufficiently with the germination requirements of the pathogen can be selected as a set with potential to prevent germination of the pathogen.

Example 28: Inhibition of a pathogen by identifying compositions that modify its germinants required for sporulation

[0330] *C. difficile* is a spore former whose germination is induced by primary bile salts, including cholate, taurocholate, glycocholate and other taurocholate derivatives [Sorg and Sonnenshein (2008) *J Bact* **190**: 2505-2512; Theriot, C.M. et al., (2013) *Nat Communications* DOI: 10.1038/ncomms4114]. Many commensal organisms encode enzymes that dehydroxylate primary bile salts to secondary bile salts, including lithocholate, deoxycholate isomers, and various conjugated bile salts. An overnight culture of the commensal of interest is incubated in a growth media containing added taurocholate or cholate. Concentrations of between 0.001% and 0.1% are tested with each growth media in a 96 well measuring growth by monitoring OD₆₀₀ in order to find a concentration of each bile salt that is not inhibitory to growth of the commensal organism of interest. For each condition in which growth was observed, an aliquot is analyzed. The microbes are removed by centrifugation, and the supernatant tested using LC-electrospray-triple quadrupole mass spectrometry, or an equivalent method, to detect the production of secondary bile acids from taurocholate and cholate. A set of reference standards, including commercially available primary and secondary bile acids, is prepared and run in parallel. A microbial composition is then selected by combining two or more commensals capable of metabolizing taurocholate and cholate and tested for the ability to prevent *C. difficile* infection in the mouse as described in the examples herein.

Example 29: Determination of nutrient utilization by a pathogen

[0331] The specific nutrient utilization capabilities of a pathogen can be determined by use of one, or a combination of methods described above. In one instance, a list of compounds which allow, enhance or are necessary for the growth of *Clostridium difficile* was determined using the method described in Example 15 above. Wells that had a final value of 1.5x the negative control well under any of the conditions tested were scored as a growth requirement. Three strains of the same pathogen species were tested using a variety of redox buffer conditions, and compounds that supported growth of any of the strains were considered a growth substrate for the pathogen species.

Example 30: Determination of nutrient utilization by a potential competitor

[0332] The specific nutrient utilization capabilities of a bacterial species which is a potential pathogen competitor can be determined by use of one or, a combination of methods described above. In one instance, a list of compounds which allow, enhance or are necessary for the growth of a potential pathogen competitor species was determined by using the method described in Example 15 above. Wells that had a final absorbance value of at least 1.5x the negative control well under any of the conditions tested were scored as a nutrient that the competitor is capable of utilizing. A potential competitor of the pathogen or combination of competitors can be chosen based on the level of overlap with nutrient utilization capabilities of the pathogen. As a general principle, the greater the overlap, the greater potential the isolate or set of isolates has as a competitor to the pathogen.

Example 31: Use of minimum nutrient threshold quantification to identify potential competitors

[0333] The minimum concentration of a nutrient or other growth requirement necessary for growth can be determined by growth in a minimal defined media assay. In this assay, a minimal defined media is constructed that lacks at least one constituent necessary for growth. The specific missing nutrient is titrated into the defined media by two fold dilutions along a row of a 96-well plate. The potential competitor of a particular pathogen is then inoculated into all wells of the plate, and the growth rate is determined as a function of the test nutrient concentration. The plate is incubated for 48 hr and growth is measured by optical density (OD) at 600 nm. The well with the lowest concentration of nutrient that allows growth is determined to be the minimum threshold concentration. Potential competitors of the pathogen can be chosen by virtue of having a minimum threshold concentration below that of the pathogen.

Example 32: Use of proliferation rate quantification to identify potential competitors

[0334] The proliferation rate of a bacterial species can be determined by inoculating the species being tested into a defined media and measuring the titer at several points over a time course. In one example, a pathogen or potential competitor of the pathogen is inoculated into a minimal defined media containing the nutrient being tested. For instance, *Clostridium difficile* growth rates can be measured in defined media containing a single carbon source (Theriot et al., 2013 (*Nature*

Communications)). A variety of carbon sources are tested, with glucose as a positive control and no carbohydrate media as negative controls. The defined media used for the assay contains 0.5% (wt/vol) of the test carbohydrate, no other carbon source, and 0.125 mg/l biotin, 1 mg/l pyridoxine and pantothenate, 75 mg/l histidine, glycine and tryptophan, 150 mg/l arginine, methionine and threonine, 225 mg/l valine and isoleucine, 300 mg/l leucine, 400 mg/l cysteine, and 450 mg/l proline.

[0335] Specifically, for each carbon source tested, *C. difficile* is cultured for 14 hours in brain heart infusion medium at 37 degrees and then subcultured 1:3 into defined media containing 0.5% glucose with the described vitamins and amino acids, grown for 3 hours, centrifuged for 10 min at 2000 x g, and resuspended in equal volume of anaerobically equilibrated PBS buffer. 100 µl of the suspension is used to inoculate defined media containing 0.5% test carbohydrate and described vitamins and amino acids. Samples are incubated shaking at 200 r.p.m. OD₆₀₀ is monitored hourly for 24 hours in a Thermo Scientific Spectronic 20D+ apparatus. The specific growth rate is determined as growth rate = $\ln(X/X_0)/T$, where X is the OD₆₀₀ value during the linear portion of growth and T is time (hours). Values are reported as the mean of 3 independent cultures conducted twice (see Table XX).

Table XX: Carbon utilization of *C. difficile*

Carbon source	Growth rate (1/hrs)
Sorbitol	0.62 ± 0.06
Mannitol	0.66 ± 0.10
Arabitol	0.58 ± 0.09
Xylitol	no growth observed
Gluconate	0.54 ± 0.01
Sucrose	0.52 ± 0.04
Lactate	0.55 ± 0.05
Raffinose	0.56 ± 0.03
Stachyose	0.55 ± 0.03
Galactose	no growth observed
Fructose	0.66 ± 0.12

Glucose control	0.73± 0.09
Amino Acids, No Carbohydrates	0.54 ± 0.02
No Amino Acids, No Carbohydrates	no growth observed

[0336] Alternatively, progress of growth can be determined by taking a small aliquot at each timepoint, serially diluting and plating to a nutrient rich solid media to count colonies.

[0337] Comparison can be made between the pathogen and potential competitor species to define competitors with high potential to compete with *C. difficile*. High potential competitor candidates will have a growth rate that is faster than that of the pathogen microbe.

Example 33: Use of *in vitro* assay to test potential bacterial competitor consortia for functionality

[0338] 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*. 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 34: Selection of species to test in vivo for pathogen inhibition

[0339] Based on the principles of identifying bacterial compositions of species that compete for resources with a pathogen or pathobiont, one builds sets of species that are likely to displace a pathogen or pathobiont in a host gut. Mechanisms include competition between a set of organisms and a pathogen or pathobiont based on nutrient utilization, minimum nutrient threshold for a given important nutrient or subset of important nutrients, proliferation rate, germinant requirements or general mechanisms understood through the in vitro competition assay. Additional other mechanisms are recognized by one skilled in the art in light of the teachings described herein and thus the mechanisms described should not be viewed as limiting the scope of this disclosure. The bacterial combinations can then be prepared as described and tested in vivo in an appropriate host model as described herein.

Example 35: Use of *in vivo* mouse model to test potential bacterial competitor consortia for functionality

[0340] Combinations of bacterial species to test in a mouse model of *Clostridium difficile* associated disease were chosen by use of a screen for nutrient utilization of both the pathogen and 17 potential competitors of the pathogen using the methods described herein. The use of nutrients used by each of the potential competitors was cross-referenced to the list of nutrients used by any of the *Clostridium difficile* strains tested under any condition tested. The 17 strains were rank-ordered for overlap with the list of *Clostridium difficile* nutrients. Two and three species combinations were chosen from the top three species (*Clostridium hylemonae*, *Blautia producta*, *Clostridium butyricum*) ranked for overlap with *Clostridium difficile* nutrients. Four species combinations were chosen from species that ranked in the top 50% when rank ordered for overlap with *Clostridium difficile* nutrients. A three species combination with reduced overlap was also chosen based on a rank ordering of nutrient utilization by *Clostridium difficile*.

[0341] To test the therapeutic potential of the bacterial compositions 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. A positive control group receives 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 empty 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. 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).

[0342] 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 treatments are specified in Table W and the results are reported in Table Y.

Table W: Treatment compositions tested C. difficile mouse model.

Treatment	Vehicle (PBS)	10% Fecal Suspension	Collinsella_aerofaciens	Clostridium_tertium	Clostridium_disporicum	Clostridium_innocuum	Clostridium_mayombel	Clostridium_butyricum	Coprococcus_comes	Clostridium_hylemonae	Clostridium_bolteae	Clostridium_symbiosum	Clostridium_orbiscindens	Lachnospiraceae_bacterium_5_1_57FAA	Blautia_producta	Ruminococcus_gnavus	Ruminococcus_bromii	Eubacterium_rectale
1	x																	
2		x																
6								x		x					x			
3			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
9													x	x				x
8								x							x			
7								x		x								
4			x					x		x					x			
5			x			x			x								x	

Table Y: Results of bacterial compositions tested in a C. difficile mouse model.

Treatment	Dose	Cumulative Mortality (%)	Avg. Minimum Relative Weight	Avg. Maximum Clinical Score (Death = 4)
1	-	40	0.87	2.8
2	5.8e8 cfu	0	0.99	0
3	1e8 cfu/OTU	0	0.98	0
4	1e8 cfu/OTU	10	0.84	2.1
5	1e8 cfu/OTU	10	0.84	2.2

6	1e8 cfu/OTU	0	0.87	2
7	1e8 cfu/OTU	20	0.91	1.7
8	1e8 cfu/OTU	40	0.82	2.8
9	1e8 cfu/OTU	0	0.90	1

Example 36: Selection of Media for Growth

[0343] It is important to select 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 are below. In the case of plating on solid media, 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. Liquid media include (can be adapted to solid media by addition of 1.5% agar):

- 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.
- Sweet GAM [Gifu Anaerobic Medium (GAM, Nissui)] modified, supplemented with glucose, cellobiose, maltose, L-arabinose, fructose, fructooligosaccharides/inulin, mannitol and sodium lactate)
- Brucella Blood Broth (BBA, Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010)
- PEA sheep blood (Anaerobe Systems; 5% Sheep Blood with Phenylethyl Alcohol)
- Egg Yolk Broth (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010)
- Sulfite polymyxin milk (Mevissen-Verhage et al., J. Clin. Microbiol. 25:285-289 (1987))
- Mucin Broth (Derrien et al., IJSEM 54: 1469-1476 (2004))
- Polygalacturonate Broth (Jensen & Canale-Parola, Appl. Environ. Microbiol. 52:880-997 (1986))
- M2GSC (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010)

- M2 Broth (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%)
- Sweet B - Brain Heart Infusion Broth (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).
- PY-salicin (peptone-yeast extract agar supplemented with salicin) (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010).
- 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, and after autoclaving add 50mL of 8% NaHCO₃ solution and 50mL 1M MOPS-KOH (pH 7).
- Noack-Blaut Eubacterium Broth (See Noack et al. J. Nutr. (1998) 128:1385-1391)
- BHIS az1/ge2 - BHIS az/ge agar (Reeves et. al. Infect. Immun. 80:3786-3794 (2012)) [Brain Heart Infusion Broth (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]
- BHIS CInM az1/ge2- BHIS CInM [Brain Heart Infusion Broth (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 37: High throughput screening of binary pairs

[0344] Construction of binary pairs in a high-throughput 96-well format. To allow high-throughput screening of binary pairs, vials of -80 °C glycerol stock banks

were thawed and diluted to 1×10^8 CFU/mL. Each strain was then diluted 10x (to a final concentration of 1×10^7 CFU/mL of each strain) into 200 μ L of PBS + 15% glycerol in the wells of a 96-well plate. Plates were then frozen at -80°C . When needed, plates were removed from -80°C and thawed at room temperature under anaerobic conditions when testing in an In vitro inhibition assay with *Clostridium difficile*.

[0345] Construction of ternary combinations in a high-throughput 96-well format. To allow high-throughput screening of ternary combinations, vials of -80°C glycerol stock banks were thawed and diluted to 1×10^8 CFU/mL. Each strain was then diluted 10x (to a final concentration of 1×10^7 CFU/mL of each strain) into 200 μ L 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 an In vitro inhibition assay with *Clostridium difficile*.

[0346] Construction of an In vitro inhibition Assay to Screen for Ecobiotic™ compositions Inhibitory to the Growth of *Clostridium difficile*. Inhibition of *C. difficile* in this assay may result from competition for nutrients; other mechanisms of inhibition may contribute, as the assay is not limited to a single mechanism.

[0347] An overnight culture of *Clostridium difficile* was 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 was 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 was then aliquoted to wells of a 96-well plate (180 μ L to each well). 20 μ L of a unique binary pair of potential inhibitory species was then added to each well at a final concentration of 1×10^6 CFU/mL of each species. Alternatively the assay can be tested with binary pairs at different initial concentrations (1×10^9 CFU/mL, 1×10^8 CFU/mL, 1×10^7 CFU/mL, 1×10^5 CFU/mL, 1×10^4 CFU/mL, 1×10^3 CFU/mL, 1×10^2 CFU/mL). Control wells only inoculated with *C. difficile* were included for a comparison to the growth of *C. difficile* without inhibition. Additional wells were used for controls that either inhibit or do not inhibit the growth of *C. difficile*. One example of a positive control that inhibits growth was a combination of *Blautia*

producta, *Clostridium bifermentans* and *Escherichia coli*. One example of a control that shows reduced inhibition of *C. difficile* growth as a combination of *Bacteroides thetaiotaomicron*, *Bacteroides ovatus* and *Bacteroides vulgatus*. Plates were wrapped with parafilm and incubated for 24 hr at 37 °C under anaerobic conditions. After 24 hr the wells containing *C. difficile* alone were serially diluted and plated to determine titer. The 96-well plate was then frozen at -80C before quantifying *C. difficile* by qPCR assay.

[0348] Construction of an in vitro inhibition Assay to Screen for bacterial compositions that are inhibitory to the growth of *Clostridium difficile* by diffusion mediated mechanisms. The In vitro inhibition assay described above was modified by using a 0.22 uM filter insert (Millipore™ MultiScreen™ 96-Well Assay Plates - Item MAGVS2210) in 96-well format to physically separate *C. difficile* from the bacterial compositions. The *C. difficile* was aliquoted into the 96-well plate while the bacterial compositions were aliquoted into media on the filter overlay. The nutrient media as in contact on both sides of the 0.22 uM filter, allowing exchange of nutrients, small molecules and many macromolecules (e.g., bacteriocins, cell-surface proteins, or polysaccharides) by diffusion. In this embodiment, after 24 hr incubation, the filter insert containing the bacterial compositions was removed. The plate containing *C. difficile* was then transferred to a 96-well plate reader suitable for measuring optical density (OD) at 600 nm. The growth of *C. difficile* in the presence of different bacterial compositions was compared based on the OD measurement.

[0349] Construction of an In vitro inhibition Assay to Screen for bacterial compositions inhibitory to the growth of *Clostridium difficile* using *Clostridium difficile* selective media for quantification. The In vitro inhibition assay described above can be modified to determine final *C. difficile* titer by serially diluting and plating to *C. difficile* selective media (Bloedt et al 2009) such as CCFA (cycloserine cefoxitin fructose agar, Anaerobe Systems), CDSA (*Clostridium difficile* selective agar, which is cycloserine cefoxitin mannitol agar, Becton Dickinson).

Example 37. Quantification of *C. difficile* using Quantitative PCR (qPCR)

Standard Curve Preparation

[0350] The standard curve was generated from a well on each assay plate containing only pathogenic *C. difficile* grown in SweetB + FosIn media as provided herein and quantified by selective spot plating. Serial dilutions of the culture were

performed in sterile phosphate-buffered saline. Genomic DNA was extracted from the standard curve samples along with the other wells.

Genomic DNA Extraction

[0351] Genomic DNA was extracted from 5 µl of each sample using a dilution, freeze/thaw, and heat lysis protocol. 5 µL of thawed samples were added to 45 µL of UltraPure water (Life Technologies, Carlsbad, CA) and mixed by pipetting. The plates with diluted samples were frozen at -20 °C until use for qPCR which includes a heated lysis step prior to amplification. Alternatively the genomic DNA could be 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.

qPCR Composition and Conditions

[0352] The qPCR reaction mixture contained 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 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 was aliquoted to wells of a Hard-shell Low-Profile Thin Wall 96-well Skirted PCR Plate (BioRad, Hercules, CA). To this reaction mixture, 2 µl of diluted, frozen, and thawed samples were added and the plate sealed with a Microseal 'B' Adhesive Seal (BioRad, Hercules, CA). The qPCR was performed on a BioRad C1000™ Thermal Cycler equipped with a CFX96™ Real-Time System (BioRad, Hercules, CA). The thermocycling conditions were 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 could be performed with other standard methods known to those skilled in the art.

Data Analysis

[0353] The C_q value for each well on the FAM channel was determined by the CFX Manager™ 3.0 software. The log₁₀(cfu/mL) of *C. difficile* each experimental sample was calculated by inputting a given sample's C_q value into a 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 log inhibition was calculated for each sample by subtracting the log₁₀(cfu/mL) of *C. difficile* in the sample from the log₁₀(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 was calculated for all replicates for each composition.

[0354] A histogram of the range and standard deviation of each composition was plotted. Ranges or standard deviations of the log inhibitions that were distinct from the overall distribution were examined as possible outliers. If the removal of a single log inhibition datum from one of the binary pairs that were identified in the histograms would bring the range or standard deviation in line with those from the majority of the samples, that datum was removed as an outlier, and the mean log inhibition was recalculated.

[0355] The pooled variance of all samples evaluated in the assay was estimated as the average of the sample variances weighted by the sample's degrees of freedom. The pooled standard error was 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 were determined by multiplying the pooled standard error to the z score corresponding to a given percentage threshold. Mean log inhibitions outside the confidence interval were considered to be inhibitory if positive or stimulatory if negative with the percent confidence corresponding to the interval used. Samples with mean log inhibition greater than the 99% confidence interval (C.I.) of the null hypothesis are reported as +++, those with a 95% < C.I. < 99% as ++, those with a 90% < C.I. < 95% as +, those with a 80% < C.I. < 90% as + while samples with mean log inhibition less than than the 99% confidence interval (C.I.) of the null hypothesis are reported as ---, those with a 95% < C.I. < 99% as ---, those with a 90% < C.I. < 95% as --, those with a 80% < C.I. < 90% as -.

[0356] Many binary pairs inhibit *C. difficile* as shown in Table 5. 622 of 989 combinations show inhibition with a confidence interval >80%; 545 of 989 with a C.I. >

90%; 507 of 989 with a C.I. > 95%; 430 of 989 with a C.I. of >99%. Non-limiting but exemplary binary pairs include those with mean log reduction greater than 0.366, e.g. *Allistipes shahii* paired with *Blautia producta*, *Clostridium hathawayi*, or *Colinsella aerofaciens*, or *Clostridium mayombeii* paired with *C. innocuum*, *C. tertium*, *Colinsella aerofaciens*, or any of the other 424 combinations shown in Table 5. Equally important, the In vitro inhibition assay describes binary pairs that do not effectively inhibit *C. difficile*. 188 of 989 combinations promote growth with >80% confidence; 52 of 989 show a lack of inhibition with >90% confidence; 22 of 989 show a lack of inhibition with >95% confidence; 3 of 989, including *B. producta* combined with *Coprococcus catus*, *Allistipes shahii* combined with *Dorea formicigenerans*, and *Eubacterium rectale* combined with *Roseburia intestinalis*, show a lack of inhibition with >99% confidence. 249 of 989 combinations are neutral in the assay, meaning they neither promote nor inhibit *C. difficile* growth to the limit of measurement.

[0357] 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 --- ($95\% < \text{C.I.} < 99\%$), and those with mean log inhibition less than -0.312 as ---- ($99\% < \text{C.I.}$).

[0358] The In vitro inhibition assay 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 6 with a score of ++++, such as *Colinsella aerofaciens*, *Coprococcus comes*, and *Blautia producta*. Equally important, the In vitro inhibition 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.

[0359] 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.

[0360] Unless otherwise indicated, the term “at least” preceding a series of elements is to be understood to refer to every element in the series.

[0361] 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.

[0362] 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: List of Operational Taxonomic Units (OTU) with taxonomic assignments made to Genus, Species, and Phylogenetic Clade

[0363] 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.

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

OTU	SEQ ID Number	Public DB Accession	Clade	Spore Former	Pathogen Status
<i>Clostridium baratii</i>	555	NR_029229	clade_223	Y	N
<i>Clostridium colicanis</i>	576	FJ957863	clade_223	Y	N
<i>Clostridium paraputrificum</i>	611	AB536771	clade_223	Y	N
<i>Clostridium sardiniense</i>	621	NR_041006	clade_223	Y	N
<i>Eubacterium budayi</i>	837	NR_024682	clade_223	Y	N
<i>Eubacterium moniliiforme</i>	851	HF558373	clade_223	Y	N
<i>Eubacterium multiforme</i>	852	NR_024683	clade_223	Y	N
<i>Eubacterium nitritogenes</i>	853	NR_024684	clade_223	Y	N
<i>Anoxybacillus flavithermus</i>	173	NR_074667	clade_238	Y	N
<i>Bacillus aerophilus</i>	196	NR_042339	clade_238	Y	N
<i>Bacillus aestuarii</i>	197	GQ980243	clade_238	Y	N
<i>Bacillus amyloliquefaciens</i>	199	NR_075005	clade_238	Y	N
<i>Bacillus anthracis</i>	200	AAEN01000020	clade_238	Y	Category -A
<i>Bacillus atrophaeus</i>	201	NR_075016	clade_238	Y	OP
<i>Bacillus badius</i>	202	NR_036893	clade_238	Y	OP
<i>Bacillus cereus</i>	203	ABDJ01000015	clade_238	Y	OP
<i>Bacillus circulans</i>	204	AB271747	clade_238	Y	OP
<i>Bacillus firmus</i>	207	NR_025842	clade_238	Y	OP
<i>Bacillus flexus</i>	208	NR_024691	clade_238	Y	OP
<i>Bacillus fordii</i>	209	NR_025786	clade_238	Y	OP
<i>Bacillus halmapalus</i>	211	NR_026144	clade_238	Y	OP
<i>Bacillus herbersteinensis</i>	213	NR_042286	clade_238	Y	OP
<i>Bacillus idriensis</i>	215	NR_043268	clade_238	Y	OP
<i>Bacillus lentus</i>	216	NR_040792	clade_238	Y	OP
<i>Bacillus licheniformis</i>	217	NC_006270	clade_238	Y	OP
<i>Bacillus megaterium</i>	218	GU252124	clade_238	Y	OP
<i>Bacillus nealsonii</i>	219	NR_044546	clade_238	Y	OP
<i>Bacillus niabensis</i>	220	NR_043334	clade_238	Y	OP
<i>Bacillus niacini</i>	221	NR_024695	clade_238	Y	OP
<i>Bacillus pocheonensis</i>	222	NR_041377	clade_238	Y	OP
<i>Bacillus pumilus</i>	223	NR_074977	clade_238	Y	OP
<i>Bacillus safensis</i>	224	JQ624766	clade_238	Y	OP
<i>Bacillus simplex</i>	225	NR_042136	clade_238	Y	OP
<i>Bacillus sonorensis</i>	226	NR_025130	clade_238	Y	OP
<i>Bacillus</i> sp. 10403023 MM10403188	227	CAET01000089	clade_238	Y	OP
<i>Bacillus</i> sp. 2_A_57_CT2	230	ACWD01000095	clade_238	Y	OP
<i>Bacillus</i> sp. 2008724126	228	GU252108	clade_238	Y	OP
<i>Bacillus</i> sp. 2008724139	229	GU252111	clade_238	Y	OP
<i>Bacillus</i> sp. 7_16AIA	231	FN397518	clade_238	Y	OP
<i>Bacillus</i> sp. AP8	233	JX101689	clade_238	Y	OP
<i>Bacillus</i> sp. B27(2008)	234	EU362173	clade_238	Y	OP
<i>Bacillus</i> sp. BT1B_CT2	235	ACWC01000034	clade_238	Y	OP
<i>Bacillus</i> sp. GB1.1	236	FJ897765	clade_238	Y	OP
<i>Bacillus</i> sp. GB9	237	FJ897766	clade_238	Y	OP
<i>Bacillus</i> sp. HU19.1	238	FJ897769	clade_238	Y	OP
<i>Bacillus</i> sp. HU29	239	FJ897771	clade_238	Y	OP
<i>Bacillus</i> sp. HU33.1	240	FJ897772	clade_238	Y	OP
<i>Bacillus</i> sp. JC6	241	JF824800	clade_238	Y	OP
<i>Bacillus</i> sp. oral taxon F79	248	HM099654	clade_238	Y	OP
<i>Bacillus</i> sp. SRC_DSF1	243	GU797283	clade_238	Y	OP
<i>Bacillus</i> sp. SRC_DSF10	242	GU797292	clade_238	Y	OP
<i>Bacillus</i> sp. SRC_DSF2	244	GU797284	clade_238	Y	OP

OTU	SEQ ID Number	Public DB Accession	Clade	Spore Former	Pathogen Status
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
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

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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
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	1050	ADLB01000035	clade_360	Y	N

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2_1_46FAA					
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
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

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Clostridium citroniae	569	ADLJ01000059	clade_408	Y	N
Clostridium clostridioforme	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
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

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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
Coprobacillus cateniformis	670	AB030218	clade_469	Y	N
Coprobacillus 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
Coprobacillus 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
Clostridium clariflavum	570	NR_041235	clade_495	Y	N

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<i>Clostridium stercorarium</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 metalliredigens</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
<i>Lutispora thermophila</i>	1191	NR_041236	clade_564	Y	N

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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
Butyricococcus 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 licherasiae	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 riegliei	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

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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
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
Photobacterium 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

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Ochrobactrum pseudintermedium	1362	DQ365921	clade_159	N	N
Prevotella genomsp. C2	1496	AY278625	clade_164	N	N
Prevotella multisaccharivorax	1509	AFJE01000016	clade_164	N	N
Prevotella sp. oral clone IDR_CEC_0055	1543	AY550997	clade_164	N	N
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 genomsp. 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

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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
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 genomosp. 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	ADBFO1000003	clade_185	N	N
Neisseria genomosp. 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 aeria	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 tuberculoostearicum	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	1529	AY923148	clade_206	N	N

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ASCG10					
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 genomosp. 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 genomosp. C1	56	AY278610	clade_212	N	N
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

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Microbacterium chocolatum	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
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	AFZ01000142	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

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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
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. KLDS 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 tucseti	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

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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
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

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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
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

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<i>Agrobacterium tumefaciens</i>	116	AJ389893	clade_316	N	N
<i>Corynebacterium argenteoroseum</i>	685	EF463055	clade_317	N	N
<i>Corynebacterium diphtheriae</i>	693	NC_002935	clade_317	N	OP
<i>Corynebacterium pseudotuberculosis</i>	715	NR_037070	clade_317	N	N
<i>Corynebacterium renale</i>	717	NR_037069	clade_317	N	N
<i>Corynebacterium ulcerans</i>	731	NR_074467	clade_317	N	N
<i>Aurantimonas coralicida</i>	191	AY065627	clade_318	N	N
<i>Aureimonas altamirensis</i>	192	FN658986	clade_318	N	N
<i>Lactobacillus acidipiscis</i>	1066	NR_024718	clade_320	N	N
<i>Lactobacillus salivarius</i>	1117	AEBA01000145	clade_320	N	N
<i>Lactobacillus</i> sp. KLD5 1.0719	1134	EU600923	clade_320	N	N
<i>Lactobacillus buchneri</i>	1073	ACGH01000101	clade_321	N	N
<i>Lactobacillus genomsp. C1</i>	1086	AY278619	clade_321	N	N
<i>Lactobacillus genomsp. C2</i>	1087	AY278620	clade_321	N	N
<i>Lactobacillus hilgardii</i>	1089	ACGP01000200	clade_321	N	N
<i>Lactobacillus kefir</i>	1096	NR_042230	clade_321	N	N
<i>Lactobacillus parabuchneri</i>	1105	NR_041294	clade_321	N	N
<i>Lactobacillus parakefir</i>	1107	NR_029039	clade_321	N	N
<i>Lactobacillus curvatus</i>	1079	NR_042437	clade_322	N	N
<i>Lactobacillus sakei</i>	1116	DQ989236	clade_322	N	N
<i>Aneurinibacillus aneurinilyticus</i>	167	AB101592	clade_323	N	N
<i>Aneurinibacillus danicus</i>	168	NR_028657	clade_323	N	N
<i>Aneurinibacillus migulanus</i>	169	NR_036799	clade_323	N	N
<i>Aneurinibacillus terranovensis</i>	170	NR_042271	clade_323	N	N
<i>Staphylococcus aureus</i>	1757	CP002643	clade_325	N	Category -B
<i>Staphylococcus auricularis</i>	1758	JQ624774	clade_325	N	
<i>Staphylococcus capitis</i>	1759	ACFR01000029	clade_325	N	N
<i>Staphylococcus caprae</i>	1760	ACRH01000033	clade_325	N	N
<i>Staphylococcus carnosus</i>	1761	NR_075003	clade_325	N	N
<i>Staphylococcus cohnii</i>	1762	JN175375	clade_325	N	N
<i>Staphylococcus condiment</i>	1763	NR_029345	clade_325	N	N
<i>Staphylococcus epidermidis</i>	1764	ACHE01000056	clade_325	N	N
<i>Staphylococcus equorum</i>	1765	NR_027520	clade_325	N	N
<i>Staphylococcus haemolyticus</i>	1767	NC_007168	clade_325	N	N
<i>Staphylococcus hominis</i>	1768	AM157418	clade_325	N	N
<i>Staphylococcus lugdunensis</i>	1769	AEQA01000024	clade_325	N	N
<i>Staphylococcus pasteurii</i>	1770	FJ189773	clade_325	N	N
<i>Staphylococcus pseudintermedius</i>	1771	CP002439	clade_325	N	N
<i>Staphylococcus saccharolyticus</i>	1772	NR_029158	clade_325	N	N
<i>Staphylococcus saprophyticus</i>	1773	NC_007350	clade_325	N	N
<i>Staphylococcus</i> sp. clone bottae7	1777	AF467424	clade_325	N	N
<i>Staphylococcus</i> sp. H292	1775	AB177642	clade_325	N	N
<i>Staphylococcus</i> sp. H780	1776	AB177644	clade_325	N	N
<i>Staphylococcus succinus</i>	1778	NR_028667	clade_325	N	N
<i>Staphylococcus warneri</i>	1780	ACPZ01000009	clade_325	N	N
<i>Staphylococcus xylosus</i>	1781	AY395016	clade_325	N	N
<i>Cardiobacterium hominis</i>	490	ACKY01000036	clade_326	N	N
<i>Cardiobacterium valvarum</i>	491	NR_028847	clade_326	N	N

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<i>Pseudomonas fluorescens</i>	1593	AY622220	clade_326	N	N
<i>Pseudomonas gessardii</i>	1594	FJ943496	clade_326	N	N
<i>Pseudomonas monteilii</i>	1596	NR_024910	clade_326	N	N
<i>Pseudomonas poae</i>	1597	GU188951	clade_326	N	N
<i>Pseudomonas putida</i>	1599	AF094741	clade_326	N	N
<i>Pseudomonas</i> sp. G1229	1601	DQ910482	clade_326	N	N
<i>Pseudomonas tolaasii</i>	1604	AF320988	clade_326	N	N
<i>Pseudomonas viridiflava</i>	1605	NR_042764	clade_326	N	N
<i>Listeria grayi</i>	1185	ACCR02000003	clade_328	N	OP
<i>Listeria innocua</i>	1186	JF967625	clade_328	N	N
<i>Listeria ivanovii</i>	1187	X56151	clade_328	N	N
<i>Listeria monocytogenes</i>	1188	CP002003	clade_328	N	Category -B
<i>Listeria welshimeri</i>	1189	AM263198	clade_328	N	OP
<i>Capnocytophaga</i> sp. oral clone ASCH05	484	AY923149	clade_333	N	N
<i>Capnocytophaga sputigena</i>	489	ABZV01000054	clade_333	N	N
<i>Leptotrichia genomosp.</i> C1	1166	AY278621	clade_334	N	N
<i>Leptotrichia shahii</i>	1169	AY029806	clade_334	N	N
<i>Leptotrichia</i> sp. neutropenicPatient	1170	AF189244	clade_334	N	N
<i>Leptotrichia</i> sp. oral clone GT018	1171	AY349384	clade_334	N	N
<i>Leptotrichia</i> sp. oral clone GT020	1172	AY349385	clade_334	N	N
<i>Bacteroides</i> sp. 20_3	296	ACRQ01000064	clade_335	N	N
<i>Bacteroides</i> sp. 3_1_19	307	ADCJ01000062	clade_335	N	N
<i>Bacteroides</i> sp. 3_2_5	311	ACIB01000079	clade_335	N	N
<i>Parabacteroides distasonis</i>	1416	CP000140	clade_335	N	N
<i>Parabacteroides goldsteinii</i>	1417	AY974070	clade_335	N	N
<i>Parabacteroides gordonii</i>	1418	AB470344	clade_335	N	N
<i>Parabacteroides</i> sp. D13	1421	ACPW01000017	clade_335	N	N
<i>Capnocytophaga genomosp.</i> C1	477	AY278613	clade_336	N	N
<i>Capnocytophaga ochracea</i>	480	AEOH01000054	clade_336	N	N
<i>Capnocytophaga</i> sp. GEJ8	481	GU561335	clade_336	N	N
<i>Capnocytophaga</i> sp. oral strain A47ROY	486	AY005077	clade_336	N	N
<i>Capnocytophaga</i> sp. S1b	482	U42009	clade_336	N	N
<i>Paraprevotella clara</i>	1426	AFFY01000068	clade_336	N	N
<i>Bacteroides heparinolyticus</i>	282	JN867284	clade_338	N	N
<i>Prevotella heparinolytica</i>	1500	GQ422742	clade_338	N	N
<i>Treponema genomosp.</i> P4 oral clone MB2_G19	1928	DQ003618	clade_339	N	N
<i>Treponema genomosp.</i> P6 oral clone MB4_G11	1930	DQ003625	clade_339	N	N
<i>Treponema</i> sp. oral taxon 254	1952	GU408803	clade_339	N	N
<i>Treponema</i> sp. oral taxon 508	1956	GU413616	clade_339	N	N
<i>Treponema</i> sp. oral taxon 518	1957	GU413640	clade_339	N	N
<i>Chlamydia muridarum</i>	502	AE002160	clade_341	N	OP
<i>Chlamydia trachomatis</i>	504	U68443	clade_341	N	OP
<i>Chlamydia psittaci</i>	503	NR_036864	clade_342	N	Category -B
<i>Chlamydophila pneumoniae</i>	509	NC_002179	clade_342	N	OP
<i>Chlamydophila psittaci</i>	510	D85712	clade_342	N	OP

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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 lincolnii	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 genomosp. P1 oral clone MB5_P17	1976	DQ003631	clade_358	N	N
Veillonella parvula	1978	ADFU01000009	clade_358	N	N
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

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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 barnesiae	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
Bacteroides sp. 2_1_22	297	ACPD01000117	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

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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
Lactobacillus gasseri	1084	AC0Z01000018	clade_398	N	N
Lactobacillus hominis	1090	FR681902	clade_398	N	N
Lactobacillus iners	1091	AEKJ01000002	clade_398	N	N
Lactobacillus johnsonii	1093	AE017198	clade_398	N	N
Lactobacillus senioris	1119	AB602570	clade_398	N	N
Lactobacillus sp. oral clone HT002	1135	AY349382	clade_398	N	N

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Weissella beninensis	2006	EU439435	clade_398	N	N
Sphingomonas echinoides	1744	NR_024700	clade_399	N	N
Sphingomonas sp. oral taxon A09	1747	HM099639	clade_399	N	N
Sphingomonas sp. oral taxon F71	1748	HM099645	clade_399	N	N
Zymomonas mobilis	2032	NR_074274	clade_399	N	N
Arcanobacterium haemolyticum	174	NR_025347	clade_400	N	N
Arcanobacterium pyogenes	175	GU585578	clade_400	N	N
Trueperella pyogenes	1962	NR_044858	clade_400	N	N
Lactococcus garvieae	1144	AF061005	clade_401	N	N
Lactococcus lactis	1145	CP002365	clade_401	N	N
Brevibacterium mcbrellneri	424	ADNU01000076	clade_402	N	N
Brevibacterium paucivorans	425	EU086796	clade_402	N	N
Brevibacterium sp. JC43	428	JF824806	clade_402	N	N
Selenomonas artemidis	1692	HM596274	clade_403	N	N
Selenomonas sp. FOBR9	1704	HQ616378	clade_403	N	N
Selenomonas sp. oral taxon 137	1715	AENV01000007	clade_403	N	N
Desmospora activa	751	AM940019	clade_404	N	N
Desmospora sp. 8437	752	AFHT01000143	clade_404	N	N
Paenibacillus sp. oral taxon F45	1407	HM099647	clade_404	N	N
Corynebacterium ammoniagenes	682	ADNS01000011	clade_405	N	N
Corynebacterium aurimucosum	687	ACLH01000041	clade_405	N	N
Corynebacterium bovis	688	AF537590	clade_405	N	N
Corynebacterium canis	689	GQ871934	clade_405	N	N
Corynebacterium casei	690	NR_025101	clade_405	N	N
Corynebacterium durum	694	Z97069	clade_405	N	N
Corynebacterium efficiens	695	ACLI01000121	clade_405	N	N
Corynebacterium falsenii	696	Y13024	clade_405	N	N
Corynebacterium flavescens	697	NR_037040	clade_405	N	N
Corynebacterium glutamicum	701	BA000036	clade_405	N	N
Corynebacterium jeikeium	704	ACYW01000001	clade_405	N	OP
Corynebacterium kroppenstedtii	705	NR_026380	clade_405	N	N
Corynebacterium lipophiloflavum	706	ACHJ01000075	clade_405	N	N
Corynebacterium matruchotii	709	ACSH02000003	clade_405	N	N
Corynebacterium minutissimum	710	X82064	clade_405	N	N
Corynebacterium resistens	718	ADGN01000058	clade_405	N	N
Corynebacterium simulans	720	AF537604	clade_405	N	N
Corynebacterium singulare	721	NR_026394	clade_405	N	N
Corynebacterium sp. 1 ex sheep	722	Y13427	clade_405	N	N
Corynebacterium sp. NML 99_0018	726	GU238413	clade_405	N	N
Corynebacterium striatum	727	ACGE01000001	clade_405	N	OP
Corynebacterium urealyticum	732	X81913	clade_405	N	OP
Corynebacterium variabile	734	NR_025314	clade_405	N	N
Aerococcus sanguinicola	98	AY837833	clade_407	N	N

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Aerococcus urinae	99	CP002512	clade_407	N	N
Aerococcus urinaeequi	100	NR_043443	clade_407	N	N
Aerococcus viridans	101	ADNT01000041	clade_407	N	N
Fusobacterium naviforme	898	HQ223106	clade_408	N	N
Moryella indoligenes	1268	AF527773	clade_408	N	N
Selenomonas genomosp. P5	1697	AY341820	clade_410	N	N
Selenomonas sp. oral clone IQ048	1710	AY349408	clade_410	N	N
Selenomonas sputigena	1717	ACKP02000033	clade_410	N	N
Hyphomicrobium sulfonivorans	1007	AY468372	clade_411	N	N
Methylocella silvestris	1228	NR_074237	clade_411	N	N
Legionella pneumophila	1153	NC_002942	clade_412	N	OP
Lactobacillus coryniformis	1077	NR_044705	clade_413	N	N
Arthrobacter agilis	178	NR_026198	clade_414	N	N
Arthrobacter arilaitensis	179	NR_074608	clade_414	N	N
Arthrobacter bergerei	180	NR_025612	clade_414	N	N
Arthrobacter globiformis	181	NR_026187	clade_414	N	N
Arthrobacter nicotianae	182	NR_026190	clade_414	N	N
Mycobacterium abscessus	1269	AGQU01000002	clade_418	N	OP
Mycobacterium chelonae	1273	AB548610	clade_418	N	OP
Bacteroides salanitronis	291	CP002530	clade_419	N	N
Paraprevotella xylaniphila	1427	AFBR01000011	clade_419	N	N
Barnesiella intestinihominis	336	AB370251	clade_420	N	N
Barnesiella viscericola	337	NR_041508	clade_420	N	N
Parabacteroides sp. NS31_3	1422	JN029805	clade_420	N	N
Porphyromonadaceae bacterium NML 060648	1470	EF184292	clade_420	N	N
Tannerella forsythia	1913	CP003191	clade_420	N	N
Tannerella sp. 6_1_58FAA_CT1	1914	ACWX01000068	clade_420	N	N
Mycoplasma amphoriforme	1311	AY531656	clade_421	N	N
Mycoplasma genitalium	1317	L43967	clade_421	N	N
Mycoplasma pneumoniae	1322	NC_000912	clade_421	N	N
Mycoplasma penetrans	1321	NC_004432	clade_422	N	N
Ureaplasma parvum	1966	AE002127	clade_422	N	N
Ureaplasma urealyticum	1967	AAYN01000002	clade_422	N	N
Treponema genomosp. P1	1927	AY341822	clade_425	N	N
Treponema sp. oral taxon 228	1943	GU408580	clade_425	N	N
Treponema sp. oral taxon 230	1944	GU408603	clade_425	N	N
Treponema sp. oral taxon 231	1945	GU408631	clade_425	N	N
Treponema sp. oral taxon 232	1946	GU408646	clade_425	N	N
Treponema sp. oral taxon 235	1947	GU408673	clade_425	N	N
Treponema sp. ovine footrot	1959	AJ010951	clade_425	N	N
Treponema vincentii	1960	ACYH01000036	clade_425	N	OP
Burkholderiales bacterium 1_1_47	452	ADCQ01000066	clade_432	N	OP
Parasutterella excrementihominis	1429	AFBP01000029	clade_432	N	N
Parasutterella secunda	1430	AB491209	clade_432	N	N
Sutterella morbirenis	1898	AJ832129	clade_432	N	N
Sutterella sanguinus	1900	AJ748647	clade_432	N	N
Sutterella sp. YIT 12072	1901	AB491210	clade_432	N	N
Sutterella stercoricanis	1902	NR_025600	clade_432	N	N
Sutterella wadsworthensis	1903	ADMF01000048	clade_432	N	N

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Propionibacterium freudenreichii	1572	NR_036972	clade_433	N	N
Propionibacterium sp. oral taxon 192	1580	GQ422728	clade_433	N	N
Tessaracoccus sp. oral taxon F04	1917	HM099640	clade_433	N	N
Peptoniphilus ivorii	1445	Y07840	clade_434	N	N
Peptoniphilus sp. gpac007	1448	AM176517	clade_434	N	N
Peptoniphilus sp. gpac018A	1449	AM176519	clade_434	N	N
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 satellites	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 bemidjiensis	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

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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
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

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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 mantanii	1283	FJ042897	clade_46	N	OP
Mycobacterium marinum	1284	NC_010612	clade_46	N	OP
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

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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
Fusobacterium ulcerans	921	ACDH01000090	clade_470	N	N
Fusobacterium varium	922	ACIE01000009	clade_470	N	N
Mycoplasma arthritidis	1312	NC_011025	clade_473	N	N
Mycoplasma faucium	1314	NR_024983	clade_473	N	N
Mycoplasma hominis	1318	AF443616	clade_473	N	N
Mycoplasma orale	1319	AY796060	clade_473	N	N
Mycoplasma salivarium	1324	M24661	clade_473	N	N
Mitsuokella jalaludinii	1247	NR_028840	clade_474	N	N
Mitsuokella multacida	1248	ABWK02000005	clade_474	N	N
Mitsuokella sp. oral taxon 521	1249	GU413658	clade_474	N	N
Mitsuokella sp. oral taxon G68	1250	GU432166	clade_474	N	N
Selenomonas genomosp. C1	1695	AY278627	clade_474	N	N
Selenomonas genomosp. P8 oral clone MB5_P06	1700	DQ003628	clade_474	N	N
Selenomonas ruminantium	1703	NR_075026	clade_474	N	N
Veillonellaceae bacterium oral taxon 131	1994	GU402916	clade_474	N	N
Alloscardovia omnicolens	139	NR_042583	clade_475	N	N
Alloscardovia sp. OB7196	140	AB425070	clade_475	N	N
Bifidobacterium urinalis	366	AJ278695	clade_475	N	N
Prevotella loescheii	1503	JN867231	clade_48	N	N
Prevotella sp. oral clone ASCG12	1530	DQ272511	clade_48	N	N
Prevotella sp. oral clone GU027	1540	AY349398	clade_48	N	N
Prevotella sp. oral taxon 472	1553	ACZS01000106	clade_48	N	N
Selenomonas diana	1693	GQ422719	clade_480	N	N
Selenomonas flueggei	1694	AF287803	clade_480	N	N
Selenomonas genomosp. C2	1696	AY278628	clade_480	N	N
Selenomonas genomosp. P6 oral clone MB3_C41	1698	DQ003636	clade_480	N	N
Selenomonas genomosp. P7 oral clone MB5_C08	1699	DQ003627	clade_480	N	N
Selenomonas infelix	1701	AF287802	clade_480	N	N
Selenomonas noxia	1702	GU470909	clade_480	N	N

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Selenomonas sp. oral clone FT050	1705	AY349403	clade_480	N	N
Selenomonas sp. oral clone GI064	1706	AY349404	clade_480	N	N
Selenomonas sp. oral clone GT010	1707	AY349405	clade_480	N	N
Selenomonas sp. oral clone HU051	1708	AY349406	clade_480	N	N
Selenomonas sp. oral clone IK004	1709	AY349407	clade_480	N	N
Selenomonas sp. oral clone JI021	1711	AY349409	clade_480	N	N
Selenomonas sp. oral clone JS031	1712	AY349410	clade_480	N	N
Selenomonas sp. oral clone OH4A	1713	AY947498	clade_480	N	N
Selenomonas sp. oral clone P2PA_80 P4	1714	AY207052	clade_480	N	N
Selenomonas sp. oral taxon 149	1716	AEEJ01000007	clade_480	N	N
Veillonellaceae bacterium oral taxon 155	1995	GU470897	clade_480	N	N
Agrococcus jenensis	117	NR_026275	clade_484	N	N
Microbacterium gubbeenense	1232	NR_025098	clade_484	N	N
Pseudoclavibacter sp. Timone	1590	FJ375951	clade_484	N	N
Tropheryma whipplei	1961	BX251412	clade_484	N	N
Zimmermannella bifida	2031	AB012592	clade_484	N	N
Legionella hackeliae	1151	M36028	clade_486	N	OP
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.	540	CP001850	clade_495	N	N

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BVAB3					
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 genomosp. C1	894	AY278616	clade_497	N	N
Fusobacterium genomosp. C2	895	AY278617	clade_497	N	N
Fusobacterium periodonticum	902	ACJY01000002	clade_497	N	N
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

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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
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

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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	ABY001000217	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
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 genomosp. P1	258	AY341819	clade_529	N	N
Bacteroidales genomosp. P2 oral clone MB1_G13	259	DQ003613	clade_529	N	N
Bacteroidales genomosp. P3 oral clone MB1_G34	260	DQ003615	clade_529	N	N
Bacteroidales genomosp. P4 oral clone MB2_G17	261	DQ003617	clade_529	N	N
Bacteroidales genomosp. P5 oral clone MB2_P04	262	DQ003619	clade_529	N	N
Bacteroidales genomosp. P6 oral clone MB3_C19	263	DQ003634	clade_529	N	N

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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
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

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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
Macrococcus 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
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

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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 piscicola	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
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 phI	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

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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 genomosp. 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
Nocardiosis 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
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

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<i>Thermus aquaticus</i>	1923	NR_025900	clade_589	N	N
<i>Actinomyces</i> sp. c109	81	AB167239	clade_590	N	N
<i>Syntrophomonadaceae</i> genomsp. P1	1912	AY341821	clade_590	N	N
<i>Anaerobaculum hydrogeniformans</i>	141	ACJX02000009	clade_591	N	N
<i>Microcystis aeruginosa</i>	1246	NC_010296	clade_592	N	N
<i>Prochlorococcus marinus</i>	1567	CP000551	clade_592	N	N
<i>Methanobrevibacter acididurans</i>	1208	NR_028779	clade_593	N	N
<i>Methanobrevibacter arboriphilus</i>	1209	NR_042783	clade_593	N	N
<i>Methanobrevibacter curvatus</i>	1210	NR_044796	clade_593	N	N
<i>Methanobrevibacter cuticularis</i>	1211	NR_044776	clade_593	N	N
<i>Methanobrevibacter filiformis</i>	1212	NR_044801	clade_593	N	N
<i>Methanobrevibacter woesei</i>	1220	NR_044788	clade_593	N	N
<i>Roseiflexus castenholzii</i>	1642	CP000804	clade_594	N	N
<i>Methanobrevibacter olleyae</i>	1215	NR_043024	clade_595	N	N
<i>Methanobrevibacter ruminantium</i>	1217	NR_042784	clade_595	N	N
<i>Methanobrevibacter wolnii</i>	1221	NR_044790	clade_595	N	N
<i>Methanosphaera stadtmanae</i>	1222	AY196684	clade_595	N	N
<i>Chloroflexi</i> genomsp. P1	511	AY331414	clade_596	N	N
<i>Halorubrum lipolyticum</i>	992	AB477978	clade_597	N	N
<i>Methanobacterium formicicum</i>	1207	NR_025028	clade_597	N	N
<i>Acidilobus saccharovorans</i>	24	AY350586	clade_598	N	N
<i>Hyperthermus butylicus</i>	1006	CP000493	clade_598	N	N
<i>Ignicoccus islandicus</i>	1011	X99562	clade_598	N	N
<i>Metallosphaera sedula</i>	1206	D26491	clade_598	N	N
<i>Thermophilum pendens</i>	1922	X14835	clade_598	N	N
<i>Prevotella melaninogenica</i>	1506	CP002122	clade_6	N	N
<i>Prevotella</i> sp. ICM1	1520	HQ616385	clade_6	N	N
<i>Prevotella</i> sp. oral clone FU048	1535	AY349393	clade_6	N	N
<i>Prevotella</i> sp. oral clone GI030	1537	AY349395	clade_6	N	N
<i>Prevotella</i> sp. SEQ116	1526	JN867246	clade_6	N	N
<i>Streptococcus anginosus</i>	1787	AECT01000011	clade_60	N	N
<i>Streptococcus milleri</i>	1812	X81023	clade_60	N	N
<i>Streptococcus</i> sp. 16362	1829	JN590019	clade_60	N	N
<i>Streptococcus</i> sp. 69130	1832	X78825	clade_60	N	N
<i>Streptococcus</i> sp. AC15	1833	HQ616356	clade_60	N	N
<i>Streptococcus</i> sp. CM7	1839	HQ616373	clade_60	N	N
<i>Streptococcus</i> sp. OBRC6	1847	HQ616352	clade_60	N	N
<i>Burkholderia ambifaria</i>	442	AAUZ01000009	clade_61	N	OP
<i>Burkholderia cenocepacia</i>	443	AAHI01000060	clade_61	N	OP
<i>Burkholderia cepacia</i>	444	NR_041719	clade_61	N	OP
<i>Burkholderia mallei</i>	445	CP000547	clade_61	N	Category -B
<i>Burkholderia multivorans</i>	446	NC_010086	clade_61	N	OP
<i>Burkholderia oklahomensis</i>	447	DQ108388	clade_61	N	OP
<i>Burkholderia pseudomallei</i>	448	CP001408	clade_61	N	Category -B
<i>Burkholderia rhizoxinica</i>	449	HQ005410	clade_61	N	OP
<i>Burkholderia</i> sp. 383	450	CP000151	clade_61	N	OP

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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	ACW01000065	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 kefiranoferiens	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
Prevotella genomsp. C1	1495	AY278624	clade_82	N	N
Prevotella sp. CM38	1519	HQ610181	clade_82	N	N

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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

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Haemophilus sputorum	988	AFNK01000005	clade_88	N	N
Histophilus somni	1003	AF549387	clade_88	N	N
Mannheimia haemolytica	1195	ACZX01000102	clade_88	N	N
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

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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
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 grimontii	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

OTU	SEQ ID Number	Public DB Accession	Clade	Spore Former	Pathogen Status
<i>Pasteurella dagmatis</i>	1434	ACZR01000003	clade_92	N	N
<i>Pasteurella multocida</i>	1435	NC_002663	clade_92	N	N
<i>Plesiomonas shigelloides</i>	1469	X60418	clade_92	N	N
<i>Raoultella ornithinolytica</i>	1617	AB364958	clade_92	N	N
<i>Raoultella planticola</i>	1618	AF129443	clade_92	N	N
<i>Raoultella terrigena</i>	1619	NR_037085	clade_92	N	N
<i>Salmonella bongori</i>	1683	NR_041699	clade_92	N	Category -B
<i>Salmonella enterica</i>	1672	NC_011149	clade_92	N	Category -B
<i>Salmonella enterica</i>	1673	NC_011205	clade_92	N	Category -B
<i>Salmonella enterica</i>	1674	DQ344532	clade_92	N	Category -B
<i>Salmonella enterica</i>	1675	ABEH02000004	clade_92	N	Category -B
<i>Salmonella enterica</i>	1676	ABAK02000001	clade_92	N	Category -B
<i>Salmonella enterica</i>	1677	NC_011080	clade_92	N	Category -B
<i>Salmonella enterica</i>	1678	EU118094	clade_92	N	Category -B
<i>Salmonella enterica</i>	1679	NC_011094	clade_92	N	Category -B
<i>Salmonella enterica</i>	1680	AE014613	clade_92	N	Category -B
<i>Salmonella enterica</i>	1682	ABFH02000001	clade_92	N	Category -B
<i>Salmonella enterica</i>	1684	ABEM01000001	clade_92	N	Category -B
<i>Salmonella enterica</i>	1685	ABAM02000001	clade_92	N	Category -B
<i>Salmonella typhimurium</i>	1681	DQ344533	clade_92	N	Category -B
<i>Salmonella typhimurium</i>	1686	AF170176	clade_92	N	Category -B
<i>Serratia fonticola</i>	1718	NR_025339	clade_92	N	N
<i>Serratia liquefaciens</i>	1719	NR_042062	clade_92	N	N
<i>Serratia marcescens</i>	1720	GU826157	clade_92	N	N
<i>Serratia odorifera</i>	1721	ADBY01000001	clade_92	N	N
<i>Serratia proteamaculans</i>	1722	AAUN01000015	clade_92	N	N
<i>Shigella boydii</i>	1724	AAKA01000007	clade_92	N	Category -B
<i>Shigella dysenteriae</i>	1725	NC_007606	clade_92	N	Category -B
<i>Shigella flexneri</i>	1726	AE005674	clade_92	N	Category -B
<i>Shigella sonnei</i>	1727	NC_007384	clade_92	N	Category -B
<i>Tatumella ptyseos</i>	1916	NR_025342	clade_92	N	N
<i>Trabulsiella guamensis</i>	1925	AY373830	clade_92	N	N
<i>Yersinia aldovae</i>	2019	AJ871363	clade_92	N	OP
<i>Yersinia aleksiciae</i>	2020	AJ627597	clade_92	N	OP
<i>Yersinia bercovieri</i>	2021	AF366377	clade_92	N	OP
<i>Yersinia enterocolitica</i>	2022	FR729477	clade_92	N	Category -B

OTU	SEQ ID Number	Public DB Accession	Clade	Spore Former	Pathogen Status
<i>Yersinia frederiksenii</i>	2023	AF366379	clade_92	N	OP
<i>Yersinia intermedia</i>	2024	AF366380	clade_92	N	OP
<i>Yersinia kristensenii</i>	2025	ACCA01000078	clade_92	N	OP
<i>Yersinia mollaretii</i>	2026	NR_027546	clade_92	N	OP
<i>Yersinia pestis</i>	2027	AE013632	clade_92	N	Category -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 sp. KEM232</i>	1341	GQ203291	clade_94	N	N
<i>Neisseria sp. oral clone AP132</i>	1344	AY005027	clade_94	N	N
<i>Neisseria sp. oral strain B33KA</i>	1346	AY005028	clade_94	N	N
<i>Neisseria sp. oral taxon 014</i>	1347	ADEA01000039	clade_94	N	N
<i>Neisseria sp. TM10_1</i>	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. C1</i>	1798	AY278629	clade_98	N	N
<i>Streptococcus genomosp. C2</i>	1799	AY278630	clade_98	N	N
<i>Streptococcus genomosp. C3</i>	1800	AY278631	clade_98	N	N
<i>Streptococcus genomosp. C4</i>	1801	AY278632	clade_98	N	N
<i>Streptococcus genomosp. C5</i>	1802	AY278633	clade_98	N	N
<i>Streptococcus genomosp. C6</i>	1803	AY278634	clade_98	N	N
<i>Streptococcus genomosp. C7</i>	1804	AY278635	clade_98	N	N
<i>Streptococcus genomosp. C8</i>	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

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<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
<i>Streptococcus pasteurianus</i>	1818	AP012054	clade_98	N	N
<i>Streptococcus peroris</i>	1819	AEVF01000016	clade_98	N	N
<i>Streptococcus pneumoniae</i>	1820	AE008537	clade_98	N	N
<i>Streptococcus porcinus</i>	1821	EF121439	clade_98	N	N
<i>Streptococcus pseudopneumoniae</i>	1822	FJ827123	clade_98	N	N
<i>Streptococcus pseudoporcinus</i>	1823	AENS01000003	clade_98	N	N
<i>Streptococcus pyogenes</i>	1824	AE006496	clade_98	N	OP
<i>Streptococcus rattii</i>	1825	X58304	clade_98	N	N
<i>Streptococcus sanguinis</i>	1827	NR_074974	clade_98	N	N
<i>Streptococcus sinensis</i>	1828	AF432857	clade_98	N	N
<i>Streptococcus</i> sp. 2_1_36FAA	1831	ACO101000028	clade_98	N	N
<i>Streptococcus</i> sp. 2285_97	1830	AJ131965	clade_98	N	N
<i>Streptococcus</i> sp. ACS2	1834	HQ616360	clade_98	N	N
<i>Streptococcus</i> sp. AS20	1835	HQ616366	clade_98	N	N
<i>Streptococcus</i> sp. BS35a	1836	HQ616369	clade_98	N	N
<i>Streptococcus</i> sp. C150	1837	ACRI01000045	clade_98	N	N
<i>Streptococcus</i> sp. CM6	1838	HQ616372	clade_98	N	N
<i>Streptococcus</i> sp. ICM10	1840	HQ616389	clade_98	N	N
<i>Streptococcus</i> sp. ICM12	1841	HQ616390	clade_98	N	N
<i>Streptococcus</i> sp. ICM2	1842	HQ616386	clade_98	N	N
<i>Streptococcus</i> sp. ICM4	1844	HQ616387	clade_98	N	N
<i>Streptococcus</i> sp. ICM45	1843	HQ616394	clade_98	N	N
<i>Streptococcus</i> sp. M143	1845	ACRK01000025	clade_98	N	N
<i>Streptococcus</i> sp. M334	1846	ACRL01000052	clade_98	N	N
<i>Streptococcus</i> sp. oral clone ASB02	1849	AY923121	clade_98	N	N
<i>Streptococcus</i> sp. oral clone ASCA03	1850	DQ272504	clade_98	N	N
<i>Streptococcus</i> sp. oral clone ASCA04	1851	AY923116	clade_98	N	N
<i>Streptococcus</i> sp. oral clone ASCA09	1852	AY923119	clade_98	N	N
<i>Streptococcus</i> sp. oral clone ASCB04	1853	AY923123	clade_98	N	N
<i>Streptococcus</i> sp. oral clone ASCB06	1854	AY923124	clade_98	N	N
<i>Streptococcus</i> sp. oral clone ASCC04	1855	AY923127	clade_98	N	N
<i>Streptococcus</i> sp. oral clone ASCC05	1856	AY923128	clade_98	N	N
<i>Streptococcus</i> sp. oral clone ASCC12	1857	DQ272507	clade_98	N	N
<i>Streptococcus</i> sp. oral clone ASCD01	1858	AY923129	clade_98	N	N
<i>Streptococcus</i> sp. oral clone ASCD09	1859	AY923130	clade_98	N	N
<i>Streptococcus</i> sp. oral clone ASCD10	1860	DQ272509	clade_98	N	N
<i>Streptococcus</i> sp. oral clone	1861	AY923134	clade_98	N	N

OTU	SEQ ID Number	Public DB Accession	Clade	Spore Former	Pathogen Status
ASCE03					
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 ASCE06	1864	AY923135	clade_98	N	N
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	AEK001000008	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

Lachnospiraceae sp	1	0
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Table 3 Mortality and weight change in mice challenged with *C. difficile* with or without ethanol treated, spore product 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 feces 1x	0	2.3%
EtOH-treated feces 0.1x	0	2.4%
EtOH-treated feces 0.01x	0	-3%
heat-treated feces	0	0.1%

Table 5: Binary pair inhibition of *C. difficile*

SPC1	SPC2	OTU1	OTU2	Result
SPC10414	SPC10414	Alistipes_shahii	Alistipes_shahii	
SPC10211	SPC10414	Bacteroides_caccae	Alistipes_shahii	
SPC10213	SPC10414	Bacteroides_eggerthii	Alistipes_shahii	--
SPC10030	SPC10414	Bacteroides_ovatus	Alistipes_shahii	
SPC00006	SPC10414	Bacteroides_sp_1_1_6	Alistipes_shahii	++++
SPC00007	SPC10414	Bacteroides_sp_3_1_23	Alistipes_shahii	+
SPC10019	SPC10414	Bacteroides_sp_D20	Alistipes_shahii	-
SPC00005	SPC10414	Bacteroides_vulgatus	Alistipes_shahii	+++
SPC10081	SPC10414	Bacteroides_vulgatus	Alistipes_shahii	+
SPC10301	SPC10414	Bifidobacterium_adolescentis	Alistipes_shahii	++++
SPC10298	SPC10414	Bifidobacterium_pseudocatenulatum	Alistipes_shahii	
SPC00021	SPC10414	Blautia_producta	Alistipes_shahii	++++
SPC10403	SPC10414	Blautia_schinkii	Alistipes_shahii	
SPC10243	SPC10414	Clostridium_hathewayi	Alistipes_shahii	++++
SPC00026	SPC10414	Clostridium_nexile	Alistipes_shahii	
SPC00027	SPC10414	Clostridium_sp_HGF2	Alistipes_shahii	
SPC10355	SPC10414	Clostridium_symbiosum	Alistipes_shahii	
SPC10097	SPC10414	Collinsella_aerofaciens	Alistipes_shahii	++++
SPC00009	SPC10414	Coprobacillus_sp_D7	Alistipes_shahii	++++
SPC00080	SPC10414	Coprococcus_catus	Alistipes_shahii	-
SPC10304	SPC10414	Coprococcus_comes	Alistipes_shahii	
SPC00018	SPC10414	Dorea_formicigenerans	Alistipes_shahii	----
SPC00057	SPC10414	Dorea_longicatena	Alistipes_shahii	++++
SPC00008	SPC10414	Enterococcus_faecalis	Alistipes_shahii	++++
SPC10001	SPC10414	Erysipelotrichaceae_bacterium	Alistipes_shahii	---
SPC00001	SPC10414	Escherichia_coli	Alistipes_shahii	++++
SPC10110	SPC10414	Escherichia_coli	Alistipes_shahii	++++
SPC00022	SPC10414	Eubacterium_eligens	Alistipes_shahii	--
SPC10363	SPC10414	Eubacterium_rectale	Alistipes_shahii	
SPC00054	SPC10414	Faecalibacterium_prausnitzii	Alistipes_shahii	
SPC10386	SPC10414	Faecalibacterium_prausnitzii	Alistipes_shahii	+
SPC10390	SPC10414	Lachnospiraceae_bacterium_5_1_57FAA	Alistipes_shahii	
SPC00056	SPC10414	Odoribacter_splanchnicus	Alistipes_shahii	
SPC10388	SPC10414	Odoribacter_splanchnicus	Alistipes_shahii	
SPC10048	SPC10414	Parabacteroides_merdae	Alistipes_shahii	
SPC00061	SPC10414	Roseburia_intestinalis	Alistipes_shahii	-
SPC10197	SPC10414	Ruminococcus_obeum	Alistipes_shahii	
SPC10233	SPC10414	Ruminococcus_torques	Alistipes_shahii	

SPC1	SPC2	OTU1	OTU2	Result
SPC00015	SPC10414	Streptococcus_thermophilus	Alistipes_shahii	
SPC10211	SPC10211	Bacteroides_caccae	Bacteroides_caccae	++++
SPC10030	SPC10211	Bacteroides_ovatus	Bacteroides_caccae	
SPC00006	SPC10211	Bacteroides_sp_1_1_6	Bacteroides_caccae	++++
SPC00007	SPC10211	Bacteroides_sp_3_1_23	Bacteroides_caccae	+++
SPC10019	SPC10211	Bacteroides_sp_D20	Bacteroides_caccae	+++
SPC00005	SPC10211	Bacteroides_vulgatus	Bacteroides_caccae	++++
SPC10081	SPC10211	Bacteroides_vulgatus	Bacteroides_caccae	+
SPC00021	SPC10211	Blautia_producta	Bacteroides_caccae	++++
SPC00026	SPC10211	Clostridium_nexile	Bacteroides_caccae	
SPC00027	SPC10211	Clostridium_sp_HGF2	Bacteroides_caccae	
SPC10097	SPC10211	Collinsella_aerofaciens	Bacteroides_caccae	++++
SPC00009	SPC10211	Coprobacillus_sp_D7	Bacteroides_caccae	+++
SPC00080	SPC10211	Coprococcus_catus	Bacteroides_caccae	++++
SPC00018	SPC10211	Dorea_formicigenerans	Bacteroides_caccae	+++
SPC00057	SPC10211	Dorea_longicatena	Bacteroides_caccae	
SPC00008	SPC10211	Enterococcus_faecalis	Bacteroides_caccae	++++
SPC10001	SPC10211	Erysipelotrichaceae_bacterium	Bacteroides_caccae	++
SPC00001	SPC10211	Escherichia_coli	Bacteroides_caccae	++++
SPC10110	SPC10211	Escherichia_coli	Bacteroides_caccae	++++
SPC00022	SPC10211	Eubacterium_eligens	Bacteroides_caccae	++
SPC00054	SPC10211	Faecalibacterium_prausnitzii	Bacteroides_caccae	-
SPC00056	SPC10211	Odoribacter_splanchnicus	Bacteroides_caccae	
SPC10048	SPC10211	Parabacteroides_merdae	Bacteroides_caccae	+
SPC00061	SPC10211	Roseburia_intestinalis	Bacteroides_caccae	+
SPC10197	SPC10211	Ruminococcus_obeum	Bacteroides_caccae	++++
SPC00015	SPC10211	Streptococcus_thermophilus	Bacteroides_caccae	++
SPC10211	SPC10213	Bacteroides_caccae	Bacteroides_eggerthii	++++
SPC10213	SPC10213	Bacteroides_eggerthii	Bacteroides_eggerthii	++++
SPC10030	SPC10213	Bacteroides_ovatus	Bacteroides_eggerthii	
SPC00006	SPC10213	Bacteroides_sp_1_1_6	Bacteroides_eggerthii	+++
SPC00007	SPC10213	Bacteroides_sp_3_1_23	Bacteroides_eggerthii	++
SPC10019	SPC10213	Bacteroides_sp_D20	Bacteroides_eggerthii	
SPC00005	SPC10213	Bacteroides_vulgatus	Bacteroides_eggerthii	++++
SPC10081	SPC10213	Bacteroides_vulgatus	Bacteroides_eggerthii	+
SPC00021	SPC10213	Blautia_producta	Bacteroides_eggerthii	++++
SPC00026	SPC10213	Clostridium_nexile	Bacteroides_eggerthii	
SPC00027	SPC10213	Clostridium_sp_HGF2	Bacteroides_eggerthii	-
SPC10097	SPC10213	Collinsella_aerofaciens	Bacteroides_eggerthii	++++
SPC00009	SPC10213	Coprobacillus_sp_D7	Bacteroides_eggerthii	
SPC00080	SPC10213	Coprococcus_catus	Bacteroides_eggerthii	+
SPC00018	SPC10213	Dorea_formicigenerans	Bacteroides_eggerthii	

SPC1	SPC2	OTU1	OTU2	Result
SPC00057	SPC10213	Dorea_longicatena	Bacteroides_eggerthii	-
SPC00008	SPC10213	Enterococcus_faecalis	Bacteroides_eggerthii	++++
SPC10001	SPC10213	Erysipelotrichaceae_bacterium	Bacteroides_eggerthii	
SPC00001	SPC10213	Escherichia_coli	Bacteroides_eggerthii	++++
SPC10110	SPC10213	Escherichia_coli	Bacteroides_eggerthii	++++
SPC00022	SPC10213	Eubacterium_eligens	Bacteroides_eggerthii	
SPC00054	SPC10213	Faecalibacterium_prausnitzii	Bacteroides_eggerthii	
SPC00056	SPC10213	Odoribacter_splanchnicus	Bacteroides_eggerthii	
SPC10048	SPC10213	Parabacteroides_merdae	Bacteroides_eggerthii	
SPC00061	SPC10213	Roseburia_intestinalis	Bacteroides_eggerthii	
SPC10197	SPC10213	Ruminococcus_oboeum	Bacteroides_eggerthii	++++
SPC00015	SPC10213	Streptococcus_thermophilus	Bacteroides_eggerthii	
SPC10030	SPC10030	Bacteroides_ovatus	Bacteroides_ovatus	+++
SPC00006	SPC10030	Bacteroides_sp_1_1_6	Bacteroides_ovatus	++++
SPC00007	SPC10030	Bacteroides_sp_3_1_23	Bacteroides_ovatus	
SPC10019	SPC10030	Bacteroides_sp_D20	Bacteroides_ovatus	-
SPC00005	SPC10030	Bacteroides_vulgatus	Bacteroides_ovatus	+
SPC00021	SPC10030	Blautia_producta	Bacteroides_ovatus	++++
SPC00026	SPC10030	Clostridium_nexile	Bacteroides_ovatus	
SPC00027	SPC10030	Clostridium_sp_HGF2	Bacteroides_ovatus	
SPC00009	SPC10030	Coprobacillus_sp_D7	Bacteroides_ovatus	
SPC00080	SPC10030	Coprococcus_catus	Bacteroides_ovatus	
SPC00018	SPC10030	Dorea_formicigenerans	Bacteroides_ovatus	
SPC00057	SPC10030	Dorea_longicatena	Bacteroides_ovatus	-
SPC00008	SPC10030	Enterococcus_faecalis	Bacteroides_ovatus	++++
SPC10001	SPC10030	Erysipelotrichaceae_bacterium	Bacteroides_ovatus	
SPC00001	SPC10030	Escherichia_coli	Bacteroides_ovatus	++++
SPC00022	SPC10030	Eubacterium_eligens	Bacteroides_ovatus	-
SPC00054	SPC10030	Faecalibacterium_prausnitzii	Bacteroides_ovatus	
SPC00056	SPC10030	Odoribacter_splanchnicus	Bacteroides_ovatus	
SPC00061	SPC10030	Roseburia_intestinalis	Bacteroides_ovatus	
SPC00015	SPC10030	Streptococcus_thermophilus	Bacteroides_ovatus	++
SPC00006	SPC00006	Bacteroides_sp_1_1_6	Bacteroides_sp_1_1_6	++++
SPC00005	SPC00006	Bacteroides_vulgatus	Bacteroides_sp_1_1_6	++++
SPC00001	SPC00006	Escherichia_coli	Bacteroides_sp_1_1_6	++++
SPC00006	SPC00007	Bacteroides_sp_1_1_6	Bacteroides_sp_3_1_23	++++
SPC00007	SPC00007	Bacteroides_sp_3_1_23	Bacteroides_sp_3_1_23	
SPC00005	SPC00007	Bacteroides_vulgatus	Bacteroides_sp_3_1_23	+++
SPC00001	SPC00007	Escherichia_coli	Bacteroides_sp_3_1_23	++++
SPC00006	SPC10019	Bacteroides_sp_1_1_6	Bacteroides_sp_D20	++++
SPC00007	SPC10019	Bacteroides_sp_3_1_23	Bacteroides_sp_D20	++++
SPC10019	SPC10019	Bacteroides_sp_D20	Bacteroides_sp_D20	

SPC1	SPC2	OTU1	OTU2	Result
SPC00005	SPC10019	Bacteroides_vulgatus	Bacteroides_sp_D20	+
SPC00021	SPC10019	Blautia_producta	Bacteroides_sp_D20	++++
SPC00026	SPC10019	Clostridium_nexile	Bacteroides_sp_D20	-
SPC00027	SPC10019	Clostridium_sp_HGF2	Bacteroides_sp_D20	
SPC00009	SPC10019	Coprobacillus_sp_D7	Bacteroides_sp_D20	
SPC00080	SPC10019	Coprococcus_catus	Bacteroides_sp_D20	
SPC00018	SPC10019	Dorea_formicigenerans	Bacteroides_sp_D20	-
SPC00057	SPC10019	Dorea_longicatena	Bacteroides_sp_D20	
SPC00008	SPC10019	Enterococcus_faecalis	Bacteroides_sp_D20	++++
SPC10001	SPC10019	Erysipelotrichaceae_bacterium	Bacteroides_sp_D20	
SPC00001	SPC10019	Escherichia_coli	Bacteroides_sp_D20	++++
SPC00022	SPC10019	Eubacterium_eligens	Bacteroides_sp_D20	-
SPC00054	SPC10019	Faecalibacterium_prausnitzii	Bacteroides_sp_D20	
SPC00056	SPC10019	Odoribacter_splanchnicus	Bacteroides_sp_D20	
SPC00061	SPC10019	Roseburia_intestinalis	Bacteroides_sp_D20	-
SPC00015	SPC10019	Streptococcus_thermophilus	Bacteroides_sp_D20	+
SPC10030	SPC10081	Bacteroides_ovatus	Bacteroides_vulgatus	
SPC00006	SPC10081	Bacteroides_sp_1_1_6	Bacteroides_vulgatus	
SPC00007	SPC10081	Bacteroides_sp_3_1_23	Bacteroides_vulgatus	-
SPC10019	SPC10081	Bacteroides_sp_D20	Bacteroides_vulgatus	
SPC00005	SPC00005	Bacteroides_vulgatus	Bacteroides_vulgatus	+
SPC00005	SPC10081	Bacteroides_vulgatus	Bacteroides_vulgatus	++
SPC10081	SPC10081	Bacteroides_vulgatus	Bacteroides_vulgatus	
SPC00021	SPC10081	Blautia_producta	Bacteroides_vulgatus	++++
SPC00026	SPC10081	Clostridium_nexile	Bacteroides_vulgatus	
SPC00027	SPC10081	Clostridium_sp_HGF2	Bacteroides_vulgatus	+++
SPC00009	SPC10081	Coprobacillus_sp_D7	Bacteroides_vulgatus	-
SPC00080	SPC10081	Coprococcus_catus	Bacteroides_vulgatus	++
SPC00018	SPC10081	Dorea_formicigenerans	Bacteroides_vulgatus	
SPC00057	SPC10081	Dorea_longicatena	Bacteroides_vulgatus	
SPC00008	SPC10081	Enterococcus_faecalis	Bacteroides_vulgatus	++++
SPC10001	SPC10081	Erysipelotrichaceae_bacterium	Bacteroides_vulgatus	
SPC00001	SPC00005	Escherichia_coli	Bacteroides_vulgatus	++++
SPC00001	SPC10081	Escherichia_coli	Bacteroides_vulgatus	++++
SPC00022	SPC10081	Eubacterium_eligens	Bacteroides_vulgatus	
SPC00054	SPC10081	Faecalibacterium_prausnitzii	Bacteroides_vulgatus	
SPC00056	SPC10081	Odoribacter_splanchnicus	Bacteroides_vulgatus	
SPC10048	SPC10081	Parabacteroides_merdae	Bacteroides_vulgatus	+
SPC00061	SPC10081	Roseburia_intestinalis	Bacteroides_vulgatus	
SPC00015	SPC10081	Streptococcus_thermophilus	Bacteroides_vulgatus	--
SPC10211	SPC10301	Bacteroides_caccae	Bifidobacterium_adolescentis	++++

SPC1	SPC2	OTU1	OTU2	Result
SPC10213	SPC10301	Bacteroides_eggerthii	Bifidobacterium_adolescentis	++++
SPC10030	SPC10301	Bacteroides_ovatus	Bifidobacterium_adolescentis	++++
SPC00006	SPC10301	Bacteroides_sp_1_1_6	Bifidobacterium_adolescentis	++++
SPC00007	SPC10301	Bacteroides_sp_3_1_23	Bifidobacterium_adolescentis	++++
SPC10019	SPC10301	Bacteroides_sp_D20	Bifidobacterium_adolescentis	++++
SPC00005	SPC10301	Bacteroides_vulgatus	Bifidobacterium_adolescentis	++++
SPC10081	SPC10301	Bacteroides_vulgatus	Bifidobacterium_adolescentis	++++
SPC10301	SPC10301	Bifidobacterium_adolescentis	Bifidobacterium_adolescentis	++++
SPC10298	SPC10301	Bifidobacterium_pseudocatenulatum	Bifidobacterium_adolescentis	++++
SPC00021	SPC10301	Blautia_producta	Bifidobacterium_adolescentis	++++
SPC10243	SPC10301	Clostridium_hathewayi	Bifidobacterium_adolescentis	++++
SPC00026	SPC10301	Clostridium_nexile	Bifidobacterium_adolescentis	++++
SPC00027	SPC10301	Clostridium_sp_HGF2	Bifidobacterium_adolescentis	++++
SPC10097	SPC10301	Collinsella_aerofaciens	Bifidobacterium_adolescentis	++++
SPC00009	SPC10301	Coprobacillus_sp_D7	Bifidobacterium_adolescentis	++++
SPC00080	SPC10301	Coprococcus_catus	Bifidobacterium_adolescentis	++++
SPC00018	SPC10301	Dorea_formicigenerans	Bifidobacterium_adolescentis	++++
SPC00057	SPC10301	Dorea_longicatena	Bifidobacterium_adolescentis	++++
SPC00008	SPC10301	Enterococcus_faecalis	Bifidobacterium_adolescentis	++++
SPC10001	SPC10301	Erysipelotrichaceae_bacterium	Bifidobacterium_adolescentis	++++
SPC00001	SPC10301	Escherichia_coli	Bifidobacterium_adolescentis	++++
SPC10110	SPC10301	Escherichia_coli	Bifidobacterium_adolescentis	++++
SPC00022	SPC10301	Eubacterium_eligens	Bifidobacterium_adolescentis	++++
SPC00054	SPC10301	Faecalibacterium_prausnitzii	Bifidobacterium_adolescentis	+
SPC00056	SPC10301	Odoribacter_splanchnicus	Bifidobacterium_adolescentis	+++
SPC10048	SPC10301	Parabacteroides_merdae	Bifidobacterium_adolescentis	++++
SPC00061	SPC10301	Roseburia_intestinalis	Bifidobacterium_adolescentis	+++

SPC1	SPC2	OTU1	OTU2	Result
SPC10197	SPC10301	Ruminococcus_obeum	Bifidobacterium_adolescentis	++++
SPC10233	SPC10301	Ruminococcus_torques	Bifidobacterium_adolescentis	++++
SPC00015	SPC10301	Streptococcus_thermophilus	Bifidobacterium_adolescentis	++++
SPC10211	SPC10298	Bacteroides_caccae	Bifidobacterium_pseudocatenulatum	++++
SPC10213	SPC10298	Bacteroides_eggerthii	Bifidobacterium_pseudocatenulatum	++++
SPC10030	SPC10298	Bacteroides_ovatus	Bifidobacterium_pseudocatenulatum	++++
SPC00006	SPC10298	Bacteroides_sp_1_1_6	Bifidobacterium_pseudocatenulatum	++++
SPC00007	SPC10298	Bacteroides_sp_3_1_23	Bifidobacterium_pseudocatenulatum	++++
SPC10019	SPC10298	Bacteroides_sp_D20	Bifidobacterium_pseudocatenulatum	--
SPC00005	SPC10298	Bacteroides_vulgatus	Bifidobacterium_pseudocatenulatum	++++
SPC10081	SPC10298	Bacteroides_vulgatus	Bifidobacterium_pseudocatenulatum	++++
SPC10298	SPC10298	Bifidobacterium_pseudocatenulatum	Bifidobacterium_pseudocatenulatum	++++
SPC00021	SPC10298	Blautia_producta	Bifidobacterium_pseudocatenulatum	+
SPC10243	SPC10298	Clostridium_hathewayi	Bifidobacterium_pseudocatenulatum	++++
SPC00026	SPC10298	Clostridium_nexile	Bifidobacterium_pseudocatenulatum	++++
SPC00027	SPC10298	Clostridium_sp_HGF2	Bifidobacterium_pseudocatenulatum	+++
SPC10097	SPC10298	Collinsella_aerofaciens	Bifidobacterium_pseudocatenulatum	++++
SPC00009	SPC10298	Coprobacillus_sp_D7	Bifidobacterium_pseudocatenulatum	+++
SPC00080	SPC10298	Coprococcus_catus	Bifidobacterium_pseudocatenulatum	
SPC00018	SPC10298	Dorea_formicigenerans	Bifidobacterium_pseudocatenulatum	+++
SPC00057	SPC10298	Dorea_longicatena	Bifidobacterium_pseudocatenulatum	++++
SPC00008	SPC10298	Enterococcus_faecalis	Bifidobacterium_pseudocatenulatum	++++
SPC10001	SPC10298	Erysipelotrichaceae_bacterium	Bifidobacterium_pseudocatenulatum	
SPC00001	SPC10298	Escherichia_coli	Bifidobacterium_pseudocatenulatum	++++
SPC10110	SPC10298	Escherichia_coli	Bifidobacterium_pseudocatenulatum	++++
SPC00022	SPC10298	Eubacterium_eligens	Bifidobacterium_pseudocatenulatum	++++
SPC00054	SPC10298	Faecalibacterium_prausnitzii	Bifidobacterium_pseudocatenulatum	++

SPC1	SPC2	OTU1	OTU2	Result
SPC00056	SPC10298	Odoribacter_splanchnicus	Bifidobacterium_pseudocat enulatum	+
SPC10048	SPC10298	Parabacteroides_merdae	Bifidobacterium_pseudocat enulatum	++++
SPC00061	SPC10298	Roseburia_intestinalis	Bifidobacterium_pseudocat enulatum	+++
SPC10197	SPC10298	Ruminococcus_obeum	Bifidobacterium_pseudocat enulatum	++++
SPC10233	SPC10298	Ruminococcus_torques	Bifidobacterium_pseudocat enulatum	++++
SPC00015	SPC10298	Streptococcus_thermophilus	Bifidobacterium_pseudocat enulatum	++++
SPC10414	SPC10415	Alistipes_shahii	Blautia_producta	
SPC10211	SPC10415	Bacteroides_caccae	Blautia_producta	+
SPC10213	SPC10415	Bacteroides_eggerthii	Blautia_producta	
SPC10030	SPC10415	Bacteroides_ovatus	Blautia_producta	-
SPC00006	SPC00021	Bacteroides_sp_1_1_6	Blautia_producta	++++
SPC00006	SPC10415	Bacteroides_sp_1_1_6	Blautia_producta	++++
SPC00007	SPC00021	Bacteroides_sp_3_1_23	Blautia_producta	++++
SPC00007	SPC10415	Bacteroides_sp_3_1_23	Blautia_producta	++
SPC10019	SPC10415	Bacteroides_sp_D20	Blautia_producta	
SPC00005	SPC00021	Bacteroides_vulgatus	Blautia_producta	++++
SPC00005	SPC10415	Bacteroides_vulgatus	Blautia_producta	++++
SPC10081	SPC10415	Bacteroides_vulgatus	Blautia_producta	++++
SPC10301	SPC10415	Bifidobacterium_adolescentis	Blautia_producta	++++
SPC10298	SPC10415	Bifidobacterium_pseudocatenulatu m	Blautia_producta	
SPC00021	SPC00021	Blautia_producta	Blautia_producta	++++
SPC00021	SPC10415	Blautia_producta	Blautia_producta	++++
SPC10415	SPC10415	Blautia_producta	Blautia_producta	+
SPC10415	SPC10415	Blautia_producta	Blautia_producta	++++
SPC10403	SPC10415	Blautia_schinkii	Blautia_producta	
SPC10256	SPC10415	Clostridium_butyricum	Blautia_producta	++++
SPC10358	SPC10415	Clostridium_orbiscindens	Blautia_producta	++++
SPC10325	SPC10415	Clostridium_bolteae	Blautia_producta	++++
SPC10167	SPC10415	Clostridium_disporicum	Blautia_producta	++++
SPC10243	SPC10415	Clostridium_hathewayi	Blautia_producta	+++
SPC10313	SPC10415	Clostridium_hylemonae	Blautia_producta	++++
SPC10202	SPC10415	Clostridium_innocuum	Blautia_producta	++++
SPC10238	SPC10415	Clostridium_mayombeii	Blautia_producta	++++
SPC00026	SPC10415	Clostridium_nexile	Blautia_producta	-
SPC00027	SPC10415	Clostridium_sp_HGF2	Blautia_producta	
SPC10355	SPC10415	Clostridium_symbiosum	Blautia_producta	
SPC10355	SPC10415	Clostridium_symbiosum	Blautia_producta	++++
SPC10155	SPC10415	Clostridium_tertium	Blautia_producta	++++

SPC1	SPC2	OTU1	OTU2	Result
SPC10097	SPC10415	Collinsella_aerofaciens	Blautia_producta	++++
SPC10097	SPC10415	Collinsella_aerofaciens	Blautia_producta	++++
SPC00009	SPC00021	Coprobacillus_sp_D7	Blautia_producta	++++
SPC00009	SPC10415	Coprobacillus_sp_D7	Blautia_producta	++++
SPC00080	SPC10415	Coprococcus_catus	Blautia_producta	----
SPC10304	SPC10415	Coprococcus_comes	Blautia_producta	
SPC10304	SPC10415	Coprococcus_comes	Blautia_producta	++++
SPC00018	SPC00021	Dorea_formicigenerans	Blautia_producta	++++
SPC00018	SPC10415	Dorea_formicigenerans	Blautia_producta	--
SPC00057	SPC10415	Dorea_longicatena	Blautia_producta	+++
SPC00008	SPC00021	Enterococcus_faecalis	Blautia_producta	++++
SPC00008	SPC10415	Enterococcus_faecalis	Blautia_producta	++++
SPC10001	SPC10415	Erysipelotrichaceae_bacterium	Blautia_producta	---
SPC00001	SPC00021	Escherichia_coli	Blautia_producta	++++
SPC00001	SPC10415	Escherichia_coli	Blautia_producta	++++
SPC10110	SPC10415	Escherichia_coli	Blautia_producta	++++
SPC00022	SPC10415	Eubacterium_eligens	Blautia_producta	---
SPC10363	SPC10415	Eubacterium_rectale	Blautia_producta	+
SPC00054	SPC10415	Faecalibacterium_prausnitzii	Blautia_producta	
SPC10386	SPC10415	Faecalibacterium_prausnitzii	Blautia_producta	+
SPC10386	SPC10415	Faecalibacterium_prausnitzii	Blautia_producta	++++
		Lachnospiraceae_bacterium_5_1_5		
SPC10390	SPC10415	7FAA	Blautia_producta	+
		Lachnospiraceae_bacterium_5_1_5		
SPC10390	SPC10415	7FAA	Blautia_producta	++++
SPC00056	SPC10415	Odoribacter_splanchnicus	Blautia_producta	-
SPC10388	SPC10415	Odoribacter_splanchnicus	Blautia_producta	+
SPC10048	SPC10415	Parabacteroides_merdae	Blautia_producta	+++
SPC00061	SPC10415	Roseburia_intestinalis	Blautia_producta	--
SPC10468	SPC10415	Ruminococcus_gnavus	Blautia_producta	++++
SPC10197	SPC10415	Ruminococcus_obeum	Blautia_producta	
SPC10233	SPC10415	Ruminococcus_torques	Blautia_producta	
SPC00015	SPC00021	Streptococcus_thermophilus	Blautia_producta	++++
SPC00015	SPC10415	Streptococcus_thermophilus	Blautia_producta	
SPC10211	SPC10403	Bacteroides_caccae	Blautia_schinkii	
SPC10213	SPC10403	Bacteroides_eggerthii	Blautia_schinkii	--
SPC10030	SPC10403	Bacteroides_ovatus	Blautia_schinkii	-
SPC00006	SPC10403	Bacteroides_sp_1_1_6	Blautia_schinkii	+++
SPC00007	SPC10403	Bacteroides_sp_3_1_23	Blautia_schinkii	+
SPC10019	SPC10403	Bacteroides_sp_D20	Blautia_schinkii	--
SPC00005	SPC10403	Bacteroides_vulgatus	Blautia_schinkii	++
SPC10081	SPC10403	Bacteroides_vulgatus	Blautia_schinkii	
SPC10301	SPC10403	Bifidobacterium_adolescentis	Blautia_schinkii	++

SPC1	SPC2	OTU1	OTU2	Result
SPC10298	SPC10403	Bifidobacterium_pseudocatenulatum	Blautia_schinkii	-
SPC00021	SPC10403	Blautia_producta	Blautia_schinkii	++++
SPC10403	SPC10403	Blautia_schinkii	Blautia_schinkii	
SPC10243	SPC10403	Clostridium_hathewayi	Blautia_schinkii	++++
SPC00026	SPC10403	Clostridium_nexile	Blautia_schinkii	--
SPC00027	SPC10403	Clostridium_sp_HGF2	Blautia_schinkii	
SPC10355	SPC10403	Clostridium_symbiosum	Blautia_schinkii	
SPC10097	SPC10403	Collinsella_aerofaciens	Blautia_schinkii	++++
SPC00009	SPC10403	Coprobacillus_sp_D7	Blautia_schinkii	++++
SPC00080	SPC10403	Coprococcus_catus	Blautia_schinkii	---
SPC10304	SPC10403	Coprococcus_comes	Blautia_schinkii	+
SPC00018	SPC10403	Dorea_formicigenerans	Blautia_schinkii	
SPC00057	SPC10403	Dorea_longicatena	Blautia_schinkii	+++
SPC00008	SPC10403	Enterococcus_faecalis	Blautia_schinkii	++++
SPC10001	SPC10403	Erysipelotrichaceae_bacterium	Blautia_schinkii	---
SPC00001	SPC10403	Escherichia_coli	Blautia_schinkii	++++
SPC10110	SPC10403	Escherichia_coli	Blautia_schinkii	++++
SPC00022	SPC10403	Eubacterium_eligens	Blautia_schinkii	-
SPC10363	SPC10403	Eubacterium_rectale	Blautia_schinkii	+
SPC00054	SPC10403	Faecalibacterium_prausnitzii	Blautia_schinkii	
SPC10386	SPC10403	Faecalibacterium_prausnitzii	Blautia_schinkii	
SPC10390	SPC10403	Lachnospiraceae_bacterium_5_1_57FAA	Blautia_schinkii	
SPC00056	SPC10403	Odoribacter_splanchnicus	Blautia_schinkii	-
SPC10388	SPC10403	Odoribacter_splanchnicus	Blautia_schinkii	
SPC10048	SPC10403	Parabacteroides_merdae	Blautia_schinkii	
SPC00061	SPC10403	Roseburia_intestinalis	Blautia_schinkii	-
SPC10197	SPC10403	Ruminococcus_obaeum	Blautia_schinkii	
SPC10233	SPC10403	Ruminococcus_torques	Blautia_schinkii	
SPC00015	SPC10403	Streptococcus_thermophilus	Blautia_schinkii	
SPC10256	SPC10256	Clostridium_butyricum	Clostridium_butyricum	++++
SPC10167	SPC10256	Clostridium_disporicum	Clostridium_butyricum	++++
SPC10202	SPC10256	Clostridium_innocuum	Clostridium_butyricum	++++
SPC10238	SPC10256	Clostridium_mayombei	Clostridium_butyricum	++++
SPC10155	SPC10256	Clostridium_tertium	Clostridium_butyricum	++++
SPC10097	SPC10256	Collinsella_aerofaciens	Clostridium_butyricum	++++
SPC10304	SPC10256	Coprococcus_comes	Clostridium_butyricum	++++
SPC10256	SPC10358	Clostridium_butyricum	Clostridium_orbiscindens	++++
SPC10358	SPC10358	Clostridium_orbiscindens	Clostridium_orbiscindens	+
SPC10325	SPC10358	Clostridium_bolteae	Clostridium_orbiscindens	++++
SPC10167	SPC10358	Clostridium_disporicum	Clostridium_orbiscindens	++++
SPC10313	SPC10358	Clostridium_hylemonae	Clostridium_orbiscindens	

SPC1	SPC2	OTU1	OTU2	Result
SPC10202	SPC10358	Clostridium_innocuum	Clostridium orbiscindens	++++
SPC10238	SPC10358	Clostridium_mayombeii	Clostridium orbiscindens	++++
SPC10355	SPC10358	Clostridium_symbiosum	Clostridium orbiscindens	++++
SPC10155	SPC10358	Clostridium_tertium	Clostridium orbiscindens	++++
SPC10097	SPC10358	Collinsella_aerofaciens	Clostridium orbiscindens	++++
SPC10304	SPC10358	Coprococcus_comes	Clostridium orbiscindens	++++
SPC10386	SPC10358	Faecalibacterium_prausnitzii	Clostridium orbiscindens	
SPC10256	SPC10325	Clostridium_butyricum	Clostridium_bolteae	++++
SPC10325	SPC10325	Clostridium_bolteae	Clostridium_bolteae	++++
SPC10167	SPC10325	Clostridium_disporicum	Clostridium_bolteae	++++
SPC10313	SPC10325	Clostridium_hylemonae	Clostridium_bolteae	
SPC10202	SPC10325	Clostridium_innocuum	Clostridium_bolteae	++++
SPC10238	SPC10325	Clostridium_mayombeii	Clostridium_bolteae	++++
SPC10355	SPC10325	Clostridium_symbiosum	Clostridium_bolteae	++++
SPC10155	SPC10325	Clostridium_tertium	Clostridium_bolteae	++++
SPC10097	SPC10325	Collinsella_aerofaciens	Clostridium_bolteae	++++
SPC10304	SPC10325	Coprococcus_comes	Clostridium_bolteae	++++
SPC10167	SPC10167	Clostridium_disporicum	Clostridium_disporicum	+++
SPC10202	SPC10167	Clostridium_innocuum	Clostridium_disporicum	+++
SPC10155	SPC10167	Clostridium_tertium	Clostridium_disporicum	++++
SPC10097	SPC10167	Collinsella_aerofaciens	Clostridium_disporicum	-
SPC10211	SPC10243	Bacteroides_caccae	Clostridium_hathewayi	++++
SPC10213	SPC10243	Bacteroides_eggerthii	Clostridium_hathewayi	++++
SPC10030	SPC10243	Bacteroides_ovatus	Clostridium_hathewayi	++++
SPC00006	SPC10243	Bacteroides_sp_1_1_6	Clostridium_hathewayi	++++
SPC00007	SPC10243	Bacteroides_sp_3_1_23	Clostridium_hathewayi	++++
SPC10019	SPC10243	Bacteroides_sp_D20	Clostridium_hathewayi	++++
SPC00005	SPC10243	Bacteroides_vulgatus	Clostridium_hathewayi	++++
SPC10081	SPC10243	Bacteroides_vulgatus	Clostridium_hathewayi	++++
SPC00021	SPC10243	Blautia_producta	Clostridium_hathewayi	++++
SPC10243	SPC10243	Clostridium_hathewayi	Clostridium_hathewayi	++++
SPC00026	SPC10243	Clostridium_nexile	Clostridium_hathewayi	
SPC00027	SPC10243	Clostridium_sp_HGF2	Clostridium_hathewayi	
SPC10097	SPC10243	Collinsella_aerofaciens	Clostridium_hathewayi	++++
SPC00009	SPC10243	Coprobacillus_sp_D7	Clostridium_hathewayi	++++
SPC00080	SPC10243	Coprococcus_catus	Clostridium_hathewayi	+++
SPC00018	SPC10243	Dorea_formicigenerans	Clostridium_hathewayi	++++
SPC00057	SPC10243	Dorea_longicatena	Clostridium_hathewayi	+
SPC00008	SPC10243	Enterococcus_faecalis	Clostridium_hathewayi	++++
SPC10001	SPC10243	Erysipelotrichaceae_bacterium	Clostridium_hathewayi	++++
SPC00001	SPC10243	Escherichia_coli	Clostridium_hathewayi	++++
SPC10110	SPC10243	Escherichia_coli	Clostridium_hathewayi	++++

SPC1	SPC2	OTU1	OTU2	Result
SPC00022	SPC10243	Eubacterium_eligens	Clostridium_hathewayi	+
SPC00054	SPC10243	Faecalibacterium_prausnitzii	Clostridium_hathewayi	
SPC00056	SPC10243	Odoribacter_splanchnicus	Clostridium_hathewayi	
SPC10048	SPC10243	Parabacteroides_merdae	Clostridium_hathewayi	+
SPC00061	SPC10243	Roseburia_intestinalis	Clostridium_hathewayi	+++
SPC10197	SPC10243	Ruminococcus_obeum	Clostridium_hathewayi	++++
SPC10233	SPC10243	Ruminococcus_torques	Clostridium_hathewayi	++++
SPC00015	SPC10243	Streptococcus_thermophilus	Clostridium_hathewayi	++
SPC10256	SPC10313	Clostridium_butyricum	Clostridium_hylemonae	++++
SPC10325	SPC10313	Clostridium_bolteae	Clostridium_hylemonae	
SPC10167	SPC10313	Clostridium_disporicum	Clostridium_hylemonae	
SPC10313	SPC10313	Clostridium_hylemonae	Clostridium_hylemonae	
SPC10202	SPC10313	Clostridium_innocuum	Clostridium_hylemonae	++++
SPC10238	SPC10313	Clostridium_mayombeii	Clostridium_hylemonae	++++
SPC10155	SPC10313	Clostridium_tertium	Clostridium_hylemonae	++++
SPC10097	SPC10313	Collinsella_aerofaciens	Clostridium_hylemonae	+++
SPC10304	SPC10313	Coprococcus_comes	Clostridium_hylemonae	+
SPC10167	SPC10202	Clostridium_disporicum	Clostridium_innocuum	+++
SPC10202	SPC10202	Clostridium_innocuum	Clostridium_innocuum	++++
SPC10238	SPC10202	Clostridium_mayombeii	Clostridium_innocuum	++++
SPC10155	SPC10202	Clostridium_tertium	Clostridium_innocuum	++++
SPC10097	SPC10202	Collinsella_aerofaciens	Clostridium_innocuum	+++
SPC10256	SPC10238	Clostridium_butyricum	Clostridium_mayombeii	++++
SPC10167	SPC10238	Clostridium_disporicum	Clostridium_mayombeii	++++
SPC10202	SPC10238	Clostridium_innocuum	Clostridium_mayombeii	++++
SPC10238	SPC10238	Clostridium_mayombeii	Clostridium_mayombeii	++++
SPC10155	SPC10238	Clostridium_tertium	Clostridium_mayombeii	++++
SPC10097	SPC10238	Collinsella_aerofaciens	Clostridium_mayombeii	++++
SPC00006	SPC00026	Bacteroides_sp_1_1_6	Clostridium_nexile	++++
SPC00007	SPC00026	Bacteroides_sp_3_1_23	Clostridium_nexile	++++
SPC00005	SPC00026	Bacteroides_vulgatus	Clostridium_nexile	++++
SPC00021	SPC00026	Blautia_producta	Clostridium_nexile	++++
SPC00026	SPC00026	Clostridium_nexile	Clostridium_nexile	++
SPC00009	SPC00026	Coprobacillus_sp_D7	Clostridium_nexile	
SPC00018	SPC00026	Dorea_formicigenerans	Clostridium_nexile	
SPC00008	SPC00026	Enterococcus_faecalis	Clostridium_nexile	++++
SPC00001	SPC00026	Escherichia_coli	Clostridium_nexile	++++
SPC00022	SPC00026	Eubacterium_eligens	Clostridium_nexile	+
SPC00015	SPC00026	Streptococcus_thermophilus	Clostridium_nexile	+
SPC00006	SPC00027	Bacteroides_sp_1_1_6	Clostridium_sp_HGF2	++++
SPC00007	SPC00027	Bacteroides_sp_3_1_23	Clostridium_sp_HGF2	++++
SPC00005	SPC00027	Bacteroides_vulgatus	Clostridium_sp_HGF2	++

SPC1	SPC2	OTU1	OTU2	Result
SPC00021	SPC00027	Blautia_producta	Clostridium_sp_HGF2	++++
SPC00026	SPC00027	Clostridium_nexile	Clostridium_sp_HGF2	++++
SPC00027	SPC00027	Clostridium_sp_HGF2	Clostridium_sp_HGF2	++++
SPC00009	SPC00027	Coprobacillus_sp_D7	Clostridium_sp_HGF2	-
SPC00018	SPC00027	Dorea_formicigenerans	Clostridium_sp_HGF2	
SPC00008	SPC00027	Enterococcus_faecalis	Clostridium_sp_HGF2	++++
SPC00001	SPC00027	Escherichia_coli	Clostridium_sp_HGF2	++++
SPC00022	SPC00027	Eubacterium_eligens	Clostridium_sp_HGF2	
SPC00015	SPC00027	Streptococcus_thermophilus	Clostridium_sp_HGF2	+
SPC10211	SPC10355	Bacteroides_caccae	Clostridium_symbiosum	+++
SPC10213	SPC10355	Bacteroides_eggerthii	Clostridium_symbiosum	++++
SPC10030	SPC10355	Bacteroides_ovatus	Clostridium_symbiosum	
SPC00006	SPC10355	Bacteroides_sp_1_1_6	Clostridium_symbiosum	++++
SPC00007	SPC10355	Bacteroides_sp_3_1_23	Clostridium_symbiosum	++++
SPC10019	SPC10355	Bacteroides_sp_D20	Clostridium_symbiosum	
SPC00005	SPC10355	Bacteroides_vulgatus	Clostridium_symbiosum	+++
SPC10081	SPC10355	Bacteroides_vulgatus	Clostridium_symbiosum	
SPC10301	SPC10355	Bifidobacterium_adolescentis	Clostridium_symbiosum	++++
SPC10298	SPC10355	Bifidobacterium_pseudocatenulatum	Clostridium_symbiosum	+
SPC00021	SPC10355	Blautia_producta	Clostridium_symbiosum	++++
SPC10256	SPC10355	Clostridium_butyricum	Clostridium_symbiosum	++++
SPC10358	SPC10355	Clostridium_orbiscindens	Clostridium_symbiosum	++++
SPC10325	SPC10355	Clostridium_bolteae	Clostridium_symbiosum	++++
SPC10167	SPC10355	Clostridium_disporicum	Clostridium_symbiosum	++++
SPC10243	SPC10355	Clostridium_hathewayi	Clostridium_symbiosum	++++
SPC10313	SPC10355	Clostridium_hylemonae	Clostridium_symbiosum	+++
SPC10202	SPC10355	Clostridium_innocuum	Clostridium_symbiosum	++++
SPC10238	SPC10355	Clostridium_mayombeii	Clostridium_symbiosum	++++
SPC00026	SPC10355	Clostridium_nexile	Clostridium_symbiosum	+
SPC00027	SPC10355	Clostridium_sp_HGF2	Clostridium_symbiosum	
SPC10355	SPC10355	Clostridium_symbiosum	Clostridium_symbiosum	+
SPC10355	SPC10355	Clostridium_symbiosum	Clostridium_symbiosum	++++
SPC10155	SPC10355	Clostridium_tertium	Clostridium_symbiosum	+
SPC10097	SPC10355	Collinsella_aerofaciens	Clostridium_symbiosum	+++
SPC10097	SPC10355	Collinsella_aerofaciens	Clostridium_symbiosum	++++
SPC00009	SPC10355	Coprobacillus_sp_D7	Clostridium_symbiosum	
SPC00080	SPC10355	Coprococcus_catus	Clostridium_symbiosum	-
SPC10304	SPC10355	Coprococcus_comes	Clostridium_symbiosum	
SPC10304	SPC10355	Coprococcus_comes	Clostridium_symbiosum	++++
SPC00018	SPC10355	Dorea_formicigenerans	Clostridium_symbiosum	
SPC00057	SPC10355	Dorea_longicatena	Clostridium_symbiosum	++++

SPC1	SPC2	OTU1	OTU2	Result
SPC00008	SPC10355	Enterococcus_faecalis	Clostridium_symbiosum	++++
SPC10001	SPC10355	Erysipelotrichaceae_bacterium	Clostridium_symbiosum	
SPC00001	SPC10355	Escherichia_coli	Clostridium_symbiosum	++++
SPC10110	SPC10355	Escherichia_coli	Clostridium_symbiosum	++++
SPC00022	SPC10355	Eubacterium_eligens	Clostridium_symbiosum	+
SPC00054	SPC10355	Faecalibacterium_prausnitzii	Clostridium_symbiosum	
SPC00056	SPC10355	Odoribacter_splanchnicus	Clostridium_symbiosum	
SPC10048	SPC10355	Parabacteroides_merdae	Clostridium_symbiosum	-
SPC00061	SPC10355	Roseburia_intestinalis	Clostridium_symbiosum	--
SPC10197	SPC10355	Ruminococcus_obeum	Clostridium_symbiosum	++++
SPC10233	SPC10355	Ruminococcus_torques	Clostridium_symbiosum	++
SPC00015	SPC10355	Streptococcus_thermophilus	Clostridium_symbiosum	
SPC10167	SPC10155	Clostridium_disporicum	Clostridium_tertium	++++
SPC10155	SPC10155	Clostridium_tertium	Clostridium_tertium	++++
SPC10097	SPC10155	Collinsella_aerofaciens	Clostridium_tertium	
SPC10030	SPC10097	Bacteroides_ovatus	Collinsella_aerofaciens	++++
SPC00006	SPC10097	Bacteroides_sp_1_1_6	Collinsella_aerofaciens	++++
SPC00007	SPC10097	Bacteroides_sp_3_1_23	Collinsella_aerofaciens	++++
SPC10019	SPC10097	Bacteroides_sp_D20	Collinsella_aerofaciens	++++
SPC00005	SPC10097	Bacteroides_vulgatus	Collinsella_aerofaciens	++++
SPC10081	SPC10097	Bacteroides_vulgatus	Collinsella_aerofaciens	++++
SPC00021	SPC10097	Blautia_producta	Collinsella_aerofaciens	++++
SPC00026	SPC10097	Clostridium_nexile	Collinsella_aerofaciens	+
SPC00027	SPC10097	Clostridium_sp_HGF2	Collinsella_aerofaciens	++++
SPC10155	SPC10097	Clostridium_tertium	Collinsella_aerofaciens	
SPC10097	SPC10097	Collinsella_aerofaciens	Collinsella_aerofaciens	++++
SPC10097	SPC10097	Collinsella_aerofaciens	Collinsella_aerofaciens	
SPC00009	SPC10097	Coprobacillus_sp_D7	Collinsella_aerofaciens	+++
SPC00080	SPC10097	Coprococcus_catus	Collinsella_aerofaciens	++++
SPC00018	SPC10097	Dorea_formicigenerans	Collinsella_aerofaciens	++
SPC00057	SPC10097	Dorea_longicatena	Collinsella_aerofaciens	++++
SPC00008	SPC10097	Enterococcus_faecalis	Collinsella_aerofaciens	++++
SPC10001	SPC10097	Erysipelotrichaceae_bacterium	Collinsella_aerofaciens	++++
SPC00001	SPC10097	Escherichia_coli	Collinsella_aerofaciens	++++
SPC00022	SPC10097	Eubacterium_eligens	Collinsella_aerofaciens	+++
SPC00054	SPC10097	Faecalibacterium_prausnitzii	Collinsella_aerofaciens	+++
SPC00056	SPC10097	Odoribacter_splanchnicus	Collinsella_aerofaciens	+++
SPC10048	SPC10097	Parabacteroides_merdae	Collinsella_aerofaciens	++++
SPC00061	SPC10097	Roseburia_intestinalis	Collinsella_aerofaciens	++
SPC00015	SPC10097	Streptococcus_thermophilus	Collinsella_aerofaciens	+
SPC00006	SPC00009	Bacteroides_sp_1_1_6	Coprobacillus_sp_D7	+++
SPC00007	SPC00009	Bacteroides_sp_3_1_23	Coprobacillus_sp_D7	

SPC1	SPC2	OTU1	OTU2	Result
SPC00005	SPC00009	Bacteroides_vulgatus	Coprobacillus_sp_D7	+
SPC00009	SPC00009	Coprobacillus_sp_D7	Coprobacillus_sp_D7	-
SPC00008	SPC00009	Enterococcus_faecalis	Coprobacillus_sp_D7	++++
SPC00001	SPC00009	Escherichia_coli	Coprobacillus_sp_D7	++
SPC00006	SPC00080	Bacteroides_sp_1_1_6	Coprococcus_catus	++++
SPC00007	SPC00080	Bacteroides_sp_3_1_23	Coprococcus_catus	
SPC00005	SPC00080	Bacteroides_vulgatus	Coprococcus_catus	+
SPC00021	SPC00080	Blautia_producta	Coprococcus_catus	++++
SPC00026	SPC00080	Clostridium_nexile	Coprococcus_catus	
SPC00027	SPC00080	Clostridium_sp_HGF2	Coprococcus_catus	---
SPC00009	SPC00080	Coprobacillus_sp_D7	Coprococcus_catus	---
SPC00080	SPC00080	Coprococcus_catus	Coprococcus_catus	
SPC00018	SPC00080	Dorea_formicigenerans	Coprococcus_catus	
SPC00057	SPC00080	Dorea_longicatena	Coprococcus_catus	
SPC00008	SPC00080	Enterococcus_faecalis	Coprococcus_catus	++++
SPC00001	SPC00080	Escherichia_coli	Coprococcus_catus	++++
SPC00022	SPC00080	Eubacterium_eligens	Coprococcus_catus	
SPC00054	SPC00080	Faecalibacterium_prausnitzii	Coprococcus_catus	
SPC00056	SPC00080	Odoribacter_splanchnicus	Coprococcus_catus	
SPC00061	SPC00080	Roseburia_intestinalis	Coprococcus_catus	
SPC00015	SPC00080	Streptococcus_thermophilus	Coprococcus_catus	
SPC10211	SPC10304	Bacteroides_caccae	Coprococcus_comes	+++
SPC10213	SPC10304	Bacteroides_eggerthii	Coprococcus_comes	+++
SPC10030	SPC10304	Bacteroides_ovatus	Coprococcus_comes	
SPC00006	SPC10304	Bacteroides_sp_1_1_6	Coprococcus_comes	+++
SPC00007	SPC10304	Bacteroides_sp_3_1_23	Coprococcus_comes	++++
SPC10019	SPC10304	Bacteroides_sp_D20	Coprococcus_comes	
SPC00005	SPC10304	Bacteroides_vulgatus	Coprococcus_comes	++++
SPC10081	SPC10304	Bacteroides_vulgatus	Coprococcus_comes	
SPC10301	SPC10304	Bifidobacterium_adolescentis	Coprococcus_comes	++++
SPC10298	SPC10304	Bifidobacterium_pseudocatenulatum	Coprococcus_comes	++++
SPC00021	SPC10304	Blautia_producta	Coprococcus_comes	++++
SPC10256	SPC10304	Clostridium_butyricum	Coprococcus_comes	++++
SPC10167	SPC10304	Clostridium_disporicum	Coprococcus_comes	++++
SPC10243	SPC10304	Clostridium_hathewayi	Coprococcus_comes	++++
SPC10313	SPC10304	Clostridium_hylemonae	Coprococcus_comes	+
SPC10202	SPC10304	Clostridium_innocuum	Coprococcus_comes	++++
SPC10238	SPC10304	Clostridium_mayombei	Coprococcus_comes	++++
SPC00026	SPC10304	Clostridium_nexile	Coprococcus_comes	
SPC00027	SPC10304	Clostridium_sp_HGF2	Coprococcus_comes	
SPC10155	SPC10304	Clostridium_tertium	Coprococcus_comes	++++

SPC1	SPC2	OTU1	OTU2	Result
SPC10097	SPC10304	Collinsella_aerofaciens	Coprococcus_comes	++++
SPC10097	SPC10304	Collinsella_aerofaciens	Coprococcus_comes	+++
SPC00009	SPC10304	Coprobacillus_sp_D7	Coprococcus_comes	+++
SPC00080	SPC10304	Coprococcus_catus	Coprococcus_comes	--
SPC10304	SPC10304	Coprococcus_comes	Coprococcus_comes	
SPC10304	SPC10304	Coprococcus_comes	Coprococcus_comes	++
SPC00018	SPC10304	Dorea_formicigenerans	Coprococcus_comes	
SPC00057	SPC10304	Dorea_longicatena	Coprococcus_comes	
SPC00008	SPC10304	Enterococcus_faecalis	Coprococcus_comes	++++
SPC10001	SPC10304	Erysipelotrichaceae_bacterium	Coprococcus_comes	-
SPC00001	SPC10304	Escherichia_coli	Coprococcus_comes	++++
SPC10110	SPC10304	Escherichia_coli	Coprococcus_comes	++++
SPC00022	SPC10304	Eubacterium_eligens	Coprococcus_comes	++
SPC00054	SPC10304	Faecalibacterium_prausnitzii	Coprococcus_comes	
SPC00056	SPC10304	Odoribacter_splanchnicus	Coprococcus_comes	
SPC10048	SPC10304	Parabacteroides_merdae	Coprococcus_comes	-
SPC00061	SPC10304	Roseburia_intestinalis	Coprococcus_comes	-
SPC10197	SPC10304	Ruminococcus_obesum	Coprococcus_comes	++++
SPC10233	SPC10304	Ruminococcus_torques	Coprococcus_comes	++++
SPC00015	SPC10304	Streptococcus_thermophilus	Coprococcus_comes	++
SPC00006	SPC00018	Bacteroides_sp_1_1_6	Dorea_formicigenerans	+++
SPC00007	SPC00018	Bacteroides_sp_3_1_23	Dorea_formicigenerans	
SPC00005	SPC00018	Bacteroides_vulgatus	Dorea_formicigenerans	++
SPC00009	SPC00018	Coprobacillus_sp_D7	Dorea_formicigenerans	-
SPC00018	SPC00018	Dorea_formicigenerans	Dorea_formicigenerans	--
SPC00008	SPC00018	Enterococcus_faecalis	Dorea_formicigenerans	++++
SPC00001	SPC00018	Escherichia_coli	Dorea_formicigenerans	++
SPC00015	SPC00018	Streptococcus_thermophilus	Dorea_formicigenerans	
SPC00006	SPC00057	Bacteroides_sp_1_1_6	Dorea_longicatena	++++
SPC00007	SPC00057	Bacteroides_sp_3_1_23	Dorea_longicatena	+++
SPC00005	SPC00057	Bacteroides_vulgatus	Dorea_longicatena	++++
SPC00021	SPC00057	Blautia_producta	Dorea_longicatena	++++
SPC00026	SPC00057	Clostridium_nexile	Dorea_longicatena	
SPC00027	SPC00057	Clostridium_sp_HGF2	Dorea_longicatena	--
SPC00009	SPC00057	Coprobacillus_sp_D7	Dorea_longicatena	
SPC00018	SPC00057	Dorea_formicigenerans	Dorea_longicatena	++
SPC00057	SPC00057	Dorea_longicatena	Dorea_longicatena	-
SPC00008	SPC00057	Enterococcus_faecalis	Dorea_longicatena	++++
SPC00001	SPC00057	Escherichia_coli	Dorea_longicatena	++++
SPC00022	SPC00057	Eubacterium_eligens	Dorea_longicatena	++
SPC00054	SPC00057	Faecalibacterium_prausnitzii	Dorea_longicatena	-
SPC00056	SPC00057	Odoribacter_splanchnicus	Dorea_longicatena	

SPC1	SPC2	OTU1	OTU2	Result
SPC00015	SPC00057	Streptococcus_thermophilus	Dorea_longicatena	+
SPC00006	SPC00008	Bacteroides_sp_1_1_6	Enterococcus_faecalis	++++
SPC00007	SPC00008	Bacteroides_sp_3_1_23	Enterococcus_faecalis	++++
SPC00005	SPC00008	Bacteroides_vulgatus	Enterococcus_faecalis	++++
SPC00008	SPC00008	Enterococcus_faecalis	Enterococcus_faecalis	++++
SPC00001	SPC00008	Escherichia_coli	Enterococcus_faecalis	++++
SPC00006	SPC10001	Bacteroides_sp_1_1_6	Erysipelotrichaceae_bacterium	++++
SPC00007	SPC10001	Bacteroides_sp_3_1_23	Erysipelotrichaceae_bacterium	
SPC00005	SPC10001	Bacteroides_vulgatus	Erysipelotrichaceae_bacterium	+
SPC00021	SPC10001	Blautia_producta	Erysipelotrichaceae_bacterium	++++
SPC00026	SPC10001	Clostridium_nexile	Erysipelotrichaceae_bacterium	
SPC00027	SPC10001	Clostridium_sp_HGF2	Erysipelotrichaceae_bacterium	--
SPC00009	SPC10001	Coprobacillus_sp_D7	Erysipelotrichaceae_bacterium	-
SPC00080	SPC10001	Coprococcus_catus	Erysipelotrichaceae_bacterium	
SPC00018	SPC10001	Dorea_formicigenerans	Erysipelotrichaceae_bacterium	--
SPC00057	SPC10001	Dorea_longicatena	Erysipelotrichaceae_bacterium	
SPC00008	SPC10001	Enterococcus_faecalis	Erysipelotrichaceae_bacterium	++++
SPC10001	SPC10001	Erysipelotrichaceae_bacterium	Erysipelotrichaceae_bacterium	-
SPC00001	SPC10001	Escherichia_coli	Erysipelotrichaceae_bacterium	++++
SPC00022	SPC10001	Eubacterium_eligens	Erysipelotrichaceae_bacterium	-
SPC00054	SPC10001	Faecalibacterium_prausnitzii	Erysipelotrichaceae_bacterium	-
SPC00056	SPC10001	Odoribacter_splanchnicus	Erysipelotrichaceae_bacterium	
SPC00061	SPC10001	Roseburia_intestinalis	Erysipelotrichaceae_bacterium	-
SPC00015	SPC10001	Streptococcus_thermophilus	Erysipelotrichaceae_bacterium	
SPC10030	SPC10110	Bacteroides_ovatus	Escherichia_coli	++++
SPC00006	SPC10110	Bacteroides_sp_1_1_6	Escherichia_coli	++++
SPC00007	SPC10110	Bacteroides_sp_3_1_23	Escherichia_coli	++++
SPC10019	SPC10110	Bacteroides_sp_D20	Escherichia_coli	++++
SPC00005	SPC10110	Bacteroides_vulgatus	Escherichia_coli	++++
SPC10081	SPC10110	Bacteroides_vulgatus	Escherichia_coli	++++
SPC00021	SPC10110	Blautia_producta	Escherichia_coli	++++
SPC00026	SPC10110	Clostridium_nexile	Escherichia_coli	++++

SPC1	SPC2	OTU1	OTU2	Result
SPC00027	SPC10110	Clostridium_sp_HGF2	Escherichia_coli	++++
SPC10097	SPC10110	Collinsella_aerofaciens	Escherichia_coli	++++
SPC00009	SPC10110	Coprobacillus_sp_D7	Escherichia_coli	++
SPC00080	SPC10110	Coprococcus_catus	Escherichia_coli	++++
SPC00018	SPC10110	Dorea_formicigenerans	Escherichia_coli	++++
SPC00057	SPC10110	Dorea_longicatena	Escherichia_coli	++++
SPC00008	SPC10110	Enterococcus_faecalis	Escherichia_coli	++++
SPC10001	SPC10110	Erysipelotrichaceae_bacterium	Escherichia_coli	++++
SPC00001	SPC00001	Escherichia_coli	Escherichia_coli	++++
SPC00001	SPC10110	Escherichia_coli	Escherichia_coli	++++
SPC10110	SPC10110	Escherichia_coli	Escherichia_coli	++++
SPC00022	SPC10110	Eubacterium_eligens	Escherichia_coli	++++
SPC00054	SPC10110	Faecalibacterium_prausnitzii	Escherichia_coli	+++
SPC00056	SPC10110	Odoribacter_splanchnicus	Escherichia_coli	+++
SPC10048	SPC10110	Parabacteroides_merdae	Escherichia_coli	++++
SPC00061	SPC10110	Roseburia_intestinalis	Escherichia_coli	+++
SPC00015	SPC10110	Streptococcus_thermophilus	Escherichia_coli	+++
SPC00006	SPC00022	Bacteroides_sp_1_1_6	Eubacterium_eligens	++++
SPC00007	SPC00022	Bacteroides_sp_3_1_23	Eubacterium_eligens	
SPC00005	SPC00022	Bacteroides_vulgatus	Eubacterium_eligens	+++
SPC00021	SPC00022	Blautia_producta	Eubacterium_eligens	++++
SPC00009	SPC00022	Coprobacillus_sp_D7	Eubacterium_eligens	
SPC00018	SPC00022	Dorea_formicigenerans	Eubacterium_eligens	--
SPC00008	SPC00022	Enterococcus_faecalis	Eubacterium_eligens	++++
SPC00001	SPC00022	Escherichia_coli	Eubacterium_eligens	++
SPC00022	SPC00022	Eubacterium_eligens	Eubacterium_eligens	
SPC00015	SPC00022	Streptococcus_thermophilus	Eubacterium_eligens	
SPC10211	SPC10363	Bacteroides_caccae	Eubacterium_rectale	
SPC10213	SPC10363	Bacteroides_eggerthii	Eubacterium_rectale	
SPC10030	SPC10363	Bacteroides_ovatus	Eubacterium_rectale	
SPC00006	SPC10363	Bacteroides_sp_1_1_6	Eubacterium_rectale	++++
SPC00007	SPC10363	Bacteroides_sp_3_1_23	Eubacterium_rectale	+++
SPC10019	SPC10363	Bacteroides_sp_D20	Eubacterium_rectale	--
SPC00005	SPC10363	Bacteroides_vulgatus	Eubacterium_rectale	++++
SPC10081	SPC10363	Bacteroides_vulgatus	Eubacterium_rectale	
SPC10301	SPC10363	Bifidobacterium_adolescentis	Eubacterium_rectale	++++
SPC10298	SPC10363	Bifidobacterium_pseudocatenulatum	Eubacterium_rectale	
SPC00021	SPC10363	Blautia_producta	Eubacterium_rectale	++++
SPC10415	SPC10567	Blautia_producta	Eubacterium_rectale	++++
SPC10256	SPC10567	Clostridium_butyricum	Eubacterium_rectale	++++
SPC10358	SPC10567	Clostridium_orbiscindens	Eubacterium_rectale	+

SPC1	SPC2	OTU1	OTU2	Result
SPC10325	SPC10567	Clostridium_bolteae	Eubacterium_rectale	++
SPC10167	SPC10567	Clostridium_disporicum	Eubacterium_rectale	++++
SPC10243	SPC10363	Clostridium_hathewayi	Eubacterium_rectale	++++
SPC10313	SPC10567	Clostridium_hylemonae	Eubacterium_rectale	
SPC10202	SPC10567	Clostridium_innocuum	Eubacterium_rectale	++++
SPC10238	SPC10567	Clostridium_mayombeii	Eubacterium_rectale	++++
SPC00026	SPC10363	Clostridium_nexile	Eubacterium_rectale	-
SPC00027	SPC10363	Clostridium_sp_HGF2	Eubacterium_rectale	--
SPC10355	SPC10363	Clostridium_symbiosum	Eubacterium_rectale	++
SPC10355	SPC10567	Clostridium_symbiosum	Eubacterium_rectale	+
SPC10155	SPC10567	Clostridium_tertium	Eubacterium_rectale	++++
SPC10097	SPC10363	Collinsella_aerofaciens	Eubacterium_rectale	++++
SPC10097	SPC10567	Collinsella_aerofaciens	Eubacterium_rectale	++++
SPC00009	SPC10363	Coprobaecillus_sp_D7	Eubacterium_rectale	+++
SPC00080	SPC10363	Coprococcus_catus	Eubacterium_rectale	---
SPC10304	SPC10363	Coprococcus_comes	Eubacterium_rectale	+
SPC10304	SPC10567	Coprococcus_comes	Eubacterium_rectale	++++
SPC00018	SPC10363	Dorea_formicigenerans	Eubacterium_rectale	-
SPC00057	SPC10363	Dorea_longicatena	Eubacterium_rectale	++++
SPC00008	SPC10363	Enterococcus_faecalis	Eubacterium_rectale	++++
SPC10001	SPC10363	Erysipelotrichaceae_bacterium	Eubacterium_rectale	-
SPC00001	SPC10363	Escherichia_coli	Eubacterium_rectale	++++
SPC10110	SPC10363	Escherichia_coli	Eubacterium_rectale	++++
SPC00022	SPC10363	Eubacterium_eligens	Eubacterium_rectale	
SPC10363	SPC10363	Eubacterium_rectale	Eubacterium_rectale	+++
SPC10567	SPC10567	Eubacterium_rectale	Eubacterium_rectale	
SPC00054	SPC10363	Faecalibacterium_prausnitzii	Eubacterium_rectale	--
SPC10386	SPC10567	Faecalibacterium_prausnitzii	Eubacterium_rectale	
SPC10390	SPC10567	Lachnospiraceae_bacterium_5_1_5_7FAA	Eubacterium_rectale	+++
SPC00056	SPC10363	Odoribacter_splanchnicus	Eubacterium_rectale	-
SPC10048	SPC10363	Parabacteroides_merdae	Eubacterium_rectale	-
SPC00061	SPC10363	Roseburia_intestinalis	Eubacterium_rectale	----
SPC10470	SPC10567	Ruminococcus_bromii	Eubacterium_rectale	+
SPC10468	SPC10567	Ruminococcus_gnavus	Eubacterium_rectale	++++
SPC10197	SPC10363	Ruminococcus_obesum	Eubacterium_rectale	++
SPC10233	SPC10363	Ruminococcus_torques	Eubacterium_rectale	+
SPC00015	SPC10363	Streptococcus_thermophilus	Eubacterium_rectale	
SPC10211	SPC10386	Bacteroides_caccae	Faecalibacterium_prausnitzii	
SPC10213	SPC10386	Bacteroides_eggerthii	Faecalibacterium_prausnitzii	-
SPC10030	SPC10386	Bacteroides_ovatus	Faecalibacterium_prausnitzii	-

SPC1	SPC2	OTU1	OTU2	Result
SPC00006	SPC00054	Bacteroides_sp_1_1_6	Faecalibacterium_prausnitzii	++++
SPC00006	SPC10386	Bacteroides_sp_1_1_6	Faecalibacterium_prausnitzii	+++
SPC00007	SPC00054	Bacteroides_sp_3_1_23	Faecalibacterium_prausnitzii	++
SPC00007	SPC10386	Bacteroides_sp_3_1_23	Faecalibacterium_prausnitzii	
SPC10019	SPC10386	Bacteroides_sp_D20	Faecalibacterium_prausnitzii	--
SPC00005	SPC00054	Bacteroides_vulgatus	Faecalibacterium_prausnitzii	++++
SPC00005	SPC10386	Bacteroides_vulgatus	Faecalibacterium_prausnitzii	+++
SPC10081	SPC10386	Bacteroides_vulgatus	Faecalibacterium_prausnitzii	---
SPC10301	SPC10386	Bifidobacterium_adolescentis	Faecalibacterium_prausnitzii	+
SPC10298	SPC10386	Bifidobacterium_pseudocatenulatum	Faecalibacterium_prausnitzii	
SPC00021	SPC00054	Blautia_producta	Faecalibacterium_prausnitzii	++++
SPC00021	SPC10386	Blautia_producta	Faecalibacterium_prausnitzii	++++
SPC10256	SPC10386	Clostridium_butyricum	Faecalibacterium_prausnitzii	++++
SPC10358	SPC10386	Clostridium_orbiscindens	Faecalibacterium_prausnitzii	
SPC10325	SPC10386	Clostridium_bolteae	Faecalibacterium_prausnitzii	++
SPC10167	SPC10386	Clostridium_disporicum	Faecalibacterium_prausnitzii	
SPC10243	SPC10386	Clostridium_hathewayi	Faecalibacterium_prausnitzii	+++
SPC10313	SPC10386	Clostridium_hylemonae	Faecalibacterium_prausnitzii	
SPC10202	SPC10386	Clostridium_innocuum	Faecalibacterium_prausnitzii	++++
SPC10238	SPC10386	Clostridium_mayombeii	Faecalibacterium_prausnitzii	++++
SPC00026	SPC00054	Clostridium_nexile	Faecalibacterium_prausnitzii	
SPC00026	SPC10386	Clostridium_nexile	Faecalibacterium_prausnitzii	-
SPC00027	SPC00054	Clostridium_sp_HGF2	Faecalibacterium_prausnitzii	++
SPC00027	SPC10386	Clostridium_sp_HGF2	Faecalibacterium_prausnitzii	--
SPC10355	SPC10386	Clostridium_symbiosum	Faecalibacterium_prausnitzii	+++
SPC10355	SPC10386	Clostridium_symbiosum	Faecalibacterium_prausnitzii	++++
SPC10155	SPC10386	Clostridium_tertium	Faecalibacterium_prausnitzii	++++

SPC1	SPC2	OTU1	OTU2	Result
			Faecalibacterium_prausnitzii	
SPC10097	SPC10386	Collinsella_aerofaciens	i	++++
SPC10097	SPC10386	Collinsella_aerofaciens	i	++++
SPC00009	SPC00054	Coprobacillus_sp_D7	i	---
SPC00009	SPC10386	Coprobacillus_sp_D7	i	
SPC00080	SPC10386	Coprococcus_catus	i	---
SPC10304	SPC10386	Coprococcus_comes	i	
SPC10304	SPC10386	Coprococcus_comes	i	+++
SPC00018	SPC00054	Dorea_formicigenerans	i	
SPC00018	SPC10386	Dorea_formicigenerans	i	---
SPC00057	SPC10386	Dorea_longicatena	i	+++
SPC00008	SPC00054	Enterococcus_faecalis	i	++++
SPC00008	SPC10386	Enterococcus_faecalis	i	++++
SPC10001	SPC10386	Erysipelotrichaceae_bacterium	i	--
SPC00001	SPC00054	Escherichia_coli	i	++++
SPC00001	SPC10386	Escherichia_coli	i	++++
SPC10110	SPC10386	Escherichia_coli	i	++
SPC00022	SPC00054	Eubacterium_eligens	i	
SPC00022	SPC10386	Eubacterium_eligens	i	
SPC10363	SPC10386	Eubacterium_rectale	i	+
SPC00054	SPC00054	Faecalibacterium_prausnitzii	i	+
SPC00054	SPC10386	Faecalibacterium_prausnitzii	i	
SPC10386	SPC10386	Faecalibacterium_prausnitzii	i	+
SPC10386	SPC10386	Faecalibacterium_prausnitzii	i	
SPC10390	SPC10386	Lachnospiraceae_bacterium_5_1_5_7FAA	i	++++
SPC00056	SPC10386	Odoribacter_splanchnicus	i	--
SPC10048	SPC10386	Parabacteroides_merdae	i	-
SPC00061	SPC10386	Roseburia_intestinalis	i	

SPC1	SPC2	OTU1	OTU2	Result
SPC10197	SPC10386	Ruminococcus_obeum	Faecalibacterium_prausnitzii	
SPC10233	SPC10386	Ruminococcus_torques	Faecalibacterium_prausnitzii	
SPC00015	SPC00054	Streptococcus_thermophilus	Faecalibacterium_prausnitzii	
SPC00015	SPC10386	Streptococcus_thermophilus	Faecalibacterium_prausnitzii	
SPC10211	SPC10390	Bacteroides_caccae	Lachnospiraceae_bacterium_5_1_57FAA	
SPC10213	SPC10390	Bacteroides_eggerthii	Lachnospiraceae_bacterium_5_1_57FAA	
SPC10030	SPC10390	Bacteroides_ovatus	Lachnospiraceae_bacterium_5_1_57FAA	-
SPC00006	SPC10390	Bacteroides_sp_1_1_6	Lachnospiraceae_bacterium_5_1_57FAA	+++
SPC00007	SPC10390	Bacteroides_sp_3_1_23	Lachnospiraceae_bacterium_5_1_57FAA	
SPC10019	SPC10390	Bacteroides_sp_D20	Lachnospiraceae_bacterium_5_1_57FAA	---
SPC00005	SPC10390	Bacteroides_vulgatus	Lachnospiraceae_bacterium_5_1_57FAA	+++
SPC10081	SPC10390	Bacteroides_vulgatus	Lachnospiraceae_bacterium_5_1_57FAA	--
SPC10301	SPC10390	Bifidobacterium_adolescentis	Lachnospiraceae_bacterium_5_1_57FAA	++++
SPC10298	SPC10390	Bifidobacterium_pseudocatenulatum	Lachnospiraceae_bacterium_5_1_57FAA	
SPC00021	SPC10390	Blautia_producta	Lachnospiraceae_bacterium_5_1_57FAA	++++
SPC10415	SPC10390	Blautia_producta	Lachnospiraceae_bacterium_5_1_57FAA	++++
SPC10256	SPC10390	Clostridium_butyricum	Lachnospiraceae_bacterium_5_1_57FAA	++++
SPC10358	SPC10390	Clostridium_orbiscindens	Lachnospiraceae_bacterium_5_1_57FAA	++++
SPC10325	SPC10390	Clostridiumbolteae	Lachnospiraceae_bacterium_5_1_57FAA	++++
SPC10167	SPC10390	Clostridium_disporicum	Lachnospiraceae_bacterium_5_1_57FAA	++++
SPC10243	SPC10390	Clostridium_hathewayi	Lachnospiraceae_bacterium_5_1_57FAA	++++
SPC10313	SPC10390	Clostridium_hylemonae	Lachnospiraceae_bacterium_5_1_57FAA	++++
SPC10202	SPC10390	Clostridium_innocuum	Lachnospiraceae_bacterium_5_1_57FAA	++++
SPC10238	SPC10390	Clostridium_mayombeii	Lachnospiraceae_bacterium_5_1_57FAA	++++
SPC00026	SPC10390	Clostridium_nexile	Lachnospiraceae_bacterium_5_1_57FAA	-
SPC00027	SPC10390	Clostridium_sp_HGF2	Lachnospiraceae_bacterium_5_1_57FAA	-
SPC10355	SPC10390	Clostridium_symbiosum	Lachnospiraceae_bacterium_5_1_57FAA	+

SPC1	SPC2	OTU1	OTU2	Result
			Lachnospiraceae_bacterium	
SPC10355	SPC10390	Clostridium_symbiosum	_5_1_57FAA	++++
			Lachnospiraceae_bacterium	
SPC10155	SPC10390	Clostridium_tertium	_5_1_57FAA	++++
			Lachnospiraceae_bacterium	
SPC10097	SPC10390	Collinsella_aerofaciens	_5_1_57FAA	++++
			Lachnospiraceae_bacterium	
SPC10097	SPC10390	Collinsella_aerofaciens	_5_1_57FAA	++++
			Lachnospiraceae_bacterium	
SPC00009	SPC10390	Coprobacillus_sp_D7	_5_1_57FAA	++++
			Lachnospiraceae_bacterium	
SPC00080	SPC10390	Coprococcus_catus	_5_1_57FAA	
			Lachnospiraceae_bacterium	
SPC10304	SPC10390	Coprococcus_comes	_5_1_57FAA	
			Lachnospiraceae_bacterium	
SPC10304	SPC10390	Coprococcus_comes	_5_1_57FAA	++++
			Lachnospiraceae_bacterium	
SPC00018	SPC10390	Dorea_formicigenerans	_5_1_57FAA	--
			Lachnospiraceae_bacterium	
SPC00057	SPC10390	Dorea_longicatena	_5_1_57FAA	++++
			Lachnospiraceae_bacterium	
SPC00008	SPC10390	Enterococcus_faecalis	_5_1_57FAA	++++
			Lachnospiraceae_bacterium	
SPC10001	SPC10390	Erysipelotrichaceae_bacterium	_5_1_57FAA	---
			Lachnospiraceae_bacterium	
SPC00001	SPC10390	Escherichia_coli	_5_1_57FAA	++++
			Lachnospiraceae_bacterium	
SPC10110	SPC10390	Escherichia_coli	_5_1_57FAA	++++
			Lachnospiraceae_bacterium	
SPC00022	SPC10390	Eubacterium_eligens	_5_1_57FAA	
			Lachnospiraceae_bacterium	
SPC10363	SPC10390	Eubacterium_rectale	_5_1_57FAA	
			Lachnospiraceae_bacterium	
SPC00054	SPC10390	Faecalibacterium_prausnitzii	_5_1_57FAA	
			Lachnospiraceae_bacterium	
SPC10386	SPC10390	Faecalibacterium_prausnitzii	_5_1_57FAA	
			Lachnospiraceae_bacterium	
SPC10386	SPC10390	Faecalibacterium_prausnitzii	_5_1_57FAA	++++
			Lachnospiraceae_bacterium_5_1_57FAA	
SPC10390	SPC10390	Lachnospiraceae_bacterium_5_1_57FAA	_5_1_57FAA	
			Lachnospiraceae_bacterium_5_1_57FAA	
SPC10390	SPC10390	Lachnospiraceae_bacterium_5_1_57FAA	_5_1_57FAA	++++
			Lachnospiraceae_bacterium_5_1_57FAA	
SPC00056	SPC10390	Odoribacter_splanchnicus	_5_1_57FAA	--
			Lachnospiraceae_bacterium_5_1_57FAA	
SPC10388	SPC10390	Odoribacter_splanchnicus	_5_1_57FAA	
			Lachnospiraceae_bacterium_5_1_57FAA	
SPC10048	SPC10390	Parabacteroides_merdae	_5_1_57FAA	-
			Lachnospiraceae_bacterium_5_1_57FAA	
SPC00061	SPC10390	Roseburia_intestinalis	_5_1_57FAA	
			Lachnospiraceae_bacterium_5_1_57FAA	
SPC10197	SPC10390	Ruminococcus_obeum	_5_1_57FAA	
			Lachnospiraceae_bacterium_5_1_57FAA	
SPC10233	SPC10390	Ruminococcus_torques	_5_1_57FAA	

SPC1	SPC2	OTU1	OTU2	Result
			Lachnospiraceae_bacterium	
SPC00015	SPC10390	Streptococcus_thermophilus	_5_1_57FAA	
SPC10211	SPC10388	Bacteroides_caccae	Odoribacter_splanchnicus	
SPC10213	SPC10388	Bacteroides_eggerthii	Odoribacter_splanchnicus	-
SPC10030	SPC10388	Bacteroides_ovatus	Odoribacter_splanchnicus	--
SPC00006	SPC00056	Bacteroides_sp_1_1_6	Odoribacter_splanchnicus	++++
SPC00006	SPC10388	Bacteroides_sp_1_1_6	Odoribacter_splanchnicus	+
SPC00007	SPC00056	Bacteroides_sp_3_1_23	Odoribacter_splanchnicus	+
SPC00007	SPC10388	Bacteroides_sp_3_1_23	Odoribacter_splanchnicus	
SPC10019	SPC10388	Bacteroides_sp_D20	Odoribacter_splanchnicus	---
SPC00005	SPC00056	Bacteroides_vulgatus	Odoribacter_splanchnicus	+++
SPC00005	SPC10388	Bacteroides_vulgatus	Odoribacter_splanchnicus	+++
SPC10081	SPC10388	Bacteroides_vulgatus	Odoribacter_splanchnicus	-
SPC10301	SPC10388	Bifidobacterium_adolescentis	Odoribacter_splanchnicus	++++
		Bifidobacterium_pseudocatenulatum		
SPC10298	SPC10388		Odoribacter_splanchnicus	+++
SPC00021	SPC00056	Blautia_producta	Odoribacter_splanchnicus	++++
SPC00021	SPC10388	Blautia_producta	Odoribacter_splanchnicus	++++
SPC10243	SPC10388	Clostridium_hathewayi	Odoribacter_splanchnicus	++++
SPC00026	SPC00056	Clostridium_nexile	Odoribacter_splanchnicus	
SPC00026	SPC10388	Clostridium_nexile	Odoribacter_splanchnicus	---
SPC00027	SPC00056	Clostridium_sp_HGF2	Odoribacter_splanchnicus	
SPC00027	SPC10388	Clostridium_sp_HGF2	Odoribacter_splanchnicus	---
SPC10355	SPC10388	Clostridium_symbiosum	Odoribacter_splanchnicus	++
SPC10097	SPC10388	Collinsella_aerofaciens	Odoribacter_splanchnicus	++++
SPC00009	SPC00056	Coprobacillus_sp_D7	Odoribacter_splanchnicus	-
SPC00009	SPC10388	Coprobacillus_sp_D7	Odoribacter_splanchnicus	+++
SPC00080	SPC10388	Coprococcus_catus	Odoribacter_splanchnicus	--
SPC10304	SPC10388	Coprococcus_comes	Odoribacter_splanchnicus	
SPC00018	SPC00056	Dorea_formicigenerans	Odoribacter_splanchnicus	
SPC00018	SPC10388	Dorea_formicigenerans	Odoribacter_splanchnicus	-
SPC00057	SPC10388	Dorea_longicatena	Odoribacter_splanchnicus	++++
SPC00008	SPC00056	Enterococcus_faecalis	Odoribacter_splanchnicus	++++
SPC00008	SPC10388	Enterococcus_faecalis	Odoribacter_splanchnicus	++++
SPC10001	SPC10388	Erysipelotrichaceae_bacterium	Odoribacter_splanchnicus	--
SPC00001	SPC00056	Escherichia_coli	Odoribacter_splanchnicus	++++
SPC00001	SPC10388	Escherichia_coli	Odoribacter_splanchnicus	++++
SPC10110	SPC10388	Escherichia_coli	Odoribacter_splanchnicus	++++
SPC00022	SPC00056	Eubacterium_eligens	Odoribacter_splanchnicus	
SPC00022	SPC10388	Eubacterium_eligens	Odoribacter_splanchnicus	
SPC10363	SPC10388	Eubacterium_rectale	Odoribacter_splanchnicus	+
SPC00054	SPC00056	Faecalibacterium_prausnitzii	Odoribacter_splanchnicus	
SPC00054	SPC10388	Faecalibacterium_prausnitzii	Odoribacter_splanchnicus	-

SPC1	SPC2	OTU1	OTU2	Result
SPC10386	SPC10388	Faecalibacterium_prausnitzii	Odoribacter_splanchnicus	+
SPC00056	SPC00056	Odoribacter_splanchnicus	Odoribacter_splanchnicus	
SPC00056	SPC10388	Odoribacter_splanchnicus	Odoribacter_splanchnicus	---
SPC10388	SPC10388	Odoribacter_splanchnicus	Odoribacter_splanchnicus	+
SPC10048	SPC10388	Parabacteroides_merdae	Odoribacter_splanchnicus	
SPC00061	SPC10388	Roseburia_intestinalis	Odoribacter_splanchnicus	
SPC10197	SPC10388	Ruminococcus_obeum	Odoribacter_splanchnicus	+
SPC10233	SPC10388	Ruminococcus_torques	Odoribacter_splanchnicus	
SPC00015	SPC00056	Streptococcus_thermophilus	Odoribacter_splanchnicus	
SPC00015	SPC10388	Streptococcus_thermophilus	Odoribacter_splanchnicus	+
SPC10030	SPC10048	Bacteroides_ovatus	Parabacteroides_merdae	
SPC00006	SPC10048	Bacteroides_sp_1_1_6	Parabacteroides_merdae	++++
SPC00007	SPC10048	Bacteroides_sp_3_1_23	Parabacteroides_merdae	+++
SPC10019	SPC10048	Bacteroides_sp_D20	Parabacteroides_merdae	
SPC00005	SPC10048	Bacteroides_vulgatus	Parabacteroides_merdae	++++
SPC00021	SPC10048	Blautia_producta	Parabacteroides_merdae	++++
SPC00026	SPC10048	Clostridium_nexile	Parabacteroides_merdae	++
SPC00027	SPC10048	Clostridium_sp_HGF2	Parabacteroides_merdae	+++
SPC00009	SPC10048	Coprobaillus_sp_D7	Parabacteroides_merdae	-
SPC00080	SPC10048	Coprococcus_catus	Parabacteroides_merdae	+++
SPC00018	SPC10048	Dorea_formicigenerans	Parabacteroides_merdae	
SPC00057	SPC10048	Dorea_longicatena	Parabacteroides_merdae	
SPC00008	SPC10048	Enterococcus_faecalis	Parabacteroides_merdae	++++
SPC10001	SPC10048	Erysipelotrichaceae_bacterium	Parabacteroides_merdae	
SPC00001	SPC10048	Escherichia_coli	Parabacteroides_merdae	++++
SPC00022	SPC10048	Eubacterium_eligens	Parabacteroides_merdae	
SPC00054	SPC10048	Faecalibacterium_prausnitzii	Parabacteroides_merdae	+
SPC00056	SPC10048	Odoribacter_splanchnicus	Parabacteroides_merdae	
SPC10048	SPC10048	Parabacteroides_merdae	Parabacteroides_merdae	+++
SPC00061	SPC10048	Roseburia_intestinalis	Parabacteroides_merdae	
SPC00015	SPC10048	Streptococcus_thermophilus	Parabacteroides_merdae	
SPC00006	SPC00061	Bacteroides_sp_1_1_6	Roseburia_intestinalis	++++
SPC00007	SPC00061	Bacteroides_sp_3_1_23	Roseburia_intestinalis	+
SPC00005	SPC00061	Bacteroides_vulgatus	Roseburia_intestinalis	+
SPC00021	SPC00061	Blautia_producta	Roseburia_intestinalis	++++
SPC00026	SPC00061	Clostridium_nexile	Roseburia_intestinalis	-
SPC00027	SPC00061	Clostridium_sp_HGF2	Roseburia_intestinalis	---
SPC00009	SPC00061	Coprobaillus_sp_D7	Roseburia_intestinalis	-
SPC00018	SPC00061	Dorea_formicigenerans	Roseburia_intestinalis	
SPC00057	SPC00061	Dorea_longicatena	Roseburia_intestinalis	-
SPC00008	SPC00061	Enterococcus_faecalis	Roseburia_intestinalis	++++
SPC00001	SPC00061	Escherichia_coli	Roseburia_intestinalis	++++

SPC1	SPC2	OTU1	OTU2	Result
SPC00022	SPC00061	Eubacterium_eligens	Roseburia_intestinalis	
SPC00054	SPC00061	Faecalibacterium_prausnitzii	Roseburia_intestinalis	
SPC00056	SPC00061	Odoribacter_splanchnicus	Roseburia_intestinalis	-
SPC00061	SPC00061	Roseburia_intestinalis	Roseburia_intestinalis	
SPC00015	SPC00061	Streptococcus_thermophilus	Roseburia_intestinalis	
SPC10415	SPC10470	Blautia_producta	Ruminococcus_bromii	++++
SPC10256	SPC10470	Clostridium_butyricum	Ruminococcus_bromii	++++
SPC10358	SPC10470	Clostridium_orbiscindens	Ruminococcus_bromii	
SPC10325	SPC10470	Clostridium_bolteae	Ruminococcus_bromii	+++
SPC10167	SPC10470	Clostridium_disporicum	Ruminococcus_bromii	
SPC10313	SPC10470	Clostridium_hylemonae	Ruminococcus_bromii	
SPC10202	SPC10470	Clostridium_innocuum	Ruminococcus_bromii	++++
SPC10238	SPC10470	Clostridium_mayombeii	Ruminococcus_bromii	++++
SPC10355	SPC10470	Clostridium_symbiosum	Ruminococcus_bromii	++++
SPC10155	SPC10470	Clostridium_tertium	Ruminococcus_bromii	++++
SPC10097	SPC10470	Collinsella_aerofaciens	Ruminococcus_bromii	++++
SPC10304	SPC10470	Coprococcus_comes	Ruminococcus_bromii	++++
SPC10567	SPC10470	Eubacterium_rectale	Ruminococcus_bromii	+
SPC10386	SPC10470	Faecalibacterium_prausnitzii	Ruminococcus_bromii	
SPC10390	SPC10470	Lachnospiraceae_bacterium_5_1_5_7FAA	Ruminococcus_bromii	++++
SPC10470	SPC10470	Ruminococcus_bromii	Ruminococcus_bromii	-
SPC10468	SPC10470	Ruminococcus_gnavus	Ruminococcus_bromii	++++
SPC10415	SPC10468	Blautia_producta	Ruminococcus_gnavus	++++
SPC10256	SPC10468	Clostridium_butyricum	Ruminococcus_gnavus	++++
SPC10358	SPC10468	Clostridium_orbiscindens	Ruminococcus_gnavus	++++
SPC10325	SPC10468	Clostridium_bolteae	Ruminococcus_gnavus	++++
SPC10167	SPC10468	Clostridium_disporicum	Ruminococcus_gnavus	++++
SPC10313	SPC10468	Clostridium_hylemonae	Ruminococcus_gnavus	+++
SPC10202	SPC10468	Clostridium_innocuum	Ruminococcus_gnavus	++++
SPC10238	SPC10468	Clostridium_mayombeii	Ruminococcus_gnavus	++++
SPC10355	SPC10468	Clostridium_symbiosum	Ruminococcus_gnavus	++++
SPC10155	SPC10468	Clostridium_tertium	Ruminococcus_gnavus	++++
SPC10097	SPC10468	Collinsella_aerofaciens	Ruminococcus_gnavus	++++
SPC10304	SPC10468	Coprococcus_comes	Ruminococcus_gnavus	++++
SPC10386	SPC10468	Faecalibacterium_prausnitzii	Ruminococcus_gnavus	++++
SPC10390	SPC10468	Lachnospiraceae_bacterium_5_1_5_7FAA	Ruminococcus_gnavus	++++
SPC10470	SPC10468	Ruminococcus_bromii	Ruminococcus_gnavus	++++
SPC10468	SPC10468	Ruminococcus_gnavus	Ruminococcus_gnavus	+++
SPC10030	SPC10197	Bacteroides_ovatus	Ruminococcus_obeum	
SPC00006	SPC10197	Bacteroides_sp_1_1_6	Ruminococcus_obeum	+++
SPC00007	SPC10197	Bacteroides_sp_3_1_23	Ruminococcus_obeum	+++

SPC1	SPC2	OTU1	OTU2	Result
SPC10019	SPC10197	Bacteroides_sp_D20	Ruminococcus_obeum	
SPC00005	SPC10197	Bacteroides_vulgatus	Ruminococcus_obeum	++++
SPC10081	SPC10197	Bacteroides_vulgatus	Ruminococcus_obeum	
SPC00021	SPC10197	Blautia_producta	Ruminococcus_obeum	++++
SPC00026	SPC10197	Clostridium_nexile	Ruminococcus_obeum	-
SPC00027	SPC10197	Clostridium_sp_HGF2	Ruminococcus_obeum	--
SPC10097	SPC10197	Collinsella_aerofaciens	Ruminococcus_obeum	++++
SPC00009	SPC10197	Coprobacillus_sp_D7	Ruminococcus_obeum	+
SPC00080	SPC10197	Coprococcus_catus	Ruminococcus_obeum	
SPC00018	SPC10197	Dorea_formicigenerans	Ruminococcus_obeum	++++
SPC00057	SPC10197	Dorea_longicatena	Ruminococcus_obeum	-
SPC00008	SPC10197	Enterococcus_faecalis	Ruminococcus_obeum	++++
SPC10001	SPC10197	Erysipelotrichaceae_bacterium	Ruminococcus_obeum	
SPC00001	SPC10197	Escherichia_coli	Ruminococcus_obeum	+++
SPC10110	SPC10197	Escherichia_coli	Ruminococcus_obeum	++++
SPC00022	SPC10197	Eubacterium_eligens	Ruminococcus_obeum	+
SPC00054	SPC10197	Faecalibacterium_prausnitzii	Ruminococcus_obeum	
SPC00056	SPC10197	Odoribacter_splanchnicus	Ruminococcus_obeum	-
SPC10048	SPC10197	Parabacteroides_merdae	Ruminococcus_obeum	
SPC00061	SPC10197	Roseburia_intestinalis	Ruminococcus_obeum	
SPC10197	SPC10197	Ruminococcus_obeum	Ruminococcus_obeum	++++
SPC00015	SPC10197	Streptococcus_thermophilus	Ruminococcus_obeum	+++
SPC10211	SPC10233	Bacteroides_caccae	Ruminococcus_torques	++++
SPC10213	SPC10233	Bacteroides_eggerthii	Ruminococcus_torques	++++
SPC10030	SPC10233	Bacteroides_ovatus	Ruminococcus_torques	++++
SPC00006	SPC10233	Bacteroides_sp_1_1_6	Ruminococcus_torques	++++
SPC00007	SPC10233	Bacteroides_sp_3_1_23	Ruminococcus_torques	++++
SPC10019	SPC10233	Bacteroides_sp_D20	Ruminococcus_torques	++
SPC00005	SPC10233	Bacteroides_vulgatus	Ruminococcus_torques	++++
SPC10081	SPC10233	Bacteroides_vulgatus	Ruminococcus_torques	++++
SPC00021	SPC10233	Blautia_producta	Ruminococcus_torques	++++
SPC00026	SPC10233	Clostridium_nexile	Ruminococcus_torques	+
SPC00027	SPC10233	Clostridium_sp_HGF2	Ruminococcus_torques	
SPC10097	SPC10233	Collinsella_aerofaciens	Ruminococcus_torques	++++
SPC00009	SPC10233	Coprobacillus_sp_D7	Ruminococcus_torques	++++
SPC00080	SPC10233	Coprococcus_catus	Ruminococcus_torques	+
SPC00018	SPC10233	Dorea_formicigenerans	Ruminococcus_torques	++++
SPC00057	SPC10233	Dorea_longicatena	Ruminococcus_torques	
SPC00008	SPC10233	Enterococcus_faecalis	Ruminococcus_torques	++++
SPC10001	SPC10233	Erysipelotrichaceae_bacterium	Ruminococcus_torques	+
SPC00001	SPC10233	Escherichia_coli	Ruminococcus_torques	++++
SPC10110	SPC10233	Escherichia_coli	Ruminococcus_torques	++++

SPC1	SPC2	OTU1	OTU2	Result
SPC00022	SPC10233	Eubacterium_eligens	Ruminococcus_torques	++
SPC00054	SPC10233	Faecalibacterium_prausnitzii	Ruminococcus_torques	
SPC00056	SPC10233	Odoribacter_splanchnicus	Ruminococcus_torques	
SPC10048	SPC10233	Parabacteroides_merdae	Ruminococcus_torques	+
SPC00061	SPC10233	Roseburia_intestinalis	Ruminococcus_torques	+
SPC10197	SPC10233	Ruminococcus_obeum	Ruminococcus_torques	++++
SPC10233	SPC10233	Ruminococcus_torques	Ruminococcus_torques	++++
SPC00015	SPC10233	Streptococcus_thermophilus	Ruminococcus_torques	+
SPC00006	SPC00015	Bacteroides_sp_1_1_6	Streptococcus_thermophilus	+++
SPC00007	SPC00015	Bacteroides_sp_3_1_23	Streptococcus_thermophilus	+++
SPC00005	SPC00015	Bacteroides_vulgatus	Streptococcus_thermophilus	+
SPC00009	SPC00015	Coprobacillus_sp_D7	Streptococcus_thermophilus	+
SPC00008	SPC00015	Enterococcus_faecalis	Streptococcus_thermophilus	++++
SPC00001	SPC00015	Escherichia_coli	Streptococcus_thermophilus	+
SPC00015	SPC00015	Streptococcus_thermophilus	Streptococcus_thermophilus	

Table 6: Non-limiting but exemplary ternary combinations include those with mean log reduction greater than 0.171, e.g. any combination shown in Table 6.

SPC1	SPC2	SPC3	OTU1	OTU2	OTU3	Results
SPC1	SPC1	SPC1			Eubacterium_rectal	
0325	0415	0567	Clostridium_bolteae	Blautia producta	e	++++
SPC1	SPC1	SPC1				
0325	0355	0415	Clostridium_bolteae	Clostridium_symbiosum	Blautia producta	++++
SPC1	SPC1	SPC1			Eubacterium_rectal	
0325	0355	0567	Clostridium_bolteae	Clostridium_symbiosum	e	-
SPC1	SPC1	SPC1			Faecalibacterium_pr	
0325	0355	0386	Clostridium_bolteae	Clostridium_symbiosum	ausnitzii	-
SPC1	SPC1	SPC1			Lachnospiraceae_ba	
0325	0355	0390	Clostridium_bolteae	Clostridium_symbiosum	cterium_5_1_57FAA	
SPC1	SPC1	SPC1			Faecalibacterium_praus	
0325	0386	0415	Clostridium_bolteae	nitzii	Blautia producta	++++
SPC1	SPC1	SPC1			Faecalibacterium_praus	
0325	0386	0567	Clostridium_bolteae	nitzii	Eubacterium_rectal	
SPC1	SPC1	SPC1			Faecalibacterium_praus	
0325	0386	0390	Clostridium_bolteae	nitzii	Lachnospiraceae_ba	++++
SPC1	SPC1	SPC1			Lachnospiraceae_bacter	
0325	0390	0415	Clostridium_bolteae	ium_5_1_57FAA	Blautia producta	++++
SPC1	SPC1	SPC1			Lachnospiraceae_bacter	
0325	0390	0567	Clostridium_bolteae	ium_5_1_57FAA	Eubacterium_rectal	+
SPC1	SPC1	SPC1			e	
0355	0415	0567	Clostridium_symbiosum	Blautia producta	Eubacterium_rectal	++++
SPC1	SPC1	SPC1			e	
0355	0386	0415	Clostridium_symbiosum	Faecalibacterium_praus	Blautia producta	++++
SPC1	SPC1	SPC1			Faecalibacterium_praus	
0355	0386	0567	Clostridium_symbiosum	nitzii	Eubacterium_rectal	
SPC1	SPC1	SPC1			e	
0355	0386	0567	Clostridium_symbiosum	nitzii	Lachnospiraceae_ba	
SPC1	SPC1	SPC1			Faecalibacterium_praus	
0355	0386	0390	Clostridium_symbiosum	nitzii	Lachnospiraceae_ba	+
SPC1	SPC1	SPC1			cterium_5_1_57FAA	
0355	0390	0415	Clostridium_symbiosum	Lachnospiraceae_bacter	Blautia producta	++++
SPC1	SPC1	SPC1			ium_5_1_57FAA	
0355	0390	0567	Clostridium_symbiosum	Lachnospiraceae_bacter	Eubacterium_rectal	
SPC1	SPC1	SPC1			ium_5_1_57FAA	
0097	0415	0567	Collinsella_aerofaciens	Blautia producta	Eubacterium_rectal	
SPC1	SPC1	SPC1			e	++++
0097	0325	0415	Collinsella_aerofaciens	Clostridium_bolteae	Blautia producta	++++
SPC1	SPC1	SPC1			Clostridium_symbio	
0097	0325	0355	Collinsella_aerofaciens	Clostridium_bolteae	sum	++++
SPC1	SPC1	SPC1			Eubacterium_rectal	
0097	0325	0567	Collinsella_aerofaciens	Clostridium_bolteae	e	++++
SPC1	SPC1	SPC1			Faecalibacterium_pr	
0097	0325	0386	Collinsella_aerofaciens	Clostridium_bolteae	ausnitzii	++++
SPC1	SPC1	SPC1			Lachnospiraceae_ba	
0097	0325	0390	Collinsella_aerofaciens	Clostridium_bolteae	cterium_5_1_57FAA	++++
SPC1	SPC1	SPC1				
0097	0355	0415	Collinsella_aerofaciens	Clostridium_symbiosum	Blautia producta	++++
SPC1	SPC1	SPC1			Eubacterium_rectal	
0097	0355	0567	Collinsella_aerofaciens	Clostridium_symbiosum	e	
SPC1	SPC1	SPC1			Faecalibacterium_pr	
0097	0355	0386	Collinsella_aerofaciens	Clostridium_symbiosum	ausnitzii	

SPC1	SPC2	SPC3	OTU1	OTU2	OTU3	Results
SPC1	SPC1	SPC1			Lachnospiraceae_ba	
0097	0355	0390	Collinsella_aerofaciens	Clostridium_symbiosum	cterium_5_1_57FAA	+
SPC1	SPC1	SPC1				
0097	0304	0415	Collinsella_aerofaciens	Coprococcus_comes	Blautia producta	++++
SPC1	SPC1	SPC1				
0097	0304	0325	Collinsella_aerofaciens	Coprococcus_comes	Clostridium_bolteae	++++
SPC1	SPC1	SPC1			Clostridium_symbio	
0097	0304	0355	Collinsella_aerofaciens	Coprococcus_comes	sum	+++
SPC1	SPC1	SPC1			Eubacterium_rectal	
0097	0304	0567	Collinsella_aerofaciens	Coprococcus_comes	e	+++
SPC1	SPC1	SPC1			Faecalibacterium_pr	
0097	0304	0386	Collinsella_aerofaciens	Coprococcus_comes	ausnitzii	++++
SPC1	SPC1	SPC1			Lachnospiraceae_ba	
0097	0304	0390	Collinsella_aerofaciens	Coprococcus_comes	cterium_5_1_57FAA	+++
SPC1	SPC1	SPC1			Faecalibacterium_praus	
0097	0386	0415	Collinsella_aerofaciens	nitzii	Blautia producta	++++
SPC1	SPC1	SPC1			Faecalibacterium_praus	
0097	0386	0567	Collinsella_aerofaciens	nitzii	Eubacterium_rectal	+++
SPC1	SPC1	SPC1			Faecalibacterium_praus	
0097	0386	0390	Collinsella_aerofaciens	nitzii	Lachnospiraceae_ba	+++
SPC1	SPC1	SPC1			cterium_5_1_57FAA	
0097	0390	0415	Collinsella_aerofaciens	Lachnospiraceae_bacter	Blautia producta	++++
SPC1	SPC1	SPC1			ium_5_1_57FAA	
0097	0390	0567	Collinsella_aerofaciens	Lachnospiraceae_bacter	Eubacterium_rectal	++++
SPC1	SPC1	SPC1			ium_5_1_57FAA	
0304	0415	0567	Coprococcus_comes	Blautia producta	e	++++
SPC1	SPC1	SPC1				
0304	0325	0415	Coprococcus_comes	Clostridium_bolteae	Blautia producta	++++
SPC1	SPC1	SPC1			Clostridium_symbio	
0304	0325	0355	Coprococcus_comes	Clostridium_bolteae	sum	
SPC1	SPC1	SPC1			Eubacterium_rectal	
0304	0325	0567	Coprococcus_comes	Clostridium_bolteae	e	--
SPC1	SPC1	SPC1			Faecalibacterium_pr	
0304	0325	0386	Coprococcus_comes	Clostridium_bolteae	ausnitzii	+++
SPC1	SPC1	SPC1			Lachnospiraceae_ba	
0304	0325	0390	Coprococcus_comes	Clostridium_bolteae	cterium_5_1_57FAA	+++
SPC1	SPC1	SPC1				
0304	0355	0415	Coprococcus_comes	Clostridium_symbiosum	Blautia producta	++++
SPC1	SPC1	SPC1			Eubacterium_rectal	
0304	0355	0567	Coprococcus_comes	Clostridium_symbiosum	e	---
SPC1	SPC1	SPC1			Faecalibacterium_pr	
0304	0355	0386	Coprococcus_comes	Clostridium_symbiosum	ausnitzii	
SPC1	SPC1	SPC1			Lachnospiraceae_ba	
0304	0355	0390	Coprococcus_comes	Clostridium_symbiosum	cterium_5_1_57FAA	
SPC1	SPC1	SPC1			Faecalibacterium_praus	
0304	0386	0415	Coprococcus_comes	nitzii	Blautia producta	++++
SPC1	SPC1	SPC1			Faecalibacterium_praus	
0304	0386	0567	Coprococcus_comes	nitzii	Eubacterium_rectal	-
SPC1	SPC1	SPC1			e	
0304	0386	0390	Coprococcus_comes	Faecalibacterium_praus	Lachnospiraceae_ba	
SPC1	SPC1	SPC1			cterium_5_1_57FAA	
0304	0390	0415	Coprococcus_comes	Lachnospiraceae_bacter	Blautia producta	++++
SPC1	SPC1	SPC1			ium_5_1_57FAA	
0304	0390	0567	Coprococcus_comes	Lachnospiraceae_bacter	Eubacterium_rectal	
SPC1	SPC1	SPC1			ium_5_1_57FAA	
0304	0390	0567	Coprococcus_comes	Blautia producta	e	
SPC1	SPC1	SPC1	Faecalibacterium_praus	Blautia producta	Eubacterium_rectal	++++

SPC1	SPC2	SPC3	OTU1	OTU2	OTU3	Results
0386	0415	0567	nitzii		e	
SPC1	SPC1	SPC1	Faecalibacterium_praus	Lachnospiraceae_bacter		
0386	0390	0415	nitzii	ium_5_1_57FAA	Blautia producta	++++
SPC1	SPC1	SPC1	Faecalibacterium_praus	Lachnospiraceae_bacter	Eubacterium_rectal	
0386	0390	0567	nitzii	ium_5_1_57FAA	e	
SPC1	SPC1	SPC1	Lachnospiraceae_bacter		Eubacterium_rectal	
0390	0415	0567	ium_5_1_57FAA	Blautia producta	e	++++

Table 16. 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.

[0364] 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
Bifidobacterium dentium	clade172	N	
Alcaligenes faecalis	clade183	N	
Rothia dentocariosa	clade194	N	
Peptoniphilus lacrimalis	clade291	N	
Anaerococcus sp. gpac155	clade294	N	
Sutterella stercoricanis	clade302	N	Y
Bacteroides sp. 3_1_19	clade335	N	Y
Parabacteroides goldsteinii	clade335	N	
Bacteroides dorei	clade378	N	Y
Bacteroides massiliensis	clade378	N	
Lactobacillus iners	clade398	N	
Granulicatella adiacens	clade460	N	
Eggerthella sp. 1_3_56FAA	clade477	N	
Gordonibacter pamelaee	clade477	N	
Finegoldia magna	clade509	N	
Actinomyces nasicola	clade523	N	
Streptobacillus moniliformis	clade532	N	
Oscillospira guilliermondii	clade540	N	
Orientia tsutsugamushi	clade541	N	
Christensenella minuta	clade558	N	
Clostridium oroticum	clade96	Y	
Clostridium sp. D5	clade96	Y	
Clostridium glycyrrhizinilyticum	clade147	Y	

Coprococcus comes	clade147	Y	
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	
Lachnospiraceae bacterium	clade360	Y	Y

9_1_43BFAA			
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 biforme	clade385	Y	
Eubacterium dolichum	clade385	Y	
Coprococcus catus	clade393	Y	
Acetivibrio ethanolgignens	clade396	Y	
Anaerosporeobacter 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	
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	
Clostridium viride	clade540	Y	
Butyrivibrio crossotus	clade543	Y	
Coprococcus eutactus	clade543	Y	

Eubacterium ruminantium	clade543	Y	
Eubacterium rectale	clade568	Y	Y
Roseburia inulinivorans	clade568	Y	
Butyricicoccus pullicaecorum	clade572	Y	
Eubacterium desmolans	clade572	Y	
Papillibacter cinnamivorans	clade572	Y	
Sporobacter termitidis	clade572	Y	
Clostridium lactatifermentans	clade576	Y	

Table 18. 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 19. 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>	9×10^5	1×10^3
1	<i>Klebsiella sp. Co9935</i>	4×10^5	1×10^3
1	<i>Escherichia coli</i>	7×10^5	1×10^6
2	<i>Klebsiella sp. Co9935</i>	4×10^5	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>	1×10^5	$< 1 \times 10^4$

Table 20 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	$<2 \times 10^7$ [DC1]	2×10^{10}

Table 21. 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 (1.58E10 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 22. Titers (in cfu/g) of imipenem-resistant *M. morganii*, *P. rettgeri* and *P. pennerii* from Patients B, D & E

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 XXXI List of representative vitamins, minerals and co-factors

L-glutamine
 nickel chloride
 BaCl₂
 hemin
 potassium telurite
 Fibrinogen
 Bacto Vitamin-Free Casamino Acids
 cocarboxylase
 bovine albumin fraction V
 FeCl₂·H₂O
 L-cystine·2HCl
 Bacto Casamino Acids
 Agar
 CuSO₄
 pyridoxine
 SnCl₂·2H₂O
 sodium selenite
 CaCl₂
 NaCl
 albumin fraction V
 vitamin B₁₂
 folic acid
 ZnCl₂
 FeSO₄
 oleic acid
 Co(NO₃)₂·6H₂O
 L-cystine
 Na₂B₄O₇·10H₂O
 CaSO₄·2H₂O
 AlCl₃
 SeCl₄
 Na₂MoO₄·2H₂O
 thiamine pyrophosphate
 Pyridoxine-HCl
 MnCl₂·4H₂O
 aluminum sulphate
 Na₂HPO₄

H₃BO₃
L-cysteine·HCl·H₂O
adenine sulfate
long-chain fatty acids
KNO₃
sodium metabisulfite
sodium molybdate
CoCl₂·6H₂O
Na₂MoO₄
Castenholz Salts
NaNO₃
HCl
L-cysteine
copper sulfate
L-cysteine·HCl
thiamine·HCl
biotin
sodium chloride
thallium acetate
NiCl₂·6H₂O
NaVO₃·H₂O
nicotinamide adenine dinucleotide
nicotinic acid
Na₂MoO₄·H₂O
CuCl₂·2H₂O
FeCl₂·4H₂O
(NH₄)₂MoO₄
MnSO₄
guanine·HCl
H₂SO₄
CoCl₂
cholesterol
LiCl
pyridoxine·2HCl
Disodium ethylenediamine tetraacetic acid
Vitamin K1
KBr
alkalinized oleic acid
ZnSO₄·7H₂O
trypsin inhibitor
KI
ethanol
cobalt nitrate
Ethylenediamine tetraacetic acid
CuSO₄·5H₂O

calcium-D-pathothenate
 $\text{Fe}(\text{NO}_3)_3$
 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
Sodium pyruvate
 NaOH
p-aminobenzoic acid
a-ketoglutarate
boric acid
casein
Pyridoxine hydrochloride
Dried bovine hemoglobin
 ZnSO_4
Nicotinamide
 FeCl_3
 $\text{Fe}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$
calcium pantothenate
cyanocobalamin
nitrilotriacetic acid
Adenine
sodium tartrate
magnesium sulfate
zinc sulfate
 NaHCO_3
Glucose
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$
 $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$
riboflavin
ferric pyrophosphate
Essential growth factors V and X
Peptone
 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$
catalase
 $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$
 CuCl_2
 $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$
thiamine
 NiCl_2
sodium tungstate
iron sulfate
calcium chloride
 $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$
ACES buffer/KOH
Thioctic acid
succinate
formate

lactate
butyrate
acetate
Vitamin K
Mercaptoethane-sulfonic acid
Lipoic acid
ammonia
heme
S-Adenosylmethionine

TABLE XXX5: List of nitrogen sources.

Ammonia
Nitrite
Nitrate
Urea
Biuret
L-Alanine
L-Arginine
L-Asparagine
L-Aspartic Acid
L-Cysteine
L-Glutamic Acid
Histamine
beta-Phenylethyl- amine
Tyramine
Acetamide
Formamide
Glucuronamide
D,L-Lactamide
D-Glucosamine
D-Galactosamine
D-Mannosamine
N-Acetyl-D- Glucosamine
N-Acetyl-D- Galactosamine
N-Acetyl-D- Mannosamine
Adenine
Adenosine
Cytidine
Cytosine
Guanine
Guanosine
Thymine
Thymidine
Uracil
Uridine
Inosine
Xanthine
Xanthosine
Acid
Alloxan
Allantoin
Parabanic Acid
D,L-alpha-Amino-N- Butyric
Acid
gamma-Amino-N- Butyric Acid

epsilon-Amino-N- Caproic Acid
D,L-alpha-Amino- Caprylic Acid
delta-Amino-N- Valeric Acid
alpha-Amino-N- Valeric Acid
Ala-Asp
Ala-Gln
Ala-Glu
Ala-Gly
Ala-His
Ala-Leu
Ala-Thr
Gly-Asn
Gly-Gln
Gly-Glu
Gly-Met
Met-Ala
N-Acetyl-L- Glutamic Acid
N-Phthaloyl-L- Glutamic Acid
L-Pyroglutamic Acid
Hydroxylamine
Methylamine
N-Amylamine
N-Butylamine
Ethylamine
Ethanolamine
Ethylenediamine
Putrescine
Agmatine
L-Glutamine
Glycine
L-Histidine
L-Isoleucine
L-Leucine
L-Lysine
L-Methionine
L-Phenylalanine
L-Proline
L-Serine
L-Threonine
L-Tryptophan
L-Tyrosine
L-Valine
D-Alanine
D-Asparagine
D-Aspartic Acid
D-Glutamic Acid

D-Lysine
D-Serine
D-Valine
L-Citrulline
L-Homoserine
L-Ornithine

Table X4. Spore-forming Bacterial Species

<i>Alkaliphilus metalliredigens</i>
<i>Ammonifex degensii</i>
<i>Anaerofustis stercorihominis</i>
<i>Anaerostipes caccae</i>
<i>Anaerotruncus colihominis</i>
<i>Bacillus amyloliquefaciens</i>
<i>Bacillus anthracis</i>
<i>Bacillus cellulosilyticus</i>
<i>Bacillus cereus</i>
<i>Bacillus clausii</i>
<i>Bacillus coagulans</i>
<i>Bacillus cytotoxicus</i>
<i>Bacillus halodurans</i>
<i>Bacillus licheniformis</i>
<i>Bacillus pumilus</i>
<i>Bacillus subtilis</i>
<i>Bacillus thuringiensis</i>
<i>Bacillus weihenstephanensis</i>
<i>Blautia hansenii</i>
<i>Brevibacillus brevis</i>
<i>Bryantella formatexigens</i>
<i>Caldicellulosiruptor saccharolyticus</i>
<i>Candidatus Desulforudis audaxviator</i>
<i>Carboxydibrachium pacificum</i>

Carboxydotherrnus hydrogenoformans
Clostridium acetobutylicum
Clostridium asparagiforme
Clostridium bartlettii
Clostridium beijerinckii
Clostridium bolteae
Clostridium botulinum A str. ATCC 19397
Clostridium botulinum B str. Eklund 17B
Clostridium butyricum pathogenic E4 str. BoNT BL5262
Clostridium Carboxidivorans
Clostridium cellulolyticum
Clostridium cellulovorans
Clostridium difficile
Clostridium hathewayi
Clostridium hylemonae
Clostridium kluyveri
Clostridium leptum
Clostridium methylpentosum
Clostridium nexile
Clostridium novyi NT
Clostridium papyrosolvens
Clostridium perfringens
Clostridium phytofermentans ISDg
Clostridium scindens
Clostridium sp. 7_2_43FAA

<i>Clostridium sporogenes</i>
<i>Clostridium tetani</i>
<i>Clostridium thermocellum</i>
<i>Coprococcus comes</i>
<i>Desulfotomaculum reducens</i>
<i>Dorea longicatena</i>
<i>Eubacterium eligens</i>
<i>Eubacterium hallii</i>
<i>Eubacterium rectale</i>
<i>Eubacterium ventriosum</i>
<i>Faecalibacterium prausnitzii</i>
<i>Geobacillus kaustophilus</i>
<i>Geobacillus</i> sp. G11MC16
<i>Geobacillus thermodenitrificans</i>
<i>Heliobacterium modesticaldum</i>
<i>Lysinibacillus sphaericus</i>
<i>Oceanobacillus iheyensis</i>
<i>Paenibacillus</i> sp. JDR-2
<i>Pelotomaculum thermopropionicum</i>
<i>Roseburia intestinalis</i>
<i>Ruminococcus bromii</i>
<i>Ruminococcus gnavus</i>
<i>Ruminococcus obeum</i>
<i>Ruminococcus torques</i>
<i>Subdoligranulum variabile</i>
<i>Symbiobacterium thermophilum</i>

Thermoanaerobacter italicus
Thermoanaerobacter tengcongensis
Thermoanaerobacterium thermosaccharolyticum
Thermosinus carboxydivorans

CLAIMS

What is claimed is:

1. A composition comprising an effective amount of a first type of bacteria and a second type of bacteria formulated for oral administration to a mammalian subject, wherein the first and second type of bacteria are: (i) isolated, (ii) not identical, and (iii) independently capable of proliferating in a nutrient medium having a threshold concentration of a nutrient below that concentration required for *Clostridium difficile* proliferation.
2. The composition of claim 1, wherein the nutrient comprises a carbohydrate nutrient.
3. The composition of claim 2, wherein the carbohydrate nutrient is selected from the group consisting of glucose, mannitol, fructose, N-acetylglucosamine (NAG) and N-acetylneuraminic acid (NAN).
4. The composition of claim 1, wherein the first and second type of bacteria are independently capable of proliferating in a nutrient medium having a threshold concentration of glucose, mannitol, fructose, N-acetylglucosamine (NAG) and N-acetylneuraminic acid (NAN) below that concentration required for *Clostridium difficile* proliferation.
5. The composition of claim 1, wherein the threshold concentration is about 90% or less the concentration required for *Clostridium difficile* proliferation.
6. The composition of claim 1, wherein the threshold concentration is about 50% or less the concentration required for *Clostridium difficile* proliferation.
7. The composition of claim 1, wherein the threshold concentration is about 10%, about 5%, or about 1% the concentration required for *Clostridium difficile* proliferation.
8. The composition of claim 1, wherein the first and second type of bacteria are independently capable of proliferating at a rate at least 10% greater than *Clostridium difficile* in a nutrient medium having a concentration of a nutrient selected from the

group consisting of glucose, mannitol, fructose, N-acetylglucosamine (NAG) and N-acetylneuraminic acid (NAN).

9. The composition of claim 1, wherein the first and second type of bacteria are independently capable of proliferating at a rate at least 50% greater than *Clostridium difficile* in a nutrient medium having a concentration of a nutrient selected from the group consisting of glucose, mannitol, fructose, N-acetylglucosamine (NAG) and N-acetylneuraminic acid (NAN).

10. The composition of claim 1, wherein the nutrient comprises a vitamin nutrient.

11. The composition of claim 10, wherein the vitamin nutrient is selected from the group consisting of biotin, pantothenate and pyridoxine.

12. The composition of claim 11, wherein the first and second type of bacteria are independently capable of proliferating in a nutrient medium having a threshold concentration of biotin, pantothenate and pyridoxine below that concentration required for *Clostridium difficile* proliferation.

13. The composition of claim 11, wherein the threshold concentration is about 90% or less the concentration required for *Clostridium difficile* proliferation.

14. The composition of claim 11, wherein the threshold concentration is about 50% or less the concentration required for *Clostridium difficile* proliferation.

15. The composition of claim 11, wherein the threshold concentration is about 10% or less the concentration required for *Clostridium difficile* proliferation.

16. The composition of claim 1, wherein the first and second type of bacteria are independently capable of proliferating at a rate at least 10% greater than *Clostridium difficile* in a nutrient medium having a concentration of a nutrient selected from the group consisting of biotin, pantothenate and pyridoxine.

17. The composition of claim 1, wherein the first and second type of bacteria are independently capable of proliferating at a rate at least 50% greater than *Clostridium difficile* in a nutrient medium having a concentration of a nutrient selected from the group consisting of biotin, pantothenate and pyridoxine.

18. The composition of claim 1, wherein the first and second type of bacteria are independently capable of proliferating at a rate at least 10% greater than *Clostridium difficile* in a nutrient medium having a concentration of biotin, pantothenate, pyridoxine, glucose, mannitol, fructose, N-acetylglucosamine (NAG) and N-acetylneuramic acid (NAN).

19. A composition comprising an effective amount of a first type of bacteria and a second type of bacteria that are independently capable of proliferating at a rate at least 10% greater than *Clostridium difficile* in a nutrient medium having a limiting concentration of one or more essential carbohydrate nutrients.

20. A composition comprising an effective amount of a first type of bacteria and a second type of bacteria that are independently capable of proliferating at a rate at least 10% greater than *Clostridium difficile* in a nutrient medium having a limiting concentration of one or more essential amino acid nutrients.

21. A composition comprising an effective amount of a first type of bacteria and a second type of bacteria that are independently capable of proliferating at a rate at least 10% greater than *Clostridium difficile* in a nutrient medium having a limiting concentration of one or more essential vitamin or mineral nutrients.

22. A composition comprising an effective amount of a first type of bacteria and a second type of bacteria formulated for oral administration to a mammalian subject, wherein the first and second type of bacteria are: (i) isolated, (ii) not identical, and (iii) independently capable of saccharification at a rate at least 10% greater than *Clostridium difficile*.

23. The composition of claim 22, wherein the first and second type of bacteria are independently capable of saccharification at a rate at least 50% greater than *Clostridium difficile*.

24. The composition of claim 22, wherein the first and second type of bacteria are independently capable of saccharification of a carbohydrate polymer selected from the group consisting of a mucin, a fructan, an arabinoxylan, a lactulose, and a galactooligosaccharide.

25. A composition comprising an effective amount of a first type of bacteria and a second type of bacteria formulated for oral administration to a mammalian subject, wherein the first type of bacteria is: (i) isolated, (ii) not identical to the second type of bacteria, and (iii) independently capable of saccharification at a rate at least 10% greater than *Clostridium difficile*, and wherein the second type of bacteria is: (i) isolated, (ii) not identical to the first type of bacteria, and (iii) independently capable of glycolysis of a nutrient selected from the group consisting of glucose, mannitol, fructose, N-acetylglucosamine (NAG) and N-acetylneuramic acid (NAN), at a rate at least 10% greater than *Clostridium difficile* or at a concentration at least 10% less than *Clostridium difficile*, or the combination thereof.

26. A method for the treatment of *Clostridium difficile* infection in a mammalian subject, comprising the step of orally administering to the subject a composition comprising an effective amount of a first type of bacteria and a second type of bacteria formulated for oral administration to a mammalian subject, wherein the first type of bacteria is: (i) isolated, (ii) not identical to the second type of bacteria, and (iii) independently capable of saccharification at a rate at least 10% greater than *Clostridium difficile*, and wherein the second type of bacteria is: (i) isolated, (ii) not identical to the first type of bacteria, and (iii) independently capable of glycolysis of a nutrient selected from the group consisting of glucose, mannitol, fructose, N-acetylglucosamine (NAG) and N-acetylneuramic acid (NAN), at a rate at least 10% greater than *Clostridium difficile* or at a concentration at least 10% less than *Clostridium difficile*, or the combination thereof, under conditions such that the first type of bacteria and the second type of bacteria functionally populate the gastrointestinal tract of the subject and prevent the population of the gastrointestinal tract by *Clostridium difficile*.

27. A composition comprising an effective amount of a first type of bacteria and a second type of bacteria formulated for oral administration to a mammalian subject, wherein the first and second type of bacteria are: (i) isolated, (ii) not identical, and (iii) capable of proliferating in a nutrient medium having a threshold concentration of a carbon-containing nutrient below that concentration required for a pathogen and/or pathobiont proliferation.

28. A composition comprising an effective amount of a first type of bacteria formulated for oral administration to a mammalian subject, wherein the first type of bacteria is: (i) isolated and (ii) capable of proliferating in a nutrient medium having a threshold concentration of a carbon-containing nutrient below that concentration required for a pathogen and/or pathobiont proliferation.

29. The composition of claim 28, wherein the first type of bacteria is capable of functionally interacting with a second type of bacteria in a nutrient medium such that the threshold concentration of the carbon-containing nutrient for proliferation of the first type of bacteria is lower in the presence of the second type of bacteria than in the absence of the second type of bacteria.

30. The composition of claim 27 or 28, wherein the nutrient comprises glucose, cellobiose, fructose, mannitol, mannose, melezitose, salicin, sorbitol, sucrose, trehalose, xylose, proline, aspartic acid, serine, leucine, alanine, threonine, valine, phenylalanine, methionine, or isoleucine.

31. The composition of claim 27 or 28, wherein the nutrient comprises L-Arabinose, N-Acetyl-D-Glucosamine, D-Saccharic Acid, Succinic Acid, D-Galactose, L-Aspartic Acid, L-Proline, D-Alanine, D-Trehalose, D-Mannose, Dulcitol, D-Serine, D-Sorbitol, Glycerol, L-Fucose, D-Glucuronic Acid, D-Gluconic Acid, D, L-alpha-Glycerol-Phosphate, D-Xylose, L-Lactic Acid, Formic Acid, D-Mannitol, L-Glutamic Acid, D-Glucose-6-Phosphate, D-Galactonic Acid-gamma-Lactone, D,L-Malic Acid, D-Ribose, Tween 20, L-Rhamnose, D-Fructose, Acetic Acid, alpha-D-Glucose, Maltose, D-Melibiose, Thymidine, L-Asparagine, D-Aspartic Acid, D-Glucosaminic Acid, 1,2-Propanediol, Tween 40, alpha-Keto-Glutaric Acid, alpha-Keto-Butyric Acid, alpha-Methyl-D-Galactoside, alpha-D-Lactose, Lactulose, Sucrose, Uridine, L-Glutamine, M-Tartaric Acid, D-Glucose-1-Phosphate, D-Fructose-6-Phosphate, Tween 80, alpha-Hydroxy-Glutaric-gamma-lactone, alpha-Hydroxy Butyric Acid, beta-Methyl-D-Glucoside, Adonitol, Maltotriose, 2-Deoxy Adenosine, Adenosine, Glycyl-L-Aspartic Acid, Citric Acid, M-Inositol, D-Threonine, Fumaric Acid, Bromo Succinic Acid, Propionic Acid, Mucic Acid, Glycolic Acid, Glyoxylic Acid, D-Cellobiose, Inosine, Glycyl-L-Glutamic Acid, Tricarballic Acid, L-Serine, L-Threonine, L-Alanine, L-Alanyl-Glycine, Acetoacetic Acid, N-Acetyl-beta-D-Mannosamine, Mono Methyl Succinate, Methyl Pyruvate, D-Malic Acid, L-Malic

Acid, Glycyl-L-Proline, p-Hydroxy Phenyl Acetic Acid, m-Hydroxy Phenyl Acetic Acid, Tyramine, D-Psicose, L-Lyxose, Glucuronamide, Pyruvic Acid, L-Galactonic Acid-gamma-Lactone, D-Galacturonic Acid, Pheylethyl-amine, 2-aminoethanol, ethanol, acetaldehyde, formate, Chondroitin Sulfate C, alpha-Cyclodextrin, beta-Cyclodextrin, gamma-Cyclodextrin, Dextrin, Gelatin, Glycogen, Inulin, Laminarin, Mannan, Pectin, N-Acetyl-D-Galactosamine, N-Acetyl-Neuramic Acid, beta-D-Allose, Amygdalin, D-Arabinose, D-Arabitol, L-Arabitol, Arbutin, 2-Deoxy-D-Ribose, l-Erythritol, D-Fucose, 3-O-beta-D-Galacto-pyranosyl-D-Arabinose, Gentibiose, L-Glucose, Lactitol, D-Melezitose, Maltitol, alpha-Methyl-D-Glucoside, beta-Methyl-D-Galactoside, 3-Methyl Glucose, beta-Methyl-D-Glucuronic Acid, alpha-Methyl-D-Mannoside, beta-Methyl-D-Xyloside, Palatinose, D-Raffinose, Salicin, Sedoheptulosan, L-Sorbose, Stachyose, D-Tagatose, Turanose, Xylitol, N-Acetyl-D-Glucosaminitol, gamma-Amino Butyric Acid, delta-Amino Valeric Acid, Butyric Acid, Capric Acid, Caproic Acid, Citraconic Acid, Citramalic Acid, D-Glucosamine, 2-Hydroxy Benzoic Acid, 4-Hydroxy Benzoic Acid, beta-Hydroxy Butyric Acid, gamma-Hydroxy Butyric Acid, alpha-Keto Valeric Acid, Itaconic Acid, 5-Keto-D-Gluconic Acid, D-Lactic Acid Methyl Ester, Malonic Acid, Melibionc Acid, Oxalic Acid, Oxalomalic Acid, Quinic Acid, D-Ribino-1,4-Lacton, Sebacic Acid, Sorbic Acid, Succinamic Acid, D-Tartaric Acid, L-Tartaric Acid, Acetamide, L-Alaninamide, N-Acetyl-L-Glutamic Acid, L-Arginine, Glycine, L-Histidine, L-Homserine, Hydroxy-L-Proline, L-Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Ornithine, L-Phenylalanine, L-Pyroglutamic Acid, L-Valine, D,L-Carnithine, Sec-Butylamine, D,L-Octopamine, Putrescine, Dihydroxy Acetone, 2,3-Butanediol, 2,3-Butanone, or 3-Hydroxy 2-Butanone, and combinations thereof.

32. The composition of claim 28, wherein the first type of bacteria is selected from the group consisting of *C. disporicum*, *C. mayombeii*, *C. tertium*, *C. innocuum*, and *Collinsella aerofaciens*.

33. The composition of claim 32, wherein the threshold concentration is about 90% or less the concentration required for *Clostridium difficile* proliferation.

34. The composition of claim 32, wherein the threshold concentration is about 50% or less the concentration required for *Clostridium difficile* proliferation.

35. The composition of claim 32, wherein the threshold concentration is about 10% or less the concentration required for *Clostridium difficile* proliferation.

36. A method for the treatment of *Clostridium difficile* infection in a mammalian subject, comprising the step of orally administering to the subject a composition comprising an effective amount of a first type of bacteria and a second type of bacteria formulated for oral administration to a mammalian subject, wherein the first and second type of bacteria are: (i) isolated, (ii) not identical, and (iii) capable of proliferating in a nutrient medium having a threshold concentration of a carbon-containing nutrient below that concentration required for *Clostridium difficile* proliferation, under conditions such that the first type of bacteria and the second type of bacteria functionally populate the gastrointestinal tract of the subject and prevent the population of the gastrointestinal tract by *Clostridium difficile*.

37. A method for the treatment of *Clostridium difficile* infection in a mammalian subject, comprising the step of orally administering to the subject a composition comprising an effective amount of a first type of bacteria formulated for oral administration to a mammalian subject, wherein the first type of bacteria is: (i) isolated and (ii) capable of proliferating in a nutrient medium having a threshold concentration of a carbon-containing nutrient below that concentration required for *Clostridium difficile*, under conditions such that the first type of bacteria functionally populates the gastrointestinal tract of the subject and prevent the population of the gastrointestinal tract by *Clostridium difficile*.

38. A method for the treatment of a bacterial pathogen or bacterial pathobiont infection in a mammalian subject, comprising the step of orally administering to the subject a composition comprising an effective amount of a first type of bacteria formulated for oral administration to a mammalian subject, wherein the first type of bacteria is: (i) isolated and (ii) capable of proliferating in a nutrient medium having a threshold concentration of a nutrient below that concentration required for the bacterial pathogen or the bacterial pathobiont, under conditions such that the first type of bacteria functionally populates the gastrointestinal tract of the subject and prevents the population of the gastrointestinal tract by the bacterial pathogen or bacterial pathobiont.

39. The method of claim 38, wherein the nutrient comprises a carbon-containing nutrient.
40. The method of claim 38, wherein the nutrient comprises a nitrogen-containing nutrient.
41. The method of claim 38, wherein the nutrient comprises a phosphate-containing nutrient.
42. The method of claim 38, wherein the nutrient comprises a vitamin or mineral.
43. The composition of claim 42, wherein the vitamin is selected from the group consisting of Vitamin B1 (thiamine), Vitamin B2 (riboflavin), Vitamin B5 (pantothenic acid), Vitamin B6 (pyridoxine, pyridoxal, or pyridoxamine, or pyridoxine hydrochloride), Vitamin B7 (biotin), Vitamin B9 (folic acid), and Vitamin B12 (cobalamins, cyanocobalamin), p-Aminobenzoic acid, lipoic acid, mercaptoethane-sulfonic acid, nicotinic acid, Vitamin K, and Vitamin K1.
44. The method of claim 38, wherein the nutrient comprises boron, calcium, chromium, copper, iodine, iron, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, selenium, silicon, tin, vanadium, zinc, or combinations thereof.
45. The method of claim 38, wherein the nutrient comprises S-Adenosylmethionine, hemin, heme, ammonia, or urea.
46. A method for the treatment of a bacterial pathogen or bacterial pathobiont infection in a mammalian subject, comprising the step of orally administering to the subject a composition comprising an effective amount of a first type of bacteria formulated for oral administration to a mammalian subject, wherein the first type of bacteria is: (i) isolated and (ii) capable of proliferating in a nutrient medium having a threshold concentration of a nutrient below that concentration required for a second type of bacteria, wherein the second type of bacteria produces a required nutrient for a bacterial pathogen or the bacterial pathobiont, under conditions such that the first type of bacteria functionally populates the gastrointestinal tract of the subject and i) prevents the population of the gastrointestinal tract by the second type of bacteria,

thereby preventing the population of the gastrointestinal tract by the bacterial pathogen or bacterial pathobiont, or ii) induces production by the second type of bacteria of a cytotoxic factor and/or cytostatic factor that differentially affects the bacterial pathogen or the bacterial pathobiont more than the first type of bacteria.

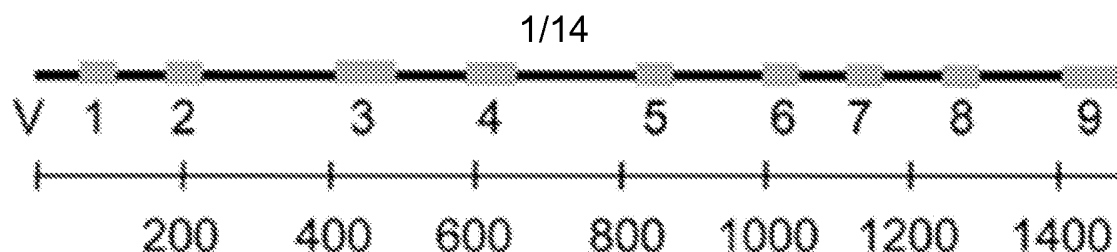


FIGURE 1A

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251 TAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAG
301 GATGACCAGCCACACTGGAAGTACGACACGGTCCAGACTCCTACGGGAGG
351 CAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
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501 ACCGGCTAACTCGTGCCCAGGCATGCGCAGGAATACGGAGGTGCAAGCGT
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651 TTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGT
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FIGURE 1B

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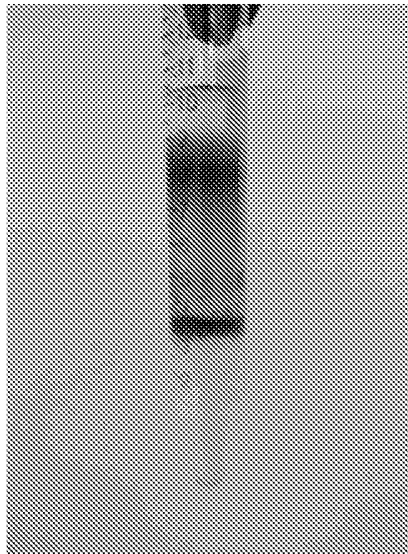


FIGURE 2

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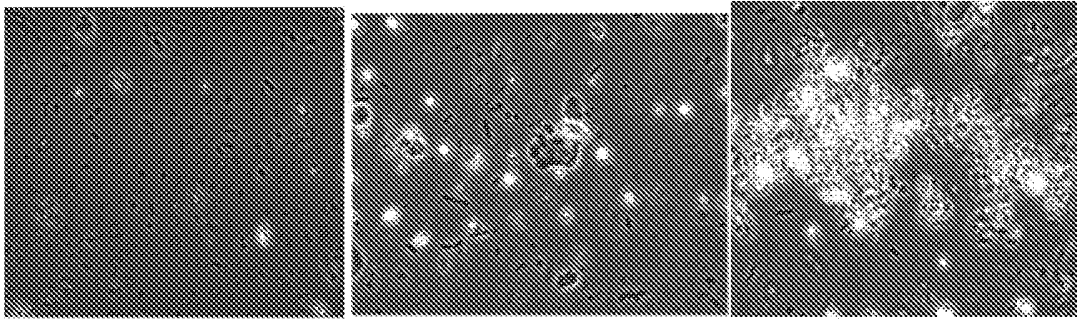


FIGURE 3

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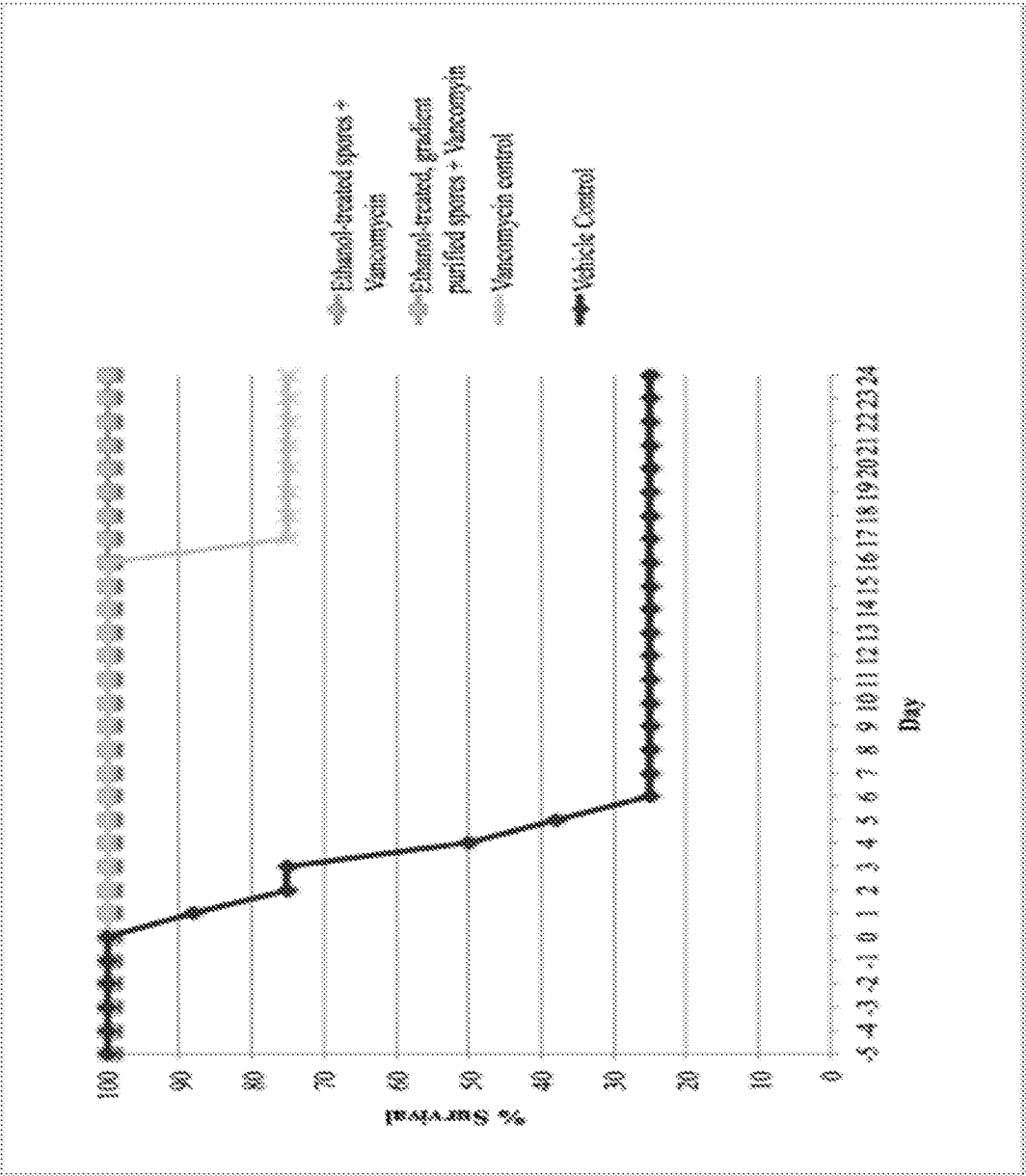


FIGURE 4

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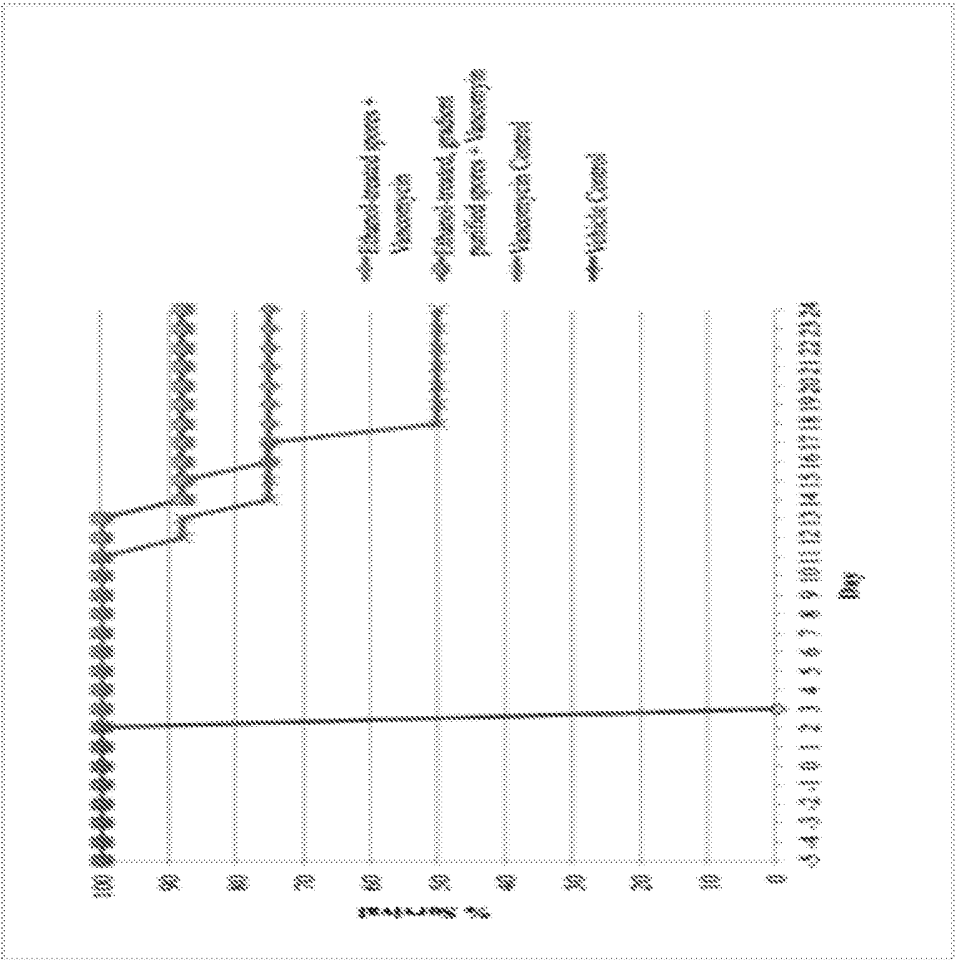


FIGURE 5

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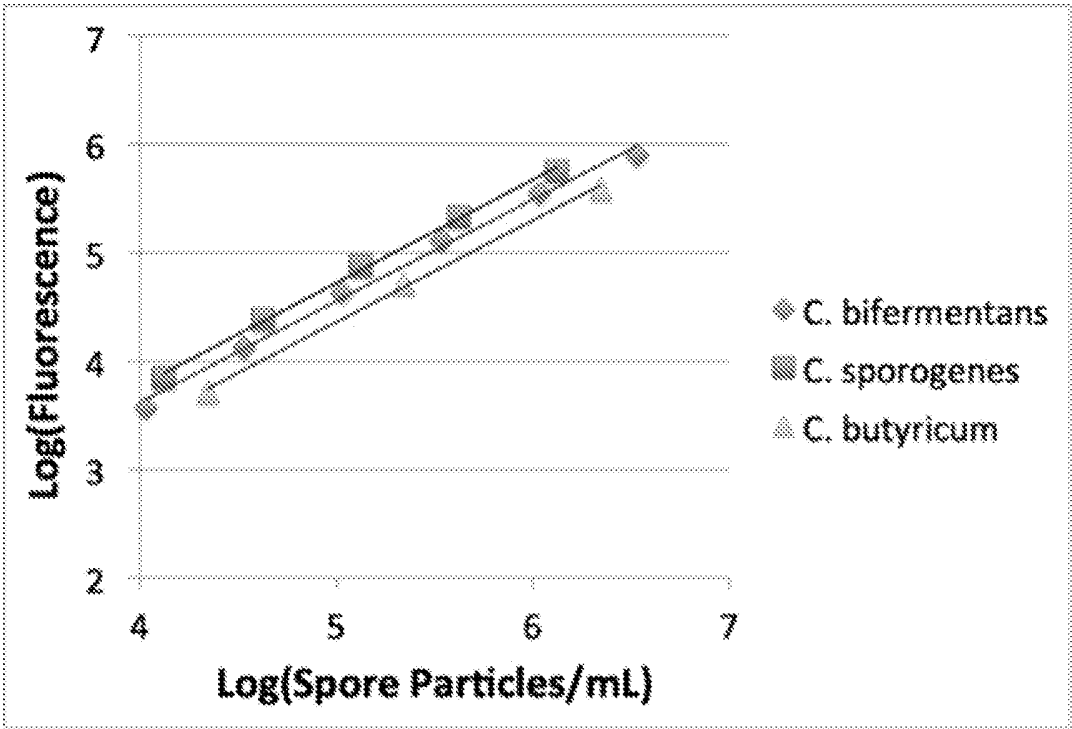


FIGURE 6

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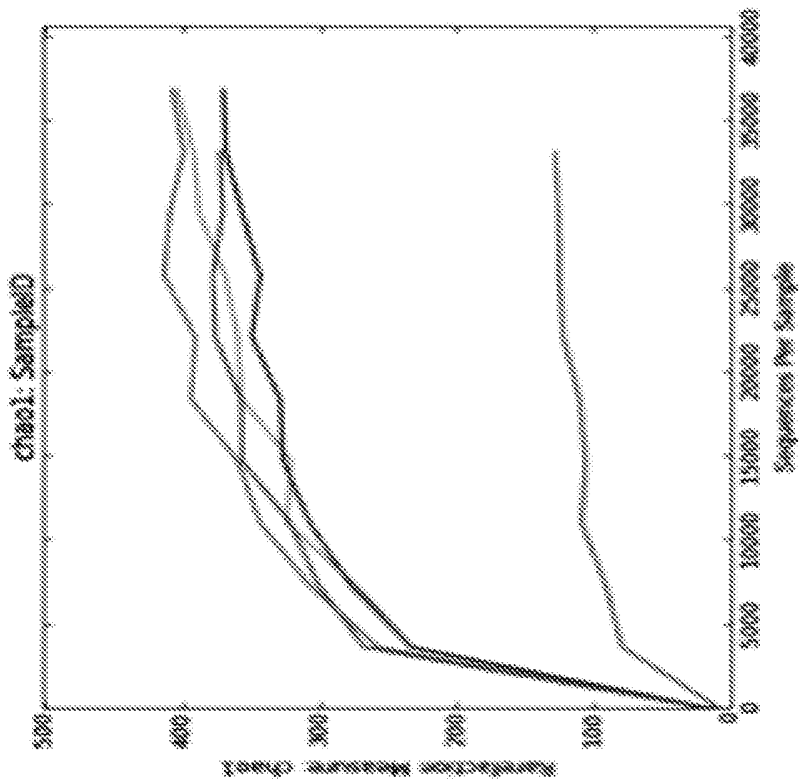


FIGURE 7

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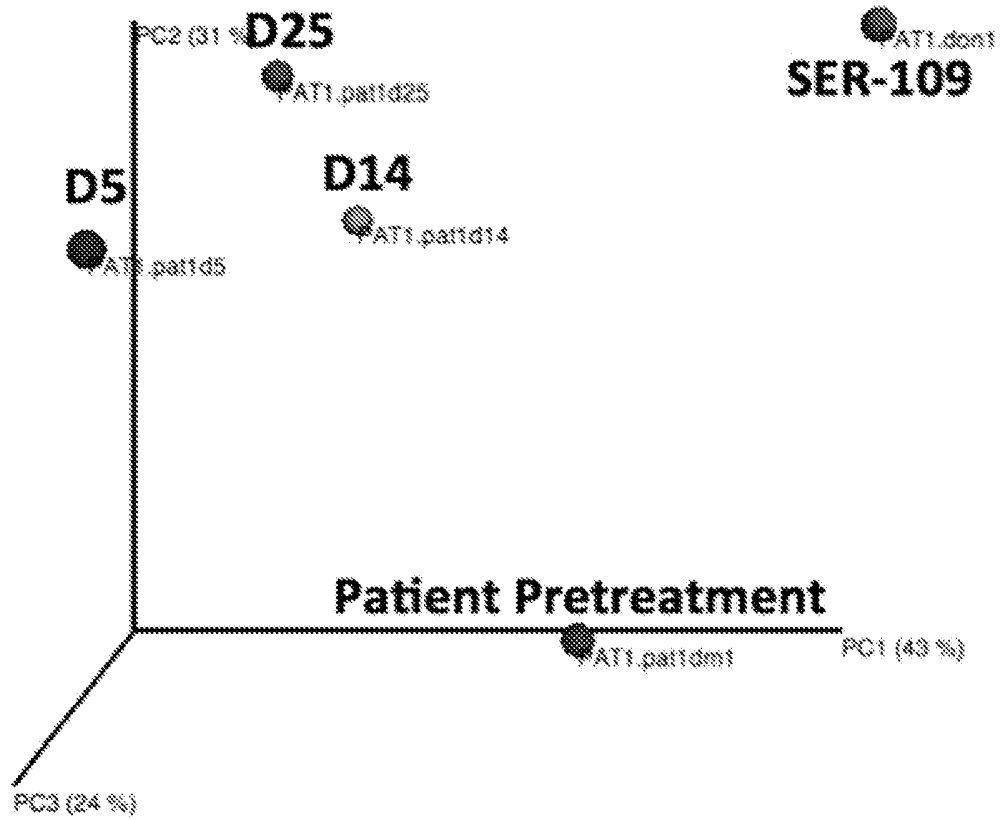


FIGURE 8

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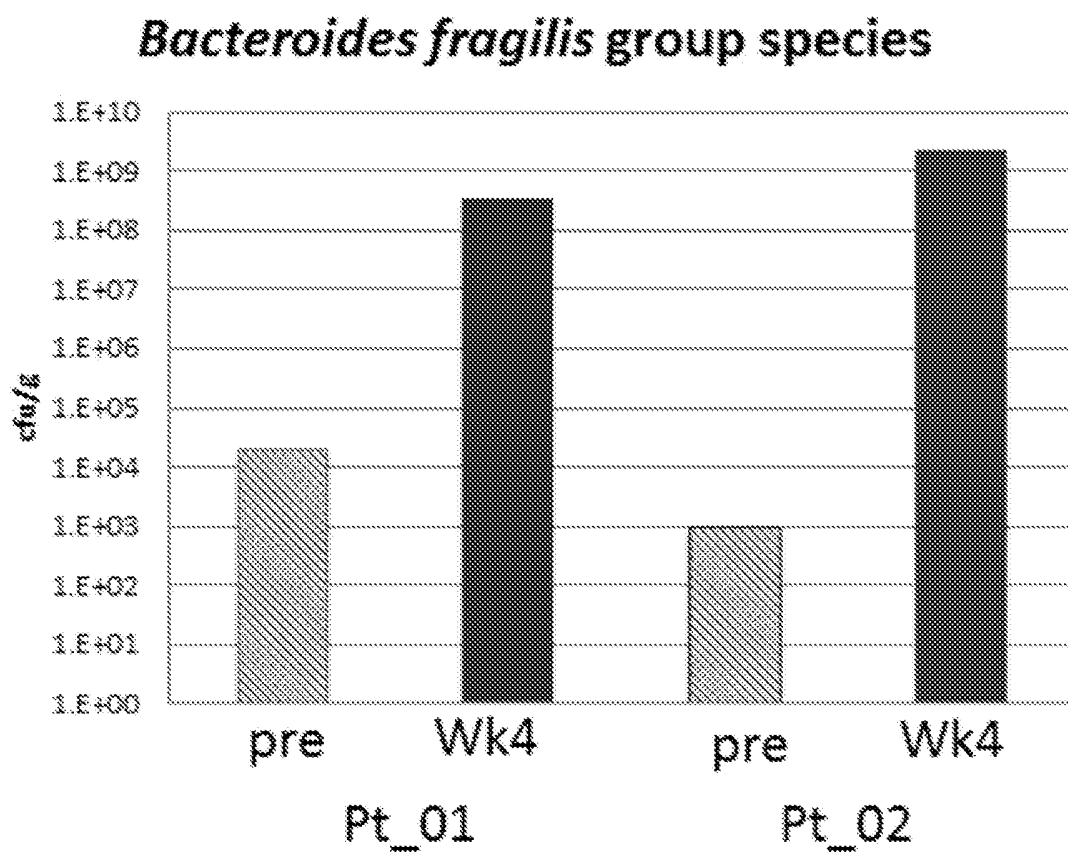


FIGURE 9

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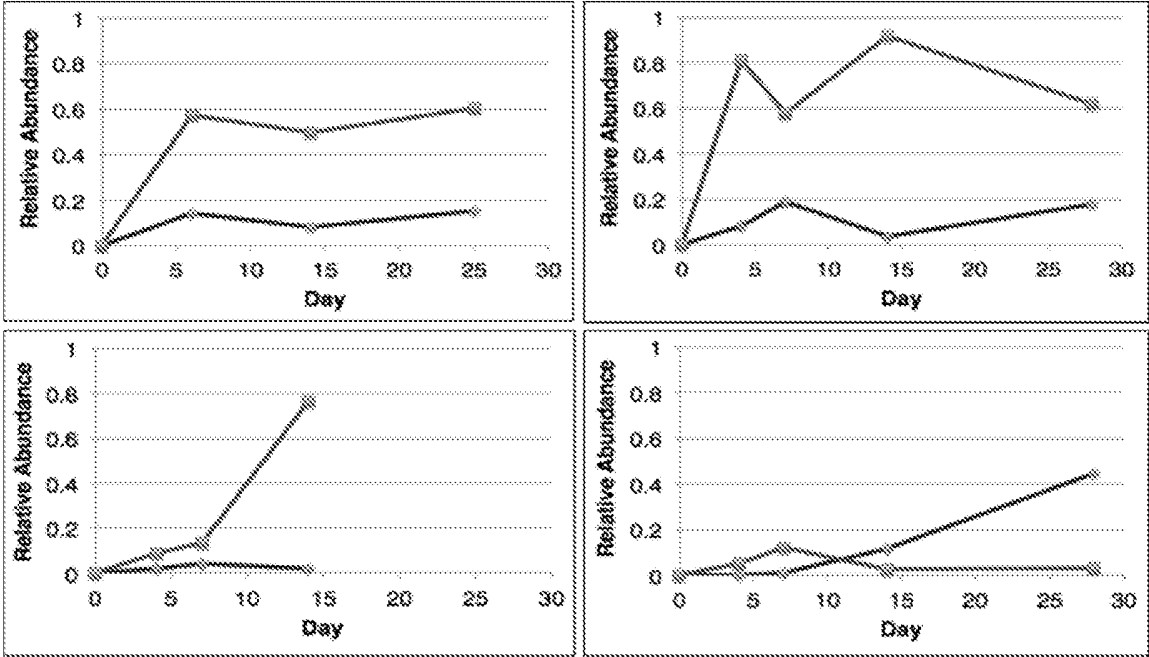


FIGURE 10

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[illegible]

FIGURE 11A

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[illegible]

FIGURE 11B

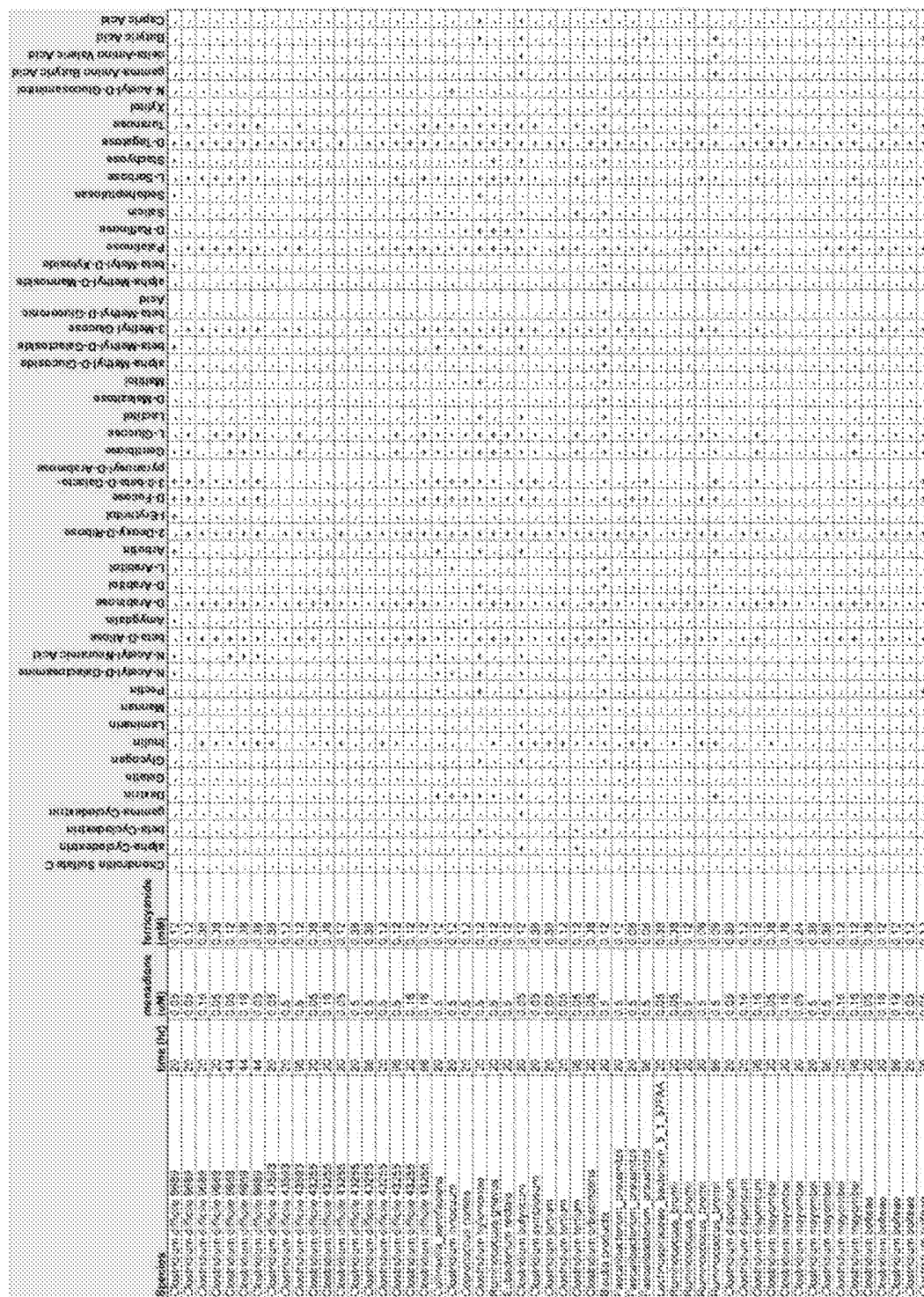


FIGURE 11C

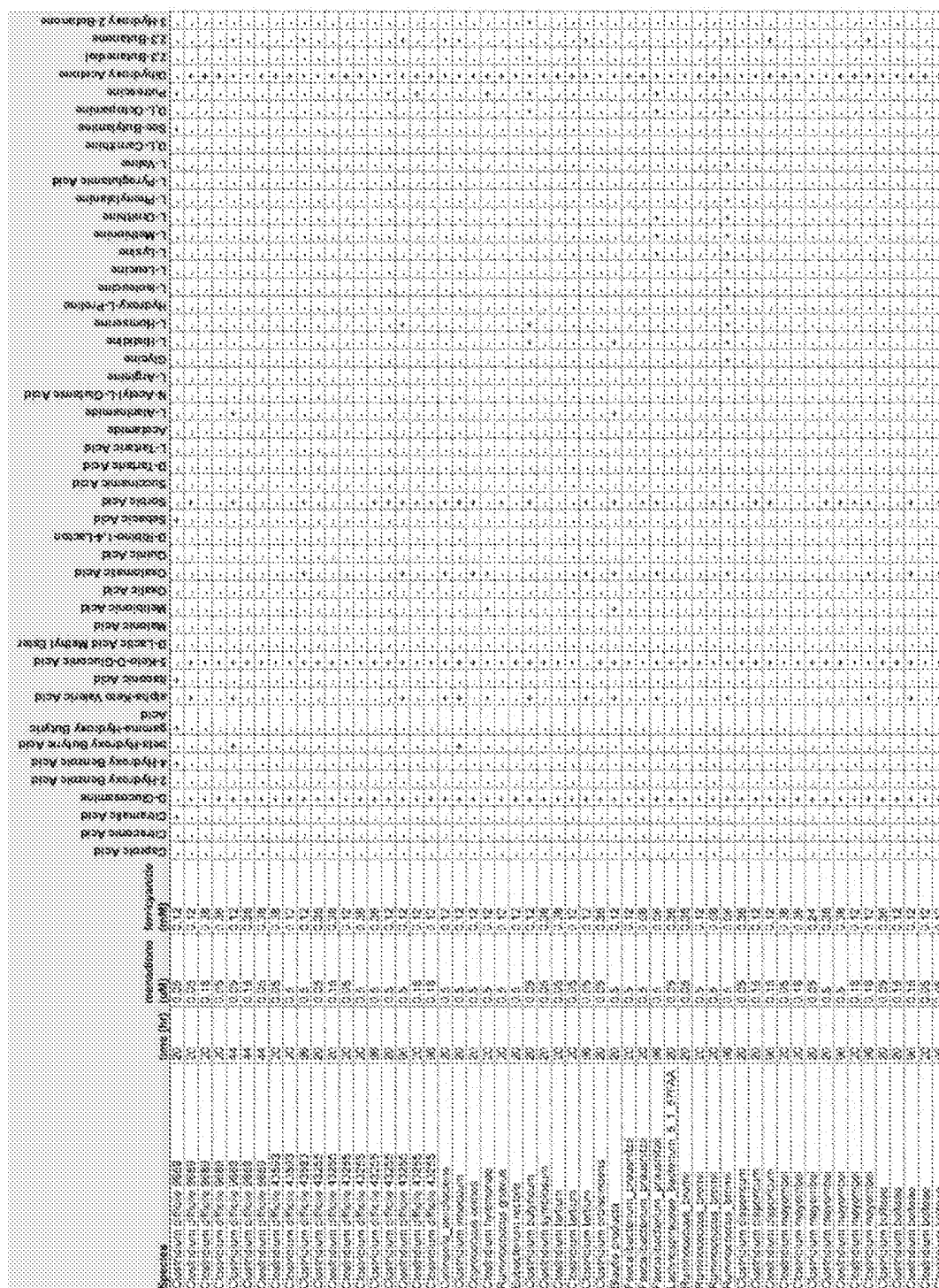


FIGURE 11D

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US14/14744

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 1/20; A61K 35/74, 35/66 (2014.01)

USPC - 435/253.6, 243; 424/93.41

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): C12N 1/20, 1/00; A61K 35/74, 35/66, 35/00 (2014.01)

USPC: 435/253.6, 243, 252.1, 252.7, 252.8, 253.4; 424/93.41, 93.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Proquest Dialog; Pubmed/Pubmed Central; Google; Google Scholar: 'Clostridium difficile,' 'C difficile,' pathogen, bacteria, pathobiont, probiotic, nutrient, vitamin, concentration, threshold, proliferate, 'Nutrient medium,' 'limiting factor,' bacteriotherapy

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y --- A	US 2004/0028689 A1 (BORODY, TJ) February 12, 2004; paragraphs [0019]-[0037], [0074], [0082]	1-3, 8-11, 16, 17, 19-29, 30/27, 30/28, 31/27, 31/28, 32, 36-46 ----- 4-7, 12-15, 18, 33-35
Y --- A	GRIMOUD, J. et al. In Vitro Screening Of Probiotic Lactic Acid Bacteria And Prebiotic Glucopoligosaccharides To Select Effective Synbiotics. Anaerobe. October 2010, Vol. 16, No. 5; pages 493-500; page 494, second column, third paragraph; page 495, second column, first paragraph to page 496, second column, third paragraph; DOI: 10.1016/j.anaerobe.2010.07.005.	1-3, 8-11, 16, 17, 19-21, 25-29, 30/27, 30/28, 31/27, 31/28, 32, 36-46 ----- 4-7, 12-15, 18, 33-35
Y --- A	US 2012/0064592 A1 (O'MULLAN, P et al.) March 15, 2012; paragraphs [0045]-[0054], [0229]	22-26
Y --- A	WILSON, K et al. Role Of Competition For Nutrients In Suppression Of Clostridium difficile By The Colonic Microflora. Infect. Immun. October 1988, Vol. 56, No. 10; pages 2610-2614; abstract; page 2611, first column, fifth paragraph to second column, second paragraph; PMID: 3417352.	8, 9, 24-26, 30/27, 30/28, 31/27, 31/28 ----- 4, 18
Y --- A	YAMAKAWA, K. et al. Enhancement Of Clostridium difficile Toxin Production In Biotin-Limited Conditions. J Med Microbiol. February 1996, Vol. 44, No. 2; pages 111-114; page 112, first column, fourth paragraph to second column, first paragraph; figure 1; DOI: 10.1099/00222615-44-2-111.	10, 11, 16, 17, 21 ----- 12
Y --- A	KARASAWA, T et al. A Defined Growth Medium For Clostridium difficile. Microbiology. February 1995, Vol. 141, No. 2; pages 371-375; page 373, first column, first paragraph; DOI: 10.1099/13500872-141-2-371.	20 ----- 5-7, 13-15, 18, 33-35

☒ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

12 May 2014 (12.05.2014)

Date of mailing of the international search report

21 MAY 2014

Name and mailing address of the ISA/US

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P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer:

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US14/14744

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

a. (means)

☐

on paper

☒

in electronic form

b. (time)

☒

in the international application as filed

☐

together with the international application in electronic form

☒

subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US14/14744

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HARRISON, F. Bacterial Cooperation In The Wild And In The Clinic: Are Pathogen Social Behaviours Relevant Outside the Laboratory? Bioessays. 27 December 2012, Vol. 35, No. 2; pages 108-112; page 108, second column, first paragraph; page 109, first column, second and third paragraphs; DOI: 10.1002/bies.201200154.	29, 46
Y --- A	IIZUKA, M et al. Elemental Diet Modulates The Growth Of Clostridium difficile In The Gut Flora. Aliment Pharmacol Ther. July 2004, Vol. 20, Suppl 1; pages 151-157; page 152, first column, second paragraph; Table 1; PMID: 15298621.	40-44 ----- 12, 18
Y	SKAAR, E. The Battle For Iron Between Bacterial Pathogens And Their Vertebrate Hosts. PLoS Pathog. 12 August 2010, Vol. 6, No. 8, entry e1000949; pages 1-4; page 3, second column, first and second paragraphs; DOI: 10.1371/journal.ppat.1000949.	45
A	KAZAMIAS, M et al. Enhanced Fermentation Of Mannitol And Release Of Cytotoxin By Clostridium difficile In Alkaline Culture Media. Appl Environ Microbiol. June 1995, Vol. 61, No. 6; pages 2425-2427; page 2425, first column, second paragraph to page 2426, first column, third paragraph; PMID: 7793964.	4, 18

摘要

本發明闡述了預防或減少致病細菌生長、增殖、和/或定殖的方法的組成含有一種或多種非致病菌引入到胃腸道並與致病菌競爭單體或聚合的碳水化合物營養素、和/或氨基酸營養素、和/或維他命營養素。