(54) HUMAN-DERIVED BACTERIA THAT INroduce PROLIFERATION OR ACCUMULATION OF REGULATORY T CELLS

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(58) Field of Classification Search
None
See application file for complete search history.

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(57) ABSTRACT
Species of human-derived bacteria belonging to the Clostridium class have been shown to induce accumulation of regulatory T cells (Treg cells) in the colon and suppress immune functions. Pharmaceutical compositions containing these bacteria can be used to prevent and treat immune-mediated diseases such as autoimmune diseases.

9 Claims, 22 Drawing Sheets
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Fig. 1C

Graphs showing the percentage of Fopx3+ cells in CD4+ T cells under different conditions:

- **C LPL**
  - GF
  - GF + huUT
  - GF + huChloro

- **SI LPL**
  - GF
  - GF + huUT
  - GF + huChloro

Fig. 1D

Graphs showing the percentage of Helios- cells in Foxp3+ cells under different conditions:

- **C LPL**
  - GF
  - GF + huUT
  - GF + huChloro

- **SI LPL**
  - GF
  - GF + huUT
  - GF + huChloro
Fig. 5A
### Fig. 8

**Cecai content from**

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Total OTUs: 153, 113, 97, 68, 89, 80, 80
HUMAN-DERIVED BACTERIA THAT INDUCE PROLIFERATION OR ACCUMULATION OF REGULATORY T CELLS

RELATED APPLICATIONS


TECHNICAL FIELD

The subject matter described herein relates to a composition of human-derived bacteria that induces proliferation, accumulation, or proliferation and accumulation of regulatory T cells and which comprises, as an active component, (a) one or more (a, at least one) human-derived bacteria that belongs to the Clostridia class, (b) culture supernatant of one or more (a, at least one) of the bacteria; (c) a physiologically active substance derived from one or more of the bacteria or (d) a combination of any two or more of the foregoing. It also relates to a method for inducing proliferation, accumulation or proliferation and accumulation of regulatory T cells. The composition, which comprises any of (a)-(d) above, is referred to as a bacterial composition. Moreover, the subject matter relates to a method for treating or preventing at least one disease or condition that is responsive to induction of regulatory T cells, such as autoimmune diseases, inflammatory diseases, and infectious diseases, by administering the bacterial composition to an individual in need thereof.

BACKGROUND

Hundreds of species of commensal microorganisms are harbored in the gastrointestinal tracts of mammals, where they interact with the host immune system. Research using germ-free (GF) animals has shown that the commensal microorganisms influence the development of the mucosal immune system, such as histogenesis of Peyer's patches (PPs) and isolated lymphoid follicles (ILFs), secretion of antimicrobial peptides from the epithelium, and accumulation of unique lymphocytes in mucosal tissues, including immunoglobulin A-producing plasma cells, intraepithelial lymphocytes, IL-17-producing CD4-positive T cells (Th17), and IL-22-producing NK-like cells (Non-Patent Literature (NPL) 1 to 7). Consequently, the presence of intestinal bacteria enhances protective functions of the mucous membranes, enabling the host to mount robust immune responses against pathogenic microbes invading the body. On the other hand, the mucosal immune system maintains unresponsiveness to dietary antigens and harmless microbes (NPL Document 3). Abnormality in the regulation of cross-talk between commensal bacteria and the immune system (intestinal dysbiosis) may lead to overly robust immune response to environmental antigens and inflammatory bowel disease (IBD) may result (NPL 8 to 10).

Recent studies have shown that individual commensal bacteria control differentiation of their specific immune cells in the mucosal immune system. For example, Bacteroides fragilis, which is a commensal bacterium in humans, specifically induces a systemic Th1 cell response and a mucosal IL-10-producing T cell response in mice, and plays a role in protecting the host from colitis, which is caused by a pathogen (NPL 3). Segmented filamentous bacteria, which are intestinal commensal bacteria in mice, induce mucosal Th17 cell response and enhance resistance against infection of gastrointestinal tracts of the host with a pathogen (NPL 11 to 13). In addition, short-chain fatty acids derived from several commensal bacteria are known to suppress intestinal inflammation (NPL 14). Moreover, it has been observed that the presence of some species of intestinal microbiota greatly influences the differentiation of regulatory T cells (hereafter referred to as “Treg cells”) which help maintain homeostasis of the immune system. Although specific species of murine bacterial commensals that can strongly stimulate Tregs have been identified (NPL 15), it is still unknown whether species of human commensal bacteria exert an equivalent influence on the human immune system. Furthermore, the human intestinal tract harbors more than a thousand bacterial species, many of which have not yet been cultured (NPL 16). It is not feasible to guess a priori which ones, if any, might have an effect on Tregs.

In order to develop drugs, dietary supplements, or foods with beneficial immune functions for human use, it is desirable to identify commensal microorganisms that naturally colonize humans and have immune-modulating properties. Furthermore, since many of the commensals in the human microbiome have yet to be cultured, it is necessary to develop methods to cultivate them so that they can be produced by traditional industrial fermentation processes and subsequently incorporated in pharmaceutical or food formulations.

CD4+ T cells are regulatory T cells that have been identified as a cell subset that suppresses immunity. A transcription factor, Foxp3, is expressed in CD4+ T cells, which are known to play an important role in maintaining immunological homeostasis (NPL 8, 9, 17, and 18). Foxp3-expressing cells are present in large numbers in the colon and only Treg cells present locally in the colon constantly express IL-10, an immunosuppressive cytokine, at a high level (NPL 19). Animals having CD4+ Foxp3+ cells from which IL-10 is specifically removed develop inflammatory bowel disease (NPL 20).

Accordingly, there is a need to identify human-derived commensal bacterial species with the ability to strongly induce Treg cells to produce IL-10 in the colon at a high level and to develop methods to culture such species. Such species could be used to enhance immunosuppression, which, in turn, can be applied to treatment of autoimmune diseases, such as inflammatory bowel disease, inflammatory diseases, allergies, or organ transplantation, among other diseases and conditions.

NON PATENT LITERATURE

The present compositions and methods have been made in view of the above-described problems in the art. Described herein are methods of identifying and culturing intestinal commensal bacteria, isolated from humans, which induce, preferably strongly induce, the proliferation, accumulation, or proliferation and accumulation of regulatory T cells. Described are compositions, also referred to as bacterial compositions, that (1) comprise (a) one or more of the identified intestinal commensal (human-derived) bacteria; (b) a culture supernatant of one or more of the bacteria; (c) one or more physiologically active substance derived from one or more of the bacteria or from one or more of the culture supernatant; (d) a combination of any two or three of (a)-(c) and (2) induce the proliferation and/or accumulation of regulatory T cells (Treg cells). Alternatively, a composition comprises (a) one or more of the identified intestinal commensal (human-derived) bacteria; (b) a culture supernatant of one or more of the bacteria; or (c) one or more physiologically active substance derived from the bacteria or from the culture supernatant, wherein the composition induces proliferation and/or accumulation of regulatory T cells. In some embodiments, the composition comprises one or more of the identified intestinal commensal (human-derived) bacteria. In some embodiments, the composition comprises a culture supernatant of one or more of the bacteria. In some embodiments, the composition comprises one or more physiologically active substance derived from the bacteria or from the culture supernatant.

In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is three or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is five or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is seven or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is twenty-three or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is twenty-three or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is twenty-three or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is twenty-three or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is twenty-three or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is twenty-three or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is twenty-three or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is twenty-three or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is twenty-three or more.
nospiraceae bacterium 5_1.57FAA, Lachnospiraceae bacterium 6_1.63FAA, Clostridium botulinum ATCC BAA-613, cf. Clostridium sp. MLG055, Erysipelotrichaceae bacterium 2_2.44A, Clostridium indolis, Anaerostipes caccae, Clostridium bolteae, Lachnospiraceae bacterium 3_1.57FAA_CT1, Anaerotruncus coliformis, Anaerotruncus coliformis DSM 17241, Ruminococcus sp. ID8, Lachnospiraceae bacterium 2_1.46FAA, Clostridium lavalense, Clostridium asparagiforme DSM 15981, Clostridium symbiosum, Clostridium symbiosum WAL-14163, Eubacterium contortum, Clostridium sp. D5, Oscillibacteraceae bacterium NML 061048, Oscillibacter valerigenes, Lachnospiraceae bacterium A4, Clostridium sp. 316002/08, and Clostridiales bacterium 1_7.47FAA, Blautia cocoides, Anaerostipes caccae DSM 14662. In some embodiments, a bacterial composition comprises a culture supernatant of at least one (a, one or more) of the bacteria described/listed herein. In some embodiments, a bacterial composition comprises a physiologically active substance derived from (a, one or more) bacteria, bacteria described/listed herein. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is three or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is five or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is 17 or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is 23 or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is 23. Bacterial compositions can comprise any bacteria (Clostridia or other bacteria) that contain DNA comprising a nucleotide sequence having sufficient homology with sequences provided herein and that exhibit substantially the same effect on regulatory T cells as that exerted by any one of the following: Clostridium saccharogena, Clostridium ramosum JCM1298, Clostridium ramosum, Flavonifractor plautii, Pseudoflavonifractor capillosus ATCC 29799, Clostridium hathewayi, Clostridium saccharolyticum WM1, Bacteroides sp. MANG, Clostridium saccharolyticum, Clostridium scindens, Lachnospiraceae bacterium 5_1.57FAA, Lachnospiraceae bacterium 6_1.63FAA, Clostridium sp. 14616, Clostridium botulinum ATCC BAA-613, cf. Clostridium sp. MLG055, Erysipelotrichaceae bacterium 2_2.44A, Clostridium indolis, Anaerostipes caccae, Clostridium bolteae, Lachnospiraceae bacterium 2_1.46FAA, Clostridium lavalense, Clostridium asparagiforme DSM 15981, Clostridium symbiosum, Clostridium symbiosum WAL-14163, Eubacterium contortum, Clostridium sp. D5, Oscillibacteraceae bacterium NML 061048, Oscillibacter valerigenes, Lachnospiraceae bacterium A4, Clostridium sp. 316002/08, and Clostridiales bacterium 1_7.47FAA, Blautia cocoides, and Anaerostipes caccae DSM 14662. In some embodiments, a bacterial composition comprises a culture supernatant of at least one (a, one or more) of the bacteria described/listed herein. In some embodiments, a bacterial composition comprises a physiologically active substance derived from (a, one or more) bacteria, bacteria described/listed herein. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is three or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is five or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is 17 or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is 23 or more. In some embodiments, the one or more bacteria or one or more physiologically active substance derived from the bacteria is 23. Bacterial compositions can comprise any bacteria (Clostridia or other bacteria) that contain DNA comprising a nucleotide sequence having sufficient homology with sequences provided herein and that exhibit substantially the same effect on regulatory T cells as that exerted by any one of the following: Clostridium saccharogena, Clostridium ramosum JCM1298, Clostridium ramosum, Flavonifractor plautii, Pseudoflavonifractor capillosus ATCC 29799, Clostridium hathewayi, Clostridium saccharolyticum WM1, Bacteroides sp. MANG, Clostridium saccharolyticum, Clostridium scindens, Lachnospiraceae bacterium 5_1.57FAA, Lachnospiraceae bacterium 6_1.63FAA, Clostridium botulinum ATCC BAA-613, cf. Clostridium sp. MLG055, Erysipelotrichaceae bacterium 2_2.44A, Clostridium indolis, Anaerostipes caccae, Clostridium bolteae, Lachnospiraceae bacterium 2_1.46FAA, Clostridium lavalense, Clostridium asparagiforme DSM 15981, Clostridium symbiosum, Clostridium symbiosum WAL-14163, Eubacterium contortum, Clostridium sp. D5, Oscillibacteraceae bacterium NML 061048, Oscillibacter valerigenes, Lachnospiraceae bacterium A4, Clostridium sp. 316002/08, and Clostridiales bacterium 1_7.47FAA, Blautia cocoides, and Anaerostipes caccae DSM 14662. In specific embodiments, the bacteria contain DNA comprising a nucleotide sequence that has at least 90% (90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) homology with one or more DNA sequence designated herein as follows: OTU136; OTU46; OTU221; OTU9; OTU296; OTU21; OTU66; OTU73; OTU174; OTU14; OTU55; OTU337; OTU352; OTU358; OTU350; OTU288; OTU334; OTU359; OTU362; or OTU367. Alternatively, bacteria contain DNA comprising a nucleotide sequence that has at least 90% (90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) homology with DNA of one or more of the following: Clostridium saccharogena, Clostridium ramosum JCM1298, Clostridium ramosum, Flavonifractor plautii, Pseudoflavonifractor capillosus ATCC 29799, Clostridium hathewayi, Clostridium saccharolyticum WM1, Bacteroides sp. MANG, Clostridium saccharolyticum, Clostridium scindens, Lachnospiraceae bacterium 5_1.57FAA, Lachnospiraceae bacterium 6_1.63FAA, Clostridium botulinum ATCC BAA-613, cf. Clostridium sp. MLG055, Erysipelotrichaceae bacterium 2_2.44A, Clostridium indolis, Anaerostipes caccae, Clostridium bolteae, Lachnospiraceae bacterium 2_1.46FAA, Clostridium lavalense, Clostridium asparagiforme DSM 15981, Clostridium symbiosum, Clostridium symbiosum WAL-14163, Eubacterium contortum, Clostridium sp. D5, Oscillibacteraceae bacterium NML.
061048, Oscillibacter valericigenes, Lachnospiraceae bacterium A4, Clostridium sp. 316002/08, and Clostridiales bacterium 1_7_47FAA, Blautia cocoides, Anaerostipes caccae DSM 14662. Any of the bacteria of the Clostridia class can be present in spore form or vegetative form.

Solution of Problem

As described herein, among the more than a thousand species of bacteria in the human microbiome, there are several species that strongly induce the accumulation of Tregs in the colon. As also described, although most bacterial species present in fecal samples from healthy individuals do not have the ability to stimulate Tregs, species that belong to the Clostridia class have the ability to cause a robust induction of Tregs in the colon. Moreover, the inventors have obtained in vitro cultures of each of the bacterial species identified and shown that inoculating mice with the in vitro cultured species also leads to a robust accumulation of Tregs in the colon.

As described herein, compositions that comprise, as an active component, (a) one or more of certain species of bacteria that belong to the Clostridia class or bacteria that contain DNA comprising a nucleotide sequence having at least 90% homology with sequences provided herein, in spore form or in vegetative form; (b) a culture supernatant of one or more such bacteria; (c) one or more physiologically active substance derived from (a) or (b); or (d) a combination of any two or three of (a), (b) and (c) and induce the proliferation and/or accumulation of regulatory T cells (Treg cells) suppress immune functions.

More specifically:

One embodiment is a composition that induces proliferation, accumulation or both proliferation and accumulation of regulatory T cells, the composition comprising, as an active component, at least one organism and/or at least one substance selected from the group consisting of: Clostridium saccharogluca, Clostridium ramosum JCM1298, Clostridium ramosum, Flavonifractor plautii, Pseudoflavonifractor capillosus ATCC 29799, Clostridium hathewayi, Clostridium saccharolyticum WM1, Bacteroides sp. MANG, Clostridium saccharolyticum, Clostridium scindens, Lachnospiraceae bacterium 5_1_57FAA, Lachnospiraceae bacterium 6_1_63FAA, Clostridium sp. 14616, Clostridium bolteae ATCC BAA-613, cf. Clostridium sp. MLG055, Erysipelotrichaceae bacterium 2_2_44A, Clostridium indolis, Anaerostipes caccae, Clostridium bolteae, Lachnospiraceae bacterium DJF_VP50, Lachnospiraceae bacterium 3_1_57FAA_C1, Anaerotrunucus Collins, Anaerotrunucus colilominis, Anaerotrunucus colilominis DSM 17241, Ruminococcus sp. ID8, Lachnospiraceae bacterium 2_1_46FAA, Clostridium lavalense, Clostridium asparagiforme DSM 15981, Clostridium symbiosus, Clostridium symbiosus WAL-14163, Eubacterium contortum, Clostridium sp. D5, Oscillospiraceae bacterium NML 061048, Oscillibacter valericigenes, Lachnospiraceae bacterium A4, Clostridium sp. 316002/08, and Clostridiales bacterium 1_7_47FAA, Blautia cocoides, Anaerostipes caccae DSM 14662; a culture supernatant of at least one of the bacteria described/listed herein, and a physiologically active substance derived from a bacterium described/listed herein.

In some embodiments, the active component is one or more of Clostridium saccharogluca, Clostridium ramosum JCM1298, Clostridium ramosum, Flavonifractor plautii, Pseudoflavonifractor capillosus ATCC 29799, Clostridium hathewayi, Clostridium saccharolyticum WM1, Bacteroides sp. MANG, Clostridium saccharolyticum, Clostridium scindens, Lachnospiraceae bacterium 5_1_57FAA, Lachnospiraceae bacterium 6_1_63FAA, Clostridium sp. 14616, Clostridium bolteae ATCC BAA-613, cf. Clostridium sp. MLG055, Erysipelotrichaceae bacterium 2_2_44A, Clostridium indolis, Anaerostipes caccae, Clostridium bolteae, Lachnospiraceae bacterium DJF_VP50, Lachnospiraceae bacterium 3_1_57FAA_C1, Anaerotrunucus Collins, Anaerotrunucus colilominis, Anaerotrunucus colilominis DSM 17241, Ruminococcus sp. ID8, Lachnospiraceae bacterium 2_1_46FAA, Clostridium lavalense, Clostridium asparagiforme DSM 15981, Clostridium symbiosus, Clostridium symbiosus WAL-14163, Eubacterium contortum, Clostridium sp. D5, Oscillospiraceae bacterium NML 061048, Oscillibacter valericigenes, Lachnospiraceae bacterium A4, Clostridium sp. 316002/08, and Clostridiales bacterium 1_7_47FAA, Blautia cocoides, Anaerostipes caccae DSM 14662 or any bacteria (such as human-derived bacteria) that contain DNA comprising at least 90% homol-
ology (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% homology) with sequences provided herein, such as, but not limited to, the nucleotide sequences designated OTU herein and listed, for example, at the pages following the last Example. In specific embodiments, the bacteria contain DNA comprising a nucleotide sequence that has at least 97%, at least 98% or at least 99% homology with one or more DNA sequence designated herein as follows: OTU136; OTU46; OTU221; OTU9; OTU296; OTU21; OTU166; OTU73; OTU174; OTU14; OTU55; OTU337; OTU314; OTU195; OTU306; OTU87; OTU86; OTU112; OTU253; OTU259; OTU281; OTU288; OTU334; OTU359; OTU362; or OTU367. Alternatively, the bacteria contain DNA comprising a nucleotide sequence that has at least 97% (97%, 98%, 99%, 100%) homology with DNA of one or more of the following: Clostridium saccharogena, Clostridium ramous JCM1298, Clostridium ramous, Flavonifractor plautii, Pseudoflavonifractor capillosus ATCC 29799, Clostridium hathewayi, Clostridium saccharolyticum WM1, Bacteroides sp. MANG, Clostridium saccharolyticum, Clostridium scindens, Lachnospiraceae bacterium 5_1_57EAA, Lachnospiraceae bacterium 6_1_63EAA, Clostridium botulinum ATCC BAA-613, cf. Clostridium sp. MLG055, Erysipelotrichaceae bacterium 2_2_44A, Clostridium indolis, Anaerostipes caccae, Clostridium botulinum, Lachnospiraceae bacterium DijVP30, Lachnospiraceae bacterium 3_1_57EAA_CTI, Anaerotrunrus colihominis, Anaerotrunrus colihominis DSM 17241, Ruminococcus sp. ID8, Lachnospiraceae bacterium 2_1_46EAA, Clostridium lavalense, Clostridium asparagiforme DSM 15981, Clostridium symbiosum, Clostridium symbiosum WAL-14163, Eubacterium contortum, Clostridium sp. D5, Oscillibacter valericigenes, Lachnospiraceae bacterium A4, Clostridium sp. 316002/08, and Clostridiales bacterium 1_7_47EAA, Blautia cocoidea, and Anaerostipes caccae DSM 14662.

In one embodiment, the composition induces regulatory T cells that are transcription factor Fox3-positive regulatory T cells or IL-10-producing regulatory T cells. In another embodiment, the composition has an immunosuppressive effect.

One embodiment is a pharmaceutical composition that induces proliferation, accumulation or both proliferation and/or accumulation of regulatory T cells and suppresses immune function. The pharmaceutical composition comprises a bacterial composition described herein and a pharmaceutically acceptable component, such as a carrier, a solvent or a diluent. In specific embodiments, such a pharmaceutical composition comprises (a) one or more species of bacteria belonging to the Clostridia class, as described herein, in spore form or in vegetative form, (b) a culture supernatant of such bacteria, (c) a physiologically active substance derived therefrom or (d) a combination of any two or three of (1), (2) and (3) and (b) a pharmaceutically acceptable component, such as carrier, a solvent or a diluent. In specific embodiments, (a) above is at least one organism or substance selected from the group consisting of: Clostridium saccharogena, Clostridium ramous JCM1298, Clostridium ramous, Flavonifractor plautii, Pseudoflavonifractor capillosus ATCC 29799, Clostridium hathewayi, Clostridium saccharolyticum WM1, Bacteroides sp. MANG, Clostridium saccharolyticum, Clostridium scindens, Lachnospiraceae bacterium 5_1_57EAA, Lachnospiraceae bacterium 6_1_63EAA, Clostridium botulinum ATCC BAA-613, cf. Clostridium sp. MLG055, Erysipelotrichaceae bacterium 2_2_44A, Clostridium indolis, Anaerostipes caccae, Clostridium botulinum, Lachnospiraceae bacterium DijVP30, Lachnospiraceae bacterium 3_1_57EAA_CTI, Anaerotrunrus colihominis, Anaerotrunrus colihominis DSM 17241, Ruminococcus sp. ID8, Lachnospiraceae bacterium 2_1_46EAA, Clostridium lavalense, Clostridium asparagiforme DSM 15981, Clostridium symbiosum, Clostridium symbiosum WAL-14163, Eubacterium contortum, Clostridium sp. D5, Oscillibacter valericigenes, Lachnospiraceae bacterium A4, Clostridium sp. 316002/08, and Clostridiales bacterium 1_7_47EAA, Blautia cocoidea, and Anaerostipes caccae DSM 14662. In some embodiments, (1) above is a culture supernatant of one or more of the bacteria. In some embodiments, (1) above is a physiologically active substance derived from one or more of the bacteria. In some embodiments, the at least one organism or substances is three or more. In some embodiments, the at least one organism or substances is five or more. In some embodiments, the at least one organism or substances is 17 or more. In some embodiments, the at least one organism or substances is 23 or more. In some embodiments, the at least one organism or substances is 23. In further embodiments, (a)(1) above is bacteria (such as human-derived bacteria) that contain DNA comprising at least 90% homology (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% homology) with sequences provided herein, such as, but not limited to, the nucleotide sequences designated OTU herein and listed, for example, at the pages following the last Example. In specific embodiments of the pharmaceutical composition, the bacteria contain DNA comprising a nucleotide sequence that has at least 97%, at least 98% or at least 99% homology with one or more DNA sequence designated herein as follows: OTU136; OTU46; OTU221; OTU9; OTU296; OTU21; OTU166; OTU73; OTU174; OTU14; OTU55; OTU337; OTU314; OTU195; OTU306; OTU87; OTU86; OTU112; OTU253; OTU259; OTU281; OTU288; OTU334; OTU359; OTU362; or OTU367. Alternatively, the bacteria in the pharmaceutical composition contain DNA comprising a nucleotide sequence that has at least 97% (97%, 98%, 99%, 100%) homology with DNA of one or more of the following: Clostridium saccharogena.
 Optionally, administration of the bacterial composition may be in combination with, or preceded by, a course of one or more antibiotics.

 Optionally, administration of the bacterial composition may be in combination with administration of at least one prebiotic substance that preferentially favors the growth of the species in the bacterial composition over the growth of other human commensal bacterial species. In one embodiment, the prebiotic substance(s) is, for example, a nongestible oligosaccharide. In specific embodiments, the one or more prebiotic substance(s) is selected from the group consisting of almond skin, inulin, oligofructose, raffinose, lactulose, pectin, hemicellulose, amylopectin, acety-Co A, biotin, beef molasses, yeast extracts, and resistant starch. Also contemplated herein is a composition that comprises the bacterial composition and at least one prebiotic substance.

 The bacterial composition may be administered in combination with a substance selected from the group consisting of corticosteroids, mesalazine, mesalamine, sulfasalazine, sulfasalazine derivatives, immunosuppressive drugs, cyclosporin A, mercaptopurine, azathiopurine, prednisone, methotrexate, antithiastamines, glucocorticoids, epinephrine, theophylline, cromolyn sodium, anti-leukotrienes, anti-cholinergic drugs for rhinitis, anti-cholinergic decongestants, mast-cell stabilizers, monoclonal anti-IgE antibodies, vaccines, anti-TNF inhibitors such as infliximab, adalimumab, certolizumab pegol, golimumab, or etanercept, and combinations thereof. Also described herein is a composition that comprises the bacterial composition and at least one substance selected from the group consisting of corticosteroids, mesalazine, mesalamine, sulfasalazine, sulfasalazine derivatives, immunosuppressive drugs, cyclosporin A, mercaptopurine, azathiopurine, prednisone, methotrexate, antithiastamines, glucocorticoids, epinephrine, theophylline, cromolyn sodium, anti-leukotrienes, anti-cholinergic drugs for rhinitis, anti-cholinergic decongestants, mast-cell stabilizers, monoclonal anti-IgE antibodies, vaccines, anti-TNF inhibitors such as infliximab, adalimumab, certolizumab pegol, golimumab, or etanercept, and combinations thereof.

 In further embodiments, the bacterial composition can be used as an adjuvant to improve the efficacy of a vaccine formulation. For example, the bacterial composition can be used as an adjuvant to a vaccine for the prophylaxis or treatment of an autoimmune disease or an allergic disease. In some embodiments, a method for prophylaxis or treatment is provided, the method comprising administering the bacterial composition and administering a vaccine.

 Assessment of the extent of induction of proliferation or accumulation of regulatory T cells that results from administration of a composition described herein can be carried out by a variety of approaches such as by measurement of the number of Foxp3-expressing Tregs in a patient sample (such as a biopsy or a blood sample), promotion of IL-10 expression, promotion of CTLA4 expression, promotion of IDO expression, suppression of IL-4 expression, or colonization of an individual with the bacterial composition. The results of such assessments are used as an index of the induction of proliferation or accumulation of regulatory T cells in the individual.

 In one embodiment, administration of a composition described herein causes induction of the regulatory T cells that are transcription factor Foxp3-positive regulatory T cells or IL-10-producing regulatory T cells.

 The composition described herein can be administered by a variety of routes and in one embodiment, is administered orally to an individual in need thereof, such as a patient in
need thereof. The composition may be administered in a number of oral forms, such as in powder, or dissolved in a liquid formulation, or enteric capsules, in sachets, or in a food matrix, such as yogurt, or a drink.

Also provided is a method to predict a subject’s response to treatment (predict whether the subject will or will not respond to treatment) with compositions of the invention. The method comprises (a) obtaining a (at least one, one or more) sample, such as a fecal sample or a colonic biopsy, from a patient before or after she or he is treated with a bacterial composition described herein; (b) measuring or determining the percentage or absolute counts in the sample of at least one bacterial species selected from the group consisting of: Clostridium saccharogumae, Clostridium ramosum JCM1298, Clostridium ramosum, Flavonifractor plautii, Pseudoflavonifractor capillosus ATCC 29799, Clostridium thauvayi, Clostridium saccharolyticum WM1, Bacteroides sp. MANG, Clostridium saccharolyticum, Clostridium scindens, Lachnospiraceae bacterium 5_1_57FAA, Lachnospiraceae bacterium 6_1_63FAA, Clostridium sp. 14616, Clostridium bolteae ATCC BAA-613, Clostridium sp. MLG055, Erysipelotrichaceae bacterium 2_2_44A, Clostridium indolis, Anaerostipes caccae, Clostridium bolteae, Lachnospiraceae bacterium DIF_VP30, Lachnospiraceae bacterium 3_1_57FAA_CT1, Anaerorarcus colihominis, Anaerorarcus colihominis DSM 17241, Ruminococcus sp. ID8, Lachnospiraceae bacterium 2_1_46FAA, Clostridium lavalense, Clostridium asparagiforme DSM 15981, Clostridium symbiosum, Clostridium symbiosum WAI-14163, Eubacterium consortum, Clostridium sp. D5, Oscillibacteraceae bacterium NML 061048, Oscillibacter valericigenes, Lachnospiraceae bacterium A4, Clostridium sp. 316002/08, and Clostridiales bacterium 1_7_47FAA, Blautia cocoides, and Anaerostipes caccae DSM 14662, thereby producing a percentage or absolute count (measurement) lower than baseline further indicates that the subject may respond favorably to administration of the bacterial composition. In some embodiments, the method further comprises administering the composition to the patient the percentage or absolute count in the sample obtained from the patient is lower than the baseline value.

EFFECTS OF COMPOSITIONS AND METHODS DESCRIBED HEREIN

The compositions described herein, which contain, as an active component, selected bacteria belonging to the Clostridia class or other bacteria, as described herein; a culture supernatant of such bacteria; a physiologically active substance derived from such bacteria; or a combination of two or three of the foregoing are excellent at inducing the proliferation or accumulation of regulatory T cells (Treg cells).

Immunity in an individual can be suppressed through administration of the subject composition, such as through ingestion of the bacterial composition in a beverage or or as a dietary supplement or through administration of a pharmaceutical composition comprising the bacterial composition. The subject composition can be used, for example, to prevent or treat autoimmune diseases, allergic diseases, infectious diseases, as well as to suppress immunological rejection in organ transplantation or the like. In addition, if a food or beverage, such as a health food, comprises the subject composition, healthy individuals can ingest the composition easily and routinely. As a result, it is possible to induce the proliferation and/or accumulation of regulatory T cells and thereby to improve immune functions.
The composition described herein provides for a natural, long-lasting, patient-friendly, and benign treatment alternative for immune-mediated conditions. For example, inflammatory bowel disease is currently managed with synthetic drugs that may have severe side effects (such as corticosteroids, TNF inhibitors), cannot be administered orally (such as TNF inhibitors), have inconvenient dosing involving several pills a day (such as mesalazine or sulfasalazine) or have limited efficacy and short-lived effects (such as currently marketed probiotics, e.g. Lactobacillus GG, Lactobacillus acidophilus, Bifidobacterium longum, etc.).

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1A is a histogram showing Foxp3 expression gated CD4 cells from colonic lamina propria (C LPL, left panel) and small intestinal lamina propria (S I LPL, right panel) of GF mice or GF mice colonized with untreated (+hUT, n=4, numbering from #A1 to #A4) or chloroform-treated (+hChloro, n=4, numbering from #B1 to #B4) human feces. Numbers above bracketed lines indicate the percentage of the population.

FIG. 1B is a histogram showing Helios expression in Foxp3+CD4+ cells from colonic lamina propria (left panel) and small intestinal lamina propria (right panel) of GF mice or GF mice colonized with untreated (+hUT) or chloroform-treated (+hChloro) human feces. Numbers above bracketed lines indicate the percentage of the population.

FIGS. 1C-1D are graphs showing, respectively, combined data for Foxp3 expression in CD4+ cells, and for Helios expression in Foxp3+CD4+ cells, from colonic lamina propria (left panel) and small intestinal lamina propria (right panel) of GF mice or GF mice colonized with untreated (+hUT) or chloroform-treated (+hChloro) human feces. Each circle in FIG. 1C and FIG. 1D represents a separate animal, and error bars indicate the SD. *P<0.05; **P<0.001, unpaired t test.

FIG. 1E shows representative flow cytometry dot plots for the intracellular expressions of IL-17 and IFN- in CD4+ cells from colonic lamina propria (upper panel) and small intestinal lamina propria (lower panel) of GF mice or GF mice colonized with untreated (+hUT) or chloroform-treated (+hChloro) human feces. The number in each quadrant indicates the percentage of the population.

FIGS. 1F-1G show, respectively, combined data of all mice for IL-17 and IFN-expression in CD4+ cells from colonic lamina propria (left panel) and small intestinal lamina propria (right panel) of GF mice or GF mice colonized with untreated (+hUT) or chloroform-treated (+hChloro) human feces. Each circle in FIG. 1F and FIG. 1G represents a separate animal, and error bars indicate the SD. *P<0.05; ns, not significant (P>0.05), unpaired t test.

FIGS. 2A-2C shows representative plots (FIG. 2A) and combined data (FIGS. 2B-2C) for Foxp3 expression in CD4+ cells (upper panel in FIG. 2A, left panel in FIG. 2B), or Helios expression in Foxp3+CD4+ cells (lower panel in FIG. 2A, right panel in FIG. 2C) for GF mice and GF mice orally inoculated (once a week for 4 weeks) with a suspension of chloroform-treated human feces that had been previously autoclaved. Numbers above bracketed lines in FIG. 2A indicate the percentage of the population. Each circle in FIG. 2B and FIG. 2C represents a separate animal, and error bars indicate the SD. ns, not significant (P>0.05), unpaired t test.

FIGS. 3A-3B shows representative plots (FIG. 3A, data of mouse #C4 is shown here) and combined data (FIG. 3B) for Foxp3 expression in CD4+ cells from colonic and small intestinal lamina propria lymphocytes for GF mice and GF mice orally inoculated with chloroform-treated human feces (+hChloro, n=7, numbering from #C1 to #C7). Numbers above bracketed lines in FIG. 3A indicate the percentage of the population. Each circle in FIG. 3B represents a separate animal, and error bars indicate the SD. **P<0.001, unpaired t test.

FIGS. 4A-4B shows representative plots (FIG. 4A) and combined data (FIG. 4B) for Foxp3 expression in CD4+ cells from colonic lamina propria (C LPL) and small intestinal lamina propria (S I LPL) for GF mice and GF (numbering from #D1 to #D6) that were co-housed with #C6 and #C7 ex-GF mice colonized with chloroform-treated human feces. Numbers above bracketed lines in FIG. 4A indicate the percentage of the population. Each circle in FIG. 4B represents a separate animal, and error bars indicate the SD. **P<0.001, unpaired t test.

FIGS. 5A-5C shows representative plots and combined data for Foxp3 expression in CD4+ cells (FIG. 5A, 5B), or Helios expression in Foxp3+CD4+ cells (FIG. 5C) from colonic lamina propria (C LPL) and small intestinal lamina propria (S I LPL) for GF mice, GF mice that were inoculated with 2000-fold (+x2000, n=4, numbering from #E1 to #E4) or 20000-fold (+x20000, n=8, numbering from #F1 to #F8) diluted fecal suspension from #C4 mouse. Numbers above bracketed lines in FIG. 5A indicate the percentage of the population. Each circle in FIG. 5B and FIG. 5C represents a separate animal, and error bars indicate the SD. *P<0.05; **P<0.001, unpaired t test.

FIGS. 6A-6D shows representative plots (FIGS. 6A, 6B) and combined data (FIGS. 6C, 6D) for Foxp3 expression in CD4+ cells (FIG. 6A, 6C), or Helios expression in Foxp3+CD4+ cells (FIGS. 6B, 6D) from colonic lamina propria (C LPL) and small intestinal lamina propria (S I LPL) for GF mice, and GF mice that were inoculated with fecal suspension of #F3 (n=5), #F7 (n=4) or #F8 (n=4) mouse. Numbers above bracketed lines in FIG. 6A and FIG. 6B indicate the percentage of the population. Each circle in FIG. 6C and FIG. 6D represents a separate animal, and error bars indicate the SD. *P<0.05; **P<0.001, unpaired t test.

FIG. 7A-7C shows representative plots (FIG. 7A) and combined data (FIGS. 7B, 7C) for Foxp3 expression in CD4+ cells (FIG. 7A, 7B) or Helios expression in Foxp3+CD4+ cells for GF mice and GF mice that were inoculated with 3 isolated strains of bacteria from cecal content of #F8 mouse (n=4, numbering from #J1 to #J4). Numbers above bracketed lines in FIG. 7A indicate the percentage of the population. Each circle in FIG. 7B and FIG. 7C represents a separate animal, and error bars indicate the SD. ns, not significant (P>0.05), unpaired t test.

FIG. 8 shows the relative abundances of OTUs having the same closest relative in each cecal sample (bacterial DNA was extracted from the cecal contents of mouse #A1, #C4, #F8, #G2, #H3, #I3, #J3 and #K3, shown in the bars). Total number of OTUs detected in each sample is depicted below the bar. The detected OTU names in sample #H3, #I3 or #K3, their closest relative and their similarity with the closest relative are depicted in the right table.

FIGS. 9A-9C shows representative plots (FIG. 9A) and combined data (FIGS. 9B, 9C) for Foxp3 expression in CD4+ cells (FIG. 9A, 9B), or Helios expression in Foxp3+CD4+ cells (FIGS. 9A, 9C) from colonic lamina propria (C LPL) and small intestinal lamina propria (S I LPL) for GF mice and GF mice that were inoculated with bacteria collections from culture plate of cecal content of #G2 mouse (n=4, numbering from #K1 to #K4). Numbers above bracketed lines in FIG. 9A indicate the percentage of the popu-
FIG. 21 shows the relative abundance of each of the 17-mix strains in the human fecal microbiota of ulcerative colitis and healthy subjects. The publicly available reads of 15 healthy and 20 ulcerative colitis subjects in the MetaHIT database were aligned to the genome of the 17 strains. The mean numbers of mapped reads in healthy and UC groups for each of the 17 strain genomes are shown. Error bars represent SEM. **P<0.05, as calculated by the Student’s t-test.

Table 1 shows the numbers of detected reads and the closest relatives for each OTU obtained from classification of sequences (3400 reads for each sample) resulting from 16s rRNA coding gene amplification and PCR metasampling of bacterial DNA extracted from the caecal contents of mouse #A1, #C4, #F8, #G2, #H3, #I3, #I3 and #K3 (classification based on sequence similarity, >97% identity to sequences in nucleic acid databases using BLAST).

Table 2 shows, for each of seventeen bacterial strains isolated from the caecal contents of mouse #F8, #G2, #I1 and #K3 using BL agar or EG agar plates, the closest relative in known species, the maximum similarity with the closest relative, its classification in the Clostridiaceae cluster, origin of mouse ID, and culture medium for isolation.

Table 3 shows, for each of 31 bacterial strains isolated from the caecal contents of mouse #F8, #G2, #I1 and #K3 using BL agar or EG agar plates, the closest relative in known species, the maximum similarity with the closest relative, the database used for BLAST search, and similarity between strains.

Table 4 shows 16S rDNA analysis for each of 31 strains that were isolated. Bacterial DNA was isolated from each of the 31 strains and the 16S rDNA of the isolates was amplified by colony-PCR. Each amplified DNA was purified, sequenced, and aligned using the ClustalW software program. Based on the sequence of 16S rDNA for each strain, their closest species, % similarity with the closest species, and the similarity to other strains are shown. Strains that were included in the 23-mix, 17-mix, 5-mixA, 5-mixB, 5-mixC and 3-mix are marked in the right hand column.

DETAILED DESCRIPTION

<Composition Having Effect of Inducing Proliferation or Accumulation of Regulatory T Cells>

Described herein is a composition that induces proliferation, accumulation of regulatory T cells or both proliferation and accumulation of regulatory T cells. The composition comprises, as an active ingredient, one or more of the following: a (at least one, one or more) organism selected from the group consisting of: Clostridium saccharogumma, Clostridium ramosum, JCM1298, Clostridium ramosum, Flavonifractor plautii, Pseudoflavonifractor capillosus ATCC 29799, Clostridium hathewayi, Clostridium saccharolyticum WM1, Bacteroides sp. MANG, Clostridium saccharolyticum, Clostridium scindens, Lachnospiraceae bacterium 5_1_57F_AAA, Lachnospiraceae bacterium 6_1_63F_AAA, Clostridium sp. 14616, Clostridium bolteae ATCC BAA-613, cf. Clostridium sp. MLG055, Erysipelotrichaceae bacterium 2_2_44A, Clostridium indolis, Anaerostipes caccae, Clostridium bolteae, Lachnospiraceae bacterium DJF_VP50, Lachnospiraceae bacterium 3_1_57F_AAA_CTI, Anaerotruncus colihominis, Anaerotruncus colihominis DSM 17241, Ruminococcus sp. ID8, Lachnospiraceae bacterium 2_1_46F_AAA, Clostridium flavalense, Clostridium asparagusforme DSM 15981, Clostridium symbiosis, Clostridium symbiosis WAL-14163, Eubacterium contra, Clostridium sp. D5, Ocellispiraeaceae bacterium
Oscillibacter valericigenes, Lachnospiraceae bacterium A4, Clostridium sp. 316002/08, and Clostridiales bacterium 1_7_47FAA, Blautia coccoides, Anaerostipes caccae DSM 14662, a culture supernatant of one or more of the bacteria, a component of culture medium in which a (at least one, one or more) bacterium described herein has grown, a physiologically active substance derived from a (at least one; one or more) bacterium described herein; and a (at least one; one or more) bacterium containing DNA comprising a nucleotide sequence having at least 97% homology to the nucleotide sequence of DNA of any of the bacterial species described herein, such as those listed above. Bacteria described herein were isolated from human fecal samples using the methods outlined in Examples 19 to 28.

The term “regulatory T cells” refers to T cells that suppress an abnormal or excessive immune response and play a role in immune tolerance. The regulatory T cells are typically transcription factor Foxp3-positive CD4-positive T cells. The regulatory T cells of the present invention also include transcription factor Foxp3-negative regulatory T cells that are IL-10-producing CD4-positive T cells.

The term “induces proliferation or accumulation of regulatory T cells” refers to an effect of inducing the differentiation of immature T cells into regulatory T cells, which differentiation leads to the proliferation and/or the accumulation of regulatory T cells. Further, the meaning of “induces proliferation or accumulation of regulatory T cells” includes in vivo effects, in vitro effects, and ex vivo effects. All of the following effects are included: an effect of inducing in vivo proliferation or accumulation of regulatory T cells through administration or ingestion of the aforementioned bacteria belonging to the Clostridia class, a culture supernatant of the bacteria or supernatant component(s), or a physiologically active substance derived from the bacteria; an effect of inducing proliferation or accumulation of cultured regulatory T cells by causing the aforementioned bacteria belonging to the Clostridia class, a culture supernatant of the bacteria or supernatant component(s), or a physiologically active substance derived from the bacteria to act on the cultured regulatory T cells; and an effect of inducing proliferation or accumulation of regulatory T cells which are collected from a living organism and which are intended to be subsequently introduced into a living organism, such as the organism from which they were obtained or another organism, by causing the aforementioned bacteria belonging to the Clostridia class, a culture supernatant of the bacteria or supernatant component(s), or the physiologically active substance derived from the bacteria to act on the regulatory T cells. The effect of inducing proliferation or accumulation of regulatory T cells can be evaluated, for example, as follows. Specifically, the aforementioned bacteria belonging to the Clostridia class, a culture supernatant of the bacteria or supernatant component(s), or a physiologically active substance derived from the bacteria is orally administered to an experimental animal, such as a germ-free mouse, then CD4-positive cells in the colon are isolated, and the ratio of regulatory T cells contained in the CD4-positive cells is measured by flow cytometry (refer to Example 7).

The regulatory T cells whose proliferation or accumulation is induced by the composition of the present invention are preferably transcription factor Foxp3-positive regulatory T cells or IL-10-producing regulatory T cells.

In the present invention, “human-derived bacteria” means bacterial species that have been isolated from a fecal sample or from a gastrointestinal biopsy obtained from a human individual or whose ancestors were isolated from a fecal sample or from a gastrointestinal biopsy obtained from a human (e.g., are progeny of bacteria obtained from a fecal sample or a gastrointestinal biopsy). For example, the bacterial species may have been previously isolated from a fecal sample or from a gastrointestinal biopsy obtained from a human and cultured for a sufficient time to generate progeny. The progeny can then be further cultured or frozen. The human-derived bacteria are naturally occurring commensals that populate the gastrointestinal tract of human individuals, preferably healthy human individuals.

In the present invention, the term “Clostridia class” (as in “compositions containing bacteria belonging to the Clostridia class”) refers to a class of Gram+, obligate anaerobic bacteria belonging to the Firmicutes phylum that have the ability to form spores. It is important to note that while currently most bacteria in this class are included in the Clostridiales order, this categorization is still partly based on old methods and is likely to be redefined in the future based on new advances in sequencing technologies that are enabling sequencing of the full genomes of bacteria in this class. Table 2 provides a summary of the categorization of 17 abundant species belonging to the Clostridia class which have been identified by the inventors as strong Treg-inducers and cultured in vitro. All of these species fall, under current categorization rules, in the Clostridiales family, and belong to clusters IV, XIa, XVI, and XVIII.

The composition of the present invention may include one strain alone (only one strain) of any of the aforementioned bacterial species, but two or more strains of the bacteria can be used together. For example, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, or seventeen of the strains listed in Table 2 or Table 4, in any combination, can be used together to affect regulatory T cells. In some embodiments, the 23, 17, 5, or 3 species mixes listed in Table 4 can be used together (and administered in one or several compositions) to affect regulatory T cells. In some embodiments, the following strains can be combined (the composition comprises): strain 1 (OTU136, closest species: Clostridium saccharogena, Clostridium ramosum JCM1298), strain 3 (OTU221, closest species: Flavonifractor plautii, Pseudoflavonifractor capillosus ATCC 29799), strain 4 (OTU9, closest species: Clostridium hathewayi, Clostridium saccharolyticum WM1), strain 5 (OTU296, closest species: Cladosporium scindens, Lachnospiraceae bacterium 5_1_57FAA), and strain 6 (OTU21, closest species: Blautia coccoides, Lachnospiraceae bacterium 6_1_63FAA), strain 2 (OTU136, closest species: Clostridium coccae DSM 14662, strain 10 (OTU166, closest species: Cladosporium bolteae, Cladosporium bolteae, ATCC BAA-613), strain 8 (OTU73, closest species: Clostridium sp. MLA655, Erysipelotrichaceae bacterium 2_2_44A), strain 9 (OTU174, closest species: Cladosporium indol, Anaerostipes caccae DSM 14662), strain 10 (OTU166, closest species: Cladosporium bolteae, Cladosporium bolteae, ATCC BAA-613), strain 12 (OTU55, closest species: Lachnospiraceae bacterium DJF_VP30, Lachnospiraceae bacterium 3_1_57FAA_CTI), strain 13 (OTU337, closest species: Anaerotruncus colihominis, Anaerotruncus colihominis DSM 17241), strain 14 (OTU314, closest species: Ruminococcus sp. ID8, Lachnospiraceae bacterium 2_1_46FAA), strain 15 (OTU195, closest species: Clostridium lavalense, Clostridium asporogiforme DSM 15981), strain 16 (OTU306, closest species: Clostridium symbiosum, Clostridium symbiosum WAL-14163), strain 18 (OTU46, closest species: Clostridium ramosum, Clostridium ramosum), strain 21 (OTU87, closest species: Eubacterium contractum, Clostridium sp. D5), strain 23 (OTU152, closest species: Lachnospiraceae bacterium DJF_VP50, Lachnospiraceae bacterium
3._1.57FAA_CT1), strain 24 (OTU253, closest species: Oscillospiraceae bacterium NML 061048, Oscillibacter valericigenes), strain 25 (OTU259, closest species: Eubacterium consortium, Clostridium sp. D53), strain 26 (OTU281, closest species: Clostridium scindens, Lachnospiraceae bacterium 5.1.57FAA), strain 27 (OTU288, closest species: Lachnospiraceae bacterium 4A, Lachnospiraceae bacterium 3.1.57FAA_CT1), strain 28 (OTU344, closest species: Clostridium sp. 316002/08, Clostridiales bacterium 1.7.47FAA), and strain 29 (OTU359, closest species: Lachnospiraceae bacterium A4, Lachnospiraceae bacterium 3.1.57FAA_CT1) as described in Table 4.

In some embodiments, the following strains can be combined: strain 3 (OTU221, closest species: Flavonifractor plautii, Pseudoflavonifractor capillosus ATCC 29799), strain 7 (OUT 166, closest species: Clostridium sp., Clostridium bolteae ATCC BAA-613), strain 9 (OTU174, closest species: Clostridium indolis, Anaerostipes caccae DSM 14662), strain 15 (OTU195, closest species: Clostridium lavalense, Clostridium asparagiforme DSM 15981), and strain 28 (OTU344, closest species: Clostridium sp. 316002/08, Clostridiales bacterium 1.7.47FAA) as described in Table 4. In some embodiments, the following strains can be combined: strain 1 (OTU136, closest species: Clostridium saccharogenum, Clostridium ramosum JCM1298), strain 3 (OTU221, closest species: Flavonifractor plautii, Pseudoflavonifractor capillosus ATCC 29799), strain 4 (OTU9, closest species: Clostridium halotolerans, Clostridium saccharolyticum WM1), strain 6 (OTU21, closest species: Blautia cocoides, Lachnospiraceae bacterium 6.1.63FAA), strain 7 (OUT 166, closest species: Clostridium sp., Clostridium bolteae ATCC BAA-613), strain 8 (OTU73, closest species: Clostridium sp. MLG055, Erysipelotrichaceae bacterium 2.2.44A), strain 9 (OTU174, closest species: Clostridium indolis, Anaerostipes caccae DSM 14662), strain 13 (OUT37, closest species: Anaerotalea coliformis, Anaerobacteriaceae coliformis DSM 17241), strain 14 (OTU314, closest species: Ruminococcus sp. ID8, Lachnospiraceae bacterium 2.1.46FAA), strain 15 (OTU195, closest species: Clostridium lavalense, Clostridium asparagiforme DSM 15981), strain 16 (OTU306, closest species: Clostridium symbiosum, Clostridium symbiosum WAL-14163), strain 18 (OTU46, closest species: Clostridium ramosum, Clostridium ramosum), strain 21 (OTU87, closest species: Eubacterium consortium, Clostridium sp. D53), strain 26 (OTU281, closest species: Clostridium scindens, Lachnospiraceae bacterium 5.1.57FAA), strain 27 (OTU288, closest species: Lachnospiraceae bacterium A4, Lachnospiraceae bacterium 3.1.57FAA_CT1), strain 28 (OTU344, closest species: Clostridium sp. 316002/08, Clostridiales bacterium 1.7.47FAA), and strain 29 (OTU359, closest species: Lachnospiraceae bacterium A4, Lachnospiraceae bacterium 3.1.57FAA_CT1) as described in Table 4.

In some embodiments, the following strains can be combined: strain 3 (OTU221, closest species: Flavonifractor plautii, Pseudoflavonifractor capillosus ATCC 29799), strain 7 (OUT 166, closest species: Clostridium sp., Clostridium bolteae ATCC BAA-613), strain 9 (OTU174, closest species: Clostridium indolis, Anaerostipes caccae DSM 14662), strain 15 (OTU195, closest species: Clostridium lavalense, Clostridium asparagiforme DSM 15981), and strain 28 (OTU344, closest species: Clostridium sp. 316002/08, Clostridiales bacterium 1.7.47FAA) as described in Table 4. The use of multiple strains of the aforementioned species of bacteria, preferably belonging to the Clostridia cluster XIVa or the cluster IV in combination can bring about an excellent effect on regulatory T cells. In addition to the bacteria belonging to clusters XIVa and IV, Clostridium ramosum, Clostridium saccharogenum (belonging to cluster XVIII) and cf. Clostridium sp. MLG055 (belonging to cluster XVI) can also be used. If more than one strain of bacteria is used (e.g., one or more strain belonging to cluster XIVa, one or more strain belonging to cluster IV, one or more strain belonging to clusters XVIII or XVI or a combination of any of the foregoing), the number and ratio of strains used can vary widely. The number and ratio used can be determined based on a variety of factors (e.g., the desired effect, such as induction or inhibition of proliferation or accumulation of regulatory T cells; the disease or condition to be treated, prevented or reduced in severity; the age or gender of the recipient; the typical amounts of the strains in healthy humans). The strains can be present in a single composition, in which case they can be consumed or ingested together (in a single composition), or can be present in more than one composition (e.g., each can be in a separate composition), in which case they can be consumed individually or the compositions can be combined and the resulting combination (combined compositions) consumed or ingested. Any number or combination of the strains that proves effective (e.g., any number from one to 22, such as 1 to 20, 1 to 15, 1 to 10, 1 to 5, 1 to 3, 1 to 2, and any number therebetween or one to 23, such as 1 to 23, 3 to 23, 5 to 23, 1 to 20, 1 to 17, 3 to 17, 5 to 17, 1 to 15, 1 to 10, 1 to 5, 1 to 3, 1 to 2, and any number therebetween) can be administered. In certain embodiments of the present invention, a combination of some or all of the 22 or 23 (e.g., the 23 strains in Example 32 and Table 4) strains described in the present disclosure is used. For example, at least one, two or more, three, three or more, four, four or more, five, five or more, six, six or more, or any other number of the 22 or 23 described strains, including 22 or 23 strains, can be used. In some embodiments, the specific combinations of 3, 5, 17, or 23 strains described in Table 4 can be used (the composition comprises combinations of 3, 5, 17 or 23 strains described in Table 4). They can be used in combination with, one another, and in combination with strains not described in the cited reference.

Cells of bacteria belonging to the Clostridia class, such as these specifically described herein, can be used in spore form or in vegetative form. From the viewpoint of stability to high temperature and pressure conditions, extended shelf life, ease of handling, resistance to antibiotics, and lack of need for a cold chain storage and distribution, the bacteria
may be preferably in the form of spore. From the viewpoint of abiding by the directives of certain manufacturing organizations that do not tolerate spore contamination in their facilities, the bacteria may alternatively be produced (and later administered) in the form of vegetative cells.

The term the "physiologically active substance derived from bacteria belonging to the Clostridia class" of the present invention includes substances contained in the bacteria, secretion products of the bacteria, and metabolites of the bacteria. Such a physiologically active substance can be identified by purifying an active component from the bacteria, a culture supernatant thereof, or intestinal tract contents in the intestinal tract of a mouse in which only bacteria belonging to the Clostridia class are colonized by an already known purification method.

"Chloroform treatment" of a fecal sample obtained from a human is a method that isolates the bacteria in the fecal sample that have the ability to form spores, and is not particularly limited, as long as the spore-forming fraction is obtained by treating feces of a human with chloroform (for example, 3% chloroform), and has the effect of inducing proliferation or accumulation of regulatory T cells, including mammalian regulatory T cells such as murine regulatory T cells and human regulatory T cells.

When the aforementioned "bacteria belonging to the Clostridia class" are cultured in a medium, substances contained in the bacteria, secretion products and metabolites produced by the bacteria are released from the bacteria. The meaning of the active ingredient "culture supernatant of the bacteria" in the composition of the present invention includes such substances, secretion products, and metabolites. The culture supernatant is not particularly limited, as long as the culture supernatant has the effect of inducing proliferation or accumulation of regulatory T cells. Examples of culture supernatant include a protein fraction of the culture supernatant, a polysaccharide fraction of the culture supernatant, a lipid fraction of the culture supernatant, and a low-molecular weight metabolite fraction of the culture supernatant.

The bacterial composition may be administered in the form of a pharmaceutical composition, a dietary supplement, or a food or beverage (which may also be an animal feed), or may be used as a reagent for an animal model experiment. The pharmaceutical composition, the dietary supplement, the food or beverage, and the reagent induce proliferation or accumulation of regulatory T cells. An example presented herein revealed that regulatory T cells (Treg cells) induced by bacteria or the like belonging to the Clostridia class suppressed the proliferation of effector T-cells. The composition of the present invention can be used suitably as a composition having an immunosuppressive effect. The immunosuppressive effect can be evaluated, for example, as follows. Regulatory T cells isolated from an experimental animal, such as a mouse, to which the composition of the present invention is orally administered are caused to act on effector T-cells (CD4+CD25- cells) isolated from the spleen, and the proliferation ability thereof is measured by using the intake amount of [3H]-thymidine as an index (refer to Example 14).

The bacterial composition of the present invention can be used, for example, as a pharmaceutical composition for preventing or treating (reducing, partially or completely, the adverse effects of) an autoimmune disease, such as chronic inflammatory bowel disease, systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, or Hashimoto's disease; an allergic disease, such as a food allergy, pollinosis, or asthma; an infectious disease, such as an infection with Clostridium difficile; an inflammatory disease such as a TNF-mediated inflammatory disease (e.g., an inflammatory disease of the gastrointestinal tract, such as pouchitis, a cardiovascular inflammatory condition, such as atherosclerosis, or an inflammatory lung disease, such as chronic obstructive pulmonary disease); a pharmaceutical composition for suppressing rejection in organ transplantation or other situations in which tissue rejection might occur; a supplement, food, or beverage for improving immune functions; or a reagent for suppressing the proliferation or function of effector T-cells.

More specific examples of target diseases for which the composition is useful for treatment (reducing adverse effects or prevention) include autoimmune diseases, allergic diseases, infectious diseases, and rejection in organ transplantations, such as inflammatory bowel disease (IBD), ulcerative colitis, Crohn's disease, sprue, autoimmune arthritis, rheumatoid arthritis, Type I diabetes, multiple sclerosis, graft vs. host disease following bone marrow transplantation, osteoarthritis, juvenile chronic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, insulin dependent diabetes mellitus, thyroiditis, asthma, psoriasis, dermatitis, atopic dermatitis, graft versus host disease, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpura, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, polyclaglandular deficiency type I syndrome and polyclaglandular deficiency type II syndrome, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, anerogenic athropy, arthropathy, Reiter's disease, psoriatic arthropathy, chlamydia, yersinia and salmonella associated arthropathy, spondyloarthopathy, atheromatous disease/artersclerosis, allergic colitis, atopic allergy, food allergies such as peanut allergy, tree nut allergy, egg allergy, milk allergy, soy allergy, wheat allergy, seafood allergy, shellfish allergy, or sesame seed allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryoglobulinemia autoimmun hepatitis, Acquired Immunodeficiency Disease Syndrome, Acquired Immunodeficiency Related Diseases, Hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinemia), dilated cardiomyopathy, fibrotic lung disease, cystic fibrosis alveolitis, postinflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjogren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitis diffuse lung disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneu-
monia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hyperglicemia, type B insulin resistance with acanthosis nigricans, hyperparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthrosis, primary sclerosing cholangitis, idiopathic leucopenia, autoimmune neutropenia, renal disease NOS, glomerulonephritis, microscopic vasculitis of the kidneys, discoid lupus, erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple sclerosis (all subtypes), insulinedependent diabetes mellitus, sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture’s syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatoid fever, rheumatoid spondylitis, Still’s disease, systemic sclerosis, Takayasu’s disease, autoimmune arthritis, autoimmune thyroiditis, idiopathic thrombocytopenic purpura, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto’s disease), atrophic autoimmune hypothyroidism, primary myxoedema, phagocytic wetsit, primary vasculitis, vitiligo, allergic rhinitis (pollen allergies), anaphylaxis, pet allergies, latex allergies, drug allergies, allergic rhinoconjunctivitis, eosinophilic esophagitis, hypereosinophilic syndrome, eosinophilic gastroenteritis cutaneous lupus erythematosus, eosinophilic esophagitis, hypereosinophilic syndrome, and eosinophilic gastroenteritis, and diarrhea.

Additional examples of target diseases for which the composition is useful for treatment include colon cancer, cystic fibrosis, celiac disease, Type 2 diabetes, and autoimmune-related immunopathologies. These diseases are characterized by a reduction of Clostridium Clusters IV and XIV in the gastrointestinal microbiota.

Compositions described herein can also be used as a pharmaceutical composition for preventing or treating infectious diseases in an individual whose resistance to the infectious diseases is impaired, for example because of damage due to excessive inflammation caused by the immunity or due to an alteration of the patient’s microbiome. Examples of infectious pathogens that impair maintenance or recovery of homeostasis of a host, and which eventually bring about such immunopathological tissue damage include Salmonella, Shigella, Clostridium difficile, Mycobacterium (which cause the disease tuberculosis), protozoa (which cause malaria), filarial nematodes (which cause the disease filariasis), Schistosoma (which cause schistosomiasis), Toxoplasma (which cause the disease toxoplasmosis), Leishmania (which cause the disease leishmaniasis), HIV and HBV (which cause the disease hepatitis C and hepatitis B), and herpes simplex viruses (which cause the disease herpes).

Pharmaceutical preparations can be formulated from the bacterial compositions described by drug formulation methods known to those of skill in the art. For example, the composition can be used orally or parenterally in the form of capsules, tablets, pills, sachets, liquids, powders, granules, fine granules, film-coated preparations, pellets, troches, sublingual preparations, chewables, buccal preparations, pastes, syrups, suspensions, elixirs, emulsions, liniments, ointments, pastes, ointments, lotions, inhalations, aerosols, injections, suppositories, and the like.

For formulating these preparations, the bacterial compositions can be used in appropriate combination with carriers that are pharmacologically acceptable or acceptable for ingestion, such as in a food or beverage, including one or more of the following: sterile water, physiological saline, vegetable oil, solvent, a base material, an emulsifier, a suspending agent, a surfactant, a stabilizer, a flavoring agent, an aromatic, an excipient, a vehicle, a preservative, a binder, a diluent, a toxin adjusting agent, a soothing agent, a bulking agent, a disintegrating agent, a buffer agent, a coating agent, a lubricant, a colorant, a sweetener, a thickening agent, a flavor corrigent, a solubilizer, and other additives.

A pharmaceutical preparation or formulation and particularly a pharmaceutical preparation for oral administration, comprises an additional component that enables efficient delivery of the bacterial composition of the present invention to the colon, in order to more efficiently induce proliferation or accumulation of regulatory T cells in the colon. A variety of pharmaceutical preparations that enable the delivery of the bacterial composition to the colon can be used. Examples thereof include pH sensitive compositions, more specifically, buffered gel formulations or enteric polymers that release their contents when the pH becomes alkaline after the enteric polymers pass through the stomach. When a pH sensitive composition is used for formulating the pharmaceutical preparation, the pH sensitive composition is preferably a polymer whose pH threshold of the decomposition of the composition is between about 6.8 and about 7.5. Such a numeric value range is a range in which the pH shifts toward the alkaline side at a distal portion of the stomach, and hence is a suitable range for use in the delivery to the colon.

Another embodiment of a pharmaceutical preparation useful for delivery of the bacterial composition to the colon is one that ensures the delivery to the colon by delaying the release of the contents (e.g., the bacterial composition) by approximately 3 to 5 hours, which corresponds to the small intestinal transit time. In one embodiment of a pharmaceutical preparation for delayed release, a hydrogel is used as a shell. The hydrogel is hydrated and swells upon contact with gastrointestinal fluid, with the result that the contents are effectively released (released predominantly in the colon). Delayed release dosage units include drug-containing compositions having a material which coats or selectively coats a drug or active ingredient to be administered. Examples of such a selective coating material include in vivo degradable polymers, gradually hydrolyzable polymers, gradually water-soluble polymers, and/or enzyme degradable polymers. A wide variety of coating materials for efficiently delaying the release is available and includes, for example, cellulose-based polymers such as hydroxypropyl cellulose, acrylic acid polymers and copolymers such as methacrylic acid polymers and copolymers, and vinyl polymers and copolymers such as polyvinylpyrrolidone.

Examples of the composition enabling the delivery to the colon further include bioadhesive compositions which specifically adhere to the colonic mucosal membrane (for example, a polymer described in the specification of U.S. Pat. No. 6,368,586) and compositions into which a protease inhibitor is incorporated for protecting particularly a biopharmaceutical preparation in the gastrointestinal tracts from decomposition due to an activity of a protease.

An example of a system enabling the delivery to the colon is a system of delivering a composition to the colon by pressure change in such a way that the contents are released by utilizing pressure change caused by generation of gas in bacterial fermentation at a distal portion of the stomach. Such a system is not particularly limited, and a more specific example thereof is a capsule which has contents dispersed in
a suppository base and which is coated with a hydrophobic polymer (for example, ethyl cellulose).

Another example of the system enabling the delivery to the colon is a system of delivering a composition to the colon, the system being specifically decomposed by an enzyme (for example, a carbohydrate hydrolase or a carbohydrate reductase) present in the colon. Such a system is not particularly limited, and more specific examples thereof include systems which use food components such as non-starch polysaccharides, amylose, xanthan gum, and azopolymer.

When used as a pharmaceutical preparation, the bacterial composition may be used in combination with an already known pharmaceutical composition for use in immunosuppression. In some embodiments, the pharmaceutical preparation can comprise both the bacterial composition and the already known pharmaceutical composition. Such a known pharmaceutical composition is not particularly limited, and may be at least one therapeutic composition selected from the group consisting of corticosteroids, mesalazine, mesalazine, sulfasalazine, sulfasalazine derivatives, immunosuppressive drugs, cyclosporin A, mercaptopurine, azathioprine, prednisone, methotrexate, antistamines, glucocorticoids, epinephrine, theophylline, cromolyn sodium, anti- leukotrienes, anti-cholinergic drugs for rhinitis, anti-cholinergic decongestants, mast-cell stabilizers, monclonal anti-IgE antibodies, vaccines (preferably vaccines used for vaccination where the amount of an allergen is gradually increased), anti-TNF inhibitors such as infliximab, adalimumab, certolizumab pegol, golimumab, or etanercept, and combinations thereof. It is preferable to use these therapeutic compositions in combination with the bacterial composition described herein. The bacterial composition can also be used as an adjuvant to improve the efficacy of a vaccine formulation such as a vaccine for the prophylaxis or treatment of an autoimmune disease or an allergic disease.

The bacterial composition can be used as a food or beverage, such as a health food or beverage, a food or beverage for infants, a food or beverage for pregnant women, athletes, senior citizens or other specified group, a functional food, a beverage, a food or beverage for specified health use, a dietary supplement, a food or beverage for patients, or an animal feed. Specific examples of the foods and beverages include various beverages such as juices, refreshing beverages, tea beverages, drink preparations, jelly beverages, and functional beverages; alcoholic beverages such as beers; carbohydrate-containing foods such as rice food products, noodles, breads, and pastas; paste products such as fish hams, sausages, paste products of seafood; retort pouch products such as curries, food dressed with a thick starchy sauces, and Chinese soups; soups; dairy products such as milk, dairy beverages, ice creams, cheeses, and yogurts; fermented products such as fermented soybean pastes, yogurts, fermented beverages, and pickles; bean products; various confectionery products such as Western confectionery products including biscuits, cookies, and the like; Japanese confectionery products including steamed bean-jam buns, soft adzuki-bean jellies, and the like, candies, chewing gums, gummies, cold desserts including jellies, crème caramels, and frozen desserts; instant foods such as instant soups and instant soy-bean soups; microwavable foods; and the like. Further, the examples also include health foods and beverages prepared in the forms of powders, granules, tablets, capsules, liquids, pastes, and jellies. The composition of the present invention can be used for animals, including humans. The animals, other than humans, are not particularly limited, and the composition can be used for various livestock, poultry, pets, experimental animals, and the like. Specific examples of the animals include pigs, cattle, horses, sheep, goats, chickens, wild ducks, ostriches, domestic ducks, dogs, cats, rabbits, hamsters, mice, rats, monkeys, and the like, but the animals are not limited thereto.

Without wishing to be bound by theory, individuals in whom bacteria belonging to the group Firmicutes (the group to which the Clostridium clusters IV and XIVA belong) are relatively abundant gain more body weight than individuals in whom bacteria belonging to the group Bacteroidetes are relatively abundant is large. The bacterial composition is capable of conditioning absorption of nutrients and improving feed efficiency. From such a viewpoint, the bacterial composition can be used for promoting body weight gain, or for a high efficiency animal feed. Diseases and conditions that would benefit from body weight gain include, e.g., starvation, cancer, AIDS, gastrointestinal disorders (e.g., celiac disease, peptic ulcer, inflammatory bowel disease (Crohn’s disease and ulcerative colitis), pancreatitis, gastritis, diarrhoea, hyperthyroidism, infection, renal disease, cardiac disease, pulmonary disease, connective tissue disease, weight loss caused by medications, anorexia, Addison’s disease, dementia, depression, hypercalcemia, Parkinson’s disease and tuberculosis.

The addition of the bacterial composition to an antibiotic-free animal feed makes it possible to increase the body weight of an animal that ingests the animal feed to a level equal to or higher than that achieved by animal ingesting antibiotic-containing animal feeds, and also makes it possible to reduce pathogenic bacteria in the stomach to a level equal to those in animals consuming typical antibiotic-containing animal feeds. The bacterial composition can be used as a component of an animal feed that does not need the addition of antibiotics.

In addition, unlike conventional bacteria (Lactobacillus and Bifidobacteria) in commercial use, which are not easy to incorporate into the livestock production, the present bacterial composition in spore form can be pelleted, sprayed, or easily mixed with an animal feed and can also be added to drinking water.

Animal feed comprising the bacterial composition can be fed to a wide variety of types of animals and animals of a varying ages and can be fed at regular intervals or for a certain period (for example, at birth, during weaning, or when the animal is relocated or shipped).

The bacterial composition can be used to promote weight gain and enhance energy absorption in humans and nonhumans (e.g., farm or other food animals).

The bacterial active components of the bacterial composition can be manufactured using fermentation techniques well known in the art. In one embodiment, the active ingredients are manufactured using anaerobic fermentors, which can support the rapid growth of bacterial species belonging to the Clostridia class. The anaerobic fermentors may be, for example, stirred tank reactors or disposable wave bioreactors. Culture media such as BL media and EGM media, or similar versions of these media devoid of animal components can be used to support the growth of the bacterial species. The bacterial product can be purified and concentrated from the fermentation broth by traditional techniques, such as centrifugation and filtration, and can optionally be dried and lyophilized by techniques well known in the art.

A food or beverage comprising a bacterial composition described herein can be manufactured by manufacturing techniques well known in the technical field. One or more
components (for example, a nutrient) which are effective for the improvement of an immune function by an immunosuppressive effect may be added to the food or beverage. In addition, the food or beverage may be combined with another component or another functional food exhibiting a function other than the function of the improvement of an immune function to thereby serve as a multi-functional food or beverage.

Moreover, the bacterial composition can be incorporated into foods requiring a processing step which may destroy ordinary probiotic strains. Specifically, most commercially usable probiotic strains cannot be incorporated into foods that need to be processed, for example, by heat treatment, long term storage, freezing, mechanical stress, or high-pressure treatment (for example, extrusion forming or roll forming). On the other hand, because of the advantageous nature of forming spores, the bacterial composition described herein can be easily incorporated into such processed foods. For example, the bacterial composition in the form of spores can survive even in a dried food, and can remain living even after being ingested. The bacterial composition can withstand low-temperature sterilization processes, typically processes carried out at a temperature from about 70°C to about 100°C, both inclusive. The bacterial composition can be incorporated into dairy products that require a pasteurization step. Furthermore, the bacterial composition can withstand long-term storage of many years; high-temperature processing such as baking and boiling; low-temperature processing such as freezing and cold storage; and high-pressure treatments such as extrusion forming and roll forming.

Many types of foods that need to be processed under such harsh conditions include foods which need to be processed in a microwave oven to be edible (for example, oatmeal), foods which need to be baked to be edible (for example, a muffin), foods which need to be subjected to a sterilization high-temperature treatment for a short period of time to be edible (for example, milk), and foods which need to be heated to be drinkable (for example, hot tea).

The amount of the bacterial composition to be administrated or ingested can be determined empirically, taking into consideration such factors as the age, body weight, gender, symptoms, health conditions, of an individual who will receive it, as well as the kind of bacterial composition (for example, a pharmaceutical product, a food or beverage) to be administered or ingested. For example, the amount per administration or ingestion is generally 0.01 mg/kg body weight to 100 mg/kg body weight, and, in specific embodiments, 1 mg/kg body weight to 10 mg/kg body weight. Also described herein is a method for suppressing the immunity (reducing the immune response) of a subject, the method being characterized in that the bacteria belonging to the Clostridia class or the physiologically active substance derived from the bacteria is administered to or ingested by the subject as described above.

The bacterial composition may be administered to an individual once, or it may be administered more than once. If the composition is administered more than once, it can be administered on a regular basis (for example, once a day, once every two days, once a week, once every two weeks, once a month, once every 6 months, or once a year) or on an as needed or irregular basis. The appropriate frequency of administration (which may depend on host genetics, age, gender, and health or disease status of the subject, among other factors) may be determined empirically. For example, a patient can be administered one dose of the composition, and the levels of the bacterial strains of the composition in fecal samples obtained from the patient can be measured at different times (for example after 1 day, after 2 days, after 1 week, after 2 weeks, after 1 month). When the levels of the bacteria fall to, for example, one half of their maximum value, a second dose can be administered, and so on.

A product comprising the bacterial composition (a pharmaceutical product, a food or beverage, or a reagent) or a manual thereof may be accompanied by document or statement explaining that the product can be used to suppress the immunity (including a statement that the product has an immunosuppressive effect and a statement that the product has an effect of suppressing the proliferation of function of effector T-cells). Here, the “provision to the product or the manual thereof with the note” means that the document or statement is provided to a main body, a container, a package, or the like of the product, or the note is provided to a manual, a package insert, a leaflet, or other printed materials, which disclose information on the product.

Method for Inducing Proliferation or Accumulation of Regulatory T Cells

As described above, and as shown in Examples, administration of the bacterial composition to an individual makes it possible to induce proliferation or accumulation of regulatory T cells in the individual. This provides a method of inducing proliferation or accumulation of regulatory T cells in an individual, the method comprising: administering, to the individual, at least one substance selected from the group consisting of: (a) Clostridium saccharogena, Clostridium ramosum XCM1298, Clostridium ramosum, Flavonifractor plautii, Pseudoflavonifractor capillosus ATCC 29799, Clostridium hathewayi, Clostridium saccharolyticum WM1, Bacteroides sp. MANG, Clostridium saccharolyticum, Clostridium scindens, Lactobacillus casei 5-1_57F AA, Lactobacillus brevis 6.3_1 S3FAA, Clostridium sp. 14616, Clostridium bolteae ATCC BAA-613, cf. Clostridium sp. MI.6055, Erysipelotrichaceae bacterium 2.2_44A, Clostridium indolus, Anaerostipes caccae, Clostridium bolteae, Lactobacillus casei JDF_VP30, Lactobacillus casei bacterium 3.1_57F AA_AT1, Anaerotruncus colihominis, Anaerotruncus colihominis DSM 17241, Ruminococcus sp. ID8, Lactobacillus casei bacterium 2.1_46F AA, Clostridium lavalense, Clostridium asparagiforme DSM 15981, Clostridium symbiosum, Clostridium symbiosum WAL-14163, Bacteroides distasonis, Clostridium sp. D5, Oscillosporaceae bacterium NML 061048, Oscillibacter valericigenes, Lactobacillus casei bacterium A4, Clostridium sp. 316002/08, and Clostridiales bacterium 1.7_47F AA, Blautia cocoidea, Anaerostipes caccae DSM 14662, (b) a culture supernatant of at least one (a, one or more) of the bacteria described/listed herein; (c) a physiologically active substance derived from a (one or more, at least one) bacteria described/listed herein; or a combination of any two or three of (a), (b) and (c). The bacterial composition is administered (provided) to the individual in sufficient quantity to produce the desired effect of inducing proliferation, accumulation or both proliferation and accumulation of regulatory T cells. It may be administered to an individual in need of treatment, reduction in the severity of or prevention of at least one disease selected from an autoimmune disease, an inflammatory disease, an allergic disease, and an infectious disease.

Note that, the “individual” or “subject” may be in a healthy state or a diseased state.

The method may further comprise the optional step of administering at least one (a, one or more) antibiotic preceding, or in combination with, the bacterial composition. The antibiotic administered can be, for example, one which
facilitates recolonization of the gut by Gram-positive bacteria of the Clostridia class, such as an antibiotic that reduces Gram-negative bacteria. Examples of such antibiotics include amnoglycoside antibiotics (amikacin, gentamicin, kanamycin, neomycin, netilmicin, tobramycin, and paromomycin), cephalosporin antibiotics (cefadex, cefamandole, cefoxitin, cefpodoxim, ceftriaxone, cefdinir, cefdinir, cefpodoxim, cefotaxime, cefazidime, cefotibuten, cefotizoxime, ceftriaxone, and cefoxitin), sulfonamides, ampicillin, and streptomycin.

Moreover, a prebiotic composition such as almond skin, inulin, oligofructose, raffinose, lactulose, pectin, hemicellulose (such as xylanoglan and alpha-glucans), amylopectin, and resistant starch which are not decomposed in the upper gastrointestinal tract and promote the growth of intestinal microbes in the intestinal tract, as well as growth factors such as acetyl-Co A, biotin, beet molasses, and yeast extracts, preferentially contributes to the proliferation of the bacteria species in the composition belonging to the Clostridia class. A method of inducing proliferation and/or accumulation of regulatory T cells in an individual can comprise administering, to the individual, at least one substance selected from the above in combination with the bacterial composition. Also contemplated herein is a composition comprising the bacterial composition and a prebiotic composition.

The above-described antibiotic, and the above-described prebiotic composition or growth factor may be used in combination. Moreover, a therapeutic composition may be administered to an individual together with at least one substance selected from the group consisting of the bacterial composition, an antibiotic, and a prebiotic composition or growth factor.

A therapeutic composition can be, for example, one therapeutic composition selected from the group consisting of corticosteroids, mesalazine, mesalamine, sulfasalazine, sulfasalazine derivatives, immunosuppressive drugs, cyclosporin A, mercaptopurine, azathioprine, prednisone, methotrexate, antithistamines, glucocorticoids, epinephrine, theophylline, cromolyn sodium, anti-leukotrienes, anti-cholinergic drugs for rhinitis, anti-cholinergic decongestants, mast-cell stabilizers, monoclonal anti-IgE antibodies, vaccines (preferably, vaccines used for vaccination where the amount of an allergen is gradually increased), anti-TNF inhibitors such as infliximab, adalimumab, certolizumab pegol, golimumab, or etanercept, and combinations thereof. These therapeutic compositions can be administered prior to, in combination with or following administration of the bacterial composition and optionally, also in combination with an antibiotic, a prebiotic composition, a growth factor or any combination of an antibiotic, a prebiotic composition and a growth factor.

There is no particular limitation imposed on the combined use of the therapeutic composition with at least one substance selected from the group consisting of the bacterial composition, the “antibiotic”, and the “prebiotic composition or growth factor”. For example, the “one substance” and the therapeutic composition are administered orally or parenterally to an individual simultaneously or sequentially/individually at any appropriate time.

Whether administration of the bacterial composition induces the proliferation and/or accumulation of regulatory T cells can be determined by using, as an index, increase or reinforcement of at least one of the following: the number of regulatory T cells, the ratio of regulatory T cells in the T cell group of the colon, a function of regulatory T cells, or expression of a marker of regulatory T cells. A specific approach is measurement counts or percentage of Foxp3-expressing Tregs in a patient sample, such as a biopsy or a blood sample, promotion (enhancement) of IL-10 expression, promotion (enhancement) of CTLA4 expression, promotion (enhancement) of IDO expression, suppression of IL-4 expression, or colonization of an individual with the bacterial composition administered as the index of the induction of proliferation or accumulation of regulatory T cells.

Methods for detecting such expression include northern blotting, RT-PCR, and dot blotting for detection of gene expression at the transcription level; ELISA, radioimmunoassays, immunoblotting, immunoprecipitation, and flow cytometry for detection of gene expression at the translation level.

Samples that may be used for measuring such an index include tissues and fluids obtained from an individual, such as blood, obtained in a biopsy, or a fecal sample. <Method for Predicting Response of an Individual to the Bacteria Composition by Monitoring the Individual’s Response to Treatment with the Composition>

Also described is a method in which an amount (e.g., count) or the percentage of at least one bacterial species selected from the group consisting of: Clostridium saccharogenum, Clostridium ramosum JCM1298, Clostridium ramosum, Flavonifractor plautii, Pseudoflavonifractor capillosus ATCC 29799, Clostridium halotayi, Clostridium saccharolyticum WM1, Bacteroides sp. MANG, Clostridium saccharolyticum, Clostridium scindens, Lachnospiraceae bacterium 5_1_57F, Lachnospiraceae bacterium 6_1_63F, Clostridium bolteae ATCC BAA-613, cf. Clostridium sp. MLG055, Erysipelotrichaceae bacterium 2_2_44A, Clostridium indol, Anaerostipes caccae, Clostridium bolteae, Lachnospiraceae bacterium DJF_VP30, Lachnospiraceae bacterium 3_1_57F, Anaerococcus cecobifidus DSM 17241, Ruminococcus sp. ID8, Lachnospiraceae bacterium 2_1_46F, Clostridium lavalense, Clostridium asapragorfera DSM 15981, Clostridium symbiosum, Clostridium symbiosum WAL-14163, Eubacterium torontii, Clostridium sp. D5, Oscillosporaceae bacterium NML 061048, Oscillibacter valericigenes, Lachnospiraceae bacterium A4, Clostridium sp. 316002/08, and Clostridiales bacterium 1_7_47F, Blautia cocoides, Anaerostipes caccae DSM 14662 in a patient’s sample (e.g., a colonic biopsy or a fecal sample) is determined. When the percentage or the count of the bacteria selected from the list above is lower in an individual than a base line value obtained by performing a similar determination on a healthy individual (e.g., an individual who does not have/has not been identified as having a disease or condition for which the bacterial composition is a potential treatment such as an autoimmune disease, an allergic condition, cancer, organ rejection), it is determined that the individual is likely to be responsive to the bacterial composition. This determination can be used, for example, by a clinician to determine whether an individual or a patient is likely to benefit from treatment with the bacterial composition, or to select an individual or a patient for inclusion in a clinical trial. The clinician can then administer the bacterial composition to the individual or patient based on the determination that the individual or patient is likely to benefit from treatment. This determination can also be used as a method to monitor an individual’s response to treatment with the bacterial compositions described, wherein a higher value of the determination after treatment with the bacterial composition (compared to a determination before treatment)
indicates that the individual has responded favorably to treatment (e.g. is a positive indicator of successful colonization and enhanced immunosuppression in the individual). Optionally, the prognosis and monitoring methods described here may further comprise the step of measuring in the individual’s samples the percentages or absolute counts of other commensal species belonging to Clostridium Clusters IV and XIVa that are not present in the bacterial composition, wherein lower than baseline values before treatment indicate a higher likelihood of a positive response to treatment, and wherein an increased value after treatment indicates that the individual has responded favorably to treatment. In the prognosis and monitoring methods described here, a variety of known methods can be used for determining the composition of the microbiota. For example, 16S rRNA sequencing can be used.

<Methods for Selecting T-Reg-Inducing Organisms>

Also described is a method of obtaining bacteria capable of inducing Tregs, comprising (1) isolating the bacterial spore-forming fraction from a fecal or biopsy sample obtained from a mammal, preferably a human (e.g. by chloroform treatment or by heat treatment), (2) optionally, orally administering the spore-forming fraction to a non-human mammal, preferably a germ-free non-human mammal; (3) optionally, obtaining a fecal sample from the non-human mammal, diluting the fecal sample for example by volume by a factor of 10, 100, 1,000, or 10,000, whereby producing a diluted fecal sample, and orally administering the diluted sample to a second germ-free non-human mammal, wherein optional step (3) can be repeated more than one time, (4) plating serial dilutions, under aerobic condition or strictly anaerobic conditions, of either the spore-forming fraction obtained in (1) or a sample of intestinal contents of the non-human mammal of (3), and (5) picking a single colony from the culture plate. The colony can be further assessed for the ability of bacteria to induce proliferation of regulatory T cells and/or accumulation of regulatory T cells using known methods, such as those described in the examples.

Following are examples, which describe specific aspects. They are not intended to be limiting in any way. Note that mice used in Examples were prepared or produced as follows. In the following description, mice may be referred to as “SPF” or “GF”. These “SPF” and “GF” indicate that the mice were maintained in the absence of specific pathogenic bacteria (specific pathogen-free, SPF), and that the mice were maintained under Germ-Free (GF) conditions, respectively.

<C57BL/6, Bulb/c, and IPl mice maintained under SPF or GF conditions were purchased from Sankyo Labo Service Corporation, Inc. (Japan), JAPAN SLC, INC. (Japan), CLEA Japan, Inc. (Japan), or The Jackson Laboratory (USA). GF mice and gnotobiotic mice were bred and maintained within the gnotobiotic facility of The University of Tokyo, Yukult Central Institute for Microbiological Research, or Sankyo Labo Service Corporation, Inc. Myd88<−/−>, Rip2<−/−>, and Card9<−/−> mice were produced as described in NPL 1 to 3, and backcrossed for 8 generations or more, so that a C57BL/6 genetic background was achieved. Foxp3<GFP> mice were purchased from the Jackson Laboratory.>

To form a bicistronic locus encoding both IL10 and Venus under control of an IL10 promoter, a targeting construct was first created. Specifically, a cassette (IRES-Venus-SV40 polyA signal) cassette, refer to Non-Patent Document 4) which was made of an internal ribosome entry site (IRES), a yellow fluorescent protein (Venus), and a SV40 polyA signal (SV40 polyA) and which was arranged next to a neomycin-resistant gene (neo), was inserted between a stop codon and a polyA signal (Exon 5) of a IL10 gene. Next, the obtained targeting construct was used to cause homologous recombination with the IL10 gene region in the genome of mice. Thus, IL10<ΔΔ> mice having an IL10<ΔΔ> allele were produced (refer to FIG. 1). Note that in FIG. 1, “Δ” represents a gene coding thymidine kinase, “ΔΔ” represents the
neomycin-resistant gene, and “BamH1” represents a cleavage site by the restriction enzyme BamH1.

Genomic DNAs were extracted from the Il10<sup>−/−</sup> mice, treated with BamH1, and Southern blotted by use of a probe shown in FIG. 1. FIG. 2 shows the obtained results. Wild-type and Il10<sup>−/−</sup> alleles were detected as bands having sizes of 19 kb and 5.5 kb, respectively. Hence, it is apparent from the results obtained, the homologous recombination occurred in the genome of the Il10<sup>−/−</sup> mice.

Further, CD4<sup>+</sup>Venus<sup>+</sup> cells or CD4<sup>+</sup>Venus<sup>+</sup> cells in the colonic lamina propria of the Il10<sup>−/−</sup> mice were sorted by use of a FACSAria. Then, real-time RT-PCR was carried out on an ABI 7300 system by a method to be described later, to determine the amount of IL-10 mRNA expressed. It was found that, since the development of the IL-10 mRNA was detected only in the CD4<sup>+</sup>Venus<sup>+</sup> cells, the expression of IL-10 mRNA in the Il10<sup>−/−</sup> mice was correctly reflected in the expression of Venus. Note that the germ-free states of such Il10<sup>−/−</sup> mice were established in Central Institute for Experimental Animals (Kawasaki, Japan). The Il10<sup>−/−</sup> mice in the germ-free states were maintained in vinyl isolators in Sankyo Labo Service Corporation, Inc. (Tokyo, Japan), and used in the following Experiments.

Experiments and Analyses in Examples were Carried Out as Follows.

<Method for Colonization of Mice with Murine Bacteria and Analysis Thereof>

According to the description in NPL 5 and 6, mice in which SFB or Clostridium were colonized were produced. Cecal contents or feces of the obtained gnotobiotic mice were dissolved in sterile water or an anaerobic dilution solution. The dissolved cecal contents or feces as they were or after a chloroform treatment were orally administered to GF mice. Three strains of the Lactobacillus and 16 strains of the Bacteroides were cultured separately from each other in a BI or EG agar medium in an anaerobic manner. The cultured bacteria were harvested, suspended in an anaerobic TS broth, and orally administrated forcibly to GF mice. The state of the cultured bacteria in the mice was assessed by microscopic observation conducted on a smear preparation of fecal pellets.

<Isolation of Intestinal Lamina Propria Lymphocytes and Flow Cytometry>

The small intestine and colon were collected and opened longitudinally. The cecum was also isolated and cecal content was directly frozen at –80°C or suspended in 2 ml PBS, then added 40% glycerol (final concentration 20%), snap-frozen in liquid nitrogen and stored at –80°C until use. The colon and small intestine were washed in PBS to remove all luminal contents and shaken in Hanks’ balanced salt solution (HBSS) containing 5 mM EDTA for 20 min at 37°C. After removing epithelial cells, muscle layers and fat tissue using tweezers, the lamina propria layers were cut into small pieces and incubated with RPMI1640 containing 4% fetal bovine serum, 1 mg/ml collagenase D, 0.5 mg/ml dispase and 40 µg/ml DNase I (all Roche Diagnostics) for 1 h at 37°C in a shaking water bath. The digested tissues were washed with HBSS containing 5 mM EDTA, resuspended in 5 ml of 40% Percoll (GE Healthcare) and overlaid on 2.5 ml of 80% Percoll in a 15-ml Falcon tube. Percoll gradient separation was performed by centrifugation at 800 g for 20 min at 25°C. The lamina propria lymphocytes were collected from the interface of Percoll gradient and suspended in ice-cold PBS. For analysis of regulatory T cells, isolated lymphocytes were labeled with the LIVE/DEAD fixable violet dead cell stain kit (Invitrogen) to exclude dead cells in the analysis. The cells were washed with staining buffer containing PBS, 2% FBS, 2 mM EDTA and 0.09% NaN3 and stained surface CD4 with PE/Cy7-labeled anti-CD4 Ab (RM4-5, BD Biociences). Intracellular staining of Foxp3 and Helios was performed using the Alexa700-labeled anti-Foxp3 Ab (FIK-16s, eBioscience), Alexa647-labeled anti-Helios (2F6, eBioscience) and Foxp3 Staining Buffer Set (eBioscience). For analysis of Th1 and Th17 cells, isolated lymphocytes were stimulated for 4 hours with 50 ng/ml phorbol 12-myristate 13-acetate (PMA, Sigma) and 1 µg/ml ionomycin (Sigma) in the presence of GolgiStop (BD Biociences). After incubation for 4 hours, cells were washed in PBS, labeled with the LIVE/DEAD fixable violet dead cell stain kit and stained surface CD4 with PE/Cy7-labeled anti-CD4 Ab. Cells were washed, fixed in Cytofix/Cytoperm, permeabilized with Perm/Wash buffer (BD Biociences), and stained with the APC-labeled anti-IL-17 Ab (eBio17B7, eBioscience) and FITC-labeled anti-IFN-γ Ab (XM1G12, BD Biociences). The Ab stained cells were analyzed with a LSR Fortessa (BD Biociences), and data were analyzed using Flow Jo software (Treestar).

<Real-Time RT-PCR>

From an RNA prepared by using RNeasy Mini Kit (Qiagen), a cDNA was synthesized by use of a MMV reverse transcriptase (Promega K.K.). The cDNA obtained was analyzed by real-time RT-PCR using Power SYBR Green PCR Master Mix (Applied Biosystems) and ABI 7300 real time PCR system (Applied Biosystems), or real-time RT-PCR using SYBR Premix Ex Taq (TAKARA) and Light Cycler 480. For each sample, a value obtained was normalized for the amount of GAPDH. A primer set was designed by using Primer Express Version 3.0 (Applied Biosystems), and those exhibiting a 90% or higher sequence identity at an initial evaluation were selected. The primer set used was as follows:

- **Foxp3**
  - SEQ ID NO: 1
  - 5′-GCGCAATAGTCTCTCCACAGTT-3′
  - SEQ ID NO: 2
  - 5′-GGTCCTCGTATTGCTACCTG-3′

- **CTLA4**
  - SEQ ID NO: 3
  - 5′-CTTTTGTACGCTGCTCCTCT-3′
  - SEQ ID NO: 4
  - 5′-GGTCACCTGTATGTCTGCA-3′

- **GITR**
  - SEQ ID NO: 5
  - 5′-TGTTGCGGAAAGTGCACCA-3′
  - SEQ ID NO: 6
  - 5′-ACACGGGAGACCACACAC-3′

- **IL-10**
  - SEQ ID NO: 7
  - 5′-GATTTTAATAAGCTCCAGACCAAGGT-3′
  - SEQ ID NO: 8
  - 5′-TTCTGATGGTTGATGGAATGTC-3′

- **GAPDH**
  - SEQ ID NO: 9
  - 5′-CTCTGCTCCTAGACAAAAATG-3′
  - SEQ ID NO: 10
  - 5′-TCTCCACTTGGCCTGCA-3′
oxazolone/50% ethanol solution was intrarectally administered again to the presensitized mice under a light anesthesia. Note that the intrarectal administration was conducted by using a 3.5 F catheter.

Each mouse was analyzed daily for body weight, occult blood, bleeding visible with the naked eyes (gross blood), and the hardness of stool. Moreover, the body weight loss percentage, intestinal bleeding (no bleeding, occult blood (hemoccult*)), or bleeding visible with the naked eyes), and the hardness of stool (normal stool, loose stool, or diarrhea) were evaluated numerically, and the disease activity index (DAI) was calculated in accordance with the description in “S. Wirtz, C. Neufert, B. Weigmann, M. F. Neurath, Nat Protoc 2, S41 (2007).”

<Preparation and Culturing of Large Intestinal Epithelial Cells (IECs)> First, the colon was collected, cut open longitudinally, and rinsed with PBS. Subsequently, the colon was treated with 1 mM dithiothreitol (DTT) at 37°C for 30 minutes on a shaker, and then vortexed for one minute to disrupt the epithelial integrity. The released intestinal epithelial cells (IECs) were collected, and suspended in 5 ml of 20% Percoll. The suspension was overlaid on 2.5 ml of 80% Percoll in a 15 ml Falcon tube. Then, the tube was centrifuged at 25°C and 780 g for 20 minutes to conduct cell separation by Percoll density gradient centrifugation. Cells at the interface were collected, and used as colonic IECs (purity: 90% or higher, viability: 95%). The IECs obtained were suspended in RPMI containing 10% FBS, and 1x10⁶ cells of the IECs were cultured in a 24-well plate for 24 hours. Thereafter, the culture supernatant was collected, and measured for active TGF-β1 level by ELISA (Promega).

Meanwhile, for culturing T cells in vitro, 1.5x10⁵ MACS-purified splenic CD4⁺ T cells were cultured in each well of a round-bottomed 96-well plate, together with a 50% conditioned medium in which IECs isolated from GF mice or Clostridium-colonized mice were cultured, and with 25 ng/ml of anti-CD3 antibody and an anti-CD28 antibody (BD Bioscience) were bound to the round-bottomed plate. After a 5-day culture, the CD4⁺ T cells were collected, and subjected to a real-time PCR.

<Colitis Experimental Model> A fecal suspension from Clostridium-colonized mice was orally administered to C57BL/6 mice (2-week old), which were grown in a conventional environment for six weeks.

For preparing a DSS-induced colitis model, 2% (wt/vol) DSS (reagent grade, DSS salt, molecular weight=36 to 50 kD, manufactured by MP Biomedicals), together with drinking water, was given to the mice for six days.

Meanwhile, for preparing an oxazolone-induced colitis model, the mice were presensitized by transdermally applying, onto the mice, 150 µl of a 3% oxazolone (4-ethoxyethylene-2-phenyl-2-oxazolin-5-one, Sigma-Aldrich)/100% ethanol solution. Five days after that, 150 µl of a 1% oxazolone/50% ethanol solution was intrarectally administered again to the presensitized mice under a light anesthesia. Note that the intrarectal administration was conducted by using a 3.5 F catheter.

Each mouse was analyzed daily for body weight, occult blood, bleeding visible with the naked eyes (gross blood), and the hardness of stool. Moreover, the body weight loss percentage, intestinal bleeding (no bleeding, occult blood (hemoccult*), or bleeding visible with the naked eyes), and the hardness of stool (normal stool, loose stool, or diarrhea) were evaluated numerically, and the disease activity index (DAI) was calculated in accordance with the description in “S. Wirtz, C. Neufert, B. Weigmann, M. F. Neurath, Nat Protoc 2, S41 (2007).”

BALB/c SPF mice were inoculated with a fecal suspension from Clostridium-colonized mice (2-week-old), and grown in a conventional environment. Then, 1 µg of OVA (grade V, Sigma) and 2 mg of alum (Thermo Scientific), 0.2 ml in total, were intraperitoneal injected to the mice (at their ages of 4 weeks and 6 weeks). Sera were collected every week from the mice at the root of their tail, and OVA-specific IgE was measured by ELISA (Chondrex). Then, at their ages of 8 weeks, splenic cells were collected, inoculated in a 96-well plate at 1x10⁶ cells per well, and stimulated with OVA (100 µg/ml) for three days. Thereafter, the culture supernatant was collected, and measured for IL-4 and IL-13 levels by ELISA (R&D).

<Statistical Analysis> The difference between control and experimental groups was evaluated by the Student’s t-test.

<Chloroform Treatment and Oral Inoculation with Fecal Samples into GF Mice> Human stool (2 g) from a healthy volunteer (Japanese, male, 29 y old) was suspended with 20 ml phosphate-buffered saline (PBS) and passed through a 70 µm cell strainer to eliminate clumps and debris. Then fecal suspension was mixed with or without chloroform (final concentration 3%), and incubated in a shaking water bath for 60 min. The fecal suspensions without chloroform treatment were orally inoculated into germ-free (GF) mice (250 µl/mouse). After evaporation of chloroform by bubbling with N2 gas for 30 minutes, the aliquots containing chloroform-resistant (spore-forming) fraction of human intestinal bacteria were inoculated into IGI GF mice. Each group of ex-GF mice was separately kept in a vinyl isolator for 3 or 4 weeks.

<Co-Housing Experiment> To evaluate whether Treg-inducing human bacteria can be transmitted horizontally, IGI GF mice were co-housed for 4 weeks with ex-GF mice colonized with chloroform-treated human feces (Example 21 mice) in a vinyl isolator (6 mice, designated as mouse #D1 to #D6.

<Inoculation with Diluted Cecal Contents into GF Mice> The frozen cecal content from ex-GF mice inoculated with chloroform-treated human feces (#C 4) was suspended in 10 times volume (v/v) of PBS, passed through a 70 µm cell strainer and treated 3% chloroform. Then the suspension was diluted 2000 (for 4 mice, designated as mouse #E1 to #E4) or 20000 (for 8 mice, designated as mouse #F1 to #F8)-fold with PBS and orally inoculated into GF IGI mice (2.5x10⁵ or 2.5x10⁶ cells/250 µl/mouse). After 4 weeks, lymphocytes were collected from colon and small intestine and analyzed for Foxp3⁺ Treg cell proportion and their Helios expression. Cecal contents were frozen and stored at −80°C until use.
<Re-Colonization Experiments>
The frozen cecal content from ex-GF mice inoculated with 20000-fold dilution (#F3, 7 and 8) was suspended in 10 times volume (w/w) of PBS, passed through a 70 µm cell strainer and treated 3% chloroform. The suspensions were orally inoculated into GF IQR mice (5, 4 or 4 mice: designated as mouse #G1 to #G5, #I1 to #I4 or #K1 to #K4, respectively). After 4 weeks, colon and small intestine were collected and analyzed for Foxp3+ Treg cell proportion and their Helios expression. Cecal contents were suspended in 20% glycerol solution, snap-frozen in liquid nitrogen and stored at −80°C

<Cultured Bacteria-Colonization Experiments>
The glycerol stock of cecal content from #G2 mouse was diluted with PBS and seeded onto BL agar plate. After 48 hours, all bacterial colonies were collected by scraping the plates with a plate scraper and inoculated into GF IQR mice (4 mice, designated as mouse #K1 to #K4). Six bacterial strains were isolated from the freeze stock of cecal content from #K4 mouse using BL agar plate. These isolated strains were inoculated into GF IQR mice (4 mice, designated as mouse #I1 to #I4). (Details of the culture method are described below.)

<16S rRNA Gene Quantitative PCR Analysis>
Using a QiAamp DNA Stool mini kit (QiAGEN), bacterial genomic DNA was isolated from the human stool from a healthy volunteer as described above (human stool), cecal contents from GF mice gavage with chloroform-treated human stool (celal content of B4 mouse) or feces from SPF ICR mouse (feces of SPF mouse). The isolated DNA was used as template for quantitative PCR. The amplification program consisted of one cycle at 95°C for 1 min, followed by 50 cycles at 95°C for 10 s and 60°C for 30 s. Quantitative PCR analysis was carried out using a LightCycler 480 (Roche). Relative quantity was calculated by the ΔCt method and normalized to the amount of total bacteria. The following primer sets were used: total bacteria, 5'-GGTGGAATATGGCTCCCCG-3'(SEQ ID NO.: 45) and 5'-TACGGCTATCCCTTGAGCCT-3'(SEQ ID NO.: 46); Clostridium cluster XIVA (Clostridium cocoides subgroup), 5'-AAATGACCGTCCAGACTA-3'(SEQ ID NO.: 47) and 5'-CTTGGATGAGTCCAATTCCGGAG-3'(SEQ ID NO.: 48); Clostridium cluster IV (Clostridium leptum) 5'-CCCTTCCGGCAGSAGTA-3'(SEQ ID NO.: 49) and 5'-GAATTAACACCATATCCACTGGCT-3'(SEQ ID NO.: 50); Bacteroides, 5'-GAGAAGGAGGTGCCCCAC-3'(SEQ ID NO.: 51) and 5'-CAGCTATCTGCGCTGTTCAAG-3'(SEQ ID NO.: 52); Bifidobacterium, 5'-CGGCTGAGTAACTGCGTACC-3'(SEQ ID NO.: 53) and 5'-TGATAAGACGCACCACAAA-3'(SEQ ID NO.: 54). Note that mice gavaged with chloroform-treated human stool exhibited high levels of spore-forming bacteria, such as Clostridium clusters XIVA and IV, and a severe decrease of non-spore-forming bacteria, such as Bacteroides and Bifidobacterium, compared with the human stool before chloroform treatment.

<Isolation of DNA from Cecal Contents for 16S rRNA Gene Metasequence Analysis>
The cecal contents of A1-1, A2-4, B4-4, E-3, E-7, E-8, F-2, G-3, H-3, I-3 and J-3 were collected by centrifugation at 5000 × g for 10 min at 4°C, suspended in 10 ml of Tris-EDTA containing 10 mM Tris-HCl and 1 mM EDTA (pH 8), and then used for DNA isolation. Lysozyme (SIGMA, 15 mg/ml) was added to the cell suspension. After incubation at 37°C for 1 h with gentle mixing, a purified achromopeptidase (Wako) was added (final 2000 unit/ml) and incubated at 37°C for 30 min. Then, sodium dodexyl sulfate (final 1%) was added to the cell suspension and mixed well. Subsequently, protease K (Merck) was added (final 1 mg/ml) to the suspension and the mixture was incubated at 55°C for 1 h.

High-molecular-weight DNA was isolated and purified by phenol/chloroform extraction, ethanol, and finally polyethylene glycol precipitation.

<16S rRNA Gene Metasequence>
An aliquot of the DNA was used for PCR amplification and sequencing of bacterial 16S rRNA genes. ~330 bp amplicons, spanning variable region 1-2 (V1-2) of the gene were generated by using (i) modified primer 8F’ (5'-CCATTCATTCCGCTGGTCTCAGCACTCGAG+Barcode+aggtttatgatgatcag-3' (SEQ ID NO.: 55)) which consists of 454 adaptor sequence (underlined), and a sample specific, error correcting barcode (10 bases, bold) and the universal bacterial primer 8F and (ii) modified primer 338R (5'-CACTACATGCTGCTGGTACCTTGAGAGATCC-3' (SEQ ID NO.: 56)) which contains 454 adaptor sequence (underlined) and the bacterial primer 338R. Polymerase chain reactions were performed for each local DNA sample: each 50-µl reaction contained 40 ng of DNA, 5 µl of 10× Ex Taq buffer (TAKARA), 5 µl of 2.5 mM dNTP mixture, 0.2 µl Ex Taq and 0.2 µM of each primer. PCR conditions consisted of an initial denaturation step performed at 96°C for 2 min, followed by 20 cycles of denaturation (96°C, 30 s), annealing (55°C, 45 s) and amplification (72°C, 1 min) and final amplification step performed at 72°C for 10 min. Amplicons generated from each sample were subsequently purified using AMPure XP (Beckman Coulter). The amount of DNA was quantified using Quant-iT Picogreen dsDNA Assay Kit (Invitrogen) and TBS-380nm Fluorometer (Turner Biosystems). The amplified DNA were used as template for 454 GS Junior Roche) pyrosequencing. The sequences were performed using GS Junior Titanium emPCR Kit-Lib-L, GS Junior Titanium Sequencing Kit and GS Junior Titanium PicoTiterPlate Kit (all Roche) according to the manufacturer’s manuals (GS Junior Titanium Series, emPCR Amplification Method Manual—Lib-L and Sequencing Method Manual). Resulting sequences (3400 reads were produced for each sample) were classified into OTU on the basis of sequence similarity (~97% identity). Representative sequences from each OTU were compared with sequences in nucleic acid databases (Ribosomal Database Project) using BLAST to determine the closest relatives. Then, OTUs were classified into species on the basis of the closest relatives. All data of close relatives and the number of reads are shown in Table 1.

<Isolation of Bacterial Strains>
Bacterial strains were isolated from the cecal contents of #F8, #G2, #I1 and #K3 by plating serial dilutions of the cecal samples under aerobic condition or strictly anaerobic condition (80% N2 10% H2 10% CO2) onto BL agar (Eiken Chemical) or EG agar plates containing medium with the following components (quantities expressed per liter): Meat extract 500 ml; Proteose peptone No. 3 (10.0 g, Difeo); Yeast Extract (5.0 g, Difeo); Na2HPO4 (4.0 g); D(+)-Glucose (1.5 g); Soluble Starch (0.5 g); L-cysteine (0.2 g); L-cysteine-HCl·H2O (0.5 g); Tween80 (0.5 g); Bacto Agar (16.0 g, Difeo); defibrinated horse blood (50 ml). After culture at 37°C for 2 or 4 days, each single colony was picked up and cultured for additional 2 or 4 days at 37°C. by ABCM broth or EG agar plate. The isolated strains were collected into EG stock medium (10% DMSO) and stored at −80°C. For suspension of isolated strains to re-inoculate mice, TS medium (27.5 g of trypticase soy broth w/o
dextrose, 0.84 g of Na2CO3, 0.5 g of L-cysteine-HCl-H2O, 1000 ml of distilled water, pH adjusted to 7.2±0.2 with NaOH, then autoclaved for 15 minutes at 115 degrees Celsius). To identify the isolated strains, 16SrRNA coding gene sequences were performed. The 16S rRNA genes were amplified by colony-PCR using KOD FX (TOYOBO), 16S rRNA gene-specific primer pairs: 8F (5'-AGAGTTTGATCMTGGCTCAG-3' (SEQ ID NO.: 57)) and 519R (5'-ATTACCGCGGCTG-3' (SEQ ID NO.: 58)) for C. indolae C. boletae, Bacteroides sp. MANG, L. bacterium DIF_VPSO, A. colihominis, Ruminococcus sp. ID8, C. lavallense, C. symbiosum and E. contortum or 1513R (5'-ACGGGCTACCTTGTTACGACTT-3' (SEQ ID NO.: 59)) for C. saccharogumina, C. ramosum, F. pilautii, C. hathewayi, C. scindens, Clostridium sp. 2335, Clostridium sp. 14616 and cf Clostridium sp. MLG055 and GeneAmp PCR System 9700 (Applied Biosystems). The amplification program consisted of one cycle at 98°C, for 2 min, followed by 40 cycles at 98°C, for 10 s, 57°C, for 30 s and 68°C, for 40 s. Each amplified DNA was purified from the reaction mixture using Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare). Sequence analysis was performed using BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems) and Applied Biosystems 3730xl DNA analyzer (Applied Biosystems). The resulting sequences were compared with sequences in nucleic acid databases using BLAST to determine the closest relatives. The closest relatives and % identity of all isolated strains, information for genus-species of the closest relatives, Clostridium cluster, ID of mouse from which was derived, maximum similarity and culture medium of isolated strains were summarized in Table 2.

EXAMPLE 1

First, it was investigated whether or not accumulation of regulatory T cells (Treg cells) in the colonic lamina propria was dependent on commensal bacteria. Specifically, lymphocytes were isolated from peripheral lymph nodes (pLN) of Balb/c mice bred in the absence of specific pathogenic bacteria (SPF) or from lamina propria of the colon or the small intestine (SI) of the mice. The CD4 and Foxp3 were stained by antibodies. Then, the ratio of Foxp3+ cells in CD4+ lymphocytes was analyzed by flow cytometry. The results showed that Foxp3+ Treg cells were present at a high frequency in the lamina propria of the gastrointestinal tracts, especially in the colonic lamina propria, of the mice kept under the environment free from specific pathogenic microorganisms (SPF). In addition, it was also found that the number of the Foxp3+Treg cells in the colonic lamina propria gradually increased up to three months after their birth, whereas the number of the Foxp3+ Treg cells in the peripheral lymph nodes was basically constant from the time of two weeks after their birth.

EXAMPLE 2

Next, it was investigated whether or not the temporal accumulation of the Treg cells in the colon as found in Example 1 had a relationship with the colonization of intestinal commensal microbiota. Specifically, the expression of CD4 and the expression of Foxp3 in lymphocytes isolated from the small intestine, the colon, and the peripheral lymph nodes of mice bred under a germ-free (GF) or SPF environment (8 weeks old: Balb/c mice, IqI mice, and C57BL/6 mice) were analyzed. Similar results were obtained in three or more independent experiments.

In addition, lamina propria lymphocytes were collected from SPF mice and GF+ mice (Balb/c mice or C57BL/6 mice). CD4 and Foxp3 were stained with antibodies. Then, the lamina propria lymphocytes were analyzed by FACS. Further, lymphocytes were isolated from the lamina propria of the colon, the lamina propria of the small intestine (SI), Peyer’s patches (PPs), and mesenteric lymph nodes (MLNs) of mice (SPF C57BL/6 mice) to which antibiotics were orally administered with water for eight weeks. CD4 and Foxp3 were stained with antibodies. Then, the lymphocytes were analyzed by FACS. Similar results (the ratio of the Foxp3+ cells in the CD4+ cells of an individual mouse) were obtained in two or more independent experiments. Note that the following antibiotics were used in combination in accordance with the description in the following document:

ampicillin (A; 500 mg/L, Sigma)
vancymycin (V; 500 mg/L, NACALAI TESQUE, INC.)
methionazole (M; 1 g/L, NACALAI TESQUE, INC.)
neomycin (N; 1 g/L, NACALAI TESQUE, INC.)
Fagarsan et al., Science 298, 1424 (Nov. 15, 2002)

As is apparent from the results, the frequencies and the absolute numbers of Foxp3+CD4+ cells in the small intestine and the peripheral lymph nodes of the GF mice were equal to or greater than those of the SPF mice. In addition, the numbers of the Treg cells in the small intestinal lamina propria, Peyer’s patches, and mesenteric lymph nodes of the SPF mice to which the antibiotics were orally administered for eight weeks were equal to or greater than those of the SPF mice that had not received antibiotics. Meanwhile, the number of the Foxp3+CD4+ cells in the colonic lamina propria of the GF mice was decreased significantly in comparison with that of the SPF mice. This decrease was commonly observed among mice of different genetic backgrounds (Balb/c, IqI, and C57BL/6), as well as among mice bred in different animal facilities. In addition, it was also shown that the number of Treg cells in the colonic lamina propria of the SPF C57BL/6 mice to which the antibodies were administered was decreased significantly.

EXAMPLE 3

Next, it was directly checked whether or not the decrease in the number of the Treg cells in the colonic lamina propria of the GF+ mice shown in Example 2 was attributed to the absence of microbiota. Specifically, a fecal suspension of B6 SPF mice purchased from The Jackson Laboratory was orally administered to GF-IqI mice (conventionalization). Three weeks after the administration, lymphocytes were isolated from the colonic lamina propria, and the expression of Foxp3 in CD4+ lymphocytes was analyzed. The results showed that the number of Treg cells in the small intestinal lamina propria did not change. However, the number of the Treg cells in the colonic lamina propria increased significantly. Hence, it was shown that host-microbial interaction played an important role in the accumulation of Foxp3+ Treg cells in the colonic lamina propria, while the accumulation of the Treg cells in the small intestinal lamina propria had a different mechanism.

EXAMPLE 4

Next, the relationship between the gut-associated lymphoid tissues of mice and the number of Foxp3+ cells in the colonic lamina propria of the mice was investigated in
according with the method described in M. N. Kweon et al., J Immunol 174, 4565 (Apr. 1, 2005). Specifically, 100 μg of an extracellular domain recombinant protein (a fusion protein (LTβR-Lg) between a lymphotixin β receptor (LTβR and a Fc region of human IgG1, refer to Honda et al., J Exp Med 193, 621 (Mar. 5, 2001)) was injected intraperitoneally into pregnant C57Bl/6 mice 14 days after conception. The LTβR-Lg was again injected intraperitoneally into fetuses obtained from such mice, so that mice from which isolated lymphoid follicles (ILFs), Peyer's patches (PPs), and colonic-patches (CPs) were completely removed were produced. Then, the ratios of Foxp3+ cells in CD4+ cells in the colonic lamina propria of the mice treated with the LTβR-Lg, and mice treated with rat IgG (control) were analyzed by FACS. The results show that the ratio of the Foxp3+ cells in the colonic lamina propria of the mice deficient in isolated lymphoid follicles, Peyer's patches, and the colonic-patches (the mice treated with the LTβR-Lg) rather increased. Accordingly, it was suggested that the decrease in the Treg cells in the colonic lamina propria of the GF mice and the mice treated with the antibiotics was caused because the transmission of specific signals which promote the accumulation of Treg cells in the colonic lamina propria and which is caused by the intestinal microbes did not occur, rather than simply because of a secondary effect of disorganized gut-associated lymphoid tissues.

EXAMPLE 5

To investigate whether or not a specific intestinal flora induced the accumulation of colonic Treg cells, vancomycin as an antibiotic against Gram-positive bacteria or polymyxin B as an antibiotic against Gram-negative bacteria was administered to SPF mice (from 4 weeks of age) for four weeks, and analyzed for the ratio of Foxp3+ cells in the CD4+ cell group (1%) Foxp3+ in CD4.

The results show that the number of Treg cells in the colon of the mice to which vancomycin was administered was markedly decreased in comparison with that of the control. In contrast, no influence was observed on the number of Treg cells of the mice to which polymyxin B was administered. Those facts suggested that Gram-positive commensal bacteria played a major role in accumulation of Treg cells.

EXAMPLE 6

A recent report has suggested that spore-forming bacteria play an important role in intestinal T cells response (see V. Gaboriau-Routhiau et al., Immunity 31, 677 (Oct. 16, 2009)). In this respect, fecal microorganisms (spore-forming fraction) resistant to 3% chloroform were orally administered to GF mice, which were then analyzed for the ratio of Foxp3+ cells in the CD4+ cell group (1%) Foxp3+ in CD4.

Three weeks after the administration of the chloroform-treated feces, the number of Treg cells in the administered mice was markedly increased to the same level as those of the SPF mice and the GF mice to which the untreated feces was forcibly administered.

Accordingly, considering the results shown in Example 5 in combination, it was revealed that the specific components of the indigenous microbiota were highly likely to belong to the Gram-positive group, and that the spore-forming fraction played an important role in the induction of Treg cells.

EXAMPLE 7

Next, the species of the intestinal microbiota which induced the accumulation of Treg cells in the colon as suggested in Examples 4 to 6 were identified. Specifically, segmented filamentous bacteria (SFB), 16 strains of Bacteroides spp. (Bactero, (6 strains of B. vulgatus, 7 of the B. acidifaciens group 1, and 3 of the B. acidifaciens group 2), 3 strains of Lactobacillus (Lacto. (L. acidophilus, L. fermentum, and L. murinus)), and 46 strains of Clostridium spp. (Clot. refer to “Itoh K., and Mitsuoka, T. Characterization of clostridia isolated from feces of limited flora mice and their effect on caecal size when associated with germ-free mice. Lab. Animals 19: 111-118 (1985)”)), or microbiota collected from mice (SPE) bred under a conventional environment was orally administered to GF-Balb/c mice or GF-IQI mice. The mice were maintained in vinyl isolators for three weeks. Then, CD4 cells were isolated from the colon and the small intestine of these mice. The numbers of Treg cells in the colon and the small intestine were analyzed by flow cytometry.

The bacteria belonging to the genus Clostridium are classified by sequencing of 16S rRNA gene. As follows, specifically, the 16S rRNA genes of the bacteria were amplified by PCR using 16S rRNA gene-specific primer pairs: 5’-ACGAGTTGATCMTGCGTCAG-3’ (SEQ ID NO: 60) and 5’-ATTACCGCGGCTCAGG-3’ (SEQ ID NO: 61) (see T. Aebischer et al., Vaccination prevents Helicobacter pylori-induced alterations of the gastric flora in mice. FEBS Immunol. Med. Microbiol. 46, 221-229 (2006)). The 1.5-9kb PCR product was then introduced into pCR-Bhant Vector. The inserts were sequenced and aligned using the Chisalw software program. The resulting sequences of 16S rRNA genes derived from strain 1-41 of 46 strains of Clostridium spp. were shown in SEQ ID NO: 21-61. A phylogenetic tree was constructed by the neighbor-joining method with the resulting sequences of the 41 strains of Clostridium and those of known bacteria obtained from Genbank database using Mega software.

The results showed no effect on the number of the Treg cells in the colon was observed in the GF mice in which the segmented filamentous bacteria (SFB) were colonized. Moreover, mice in which the cocktail of three strains of Lactobacillus was colonized gave similar results. On the other hand, it was shown that the accumulation of Foxp3+ cells in the colonic lamina propria was strongly induced in the mice in which 46 strains of Clostridium spp. were colonized. Importantly, such accumulation was promoted irrespective of the genetic backgrounds of the mice, and led to the increase in number similar to that in the SPE mice although intestinal microbiota of only a single genus was colonized. It was also shown that the colonization of the Clostridium did not change the number of Treg cells in the small intestinal lamina propria. Note that, when the 16 strains of Bactericides spp. were colonized, the number of Treg cells in the colon was increased significantly. However, the extent of the increase varied depending on the genetic background of the mice in which the bacteria were colonized.

EXAMPLE 8

Next, CD4 expression, Foxp3 expression, and Helios expression in LP lymphocytes of the thymuses and the colon of SPF mice, GF mice, Lactobacillus-colonized mice, and Clostridium-colonized mice were analyzed by flow cytometry.

The results show that most Foxp3+ cells found in the SPF mice or the Clostridium-colonized mice did not express Helios. Note that Helios is a transcription factor known to be expressed in thymic-derived natural Treg cells (see A. M.
Thornton et al., J Immunol 184, 3433 (Apr. 1, 2010). Accordingly, it was suggested that most of the Treg cells in the SPF mice and the Clostridium-colonized mice were Treg cells induced in peripheral portions (so-called Treg cells).

**EXAMPLE 9**

Next, it was investigated whether or not the colonization of the Clostridium or the like had an influence on other T cells. Specifically, SFB, 16 strains of Bacteroides spp. (Bactero.), 46 strains of Clostridium spp. (Clost.), or micro-biota collected from mice bred under a conventional environment (SPF) was colonized in GF IqI mice. Three weeks later, lymphocytes in the colonic lamina propria were isolated from these mice, and stimulated with PMA (50 ng/ml) and ionomycin (1 μg/ml) for four hours in the presence of Golgistop (BD Bioscience). After the stimulation was given, intracellular cytokines were stained by using an anti-IL-17 PE antibody (TC11-18I1H10) and an anti-IFN-g FITC antibody (BD Bioscience) in accordance with the manual of a cytokine/cytoperm kit (BD Bioscience). Then, the ratio of IFN-γ+ cells or IL-17+ cells in CD4+ lymphocytes was analyzed by flow cytometry. The results show that the colonization of the Clostridium did not have any influence on Th1 cells (CD4+ IFN-γ+ cells) in the colon, and caused only a slight increase of Th17 cells (CD4+IL-17+ cells). Accordingly, it was suggested that the genus Clostridium was a genus of bacteria which specifically induced Treg cells.

**EXAMPLE 10**

It has been reported that 46 strains of Clostridium spp. exert an influence on the accumulation of CD8+ intestinal tract intraepithelial lymphocytes (IELs) in the colon. Accordingly, it is conceivable that Clostridium regulates the immune system in various aspects, and that Clostridium exhibits a marked ability to induce and maintain Treg cells especially in the colon, as described above. In addition, a kind of cytokines, transforming growth factor-β (TGF-β), is known to play an important role in regulation of Treg cell generation.

In this respect, it was examined whether or not the colonization of Clostridium provided a colonic environment rich in TGF-β. Specifically, first, the whole colons of GF mice, Clostridium-colonized mice, and Lactobacillus-colonized mice were cultured for 24 hours, and the culture supernatants thereof were measured for the concentration of active TGF-β (TGF-β1) by ELISA (the number of mice analyzed was four per group).

The results show that the amount of TGF-β produced in the colons of the Clostridium-colonized mice was significantly greater than that in colons of the GF mice and the Lactobacillus-colonized mice.

Next, intestinal epithelial cells (IECs) of GF mice and Clostridium-colonized mice were cultured for 24 hours, and the culture supernatants thereof were measured for the concentration of active TGF-β (TGF-β1) by ELISA (the number of mice analyzed was four per group).

The results show that TGF-β was detected in the culture supernatant of the IECs isolated from the Clostridium-colonized mice, whereas no TGF-β was detected in the culture supernatant of the IECs isolated from the GF mice.

Next, as described above, splenic CD4+T cells were cultured for five days together with a 50% conditioned medium in which IECs isolated from the GF mice or the Clostridium-colonized mice were cultured, and with the anti-CD3 antibody, in the presence or absence of an anti-TGF-β antibody. Then, the T cells were collected, and analyzed for expression of Foxp3 by real-time RT-PCR.

The results show that when the culture supernatant of the IECs derived from the Clostridium-colonized mice was added to the splenic CD4+T cells, differentiation into Foxp3-expressing cells was accelerated. Meanwhile, differentiation into Treg cells was inhibited by the anti-TGF-β antibody.

The expression of MMP2, MMP9, and MMP13, which are thought to contribute to the activation of latent TGF-β was investigated. The expression of indoleamine 2,3-dioxygenase (IDO), which is thought to be involved in the induction of Treg cells, was also investigated. Specifically, 46 bacterial strains of the genus Clostridium (Clost.), or three bacterial strains of the genus Lactobacillus (Lacto.) were orally administered to C57BL/6 germ-free mice. Three weeks after administration, IECs were collected, and analyzed for relative mRNA expression levels of MMP2, MMP9, MMP13, and IDO genes by real-time RT-PCR (the number of mice analyzed was three per group).

For the relationship between the activation of latent TGF-β and the above-described MMP, see D’Angelo et al., J. Biol. Chem. 276, 11347-11353, 2001; Heldinger et al., Biol. Chem. 387, 69-78, 2006; Yu et al., Genes Dev. 14, 163-176, 2000. For the relationship between IDO and the induction of Treg cells, see G. Matteoli et al., Gut 59, 595 (May, 2010).

The results show in agreement with the production of TGF-β described above, that transcription products of the genes encoding MMP2, MMP9, and MMP13 were expressed at higher levels in the IECs derived from the Clostridium-colonized mice than in those in the GF mice and in the Lactobacillus-colonized mice.

Moreover, IDO was expressed only in the Clostridium-colonized mice.

Accordingly, it was revealed that the Clostridium activated the IECs, and led to the production of TGF-β and other Treg cell-inducing molecules in the colon.

**EXAMPLE 11**

Next, it was investigated whether or not the Treg cell accumulation induced by the colonization of the Clostridium was dependent on signal transmission by pathogen-associated molecular pattern recognition receptors. Specifically, the numbers of Treg cells in the colonic lamina propria of each SPF mice of Myd88-/- (deficient in Myd88 (signaling adaptor for Toll-like receptor)), Rip2-/- (deficient in Rip2 (NOD receptor adaptor)), and Card9-/- (deficient in Card9 (essential signal transmission factor for Dectin-1 signal transmission)) were examined. In addition, Clostridium spp. were caused to be colonized in the Myd88-/- GF mice, and the change in the number of Treg cells was investigated. The results show that the number of Treg cells of each kind of the SPF mice deficient in the associated factors of the pathogen-associated molecular pattern recognition receptors did not change relative to that of wild-type mice of the same litter, which served as a control. In addition, it was found that when Clostridium spp. were colonized in GF mice deficient in Myd88, the accumulation of Treg cells in the colonic lamina propria was induced. Accordingly, it has been suggested that the mechanism of inducing the accumulation of Treg cells in the colonic lamina propria relies not on activation of recognition pathway for major pathogen-associated molecular patterns as is caused by most bacteria, but on specific commensal bacterial species.

**EXAMPLE 12**

Intestinal tract Foxp3+ Treg cells are known to exert some immunosuppressive functions through IL-10 production (re-
fer to NPL 9). Meanwhile, animals having CD4*Foxp3* cells from which IL-10 is specifically removed are known to develop inflammatory bowel disease (to NPL 18). In this respect, first, the expression of IL-10 in lymphocytes of various tissues was examined. Specifically, lymphocytes were isolated from various tissues of SPF II10**:mice, and the expression of CD4 and the expression of Venus were analyzed by flow cytometry.

Lymphocytes in the colonic lamina propria were isolated from II10**:mice, and the expression of T cell receptor β chain (TCRβ) on the surfaces of the cells was detected by FACS. Lymphocytes in the colonic lamina propria were isolated from II10**:mice. The lymphocytes were stimulated with PMA (50 ng/ml) and ionomycin (1 μM/ml) for four hours in the presence of Golgistop (BD Bioscience). Then, after the stimulation was given, intracellular cytokines were stained by using an anti-IL-17 PE antibody, an anti-IFN-γ APC antibody (1B11), and an anti-IFN-γ FITC antibody (BD Bioscience) in accordance with the manual of a cytotox/cytokine kit (BD Bioscience).

In addition, Foxp3*CD4* cells and Foxp3*CD4* cells were isolated from the spleen (SpI) of Foxp3*GFPI reporter mice, and Venus* cells were isolated from the colonic lamina propria of II10**:mice. The obtained cells were analyzed in terms of expression of predetermined genes. The gene expression was analyzed by real-time RT-PCR using a Power SYBR Green PCR Master Mix (Applied Biosystems) and an ABI 7300 real time PCR system (Applied Biosystems). Here, the value for each cell was normalized for the amount of GAPDH.

The results show that almost no Venus* cells (IL-10-producing cells) were detected in the cervical lymph nodes (peripheral lymph nodes), thymus, peripheral blood, lung, and liver of mice kept under the SPF conditions. Meanwhile, in the spleen, Peyer’s patches, and mesenteric lymph nodes thereof, Venus* cells were slightly detected. On the other hand, many Venus* cells were found in the lymphocytes in the small intestine lamina propria and colonic lamina propria. In addition, most of the Venus* cells in the intestines were positive for CD4, and also positive for T cell receptor β chain (TCRβ). It was found that the Venus* CD4*T cells expressed Foxp3 and other Treg cell-associated factors such as a cytotoxic T-lymphocyte antigen (CTLA-4) and a glucocorticoid-induced TNFR-associated protein (GITR), although the Venus*CD4*T cells showed none of the phenotypes of Th2 (IL-4-producing) and Th17 (IL-17-producing). It was shown that the expression level of CTLA-4 in the intestinal Venus* cells was higher than that in the splenic GFPI* Treg cells isolated from the Foxp3*GFPI reporter mice.

**EXAMPLE 13**

Venus* cells can be classified into at least two subsets, namely, Venus*Foxp3* double positive (DP) Treg cells and Venus*Foxp3*Treg cells on the basis of intracellular Foxp3 expression. Cells of the latter subset correspond to type 1 regulatory T cells (Tr1) (refer to NPL 8 and 9). In this respect, the Venus* cells (IL-10-producing cells) observed in Example 8 were investigated in terms of the expression of Foxp3. Specifically, the expression of CD4, Foxp3, and Venus in the lamina propria of the colon and the lamina propria of the small intestine of II10**:mice kept under GF or SPF conditions was analyzed by FACS, and the numbers of Venus* cells in the intestinal tract lamina propria were compared between SPF and GF II10**:mice.

In addition, the intracellular expression of Venus and Foxp3 in CD4 cells in various tissues of SPF II10**:mice was analyzed by flow cytometry.

In order to investigate whether or not the presence of commensal bacteria had any influence on the expression of IL-10 in regulatory cells in the gastrointestinal tracts, germ-free (GF) II10**:mice were prepared. Then, predetermined species of bacteria were caused to be colonized in the obtained GF II10**:mice. Three weeks after the species of bacteria were colonized, a CD4* cell group (V/F, Venus*Foxp3* cells; V/F, Venus*Foxp3* cells; and V/F, Venus*Foxp3* cells) in which Foxp3 and/or Venus were expressed in the colon and the small intestine was analyzed by flow cytometry.

In order to check whether or not the presence of commensal bacteria had any influence on the expression of IL-10 in regulatory cells in the gastrointestinal tracts, antibiotics were orally given with water to five or six II10**:mice per group for 10 weeks. The following antibiotics were used in combination: ampicillin (A; 500 mg/L Sigma); vancomycin (V; 500 mg/L NACALAI TESQUE, INC.); metronidazole (M; 1 g/L NACALAI TESQUE, INC.); neomycin (N; 1 g/L NACALAI TESQUE, INC.).

Then, CD4 and Foxp3* lymphocytes in the lamina propria of the colon, the lamina propria of the small intestine (SI), mesenteric lymph nodes (MLN), and Peyers’ patches (PPs) were stained with antibodies, and analyzed by FACS. The results were obtained from two or more independent experiments which gave similar results.

The results show that the small intestinal lamina propria was rich in Venus*Foxp3* cells, namely, Tr1-like cells, and that the Venus*Foxp3*DP Treg cells were present at a high frequency in the colon of the SPF mice. In contrast, although sufficient numbers of Foxp3* cells were observed also in other tissues, the expression of Venus was not observed in almost all of the cells.

In addition, it was shown that all regulatory T cell fractions of Venus*Foxp3*, Venus*Foxp3*, and Venus*Foxp3* in the colon significantly decreased under the GF conditions. Moreover, similar decrease in Venus* cells was observed also in the SPF II10**:mice treated with the antibiotics.

The colonization of Clostridium spp. strongly induced all regulatory T cell fractions of Venus*Foxp3*, Venus*Foxp3*, and Venus*Foxp3* in the colon, and the degrees of the induction thereof were equal to those in the SPF mice. In addition, it was found that the colonization of the three strains of Lactobacillus or the colonization of SFB had an extremely small influence on the number of Venus* and/or Foxp3* cells in the colon. Moreover, the colonization of 16 strains of Bacteroides spp. also induced Venus* cells, but the influence of the colonization was specific to Venus*Foxp3* Tr1-like cells. On the other hand, it was found that none of the bacterial species tested exerted any significant influence on the number of IL-10-producing cells in the small intestinal lamina propria.

Hence, it was shown that the genus Clostridium colonized in the colon or a physiologically active substance derived from the bacteria provided a signal for inducing the accumulation of IL-10** regulatory T cells in the colonic lamina propria or the expression of IL-10 in T cells. It was shown that the number of Venus* cells in the small intestine was not significantly influenced by the situation where no commensal bacteria were present or commensal bacteria were decreased, and that IL-10** regulatory cells (Tr1-like cells)
accumulated in the small intestinal lamina propria independently of commensal bacteria.

EXAMPLE 14

It was investigated whether or not Venus* cells induced by the genus *Clostridium* had an immunosuppressive function similar to that of Venus* cells in the colon of SPF mice. Specifically, CD4*CD25* cells (effector T cells, Teff cells) isolated from the spleen were seeded in a flat-bottomed 96-well plate at 2x10^5/well, and cultured for three days together with 2x10^4 splenic CD11c* cells (antigen-representing cells) subjected to 30 Gy radiation irradiation treatment, 0.5 µg/ml of an anti-CD3 antibody, and a lot of Treg cells. In addition, for the last six hours, the CD4*CD25* cells were cultured, with [3H]-thymidine (1 µCi/well) was added thereto. Note that, Treg cells used in Example 14 were CD4*GFP* T cells isolated from the spleen of Foxp3*GFP reporter mice, or CD4*Venus* T cells in the colonic lamina propria of GF II10*venus* mice in which *Clostridium* spp. were colonized or SPF II10*venus* mice. Then, proliferation of the cells was determined based on the uptake amount of [3H]-thymidine, and represented by a count per minute (cpm) value.

The results show that Venus*CD4* cells of the mice in which the genus *Clostridium* was colonized suppressed in vitro proliferation of CD25*CD4* activated T cells. The suppression activity was slightly inferior to that of GFP* cells isolated from the Foxp3*GFP reporter mice, but equal to that of Venus* cells isolated from the SPF II10*venus* mice. Accordingly, it has been shown that the genus *Clostridium* induces IL-10 expressing T cells having sufficient immunosuppressive activities, and thereby plays a critical role in maintaining immune homeostasis in the colon.

EXAMPLE 15

Next, the influence of the colonization of a large number of *Clostridium* on the local immune response and the resultant proliferation of Treg cells were investigated.

<Dextran Sulfate Sodium (DSS)-Induced Colitis Model>
First, the DSS-induced colitis model was prepared as described above, and the influence on the model mice of the inoculation of *Clostridium* and the proliferation of Treg cells was investigated. Specifically, control mice and *Clostridium*-inoculated mice were treated with 2% DSS, then observed and measured for six days for body weight loss, the hardness of stool, and bleeding, and then were evaluated numerically. In addition, on day 6, the colons were collected, dissected, and analyzed histologically by HE staining.

The results show that the symptoms of the colitis such as body weight loss and rectal bleeding were significantly suppressed in the mice having a large number of *Clostridium* (hereinafter also referred to as “*Clostridium*-abundant mice”) in comparison with the control mice (C57BL/6 mice grown in a conventional environment for six weeks and not inoculated with the fecal suspension). All the features typical for colonic inflammation, such as shortening of the colon, edema, and hemorrhage, were observed markedly in the control mice in comparison with the *Clostridium*-abundant mice. Moreover, histological features such as mucosal erosion, edema, cellular infiltration, and crypt loss were less severe in the DSS-treated *Clostridium*-abundant mice than in the control mice.

<Oxazolone-Induced Colitis Model>
Next, the oxazolone-induced colitis model was prepared as described above, and the influence on the model mice of the inoculation of *Clostridium* and the proliferation of Treg cells was investigated. Specifically, control mice and *Clostridium*-inoculated mice were sensitized with oxazolone, and subsequently the inside of the rectums thereof were treated with a 1% oxazolone/50% ethanol solution. Then, body weight loss was observed and measured. In addition, the colons were dissected, and analyzed histologically by HE staining.

The results show that the colitis proceeded along with persistent body weight loss in the control mice. Meanwhile, the body weight loss of the *Clostridium*-abundant mice was reduced. In addition, it was also revealed that portions having histological diseases such as mucosal erosion, edema, cellular infiltration, and hemorrhage were reduced in the colon of the *Clostridium*-abundant mice.

EXAMPLE 16

Next, the influence, on the systemic immune response (systemic IgE production), of the colonization of a large number of *Clostridium* and the resultant proliferation of Treg cells was investigated. Specifically, as described above, control mice and *Clostridium*-inoculated mice were immunized by administering alum-absorbed ovalbumin (OVA) twice at a 2-week interval. Then, sera were collected from these mice, and the OVA-specific IgE level thereof was investigated by ELISA. In addition, splenic cells were collected from the mice in each group, and IL-4 and IL-10 production by in-vitro OVA restimulation was investigated.

Results show that the IgE level was significantly lower in the *Clostridium*-abundant mice than in the control mice. Moreover, the IL-4 production by the OVA restimulation was reduced and the IL-10 production thereby was increased in the splenic cells of the *Clostridium*-abundant mice sensitized with OVA and alum, in comparison with those of the control mice.

Accordingly, in consideration of the results shown in Example 15 in combination, the induction of Treg cells by *Clostridium* in the colon plays an important role in local and systemic immune responses.

EXAMPLE 17

Next, GF Balb/c were colonized with three strains of *Clostridium* belonging to cluster IV (strains 22, 23 and 32). Three weeks later, colonic Foxp3*Treg cells were analyzed by FACS. Results show that gnotobiotic mice colonized with three strains of *Clostridium* showed an intermediate pattern of Treg induction between GF mice and mice inoculated with all 46 strains.

EXAMPLE 18

Next, it was investigated whether or not a spore-forming (for example, a chloroform resistant) fraction of a fecal sample obtained from humans had the effect of inducing proliferation or accumulation of regulatory T cells similar to the spore-forming fraction of the fecal sample obtained from mice.

Human stool from a healthy volunteer (Japanese, male, 29 years old) was suspended with phosphate-buffered saline (PBS), mixed with chloroform (final concentration 3%), and then incubated in a shaking water bath for 60 min. After evaporation of chloroform by bubbling with N₂ gas, the
Bacteroides -continued

SEQ ID NO: 67
5′-GGAGAGGAGGTCCCCCAG-3′

and

SEQ ID NO: 68
5′-CCCTCACGTCTGTTCAAG-3′

Results show that gavaged with chloroform-treated human stool had large amounts of spore-forming bacteria, such as Clostridium clusters XIVa and IV, and a severe decrease of non-spore-forming bacteria, such as Bacteroides, compared with the human stool before chloroform treatment.

EXAMPLE 19

Human stool (2 g) from a healthy volunteer (Japanese, male, 29 y old) was suspended with 20 ml phosphate-buffered saline (PBS), mixed with or without chloroform (final concentration 3%), and incubated in a shaking water bath for 60 min. The chloroform was then evaporated by bubbling with N2 gas for 30 min. The suspensions of untreated human feces (designated as ‘huUT’) and chloroform-treated human feces (designated as ‘hucHloro’) were orally inoculated into Germ-free (GF) mice (IQL, 8 week old) (250 μl/mouse). The suspension of huUT was inoculated into 4 GF mice, which were numbered from #A1 to #A4, and that of hucHloro was inoculated into 4 GF mice numbered from #B1 to #B4. Such GF mice which were inoculated with suspensions of feces or the like are also referred to as “ex-GF mice” hereinafter. Each group of ex-GF mice was separately kept in a vinyl isolator to avoid further microbial contamination. After 3 weeks, the small intestinal and colonic lamina propria lymphocytes from each mouse were separately collected, and examined for the expressions of surface CD4 and intracellular Foxp3, Helios, IL-17 and IFN-γ by flow cytometry. For intracellular IL-17 and IFN-γ staining, isolated lymphocytes were stimulated in vitro with PMA and ionomycin for 4 hours. Foxp3 is the transcription factor essential for the differentiation and function of Treg cells. Helios is a member of the Ikaros transcription factor family and Helios-Foxp3+ Treg cells have been suggested to be Treg cells induced in the periphery [so called induced Treg (iTreg) cells]. As shown in FIGS. 1A-D, the percentages of Foxp3+ Treg cells within CD4+ T cells in the small intestinal and colonic lamina propria of both groups of ex-GF mice were increased, compared with those in GF mice. Marked increases were also observed for the percentage of Helios-cells among Foxp3+ Treg cells in small intestine and colon in both groups of ex-GF mice. Notably, besides Foxp3+ Treg cells, a significant accumulation of IL-17-expressing CD4+ T cells (namely, Th17 cells) was observed in exGF+huUT mice, whereas it was only marginally observed in exGF+huHloro mice (FIG. 1E, F). In both groups of mice, the percentages of IFN-γ+ cells in CD4+ cells were unchanged (FIG. 1E, G).

EXAMPLE 20

To investigate whether dead bacteria also have an effect on the induction of Treg cells, the suspension of chloroform-treated human feces was autoclaved (121° C. for 20 min) and orally inoculated into GF mice (once a week for 4 weeks). After 4 weeks, mice were sacrificed, and the colonic lamina propria lymphocytes from each mouse were examined for the expression of CD4, Foxp3 and Helios by flow
cytometry. As shown in FIG. 2, the inoculation of dead bacteria exhibited no effect on the numbers of Foxp3+ cells or Helios-Foxp3+ cells. These results do not rule out the possibility that the amount of dead bacteria inoculated was not sufficient, but suggest that live bacteria are required for the induction of Treg cells.

EXAMPLE 21

To confirm the induction of Treg cells by chloroform-resistant bacteria, another stool was obtained from the same person on a different day, treated with chloroform, and inoculated into IGI GF mice (7 mice, numbered from #C1 to C7). After 3-4 weeks, mice from #C1 to #C5 were sacrificed, and the small intestinal and colonic lamina propria lymphocytes from each mouse were separately collected, and examined for the expression of CD4 and Foxp3 by flow cytometry. Consistent with the findings in Example 19, colonization with chloroform-treated human feces significantly increased the accumulation of Foxp3+CD4+ Treg cells in colonic and small intestinal lamina propria (FIG. 3). These results further support the notion that chloroform-resistant spore-forming bacteria can induce differentiation, proliferation and/or recruitment of Treg cells in intestinal lamina propria.

EXAMPLE 22

To test whether Treg cell induction by chloroform-resistant spore-forming fraction of human intestinal bacteria is horizontally transmissible, IGI GF mice (6 mice, numbered from #D1 to #D6) were cohoused for 4 weeks with mice #C6 and #C7 in the same cage in a vinyl isolator. Lamina propria lymphocytes from colon and small intestine were isolated and examined for CD4 and Foxp3. Cohoused mice exhibited a significant increase in the percentage of Foxp3+ cells among CD4+ cells (FIG. 4). Therefore, Treg cell induction by human intestinal bacteria is horizontally transmissible. These results let us assume a role of prominent components of the intestinal microbiota, rather than minor components, for the induction of Treg cells.

EXAMPLE 23

The frozen stock of cecal content from mouse #D4 was thawed, suspended in 10 times its volume (w/v) of PBS, and passed through a 70 μm cell strainer. The suspension was then treated with 3% chloroform, diluted 2000- or 20000-fold with PBS, and orally inoculated into GF IGI mice (2.5×10^6 or 2.5×10^9 bacterial cells/250 μl/head, respectively). The 2000-fold diluted sample was orally inoculated into 4 mice (designated as exGF×2000, numbered from #E1 to #E4), whereas 20000-fold diluted sample was inoculated into 8 mice (designated as exGF×20000, numbered from #F1 to #F8). After 3 weeks, the intestinal lamina propria lymphocytes were isolated and examined for CD4, Foxp3 and Helios. Both 20000- and 20000-fold diluted samples similarly induced a marked accumulation of Foxp3+CD4+ cells in the intestinal lamina propria (FIG. 5). Therefore, the dose of bacteria for oral inoculation can be minimized to less than 2.5×10^8 bacterial cells.

EXAMPLE 24

The frozen stock of cecal content from mouse #F3, #F7 or #F8 was suspended in 10 times its volume (w/v) of PBS, passed through a 70 μm cell strainer, and treated with 3% chloroform. Then, the fecal suspension from mouse #F3 was orally inoculated into 5 GF mice (numbered from #G1 to #G5), that from #F7 mouse into 4 GF mice (numbered from #H1 to #H4), and that from #F8 mouse into 4 GF mice (numbered from #I1 to #I4). After 4 weeks, lymphocytes from colonic and small intestinal lamina propria were isolated and examined for CD4, Foxp3 and Helios expression by flow cytometry. All #F, #G, and #H mice exhibited a significant increase in the percentage of Foxp3+ cells among CD4+ cells in the intestinal lamina propria compared with untreated GF mice (FIG. 6). Therefore, the Treg cell induction by human intestinal bacteria colonizing in exGF×20000 mice is also transmissible. Moreover, as shown in the later meta 16S rDNA sequencing data (FIG. 8), these mice commonly had bacteria having 16S rDNA sequence similarities with 16S rDNA sequence similarities with 20 species of known bacteria (C. ammonia, H. saccarovorans, E. fuscata, H. fihiformis, C. cladostrodiiformis, C. indolis, C. boleae, Bacteroides sp. MANG, L. bacterium DJF_PVF0, Ruminococcus sp. ID8, C. lavalenscens, C. symbiosum, E. contortum, C. saccharogum, C. ramosum, E. plantii, C. scindens, Clostridium sp. 2335, Clostridium sp. 14616 and C. Clostridium sp. MLG055).

EXAMPLE 25

A frozen stock of the cecal content from #F8 mouse was serially diluted with 0.85% NaCl under an aerobic condition and plated onto BL agar. After culture at 37° C. for 2 or 4 days, 50 single colonies were observed. Of the 50 colonies, 29 were picked up, cultured for additional 2 or 4 days at 37° C. by ABCM broth, and stored in EGI stock medium (10% DMSO) at –80°C. The genomic DNA from each colony was isolated, and 16S rRNA coding gene sequence was analyzed. The sequence of 16S rRNA of each colony revealed that the 29 colonies observed were represented by three strains, each having 100% similarity with Clostridium ramosum, 99.75% with Clostridium saccharogum, 100% with Flavonifractor plautii, 99.17% with Clostridium hathewayi, 99.23% with Clostridium scindens, or 99.66% with Clostridium sp. 2335. Within the 29 colonies that were selected from the original 50 colonies, only Clostridium saccharogum, Clostridium ramosum, and Flavonifractor plautii were present (25, 3, and 1 colonies, respectively). These 3 isolated strains were propagated, mixed and inoculated into GF IGI mice (4 mice, numbered from #J1 to #J4). After 3-4 weeks, the colonic lamina propria lymphocytes were collected, and examined for the expressions of CD4, Foxp3, and Helios by flow cytometry. Foxp3+ cells or Helios-cells were not induced or only weakly induced by the colonization of these strains of bacteria in the colon (FIG. 7). These results suggest that the combination of Clostridium saccharogum and Clostridium ramosum (both within cluster XVIII) were insufficient to induce Treg cells in the colon of mice. The effects of Flavonifractor plautii were not clear, since the strain was only represented by 1 of the 29 colonies that were selected.

EXAMPLE 26

The frozen glycerol stock of cecal content from #G2 mouse was suspended with PBS, seeded onto BL agar plate, and incubated for 48 hours, similarly to the procedure done in Example 19. Different from Example 19, all bacteria on the plate were collected by scraping with a plate scraper, suspended in TS broth and inoculated into GF IGI mice (4 mice, numbered from #K1 to #K4). It should be noted that the bacterial suspension used in this experiment included
bacteria that did not propagate but survived on the plate. After 4 weeks, lamina propria lymphocytes from colon and small intestine of K1–K4 mice were isolated and examined for CD4, Foxp3 and Helios expression. All 4 mice exhibited a significant increase in the percentages of Foxp3+ cells among CD4+ cells (FIGS. 9A, 9B) and Helios+ cells among Foxp3+ Treg cells (FIGS. 9A, 9C) in the intestinal lamina propria compared with untreated GF mice. Considering that the inoculation of mice with 6 strains of bacteria propagated on the BL agar plate failed to induce Treg cells, bacteria that did not propagate but survived on the plate might be responsible for the induction of Treg cells.

EXAMPLE 27

Bacterial DNA was extracted from the cecal contents of mouse #A1, #C4, #F8, #G2, #I3, #J3, #J3 and #K3. Variable region 1-2 (V1-2) in bacterial 16S rRNA coding gene were amplified by PCR and used as template for metasequencing. Resulting sequences (3400 reads for each sample) were classified into operational taxonomic units (OTUs) on the basis of sequence similarity (>97% identity). Representative sequences from each OTU were compared with sequences in nucleic acid databases using BLAST to determine their closest relatives in known species. The numbers of detected reads and the closest relatives for each OTU are shown in Table 1. The relative abundances of OTUs having the same closest relative in each cecal sample are shown in FIG. 8. In mouse #A1, 153 OTUs (their closest relatives were 93 species) were identified and half of them were related to Bacteroides species. In contrast, in mouse #C4, 113 OTUs were identified and most of them were related to species belonging to the family Clostridiae. In mouse #F8, #G2, #I3, #J3, #J3 and #K3, 97-68 OTUs were identified. In these mice, in which Treg cell accumulation was observed in the intestine, the majority of bacteria consisted of bacteria having 16S rDNA sequence similarities with C. hominis, U. sabarum, E. coli, H. pylori, C. ramosum, F. prausnitzii, and F. prausnitzii species. These results suggest that the combination of these three species is insufficient to induce the intestinal Treg cells accumulation.

EXAMPLE 28

Bacterial strains were isolated from the cecal contents of mouse #F8, #G2, #I3 and #K3 using BL agar or EG agar plates. Applicant-picked up 144 colonies from EG agar plates and 116 colonies from BL agar plates. BLAST search of 16S rDNA coding sequence of these clones revealed that they belonged to 17 species, and each had 93-100% similarities with C. indolis, C. bolteae, Bacteroides sp. MANG, L. bacterium DJF_VP30, A. colihominis, Ruminococcus sp. ID8, C. lavalense, C. symbiontum, E. contortum, C. saccarogena, C. ramosum, F. prausnitzii, C. scindens, Clostridium sp. 2335, Clostridium sp. 14616 and of Clostridium sp. M.L.G055. These results suggest that the combination of these three species is insufficient to induce the intestinal Treg cells accumulation.

EXAMPLE 29

Of the colonies selected in Example 28, additional colonies were picked and isolated and these strains were cultured using EG and BL media. BLAST search of 16S rDNA coding sequence of these clones revealed that they belonged to a total of 31 species (including the species mentioned in Example 28), and each had 93-100% similarities with C. ramosum, C. bolteae, C. colihominis, F. prausnitzii, C. scindens, L. bacterium DJF_VP30, A. colihominis, Ruminococcus sp. ID8, C. lavalense, C. symbiontum, E. contortum, C. saccarogena, C. ramosum, F. prausnitzii, C. bolteae, C. colihominis, C. colihominis, A. colihominis, DSM 17241, E. contortum, C. ramosum, C. bolteae, C. lavalense, C. symbiontum, DSM 15981, C. saccarogena, DSM 14616, of C. bolteae, C. colihominis, DSM 17241, A. colihominis, C. lavalense, C. symbiontum, DSM 15981, and C. ramosum. These strains were cultured on BL agar and EG agar plates. The relative abundances of OTUs having the same closest relative in each cecal sample are shown in FIG. 8. In mouse #A1, 153 OTUs (their closest relatives were 93 species) were identified and half of them were related to Bacteroides species. In contrast, in mouse #C4, 113 OTUs were identified and most of them were related to species belonging to the family Clostridiae. In mouse #F8, #G2, #I3, #J3, #J3 and #K3, 97-68 OTUs were identified. In these mice, in which Treg cell accumulation was observed in the intestine, the majority of bacteria consisted of bacteria having 16S rDNA sequence similarities with C. hominis, U. sabarum, E. coli, H. pylori, C. ramosum, F. prausnitzii, and F. prausnitzii species. These results suggest that the combination of these three species is insufficient to induce the intestinal Treg cells accumulation.

EXAMPLE 30

To investigate whether the strains in Example 29 have the ability to induce Tregs in GF mice, 31 strains on Table 3 were mixed at equal amounts of media volume using TS media and inoculated into GF mice. A detailed analysis of the 16S rDNA sequences revealed that 8 of the 31 strains overlapped with other strains (see Table 3, indicated by an asterisk), resulting in 23 distinct bacterial strains. As shown in FIG. 10, when orally administered to GF mice, the mixture of the 23 strains (23mix) induced very strong levels of Tregs (35-40%) in the colon lamina propria, >10% in the small intestine; FIG. 10). These Tregs observed with colonization by 23mix were mostly Helios+.

EXAMPLE 31

To investigate whether the abundant members of the intestinal microbiota in the chloroform-resistant fraction of human intestinal bacteria, rather than the minor members, drive the induction of Treg cells, adult GF mice were inoculated with diluted caecal samples from mice that had been inoculated with the chloroform-resistant fraction of human intestinal bacteria (+hChlo mice) as described in example 19. As shown in FIG. 11, even when the hChlo mice caecal samples were diluted (diluted 2x10^7 and 2x10^8) to create 4x2x10^7 mice and 2x10^8 mice respectively, Tregs were induced in these adult GF mice.

EXAMPLE 32

To investigate whether the mix of 23 strains in Example 30 has the ability to induce Tregs in adult GF IqQ mice more effectively than Faecalibacterium prausnitzii, a well-known
human Clostridia strain characterized for enhancing regulatory cell functions. 23 strains in table 4 were mixed in equal amounts with media to make a cocktail, which was then administered to adult IQI BALB, and B6 mice. The mixture of 17 strains was able to induce Tregs in these three mouse models.

EXAMPLE 36

To investigate whether each of the 17 strains defined in example 35 could individually induce Tregs, adult GF mice were monoclonized with one of each of the 17 strains. As shown in FIG. 16, adult GF mice monoclonized with a single strain exhibited low to intermediate levels of Treg. Importantly, no single strain induced Tregs to the same extent as the mix of 17 strains.

EXAMPLE 37

To investigate whether subsets of the 17 strains described in example 35 could induce Tregs, randomly selected combinations of 3-5 strains were made: 3-mix, 5mixA, 5-mix B, and 5-mix C, as shown in table 4, and used to inoculate adult GF mice. As shown in FIG. 17, only the 5-species mixes induced significant increases in the frequency of Treg cells, the magnitude of which was intermediate compared with that observed in 4+7-mix mice.

EXAMPLE 38

To investigate the benefits of administration of the mix of the 17 strains described in example 35 (17-mix), adult SPF mice were orally inoculated with either 17-mix or control media and assessed for the induction of Foxp3+ Treg cells three weeks later. As shown in FIG. 18, there was a significant increase in the frequency of colonic Foxp3+ Treg (CD4) cells after three weeks of treatment.

EXAMPLE 39

To evaluate the benefit of administration of 17-mix in an animal model of allergic diarrhea, adult SPF mice were orally inoculated with 17-mix or control media while being treated with ovalbumin (OVA), an inducer of allergic diarrhea. As shown in FIG. 19, the occurrence and severity of diarrhea (diarrhea score) was significantly reduced in mice fed 17-mix relative to control mice.

EXAMPLE 40

To evaluate the benefit of administration of 17-mix in an animal model of colitis. Adult SPF mice were orally inoculated with either 17-mix or control media while being treated with trinitrobenzene sulfonic acid (TNBS), a frequently used experimental inducer of colitis. As shown in FIG. 20, 17-mix mice demonstrated lower mortality than control mice on exposure to TNBS.

EXAMPLE 41

To evaluate the usefulness of the strains represented in 17-mix as a diagnostic and monitoring tool for ulcerative colitis, we examined the relative abundance of the 17 strains in healthy and ulcerative colitis (UC) human subjects using draft genomic sequences of the 17 strains and publicly available human faecal microbiome genomes generated through the European MetaHIT project. UC subjects (N=20) showed a reduction in the 17 strains compared to healthy subjects (N=15), as shown in FIG. 21.
utilizing certain human-derived bacteria belonging to the Clostridia class or a physiologically active substance or the like derived from the bacteria. Since the bacterial composition has immunosuppressive effects, the bacterial composition can be used, for example, to prevent or treat autoimmune diseases or allergic diseases, as well as to suppress immunological rejection in organ transplantation or the like. In addition, healthy individuals can easily and routinely ingest the bacterial composition, such as in food or beverage, (e.g., a health food), to improve their immune functions.

### Table 1A

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**OTU3**

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**OTU9**

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CTTCACACTGTTTTTATAAATTCTTGGTGCAGTTACACGGTATAAGCCAGCACGGTACCTAC
GCACTGAGCTATGACGAAATGGTGTCCAGTACGGATGGGCTCAACGCTGACGACGCTGAAACG

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GCTGGGTTAAGGCCCCACGGAAGACGAGCTCATGAAAGATGAACTCAGACGACGC
CATGGGACCTGAGAAGACGACGCC

OTU162 (SEQ ID NO.: 36)
GATGGACUGCTGCCGCGCTCCTAACATGCAAGTCGAACGAGATTAGTAGGACAGGGAAATT
TTCGAGCTGAATCGTATACTTATGCTGGAGACGCTGTGAGTAACGCGTGGGAAACCTG
CTUCACGGGAGGATACCTTTAAGAATGCTCAATAAACGCAAAGGACGAGTACGCG
ATGGGTCTGTTGTAAGAACTCCGCGTTAGATAGTCTCCGCGCTGATAGTACGCCG
GCAGGCTACCGCCTACCAAGACGACGCTATAGCGCCGCTGAGAGGTCGAGCGCC
CATGGGACCTGAGAAGACGACGCC

OTU161 (SEQ ID NO.: 86)
GATGGACUGCTGCCGCGCTCCTAACATGCAAGTCGAACGAGATTAGTAGGACAGGGAAATT
TTCGAGCTGAATCGTATACTTATGCTGGAGACGCTGTGAGTAACGCGTGGGAAACCTG
CTUCACGGGAGGATACCTTTAAGAATGCTCAATAAACGCAAAGGACGAGTACGCG
ATGGGTCTGTTGTAAGAACTCCGCGTTAGATAGTCTCCGCGCTGATAGTACGCCG
GCAGGCTACCGCCTACCAAGACGACGCTATAGCGCCGCTGAGAGGTCGAGCGCC
CATGGGACCTGAGAAGACGACGCC

OTU163 (SEQ ID NO.: 87)
GATGGACUGCTGCCGCGCTCCTAACATGCAAGTCGAACGAGATTAGTAGGACAGGGAAATT
TTCGAGCTGAATCGTATACTTATGCTGGAGACGCTGTGAGTAACGCGTGGGAAACCTG
CTUCACGGGAGGATACCTTTAAGAATGCTCAATAAACGCAAAGGACGAGTACGCG
ATGGGTCTGTTGTAAGAACTCCGCGTTAGATAGTCTCCGCGCTGATAGTACGCCG
GCAGGCTACCGCCTACCAAGACGACGCTATAGCGCCGCTGAGAGGTCGAGCGCC
CATGGGACCTGAGAAGACGACGCC

OTU165 (SEQ ID NO.: 88)
GATGGACUGCTGCCGCGCTCCTAACATGCAAGTCGAACGAGATTAGTAGGACAGGGAAATT
TTCGAGCTGAATCGTATACTTATGCTGGAGACGCTGTGAGTAACGCGTGGGAAACCTG
CTUCACGGGAGGATACCTTTAAGAATGCTCAATAAACGCAAAGGACGAGTACGCG
ATGGGTCTGTTGTAAGAACTCCGCGTTAGATAGTCTCCGCGCTGATAGTACGCCG
GCAGGCTACCGCCTACCAAGACGACGCTATAGCGCCGCTGAGAGGTCGAGCGCC
CATGGGACCTGAGAAGACGACGCC

OTU166 (SEQ ID NO.: 25)
GATGGACUGCTGCCGCGCTCCTAACATGCAAGTCGAACGAGATTAGTAGGACAGGGAAATT
TTCGAGCTGAATCGTATACTTATGCTGGAGACGCTGTGAGTAACGCGTGGGAAACCTG
CTUCACGGGAGGATACCTTTAAGAATGCTCAATAAACGCAAAGGACGAGTACGCG
ATGGGTCTGTTGTAAGAACTCCGCGTTAGATAGTCTCCGCGCTGATAGTACGCCG
GCAGGCTACCGCCTACCAAGACGACGCTATAGCGCCGCTGAGAGGTCGAGCGCC

OTU173 (SEQ ID NO.: 123)
GACGGAACCTGCCGCGCCGCTTACACCAAGTCAAGTCAACGAGATTAGTAGGACAGGGAAATT
CTGGTATACACACTTTAGTCGTCGACGGTGAGTACCTCGCTATAGCTCG
GGGGTATACTTGTTAGAAATGCCTCGTACCTGATACGCCGAGAAGGACGACGCCGCTGAGAGGTCGAGCGCC

ATGGGACTGAGACGACGCC

US 9,649,345 B2
OTGAAAAAATCCCGTGGTATAGGATAGGACCGCTTCTGATTTAACAGCTGATTTGAGGCTA
ACGGCCCAACAGCCAGACGTCAGTGACGCGGCGCCGTTAAGGGACGACATTCCTGAG
ACTGAGACACACCCACGTG

OTU174  (SEQ ID NO.: 27)
GATGGAATCTGTGGCGTGCTTAAACGACAGTGAGCTGAAGAACGCAATTTTTGAAG
TTTCCCCATGAGTTTCTCTTATCTGCTGACAAGGCGAGCTGATGACCGCTTCTG
GGCTATACGGGGATAAACAGTCGGAACCGTCGCTAATTACGGATAAACAGCAGAC
CGTATGGATCTGAGTGAACAAGCTCCGGCGAGTGATGCTGATGCGCTGCTGATTG
TTGCCGCGGTAAGCAGCCCAACACCCCGAGTGACGCGATGCCGCTGAGGATGCG
CACATTGGGAGCTGAGCACAGCGCCAA

OTU181  (SEQ ID NO.: 89)
GATGGAATCTGTGGCGTGCTTAAACGACAGTGAGCTGAAGAACGCAATTTTTGAAG
TTTCCCCATGAGTTTCTCTTATCTGCTGACAAGGCGAGCTGATGACCGCTTCTG
GGCTATACGGGGATAAACAGTCGGAACCGTCGCTAATTACGGATAAACAGCAGAC
CGTATGGATCTGAGTGAACAAGCTCCGGCGAGTGATGCTGATGCGCTGCTGATTG
TTGCCGCGGTAAGCAGCCCAACACCCCGAGTGACGCGATGCCGCTGAGGATGCG
CACATTGGGAGCTGAGCACAGCGCCAA

OTU182  (SEQ ID NO.: 90)
GATGGAATCTGTGGCGTGCTTAAACGACAGTGAGCTGAAGAACGCAATTTTTGAAG
TTTCCCCATGAGTTTCTCTTATCTGCTGACAAGGCGAGCTGATGACCGCTTCTG
GGCTATACGGGGATAAACAGTCGGAACCGTCGCTAATTACGGATAAACAGCAGAC
CGTATGGATCTGAGTGAACAAGCTCCGGCGAGTGATGCTGATGCGCTGCTGATTG
TTGCCGCGGTAAGCAGCCCAACACCCCGAGTGACGCGATGCCGCTGAGGATGCG
CACATTGGGAGCTGAGCACAGCGCCAA

OTU189  (SEQ ID NO.: 91)
GATGGAATCTGTGGCGTGCTTAAACGACAGTGAGCTGAAGAACGCAATTTTTGAAG
TTTCCCCATGAGTTTCTCTTATCTGCTGACAAGGCGAGCTGATGACCGCTTCTG
GGCTATACGGGGATAAACAGTCGGAACCGTCGCTAATTACGGATAAACAGCAGAC
CGTATGGATCTGAGTGAACAAGCTCCGGCGAGTGATGCTGATGCGCTGCTGATTG
TTGCCGCGGTAAGCAGCCCAACACCCCGAGTGACGCGATGCCGCTGAGGATGCG
CACATTGGGAGCTGAGCACAGCGCCAA

OTU195  (SEQ ID NO.: 32)
GATGGAATCTGTGGCGTGCTTAAACGACAGTGAGCTGAAGAACGCAATTTTTGAAG
TTTCCCCATGAGTTTCTCTTATCTGCTGACAAGGCGAGCTGATGACCGCTTCTG
GGCTATACGGGGATAAACAGTCGGAACCGTCGCTAATTACGGATAAACAGCAGAC
CGTATGGATCTGAGTGAACAAGCTCCGGCGAGTGATGCTGATGCGCTGCTGATTG
TTGCCGCGGTAAGCAGCCCAACACCCCGAGTGACGCGATGCCGCTGAGGATGCG
CACATTGGGAGCTGAGCACAGCGCCAA

OTU196  (SEQ ID NO.: 92)
GATGGAATCTGTGGCGTGCTTAAACGACAGTGAGCTGAAGAACGCAATTTTTGAAG
TTTCCCCATGAGTTTCTCTTATCTGCTGACAAGGCGAGCTGATGACCGCTTCTG
GGCTATACGGGGATAAACAGTCGGAACCGTCGCTAATTACGGATAAACAGCAGAC
CGTATGGATCTGAGTGAACAAGCTCCGGCGAGTGATGCTGATGCGCTGCTGATTG
TTGCCGCGGTAAGCAGCCCAACACCCCGAGTGACGCGATGCCGCTGAGGATGCG
ACATGGGAATCTGAGCACAGCGCCAA
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CTTCCAAGGGGACAACAGTGAGTCAGTCTATACCAGTACAGGATAGGAC
ATCCCATGATGCGAGAATTTATCGTTGAAAGAGTGGTCCGTCGCCCCATTATCTAGAG
GCCGCGTTAACGCCCACTTACGGCGATGGTAAAGCCCCACTGAGAAGGTTGACCACGGCCAC
ATTGGGACTGAGGACAGGCCCA

OTU199  (SEQ ID NO.: 93)
GATGAAACCTGGGCGGCGGCTGGCTCTAACATGAAGTCAAGTCGAACGGCGAACCTGGCTTGAGT
GGCCGAACGCGGAAGTATACATATGAACTCTCTAGGACTCGGCGAACGGCGAACCTGGCTTGAGT
CTATAGCAGTACCCGCACTATTACGAGGCTGGAAGGGTAACTCAGGGAA
CGATAGCTAAGACCCGCACTATTACGAGGCTGGAAGGGTAACTCAGGGAA
AACACTGTTAGAAGAATGCTACCTGCGCCATAGGCTTGTTGGGCGGCTAACCGGCCAC
CCAGGGGCAGATGCTAGCGGCCCTGAGGGGCGCCCACTTGGCAGACGGGACAC
GGCCGAG

OTU202  (SEQ ID NO.: 94)
GATGAAACCTGGGCGGCGGCTGGCTCTAACATGAAGTCAAGTCGAACGGCGAACCTGGCTTGAGT
TTCCGATGAGAGTTCAGTATGCTGACGGCGACGGGTAAGACGCTGTCAGTACCTGC
CTTGTACTGGGGGCAAGCAGTATTACATATGAACTCTCTAGGACTCGGCGAACGGCGAACCTGGCTTGAGT
CGATAGCTAAGACCCGCACTATTACGAGGCTGGAAGGGTAACTCAGGGAA
CATGATACGGTGGAAGAAACCTGGGCGGCTGGCTCTAACATGAAGTCAAGTCGAACGGCGAACCTGGCTTGAGT
GGGAGGGGTACGGGCTCCTGAGGCGAGATCAGGGCCCTTGGGACGGGACAC
CATGGGACTGACGACAGGCCCA

OTU204  (SEQ ID NO.: 95)
GATGAAACCTGGGCGGCGGCTGGCTCTAACATGAAGTCAAGTCGAACGGCGAACCTGGCTTGAGT
TTCCGATGAGAGTTCAGTATGCTGACGGCGACGGGTAAGACGCTGTCAGTACCTGC
CTTGTACTGGGGGCAAGCAGTATTACATATGAACTCTCTAGGACTCGGCGAACGGCGAACCTGGCTTGAGT
GGGAGGGGTACGGGCTCCTGAGGCGAGATCAGGGCCCTTGGGACGGGACAC
CATGGGACTGACGACAGGCCCA

OTU211  (SEQ ID NO.: 96)
GATGAAACCTGGGCGGCGGCTGGCTCTAACATGAAGTCAAGTCGAACGGCGAACCTGGCTTGAGT
TTCCGATGAGAGTTCAGTATGCTGACGGCGACGGGTAAGACGCTGTCAGTACCTGC
CTTGTACTGGGGGCAAGCAGTATTACATATGAACTCTCTAGGACTCGGCGAACGGCGAACCTGGCTTGAGT
GGGAGGGGTACGGGCTCCTGAGGCGAGATCAGGGCCCTTGGGACGGGACAC
CATGGGACTGACGACAGGCCCA

OTU214  (SEQ ID NO.: 97)
GATGAAACCTGGGCGGCGGCTGGCTCTAACATGAAGTCAAGTCGAACGGCGAACCTGGCTTGAGT
TTCCGATGAGAGTTCAGTATGCTGACGGCGACGGGTAAGACGCTGTCAGTACCTGC
CTTGTACTGGGGGCAAGCAGTATTACATATGAACTCTCTAGGACTCGGCGAACGGCGAACCTGGCTTGAGT
GGGAGGGGTACGGGCTCCTGAGGCGAGATCAGGGCCCTTGGGACGGGACAC
ACATGGGACTGACGACAGGCCCA
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OTU221 (SEQ ID NO.: 21)
GATGAAACCTGCGGCGTCTTTAACCACAGCAATCGAAGCTGACGCCGAGG
TTCCGTCACAGGATTAGTTACCCAGTTGGCGACGGTGATAGCGCGTAAGGGAGGACCTGC
CTGGAGAGGGGAATTAACACATCCGGAAAACGGGTCTATATCCGACATGCTGACTGGCTCG
CATGGCTCTAGCTCAAAGATTATATTCTCCTGAGATGCGCTCCTGCTATGTTAGCTAGT
AGCGGCTTTACGCCCAACCTAGGGCAACGTAGTAGGGCGACTGAGATATGACGCC
ACATTGGAGACTGAGAAGGCACG

OTU224 (SEQ ID NO.: 98)
GATGAAACCTGCGGCGTCTTTAACCACAGCAATCGAAGCTGACGCCGAGG
TTCCGATCGCGCAAGCTGATGACCTGCGACGGGTGAGTAAACGGCGAAACCTGC
CTGTACCCGGGAGTAAACATGGATATTAGGGTCATATGCAACTAAGGCGCAACGCTCA
CATGAAAGATGGACAAACTCCCGCGTTACAGGGCTGTGCCTCTGATTACCCAGTT
GACAGGGATACAGCCCTACCAAAAGGCAACGATCAGTAGGGCGACGCCACG
CATTGGACCTGAGAACAGGCAACG

OTU225 (SEQ ID NO.: 99)
GATGAAACCTGCGGCGTCTTTAACCACAGCAATCGAAGCTGACGCCGAGG
TTCCGATCGCGCAAGCTGATGACCTGCGACGGGTGAGTAAACGGCGAAACCTGC
CTGTACCCGGGAGTAAACATGGATATTAGGGTCATATGCAACTAAGGCGCAACGCTCA
CATGAAAGATGGACAAACTCCCGCGTTACAGGGCTGTGCCTCTGATTACCCAGTT
GACAGGGATACAGCCCTACCAAAAGGCAACGATCAGTAGGGCGACGCCACG
CATTGGACCTGAGAACAGGCAACG

OTU237 (SEQ ID NO.: 100)
GATGAAACCTGCGGCGTCTTTAACCACAGCAATCGAAGCTGACGCCGAGG
TTCCGATCGCGCAAGCTGATGACCTGCGACGGGTGAGTAAACGGCGAAACCTGC
CTGTACCCGGGAGTAAACATGGATATTAGGGTCATATGCAACTAAGGCGCAACGCTCA
CATGAAAGATGGACAAACTCCCGCGTTACAGGGCTGTGCCTCTGATTACCCAGTT
GACAGGGATACAGCCCTACCAAAAGGCAACGATCAGTAGGGCGACGCCACG
CATTGGACCTGAGAACAGGCAACG

OTU246 (SEQ ID NO.: 101)
GATGAAACCTGCGGCGTCTTTAACCACAGCAATCGAAGCTGACGCCGAGG
TTCCGATCGCGCAAGCTGATGACCTGCGACGGGTGAGTAAACGGCGAAACCTGC
CTGTACCCGGGAGTAAACATGGATATTAGGGTCATATGCAACTAAGGCGCAACGCTCA
CATGAAAGATGGACAAACTCCCGCGTTACAGGGCTGTGCCTCTGATTACCCAGTT
GACAGGGATACAGCCCTACCAAAAGGCAACGATCAGTAGGGCGACGCCACG
CATTGGACCTGAGAACAGGCAACG

OTU253 (SEQ ID NO.: 37)
GATGAAACCTGCGGCGTCTTTAACCACAGCAATCGAAGCTGACGCCGAGG
TTCCGATCGCGCAAGCTGATGACCTGCGACGGGTGAGTAAACGGCGAAACCTGC
CTGTACCCGGGAGTAAACATGGATATTAGGGTCATATGCAACTAAGGCGCAACGCTCA
CATGAAAGATGGACAAACTCCCGCGTTACAGGGCTGTGCCTCTGATTACCCAGTT
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GATGAACGGCGCGGTGCTTAAACAGCAAGTCAAGCAGAAGCTTGCTCAGAGATGCTTGAAGCTTTTATAGGAATT
CTCTGAGTGGAAAAATTTTGCGACTGAGCGCGAGCGAAGCAGGATGTAACGGCGTTGGTAGTAATCC
GCTCTACAGGGGATATACAGCTGAATCGCGAAGAAGGGAACAGGTAACGAGGTAGATGGAAGTGCTCAGATG
CGACCTGGAAAGCAGGCGAAGGACAGGAGGGAACAGGAGGAGGGAACAGGAGGAGGGAACAGGAGGAG
GCGTGGAAAGCAGGCGAAGGACAGGAGGGAACAGGAGGAGGGAACAGGAGGAGGGAACAGGAGGAG
ACATGACGAGCATCGAGACAGCGGCG

(SEQ ID NO.: 38)

GATGAACGGCGCGGTGCTTAAACAGCAAGTCAAGCAGAAGCTTGCTCAGAGATGCTTGAAGCTTTTATAGGAATT
CTCTGAGTGGAAAAATTTTGCGACTGAGCGCGAGCGAAGCAGGATGTAACGGCGTTGGTAGTAATCC
GCTCTACAGGGGATATACAGCTGAATCGCGAAGAAGGGAACAGGTAACGAGGTAGATGGAAGTGCTCAGATG
CGACCTGGAAAGCAGGCGAAGGACAGGAGGGAACAGGAGGAGGGAACAGGAGGAGGGAACAGGAGGAG
ACATGACGAGCATCGAGACAGCGGCG

(SEQ ID NO.: 102)

GATGAACGGCGCGGTGCTTAAACAGCAAGTCAAGCAGAAGCTTGCTCAGAGATGCTTGAAGCTTTTATAGGAATT
CTCTGAGTGGAAAAATTTTGCGACTGAGCGCGAGCGAAGCAGGATGTAACGGCGTTGGTAGTAATCC
GCTCTACAGGGGATATACAGCTGAATCGCGAAGAAGGGAACAGGTAACGAGGTAGATGGAAGTGCTCAGATG
CGACCTGGAAAGCAGGCGAAGGACAGGAGGGAACAGGAGGAGGGAACAGGAGGAGGGAACAGGAGGAG
ACATGACGAGCATCGAGACAGCGGCG

(SEQ ID NO.: 103)

GATGAACGGCGCGGTGCTTAAACAGCAAGTCAAGCAGAAGCTTGCTCAGAGATGCTTGAAGCTTTTATAGGAATT
CTCTGAGTGGAAAAATTTTGCGACTGAGCGCGAGCGAAGCAGGATGTAACGGCGTTGGTAGTAATCC
GCTCTACAGGGGATATACAGCTGAATCGCGAAGAAGGGAACAGGTAACGAGGTAGATGGAAGTGCTCAGATG
CGACCTGGAAAGCAGGCGAAGGACAGGAGGGAACAGGAGGAGGGAACAGGAGGAGGGAACAGGAGGAG
ACATGACGAGCATCGAGACAGCGGCG

(SEQ ID NO.: 104)

GATGAACGGCGCGGTGCTTAAACAGCAAGTCAAGCAGAAGCTTGCTCAGAGATGCTTGAAGCTTTTATAGGAATT
CTCTGAGTGGAAAAATTTTGCGACTGAGCGCGAGCGAAGCAGGATGTAACGGCGTTGGTAGTAATCC
GCTCTACAGGGGATATACAGCTGAATCGCGAAGAAGGGAACAGGTAACGAGGTAGATGGAAGTGCTCAGATG
CGACCTGGAAAGCAGGCGAAGGACAGGAGGGAACAGGAGGAGGGAACAGGAGGAGGGAACAGGAGGAG
ACATGACGAGCATCGAGACAGCGGCG

(SEQ ID NO.: 105)

GATGAACGGCGCGGTGCTTAAACAGCAAGTCAAGCAGAAGCTTGCTCAGAGATGCTTGAAGCTTTTATAGGAATT
CTCTGAGTGGAAAAATTTTGCGACTGAGCGCGAGCGAAGCAGGATGTAACGGCGTTGGTAGTAATCC
GCTCTACAGGGGATATACAGCTGAATCGCGAAGAAGGGAACAGGTAACGAGGTAGATGGAAGTGCTCAGATG
CGACCTGGAAAGCAGGCGAAGGACAGGAGGGAACAGGAGGAGGGAACAGGAGGAGGGAACAGGAGGAG
ACATGACGAGCATCGAGACAGCGGCG

(SEQ ID NO.: 106)

GATGAACGGCGCGGTGCTTAAACAGCAAGTCAAGCAGAAGCTTGCTCAGAGATGCTTGAAGCTTTTATAGGAATT
CTCTGAGTGGAAAAATTTTGCGACTGAGCGCGAGCGAAGCAGGATGTAACGGCGTTGGTAGTAATCC
GCTCTACAGGGGATATACAGCTGAATCGCGAAGAAGGGAACAGGTAACGAGGTAGATGGAAGTGCTCAGATG
CGACCTGGAAAGCAGGCGAAGGACAGGAGGGAACAGGAGGAGGGAACAGGAGGAGGGAACAGGAGGAG
ACATGACGAGCATCGAGACAGCGGCG

(SEQ ID NO.: 107)

GATGAACGGCGCGGTGCTTAAACAGCAAGTCAAGCAGAAGCTTGCTCAGAGATGCTTGAAGCTTTTATAGGAATT
CTCTGAGTGGAAAAATTTTGCGACTGAGCGCGAGCGAAGCAGGATGTAACGGCGTTGGTAGTAATCC
GCTCTACAGGGGATATACAGCTGAATCGCGAAGAAGGGAACAGGTAACGAGGTAGATGGAAGTGCTCAGATG
CGACCTGGAAAGCAGGCGAAGGACAGGAGGGAACAGGAGGAGGGAACAGGAGGAGGGAACAGGAGGAG
ACATGACGAGCATCGAGACAGCGGCG

(SEQ ID NO.: 108)

GATGAACGGCGCGGTGCTTAAACAGCAAGTCAAGCAGAAGCTTGCTCAGAGATGCTTGAAGCTTTTATAGGAATT
CTCTGAGTGGAAAAATTTTGCGACTGAGCGCGAGCGAAGCAGGATGTAACGGCGTTGGTAGTAATCC
GCTCTACAGGGGATATACAGCTGAATCGCGAAGAAGGGAACAGGTAACGAGGTAGATGGAAGTGCTCAGATG
CGACCTGGAAAGCAGGCGAAGGACAGGAGGGAACAGGAGGAGGGAACAGGAGGAGGGAACAGGAGGAG
ACATGACGAGCATCGAGACAGCGGCG

(SEQ ID NO.: 109)

GATGAACGGCGCGGTGCTTAAACAGCAAGTCAAGCAGAAGCTTGCTCAGAGATGCTTGAAGCTTTTATAGGAATT
CTCTGAGTGGAAAAATTTTGCGACTGAGCGCGAGCGAAGCAGGATGTAACGGCGTTGGTAGTAATCC
GCTCTACAGGGGATATACAGCTGAATCGCGAAGAAGGGAACAGGTAACGAGGTAGATGGAAGTGCTCAGATG
CGACCTGGAAAGCAGGCGAAGGACAGGAGGGAACAGGAGGAGGGAACAGGAGGAGGGAACAGGAGGAG
ACATGACGAGCATCGAGACAGCGGCG

(SEQ ID NO.: 110)

GATGAACGGCGCGGTGCTTAAACAGCAAGTCAAGCAGAAGCTTGCTCAGAGATGCTTGAAGCTTTTATAGGAATT
CTCTGAGTGGAAAAATTTTGCGACTGAGCGCGAGCGAAGCAGGATGTAACGGCGTTGGTAGTAATCC
GCTCTACAGGGGATATACAGCTGAATCGCGAAGAAGGGAACAGGTAACGAGGTAGATGGAAGTGCTCAGATG
CGACCTGGAAAGCAGGCGAAGGACAGGAGGGAACAGGAGGAGGGAACAGGAGGAGGGAACAGGAGGAG
ACATGACGAGCATCGAGACAGCGGCG

(SEQ ID NO.: 111)

GATGAACGGCGCGGTGCTTAAACAGCAAGTCAAGCAGAAGCTTGCTCAGAGATGCTTGAAGCTTTTATAGGAATT
CTCTGAGTGGAAAAATTTTGCGACTGAGCGCGAGCGAAGCAGGATGTAACGGCGTTGGTAGTAATCC
GCTCTACAGGGGATATACAGCTGAATCGCGAAGAAGGGAACAGGTAACGAGGTAGATGGAAGTGCTCAGATG
CGACCTGGAAAGCAGGCGAAGGACAGGAGGGAACAGGAGGAGGGAACAGGAGGAGGGAACAGGAGGAG
ACATGACGAGCATCGAGACAGCGGCG
ATGGTTCTGTGTGAAAACACTCCGGTGTACAGATGAGTTCCCGCTGCTGATTAGCCAGTT
GCCCAGGGCTACCCCTACAAAAGCGAGATCTGAGAAGCAGCCCTGAGAAGGCGGACCA
CATGGGACCTGAGACACGGCACA

(SEQ ID NO.: 107)
GATGAAACCTGGCCGGCTGCCCTAATACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
CTTTAGAAGATGCGAGCAATTGAGAAGCTTACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
ATACGATTGGAAACACTGCTAATACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
TAAAGGTGGATACACACAGCGATGAGGCTTAAATCGCACTTTAGGTTAGGCTAGGA
ACGGGCCCACCTTGGGGCAATCGACAGCCCTGAGAAGGCGGACCA

(SEQ ID NO.: 39)
GATGAAACCTGGCCGGCTGCCCTAATACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
CTTTAGAAGATGCGAGCAATTGAGAAGCTTACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
ATACGATTGGAAACACTGCTAATACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
TAAAGGTGGATACACACAGCGATGAGGCTTAAATCGCACTTTAGGTTAGGCTAGGA
ACGGGCCCACCTTGGGGCAATCGACAGCCCTGAGAAGGCGGACCA

(SEQ ID NO.: 108)
GATGAAACCTGGCCGGCTGCCCTAATACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
CTTTAGAAGATGCGAGCAATTGAGAAGCTTACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
ATACGATTGGAAACACTGCTAATACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
TAAAGGTGGATACACACAGCGATGAGGCTTAAATCGCACTTTAGGTTAGGCTAGGA
ACGGGCCCACCTTGGGGCAATCGACAGCCCTGAGAAGGCGGACCA

(SEQ ID NO.: 109)
GACGAACCTGGCCGGCTGCCCTAATACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
CTTTAGAAGATGCGAGCAATTGAGAAGCTTACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
ATACGATTGGAAACACTGCTAATACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
TAAAGGTGGATACACACAGCGATGAGGCTTAAATCGCACTTTAGGTTAGGCTAGGA
ACGGGCCCACCTTGGGGCAATCGACAGCCCTGAGAAGGCGGACCA

(SEQ ID NO.: 40)
GATGAAACCTGGCCGGCTGCCCTAATACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
CTTTAGAAGATGCGAGCAATTGAGAAGCTTACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
ATACGATTGGAAACACTGCTAATACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
TAAAGGTGGATACACACAGCGATGAGGCTTAAATCGCACTTTAGGTTAGGCTAGGA
ACGGGCCCACCTTGGGGCAATCGACAGCCCTGAGAAGGCGGACCA

(SEQ ID NO.: 40)
GATGAAACCTGGCCGGCTGCCCTAATACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
CTTTAGAAGATGCGAGCAATTGAGAAGCTTACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
ATACGATTGGAAACACTGCTAATACATGCAAATTGTCAGACGCTTTGCTAAAGGAGCTGCTT
TAAAGGTGGATACACACAGCGATGAGGCTTAAATCGCACTTTAGGTTAGGCTAGGA
ACGGGCCCACCTTGGGGCAATCGACAGCCCTGAGAAGGCGGACCA

ATTGGGACCTGAGACACGGCACA
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TGGCGGGTGACGCGGACCAAGACGACGTACTGCGAGCTCAAGCTGAGTACGACCGCAC
GATTGGACCTGAGAACAAGGCCCT

OTU312

{SEQ ID NO.: 114}

GATGAAACCTGCGCGGTGCTTTACCTACATGGCAACTGCGAGCTGAGTACGACCGCAC
GATTGGACCTGAGAACAAGGCCCT

OTU313

{SEQ ID NO.: 115}

GATGAAACCTGCGCGGTGCTTTACCTACATGGCAACTGCGAGCTGAGTACGACCGCAC
GATTGGACCTGAGAACAAGGCCCT

OTU314

{SEQ ID NO.: 31}

GATGAAACCTGCGCGGTGCTTTACCTACATGGCAACTGCGAGCTGAGTACGACCGCAC
GATTGGACCTGAGAACAAGGCCCT

OTU319

{SEQ ID NO.: 116}

GATGAAACCTGCGCGGTGCTTTACCTACATGGCAACTGCGAGCTGAGTACGACCGCAC
GATTGGACCTGAGAACAAGGCCCT

OTU326

{SEQ ID NO.: 117}

GATGAAACCTGCGCGGTGCTTTACCTACATGGCAACTGCGAGCTGAGTACGACCGCAC
GATTGGACCTGAGAACAAGGCCCT

OTU328

{SEQ ID NO.: 118}

GATGAAACCTGCGCGGTGCTTTACCTACATGGCAACTGCGAGCTGAGTACGACCGCAC
GATTGGACCTGAGAACAAGGCCCT
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CATGGTCTCTGACTGCCAAGATTTATTATCGTCGAGATAAGCCCTCGCTGATTGCTTGAGTTCTTTT
TGCCGCGGTATGCTGCCCATACACACCGAGTCGATATCGCGGACTGAGGCTTCCGGCGGC
ACATTTGCTGAGACACGGCCCA

 Otto333 (SEQ ID NO.: 119)
GATGAAAGCTGGCGCCGCTGCTCTAACCTACTGCAAATGCTGACCAACTCCCCTATAGAGGTT
TTCGAGATGAAATATGGGAACTGATGCTGACGGCCGCCGGTGGATGAAACTCCCTTTG
CTCAATCGGCTACATTGATGAAGTGGCCTGAGAAGGTCCAGGCGCAATCTGC
CATGGTACGGTGGAAGAAACCCGAGGTCGGGGAATGATGGTACCCCTCCCTACCTTGC
GCGGGGGTGACACCAACACGGCGAATCGAGTCCGGGCTTACCCGAAGGTGAGCCGGCCA
CATTGGGACACTGAGACACGGCCCA

 Otto334 (SEQ ID NO.: 41)
GATGAAAGCTGGCGCCGCTGCTCTAACCTACTGCAAATGCTGACCAACTCCCCTATAGAGGTT
TTCGAGATGAAATATGGGAACTGATGCTGACGGCCGCCGGTGGATGAAACTCCCTTTG
CTCAATCGGCTACATTGATGAAGTGGCCTGAGAAGGTCCAGGCGCAATCTGC
CATGGTACGGTGGAAGAAACCCGAGGTCGGGGAATGATGGTACCCCTCCCTACCTTGC
GCGGGGGTGACACCAACACGGCGAATCGAGTCCGGGCTTACCCGAAGGTGAGCCGGCCA
CATTGGGACACTGAGACACGGCCCA

 Otto337 (SEQ ID NO.: 30)
GCGGAGGCTGCTGGCGCCGCTGCTCTAACCTACTGCAAATGCTGACCAACTCCCCTATAGAGGTT
TTCGAGATGAAATATGGGAACTGATGCTGACGGCCGCCGGTGGATGAAACTCCCTTTG
CTCAATCGGCTACATTGATGAAGTGGCCTGAGAAGGTCCAGGCGCAATCTGC
CATGGTACGGTGGAAGAAACCCGAGGTCGGGGAATGATGGTACCCCTCCCTACCTTGC
GCGGGGGTGACACCAACACGGCGAATCGAGTCCGGGCTTACCCGAAGGTGAGCCGGCCA
CATTGGGACACTGAGACACGGCCCA

 Otto339 (SEQ ID NO.: 120)
GATGAAAGCTGGCGCCGCTGCTCTAACCTACTGCAAATGCTGACCAACTCCCCTATAGAGGTT
TTCGAGATGAAATATGGGAACTGATGCTGACGGCCGCCGGTGGATGAAACTCCCTTTG
CTCAATCGGCTACATTGATGAAGTGGCCTGAGAAGGTCCAGGCGCAATCTGC
CATGGTACGGTGGAAGAAACCCGAGGTCGGGGAATGATGGTACCCCTCCCTACCTTGC
GCGGGGGTGACACCAACACGGCGAATCGAGTCCGGGCTTACCCGAAGGTGAGCCGGCCA
CATTGGGACACTGAGACACGGCCCA

 Otto340 (SEQ ID NO.: 121)
GCGGAGGCTGCTGGCGCCGCTGCTCTAACCTACTGCAAATGCTGACCAACTCCCCTATAGAGGTT
TTCGAGATGAAATATGGGAACTGATGCTGACGGCCGCCGGTGGATGAAACTCCCTTTG
CTCAATCGGCTACATTGATGAAGTGGCCTGAGAAGGTCCAGGCGCAATCTGC
CATGGTACGGTGGAAGAAACCCGAGGTCGGGGAATGATGGTACCCCTCCCTACCTTGC
GCGGGGGTGACACCAACACGGCGAATCGAGTCCGGGCTTACCCGAAGGTGAGCCGGCCA
CATTGGGACACTGAGACACGGCCCA
<160> NUMBER OF SEQ ID NOS: 123

<210> SEQ ID NO 1
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, PoxP3

<400> SEQUENCE: 1
ggcaataagtt ctcggcagag ttt
ggcaataagtt ctcggcagag ttt
<220> FEATURE:
<220> OTHER INFORMATION: Primer, F0xp3
<400> SEQUENCE: 2

gggtgcacta ttgtagttact tg
  22

<210> SEQ ID NO 3
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, CTLA4
<400> SEQUENCE: 3

cctttttag cctggtcacc tct
  23

<210> SEQ ID NO 4
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, CTLA4
<400> SEQUENCE: 4

gggtcacctg tateggtcag g
  21

<210> SEQ ID NO 5
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, GITR
<400> SEQUENCE: 5

tcagtgcaag acgctgaagc a
  21

<210> SEQ ID NO 6
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, GITR
<400> SEQUENCE: 6

acacgggaag ccaacaaca
  19

<210> SEQ ID NO 7
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, IL-10
<400> SEQUENCE: 7

gattttatac agctgcgaag ccaaggt
  27

<210> SEQ ID NO 8
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, IL-10
<400> SEQUENCE: 8

cctctatgca gtgtgatgaag atgtcag
  27
<210> SEQ ID NO 9
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, GAPDH

<400> SEQUENCE: 9
cctcgtcccg tagacaaaat g 21

<210> SEQ ID NO 10
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, GAPDH

<400> SEQUENCE: 10
ttcctcacttt gccactgcaas 20

<210> SEQ ID NO 11
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, Mmp2

<400> SEQUENCE: 11
ggacattgtc tttgatggaas 20

<210> SEQ ID NO 12
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, Mmp2

<400> SEQUENCE: 12
cctgtaacgt ggtgtaactg 20

<210> SEQ ID NO 13
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, Mmp9

<400> SEQUENCE: 13
ttcttgacag tcaaatgtgg 20

<210> SEQ ID NO 14
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, Mmp9

<400> SEQUENCE: 14
gctgaaacag agagccttc 19

<210> SEQ ID NO 15
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, Mmp13
<400> SEQUENCE: 15
aggtctggat caacctcaagg

<210> SEQ ID NO: 16
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, Mmp13

<400> SEQUENCE: 16
tgccctggac cataaaaaga

<210> SEQ ID NO: 17
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, Idol

<400> SEQUENCE: 17
agaggatgcc tgaacttttgtg

<210> SEQ ID NO: 18
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, Idol

<400> SEQUENCE: 18
atatagcagag cttctcggca

<210> SEQ ID NO: 19
<211> LENGTH: 302
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU136

<400> SEQUENCE: 19
gatgaeagct gcggcgcgtgc ctataacag cagtcgaagac gcgagcactt ggtctcaggt
  gagcgaaggg tgaagtaaac ataagtaacc tgccttttag agggggataaa ctttgaaaa
  120
cgtacagcta gcgacgtcag gtaagatcgc gcacgattaa gttattaaa agtcggcaag
  cactggtgta gatgagcact tgggcgcatt acgtattgtg tsgagtaacgc gtoaccaag
  240
gcgcctgc gtaacggcacc tgagagggtg accgggccaca cttggactga gacacggccc
  300
ag
  302

<210> SEQ ID NO: 20
<211> LENGTH: 303
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU46

<400> SEQUENCE: 20
gatgaeagct gcggcgcgtgc ctataacag cagtcgaagac gcgagcactt ggtctcaggt
  gagcgaaggg tgaagtaaac ataagtaacc tgccttttag agggggataaa ctttgaaaa
  120
cgtacagcta gcgacgtcag gtaagatcgc gcacgattaa gttattaaa agtcggcaag
  cactggtgta gatgagcact tgggcgcatt acgtattgtg tsgagtaacgc gtoaccaag
  240
gcgcctgc gtaacggcacc tgagagggtg accgggccaca cttggactga gacacggccc
  300
ag
  302
-continued

gcactgtgtag aggatgagct tattggccct tagctgggtg gcgggggttac gcgccaccas 240
ggcgcagcag ttggcgcacc cctgagaggt gacgagccac actgggagct agacacgcgc 300
cag 303

<210> SEQ ID NO 21
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU221

<400> SEQUENCE: 21

gatgaaacgt ggcggcggtgc ttaacactg caagtgcaca ggcggctgca tgcagcagga 60
tccgcaacc ggaattggt ctaccgtggc gggctgtaa gcgaagcgtg aggaacctgc 120
cctggagag ggagaaaccc ttgagaagag ttgctaatac cgcagatgag agttggggtg 180
cactgtcctg acctgcaaaag atttatccct cttgagatggc ctctcgcttg attagcgtg 240
agcgcggtgt aagccgccaa ctagcagcag ctagcagcag gacgagcagc ttgacgagcc 300
acatgagac ctagacaccc ccca 324

<210> SEQ ID NO 22
<211> LENGTH: 325
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU9

<400> SEQUENCE: 22

gatgaaacgt ggcgggggtg ctttaacact gcaagtcgag cgaagcggtt tgcagtgga 60
ctttgggttg aattgaact gcattacggt cggaggggtg agtaacgcgt gggtaacctg 120
cottacgctg ggcggcaaca gcggaaactg gctgtgataa cggcataaag gcaacgggcc 180
gctgtcgctg ttgccaagaa cttcggtgtgt gtaaatagg cccgcctctg agttagcgtg 240
tgggtgggta aacgcgccaa aagcgagcag tccagtaccc actgacagag gttgacggcc 300
acatgagac ctagacaccc ccca 325

<210> SEQ ID NO 23
<211> LENGTH: 126
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU296

<400> SEQUENCE: 23

gatgaaacgt ggcggggctg ctttaacactg caagtgcaca ggaagcgcctg gcgccgactt 60
cctgcaacc gcggagcttg gcgaaggggt gcaagcgcgt gaggcaacct 120
gccctgacact gggcggataac gcggagtaaat ggtctgtaaat accgcataag accgaacgc 180
cgcagttggc gcggccggcc gcggccggt gtc峙agtag gcggcctctg gattaggtag 240
ttgccgctg aacgcgcccaa caagcgcagc atcaagccgc gacgagcagc ggcacgggcc 300
ccacatggga cttgagacac cgc 325

<210> SEQ ID NO 24
<211> LENGTH: 223
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU21
<400> SEQUENCE: 24
    gatgaacgct ggcggcgtgc ttaacactag caagtgaagc gaagctgtaa gacagatccc 60
tcgggtgtga agcttttcttg gctggagcgg ggaagggtga gtaagcgtg ggtteaactgc 120
cctatacaggg ggtataacag ttagaatagtg tgcattaaac gcaataacg cacagacacg 180
catgttctgg tggtaaagag tccgcttggta tggatgtgaac cggaccgctga ttagctagtct 240
ggagggttaa cggccacaca agggaactgg cagtagccgg cctgagaggg tgaacgagggca 300
cattgggacct gacacacggtc cca 323
</400>

<210> SEQ ID NO 25
<211> LENGTH: 322
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE: 
<223> OTHER INFORMATION: OTU166

<400> SEQUENCE: 25
    gatgaacgct ggcggcgtgc ttaacactag caagtgaagc gaagctgtaa aatgaagtgt 60
tcgggtgtga agcttttcttg gctggagcgg ggaagggtga gtaagcgtg ggtteaactgc 120
cacactgggg taaaagcttg gtaaatagagc cataagccca cagtaagcag cagtaagcag 180
ttgattgggt gtaaaaactc ggggtgttgag gaatggaatc ggtcattgatt aggcaagttg 240
cggcttgaac gggccaccaaa ggcgagacgta gtagcgccgg cgtagaggggt tagggcccaag 300
    atggggactgg agacacagccca 322
</400>

<210> SEQ ID NO 26
<211> LENGTH: 313
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE: 
<223> OTHER INFORMATION: OTU173

<400> SEQUENCE: 26
    gatgaacgct ggcggcgtgc ttaacactag caagtgaagc gaagctgtaa tagttgctgt 60
tcgggtgtga agcttttcttg gctggagcgg ggaagggtga gtaagcgtg ggtteaactgc 120
    aacgctttga aacgcagctgcc taaatcaggt aagggcagag gagggcatctc tgtcttctta 180
    aagttgggtg acaaagccaa aagatgggattc tcggctttgac tagttgattgg tagttgattgg 240
    gggcccaacaa ggcagtagtat ctagccggtc ctagggaggg gacagcccaaa atggggactgg 300
        agacacagccca 313
</400>

<210> SEQ ID NO 27
<211> LENGTH: 325
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE: 
<223> OTHER INFORMATION: OTU174

<400> SEQUENCE: 27
    gatgaacgct ggcggcgtgc ttaacactag caagtgaagc gaagctgtaa tggagagaggt 60
    ttcgggtgtga aatctcttga tgaatcagtag ggacgaggg gtaagcgcgg tggagaacct 120
    ggcctataca gggcttgataag cagctgaataac aacgcataag cgcacagataa 180
    cgctgattcc ggttgtgtaaa gctttgctcag tcagtgctgtg tggagacagttgagttgaggg 240
    ttggcttgggt gacggccacca cagggcagac tgttcgagag agtggacggtg 300
</400>
cacatggga ctgagacag gcceca

<210> SEQ ID NO: 28
<211> LENGTH: 323
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU14

<400> SEQUENCE: 28

gatgaaacct gcggcgctgc ttaacacatg caagtctagc gaagctgttt caatgaagtt 60
tccggatgga atagaaattg acttagggcg gcaaggtgga gtaacgcttg gtaaacctgc 120
cactacgct ggataacag ttgactaatg ccgaataacgc cacagggcg 180
cagtctctgg tgtgaaacc ctcggagtgg ttaagttgag cccgctcctg ctaagttggt 240
gcgaggtcag gcgcaccaaa gcgcacgatc agtagcagac ctgagagggc gcagcgcac 300
atgcgagcag cacacagccc 323

<210> SEQ ID NO: 29
<211> LENGTH: 323
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU55

<400> SEQUENCE: 29

gatgaaacct gcggcgctgc ttaacacatg caagtctagc gaagctgttt gcaggaagtt 60
tccggatgga atcggttatt ctagtgctcgc gcggcttgag gcacagtgtg gcacccgctc 120
cagtctcatgc ggtatatc tgaaaaatcg gc tgtctaggt gcacacgccgc 180
atgcgacagt gtcacactgc acgcgaggtgt cagagggctgc cgcgcttatt ttgcctggt 240
gcgaggtcag gcgcaccaaa gcgcacgatc agtagcagac ctgagagggc tcagcgcac 300
catgcgagcag cacacagccc 323

<210> SEQ ID NO: 30
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU33

<400> SEQUENCE: 30

gacgaaaccel gcggcggacgc ttaacacatg caagtctagc gcagatattcg ttggaagttg 60
tccggatgga tgaattgtaag ctagtgctcgc gcggcttgag gcacagtgtg gcacccgctc 120
tttcagaggg gtaacagccgc cagacagcgc tgaatattcg gcagtgttg gcgaggggc 180
atgccccctgc gaaacagaga gcaatcctgag gcacagtgctg ctcgcctcctt taccgctg 240
tgcgaggtcag gcgcaccaaa gcgcacgatc agtagcagac ctgagagggc ttggaagtt 300
acatgagcag tcacacagccc 325

<210> SEQ ID NO: 31
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU314

<400> SEQUENCE: 31

gatgaaacct gcggcgctgc ttaacacatg caagtctagc gaagctgttt ttcgcaattc 60
ttcggagga gggacagtgc actgacgccc ggaaggggtga gtaacgctg ggcaacctgc 120
tctatacagg gggataacgg ttagaatga gtcgtaatacg cggctaaaaac ccacgcacccg 180
catggtgtaa tgtgaacaga aagcggggta gtaacgctgg ttaggtatgttcttggtaacacc 240
ggtgagggtaa ggccgctacca aacgagcagc aagcggggta gtaacgctgg gtaacgctgc 300
cattggggac tgaacacggccc 324

<210> SEQ ID NO 32
<211> LENGTH: 325
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU195

<400> SEQUENCE: 32

gtatgatacgct gggaggctgct ctaacactagt caagtcgaac gaagcatttt agatgaagtt 60
ttcggatgga ttagaatgatc actgactggc ggaaggggta gtaacagctt gtaacactgc 120
tcctactgac gggacgcaac gattgaataa cgtcagctgc gacaggttgaca 180
goatggtacg ttaggaacaa ttcgggggtgttg gtagatgga ttcgggtgtgg attacgaagtt 240
tggccgggttaa gggccacgcc aagcgcagc tcagcagcgc aacgagcagc gtagagagttgtgaggtggggcc 300
acattgggac tgaacacggccc 325

<210> SEQ ID NO 33
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU196

<400> SEQUENCE: 33

gtatgatacgct gggaggctgct ctaacactagt caagtcgaac gaagcagctt aacgagagtt 60
ttcggatgga aagtgaggtgc actgactgtgc ggaaggggta gtaacgctgg gtaacactgc 120
tcttgctggg ggcgcgaaca gattgaaataa cggcataaag gcacgtac 180
goatgataca ttaggaacaa ttcgggggtgg gtagatgga aacagctgg acggcgtgggt ttagctagtt 240
tggcaagcta cagcgggtac ccagcgagca tcagcagcgc aacgagcagc gtagagagttgtgaggtggggcc 300
acattgggac tgaacacggccc 324

<210> SEQ ID NO 34
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU197

<400> SEQUENCE: 34

gtatgatacgct gggaggctgct ttaacactagt caagtcgaac gaaagccttt tattagattt 60
ttcgggattgc aaagtcttttg gactgacgag cggcaggggt agtatgcggt ggttaacctgg 120
ttcctactgag gggataacagc gattgaaattta cggcataag gcacgtacct 180
goatgataca ggggaaacaa ttcgggggtgttg gtagatgga aacagctgg acggcgtgggt ttagctagtt 240
tggcaagcta cagcgggtac ccagcgagca tcagcagcgc aacgagcagc gtagagagttgtgaggtggggcc 300
acattgggac tgaacacggccc 324

<210> SEQ ID NO 35
cttcggattg aaagtttttg cgactgagcg ggcgcgggct gagtaacgct cgggttaacct 120
gcctcataca gggggataac agttgaaat gcgtgctaat accgcataag accacggctac 180
cgcagttgac atgcgagaaact cgccgtgctgg ttagatagtg accgctgttc gattagctag 240
ttggtaaag ttgacggtcct cgaagccgac atccagtaac gcagctggag ggtgacgctg 300
aacatgggac ctagacacgct gcccacaa 326

<210> SEQ ID NO 39
<211> LENGTH: 327
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE: 
<223> OTHER INFORMATION: OTU281

<400> SEQUENCE: 39

gatgaaagct ggaggggtgct ctaacacatg caagtcgaaac gaagcgcttc cgcctgattt 60
tttgcggagg tgaagggcgct cgcaagctgg tgtgaagccag ctgtgggccac 120
cgccttgcaa cttgggggat ataagccgta atacggatca agacgcggaag 180
ggctgcgtgc gctgctggcct aagcagcggct ggtgcaaacatt ggcccgctgt ctgtattgct 240
agttggggtt gtaaccgggac acctagcgcga ctggacagtag ccaacaggcag aggggtacgc 300
gccacattgg gacctacagca gggccaacaa 327

<210> SEQ ID NO 40
<211> LENGTH: 322
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE: 
<223> OTHER INFORMATION: OTU288

<400> SEQUENCE: 40

gatgaaagct ggaggggtgct ctaacacatg caagtcgaaac ggaggtagtg acaggaagtt 60
tttgcggagg tgaagggcgct cgcaagctgg tgtgaagccag ctgtgggccac 120
cgccttgcaa cttgggggat ataagccgta atacggatca agacgcggaag 180
ttagaagcttg gtgaacatgcc cccagctggttc cagctctgctg ttagcagttg 240
aggggtttac gcctccacac aagcagcgcag atgaacggcctc ctggacaggtg ggagccccac 300
atgggagctc gaccaacgccc ca 322

<210> SEQ ID NO 41
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE: 
<223> OTHER INFORMATION: OTU334

<400> SEQUENCE: 41

gatgaaagct ggaggggtgct ctaacacatg caagtcgaaac gaagcatccc ataggaggttt 60
tttgcggagg tgaagggcgct cgcaagctgg tgtgaagccag ctgtgggccac 120
cgccttgcaa cttgggggat ataagccgta atacggatca agacgcggaag 180
catgtccgctc tggccacatac cccagctggttc cagctctgctg ttagcagttg 240
aggggtttac gcctccacac aagcagcgcag atgaacggcctc ctggacaggtg ggagccccac 300
atgggagctc gaccaacgccc ca 324

<210> SEQ ID NO 42
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU359

<400> SEQUENCE: 42

gatgaagtct gcggccgtgct ttaacacactg caagtcgagc gaaattatat cggaggaagt 
60
tccgatgtaa atcgctataas ttaagttggc gcggcggcg cAAaagcggc tgaacccggc  
120
cgtgactcgg ggtgtaactc tagaataggg tggcatatgcc gctgacagcgc acagctgcc  
180
atggaacacg tggaaacactc tccgttggtta cagatggtgcc ggggcgctgta ttagcaggtt 
240
ggcgaggtt aaagctacca aagcagaagt cagtagcggc ccctggagaggt gcgaacgcgc  
300
acatggcggc tggacaacgc ccgg 
324

<210> SEQ ID NO: 43
<211> LENGTH: 321
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU362

<400> SEQUENCE: 43

gatgaagtct gcggccgtgct ttaacacactg caagtcgagc gaaattatat cggaggaagt 
60
tccgatgtaa atcgctataas ttaagttggc gcggcggcg cAAaagcggc tgaacccggc  
120
cgtgactcgg ggtgtaactc tagaataggg tggcatatgcc gctgacagcgc acagctgcc  
180
atggaacacg tggaaacactc tccgttggtta cagatggtgcc ggggcgctgta ttagcaggtt 
240
ggcgaggtt aaagctacca aagcagaagt cagtagcggc ccctggagaggt gcgaacgcgc  
300
tgggaactcgc acgagccccc a  
321

<210> SEQ ID NO: 44
<211> LENGTH: 323
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU367

<400> SEQUENCE: 44

gatgaagtct gcggccgtgct ttaacacactg caagtcgagc gaaattatat cggaggaagt 
60
tccgatgtaa atcgctataas ttaagttggc gcggcggcg cAAaagcggc tgaacccggc  
120
cgtgactcgg ggtgtaactc tagaataggg tggcatatgcc gctgacagcgc acagctgcc  
180
atggaacacg tggaaacactc tccgttggtta cagatggtgcc ggggcgctgta ttagcaggtt 
240
ggcgaggtt aaagctacca aagcagaagt cagtagcggc ccctggagaggt gcgaacgcgc  
300
atggaactcgc acgagccccc caa 
323

<210> SEQ ID NO: 45
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, total bacteria

<400> SEQUENCE: 45

gtgaattag ccctgg 
17

<210> SEQ ID NO: 46
<211> LENGTH: 22

gtgaattag ccctgg 
22
<210> SEQ ID NO 47
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, Clostridium cluster XIVa (Clostridium cocooides subgroup)

<400> SEQUENCE: 47

aatgaggt actgacctaa

<210> SEQ ID NO 48
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, Clostridium cluster XIVa (Clostridium cocooides subgroup)

<400> SEQUENCE: 48

cattgagttt cattcttgcg aa

<210> SEQ ID NO 49
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, Clostridium cluster IV (Clostridium leptum)

<400> SEQUENCE: 49

ctttcgtgc cgnagtta

<210> SEQ ID NO 50
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, Clostridium cluster IV (Clostridium leptum)

<400> SEQUENCE: 50

gataaaacc acatactca ctgctt

<210> SEQ ID NO 51
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, Bacteroides

<400> SEQUENCE: 51

gagaggaagg tcccccac

<210> SEQ ID NO 52
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
OTHER INFORMATION: Primer, Bacteroides

SEQUENCE: 52

cgctacctgg tgtgttcag

SEQ ID NO: 53
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: Primer, Bifidobacterium

SEQUENCE: 53
cggtgagta atgcgtgacc

SEQ ID NO: 54
LENGTH: 18
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: Primer, Bifidobacterium

SEQUENCE: 54
tgatagggac gcacccca

SEQ ID NO: 55
LENGTH: 50
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: Modified primer 6F
FEATURE:
NAME/KEY: misc_feature
LOCATION: (30) to (31)
OTHER INFORMATION: barcode

SEQUENCE: 55
cctatctctc cctggtgtgc tcggactcag agrggttgtat ymtggctcag

SEQ ID NO: 56
LENGTH: 49
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: Modified Primer 338R

SEQUENCE: 56
cctatctctc gtggtccttg gcagctcctc tgctgctcc ctaggagt

SEQ ID NO: 57
LENGTH: 19
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: KOD FX (TOYOBO), 16S rRNA gene-specific primer, 8F

SEQUENCE: 57
gagttttgtc mtggtctcag

SEQ ID NO: 58
LENGTH: 16
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: KOD FX (TOYOBO), 16S rRNA gene-specific primer, 519R
attacgcgg ckgctg

<210> SEQ ID NO 59
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: KOD FX (TOYOBO), 16S rRNA gene-specific primer, 1513R

<400> SEQUENCE: 59
acggctacct tgttacgact t

<210> SEQ ID NO 60
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 16S rRNA gene-specific primer

<400> SEQUENCE: 60
agatgttgtat cmtggtctcg

<210> SEQ ID NO 61
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 16S rRNA gene-specific

<400> SEQUENCE: 61
attacgcgg ckgctg

<210> SEQ ID NO 62
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, total bacteria

<400> SEQUENCE: 62
ggtgastacg ttcgcgg

<210> SEQ ID NO 63
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, total bacteria

<400> SEQUENCE: 63
tacggtacct ttgtaagact t

<210> SEQ ID NO 64
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer, Clostridium cluster XIVa (Clostridium cocoides subgroup)

<400> SEQUENCE: 64
aatgacgttt acctgactaa

20
ctttgagttt cattcttgcg a

7

22

gcacaacag tggagt

6

16

18

cgtactttg gcgggtcag

5

19

19

cgtaactcgt tttgacca

5

18

10

gatacgacgt gcggcgtgc ttaacacatg caagtcagc gaagcactaa gaacgatttc
gttttgattga agcttttttg actgagcgcc gcagcgtgac taacgcgttg gtaacctgcc
tcaacaggg gataaacagtg taagtaatgc tcgtaacct gcataacgoc acagacccg
atgctctggt gtagaaaaacct cccgtgttat gagaagcctgag tgcggagttg 240
gaggttaaac gcocacgagc ggcgaagact aagagtgcggt cttgaggggt tgaagggcacc 300
atgagcctg agacacggcc cag 323

<210> SEQ ID NO: 71
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU15

<400> SEQUENCE: 71

gatgaaacgt gcggcctgtgc tttaacatcg caagctgagc gaaacatattt gagaggaagt 60
 ttccgatagaa atcctattaa gatcctagtt gcgagcctgag tgaacatctg 120
tccgagcctg ggcctatatg atgatcttat gcctagctag ccctattttg gcaagcttacag 180
catggcttg caatcctcctgc gcaagcttacag tgaacatctg gtcggagagc 240
gtcggcgagc ggcctatatg atgatcttat gcctagctag ccctattttg gcaagcttacag 300
catggcttg cagacacggcc cag 324

<210> SEQ ID NO: 72
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU23

<400> SEQUENCE: 72

gatgaaacgt gcggcctgtgc tttaacatcg caagctgagc gaaacatattt gagaggaagt 60
ttcggatagaa atcctattaa gatcctagtt gcgagcctgag tgaacatctg 120
cctagcctg ggcctatatg atgatcttat gcctagctag ccctattttg gcaagcttacag 180
catggcttg caatcctcctgc gcaagcttacag tgaacatctg gtcggagagc 240
tgcccgggtta cggcctcacc gcgagcagat cagatcagctg tggcggagc 300
catggcttg cagacacggcc cag 324

<210> SEQ ID NO: 73
<211> LENGTH: 124
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU38

<400> SEQUENCE: 73

gatgaaacgt gcggcctgtgc tttaacatcg caagctgagc gaaacatattt gagaggaagt 60
ttcggatagaa atcctattaa gatcctagtt gcgagcctgag tgaacatctg 120
tccgagcctg ggcctatatg atgatcttat gcctagctag ccctattttg gcaagcttacag 180
catggcttg caatcctcctgc gcaagcttacag tgaacatctg gtcggagagc 240
ggcctctgtgc gtcggagagc ggcctatatg atgatcttat gcctagctag ccctattttg gcaagcttacag 300
catggcttg cagacacggcc cag 324

<210> SEQ ID NO: 74
<211> LENGTH: 229
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU49
<400> SEQUENCE: 74

gatgaacgt ggccgcgggt gcctaacacg cacagcgaac gaaagcaatta aataggaagt 60
ttcgccgtgg gggagaggt ggaggtggta gtaagcggtg gataagctgc 120
cctacacgtgg ggggataaca gtaagaattg actgtaataa cgccataaag gcaacgtacc 180
gacggtacg tggagaacaac acggggttgct gtagattggag cccggctgct aaggtgccag 240
ttgccgaggtt aacggccac caaagcagac atcagtaacc gacctgagag ggtgacgggc 300
cacattgagg aagagagaccc gccgccc 328

<210> SEQ ID NO 75
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTUS7

<400> SEQUENCE: 75

gatgaacgt ggccgcgggt gcctaacatg caagtcgagc gaaagcggttt cgatgaagtt 60
ttcgccgtgg gggagaggt ggaggtggta gtaagcggtg gataagctgc 120
cctacacgtgg ggggataaca gtaagaattg actgtaataa cgccataaag gcaacgtacc 180
gacggtacg tggagaacaac acggggttgct gtagattggag cccggctgct aaggtgccag 240
gcagaggtta aagccctaca aagagcagat cagtagcggg cctgagaggg tgaacggcc 300
cacctgagct gagacagggg ccaa 324

<210> SEQ ID NO 76
<211> LENGTH: 327
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTUS9

<400> SEQUENCE: 76

gatgaacgt ggccgcgggt gcctaacatg caagtcgagc gaaagcatttt ggaaggaagt 60
ttcgccgtgg gggagaggt ggaggtggta gtaagcggtg gataagctgc 120
cctacacgg ggagaggtgg tgaagaattg actgtaataa cgccataaag gcaacgtacc 180
gacggtacg tggagaacaac acggggttgct gtagattggag cccggctgct aaggtgccag 240
ttgccgaggtt aacggccac caaagcagac atcagtaacc gacctgagag ggtgacgggc 300
cacattgagg aagagagaccc gccgccc 327

<210> SEQ ID NO 77
<211> LENGTH: 322
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTUS2

<400> SEQUENCE: 77

gatgaacgt ggccgcgggt gcctaacatg caagtcgagc ggaagttatcg agaggaagt 60
ttcgccgtgg gggagaggt ggaggtggta gtaagcggtg gataagctgc 120
cctacacgg ggagaggtgg tgaagaattg actgtaataa cgccataaag gcaacgtacc 180
gacggtacg tggagaacaac acggggttgct gtagattggag cccggctgct aaggtgccag 240
ttgccgaggtt aacggccac caaagcagac atcagtaacc gacctgagag ggtgacgggc 300
cacattgagg aagagagaccc gccgccc 327
<210> SEQ ID NO: 78
<211> LENGTH: 323
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU101

<400> SEQUENCE: 78

attggactg agacaagggcc ca

gatgaaagct ggccgctgct ctaaacatcg caagtccgag gacagcatat atagagaagtt  60
tccggtgat ggcttacatgactagtggcc ggcgcttgta gtaatcggcg gatatactgtc 120
tccacactgg ggataacag ttgaaatagta ctgctaactac ggcataaagcg cacgtgcgctg 180
catggcgcat tgtgaaacgtgctcgggttg tggatagcgct gcggcctctgtga ttgaccagtt 240
ggagggttaa ggccacacca aagcgcagct ccagtcgcaag cctggagaggg tgggggctca 300
cactgggacct gagacaagggcc ca

<210> SEQ ID NO: 79
<211> LENGTH: 322
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU111

<400> SEQUENCE: 79

attggactg ggccgctgct ctaaacatcg caagtccgag gacagcatat atagagaagtt  60
tccggtgat ggcttacatgactagtggcc ggcgcttgta gtaatcggcg gatatactgtc 120
tctagacgct tgtgcgtcgtg acagtaacttcttgc gatagcggc cacagctggc 180
attgaagcagt gttgaaacgt gcggtgtcag gccgggctcgtg aacgtgtgtg 240
ggagggttaa ggccacacca aagcgcagct ccagtcgcaag cctggagaggg tgggggctca 300
cactgggacct gagacaagggcc ca

<210> SEQ ID NO: 80
<211> LENGTH: 323
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU114

<400> SEQUENCE: 80

attggactg ggccgctgct ctaaacatcg caagtccgag gacagcatat tttcagatcc  60
tccggtgat ggcttacatgactagtggcc ggcgcttgta gtaatcggcg gatatactgtc 120
tctagacgct tgtgcgtcgtg acagtaacttcttgc gatagcggc cacagctggc 180
attgaagcagt gttgaaacgt gcggtgtcag gccgggctcgtg aacgtgtgtg 240
ggagggttaa ggccacacca aagcgcagct ccagtcgcaag cctggagaggg tgggggctca 300
cactgggacct gagacaagggcc ca

<210> SEQ ID NO: 81
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU119

<221> NAME/KEY: misc_feature
<222> LOCATION: (293)...(293)
<223> OTHER INFORMATION: n is a, c, g, or t
<400> SEQUENCE: 81

gatgaacgct gccgccgtgc ctaacacatg caagtcgaac gagcaatta agatgaagtt 60
tccggatgga atctgttagt actgatggtgcc gaaggtgtgga ttaacgcttg gataacctgc 120
cctacacatgg ggggtacaag ttagaaatag cttgctaatac cgctaaagcc cacagtgcgg 180
catggcatgttg tggtaaacaag ctcgcgtggtgc ttgatggtttgc cccgctggagcgg 240
gccgggttaac gagccgcacca aacggaaccgg ttcagtacccgc gctgcagaggg gtnaccgggc 300
acaggtggac tggagacacgccc 324

<410> SEQ ID NO: 82
<411> LENGTH: 325
<412> TYPE: DNA
<413> ORGANISM: Artificial Sequence
<420> FEATURE:
<423> OTHER INFORMATION: OTU125

<400> SEQUENCE: 82

gatgaacgct gccgccgtgc ctaacacatg caagtcgaac gagcaatta agatgaagtt 60
tccggatgga atctgttagt actgatggtgcc gaaggtgtgga ttaacgcttg gataacctgc 120
cctacacatgg ggggtacaag ttagaaatag cttgctaatac cgctaaagcc cacagtgcgg 180
catggcatgttg tggtaaacaag ctcgcgtggtgc ttgatggtttgc cccgctggagcgg 240
gccgggttaac gagccgcacca aacggaaccgg ttcagtacccgc gctgcagaggg gtnaccgggc 300
caggtggac tggagacacgccc 325

<410> SEQ ID NO: 83
<411> LENGTH: 322
<412> TYPE: DNA
<413> ORGANISM: Artificial Sequence
<420> FEATURE:
<423> OTHER INFORMATION: OTU131
<422> LOCATION: (291) .. (291)
<423> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 83

gatgaacgct gccgccgtgc ctaacacatg caagtcgaac gagcaatta agatgaagtt 60
tccggatgga atctgttagt actgatggtgcc gaaggtgtgga ttaacgcttg gataacctgc 120
cctacacatgg ggggtacaag ttagaaatag cttgctaatac cgctaaagcc cacagtgcgg 180
catggcatgttg tggtaaacaag ctcgcgtggtgc ttgatggtttgc cccgctggagcgg 240
gccgggttaac gagccgcacca aacggaaccgg ttcagtacccgc gctgcagaggg gtnaccggca 300
ttgggcatg gacacgcccc 322

<410> SEQ ID NO: 84
<411> LENGTH: 325
<412> TYPE: DNA
<413> ORGANISM: Artificial Sequence
<420> FEATURE:
<423> OTHER INFORMATION: OTU137

<400> SEQUENCE: 84

gatgaacgct gccgccgtgc ctaacacatg caagtcgaac gagcaatta agatgaagtt 60
tccggatgga atctgttagt actgatggtgcc gaaggtgtgga ttaacgcttg gataacctgc 120
cctacacatgg ggggtacaag ttagaaatag cttgctaatac cgctaaagcc cacagtgcgg 180

<410> SEQ ID NO: 85
<411> LENGTH: 325
<412> TYPE: DNA
<413> ORGANISM: Artificial Sequence
<420> FEATURE:
<423> OTHER INFORMATION: OTU137

<400> SEQUENCE: 85

gatgaacgct gccgccgtgc ctaacacatg caagtcgaac gagcaatta agatgaagtt 60
tccggatgga atctgttagt actgatggtgcc gaaggtgtgga ttaacgcttg gataacctgc 120
cctacacatgg ggggtacaag ttagaaatag cttgctaatac cgctaaagcc cacagtgcgg 180
gcatggcagt gtgtgaaaaaa ctcgggttgt gtgagatgga tcctggtcttg attaggtagt 240
tggtgggta acgccccacc aacgccccg tcagtgacg acctggaggg gtgaccggcc 300
acatggggac tgagacacgg ccca 325

<210> SEQ ID NO 85
<211> LENGTH: 325
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU144

<400> SEQUENCE: 85

<210> SEQ ID NO 86
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU161

<400> SEQUENCE: 86

gatgaaagct ggcgccgtgc ttacacatag caagtcgagc gaagcggatt cgtaggaatt 60
ttggatgga atggaaattg acctagggcc gcacggtgtga taaacggttg gttaacctgc 120
ttacacatgg ggataacag ttagaatag tctcataact gcataaagcg cacaagggcc 180
cattgccagt ttgaaaaacc tccgggtgttg taagatcttg gtagccttca ttaggtgatt 240
gttgggtaa ggcgccacac aacgcgcagc tcagtgacg acctggaggg gtgaccggca 300
catggggac agacacacgg gcca 325

<210> SEQ ID NO 87
<211> LENGTH: 122
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU163

<400> SEQUENCE: 87

gatgaaagct ggcgccgtgc ttacacatag caagtcgagc gaagttcacc gtaggaaatt 60
tcggatgga atcggtatgta ctcctgaggc gacgcyggtga ttaacacggt gtaacccgtgc 120
tcctacacg ggcacacagc ttagaatag tgtcataact gcataaagcg cacaagggcc 180
cattgccagt ttgaaaaacc tccgggtgttg taagatcttg gtagccttca ttaggtgatt 240
gttgggtaa ggcgccacac aacgcgcagc tcagtgacg acctggaggg gtgaccggca 300
catggggact agacacacgcc ca 324

<210> SEQ ID NO 88
<211> LENGTH: 227
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU165
<400> SEQUENCE: 88

gatgaacgt gcgcgcgcgc gtaacacgct gaagtcgaac ggagcacccc tgactgagtt 60

ttcggccttatt gtagaagtct gcggccgtag gtaaagcgtg agaaacctac 120
cctcagaggg ggaagcgcag gtaagcagta gcgtgaccc ctagcgggac 180
gagaaagggc gtaagctttaaat ctagcagtttg ctagccgctg ccaggggt 240
agaggttcgc gcctgaggtc ccacccgac ccagtttgagag ggtggtacgc 300
ggccagctg cagttttaga ccggcggc 327

<210> SEQ ID NO 89
<211> LENGTH: 327
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU181

<400> SEQUENCE: 99

gatgaacgt gcgcgcgcgc gtaacacgct gaagtcgaac ggagcacccc tgactgagtt 60

ttcggccttatt gtagaagtct gcggccgtag gtaaagcgtg agaaacctac 120
cctcagaggg ggaagcgcag gtaagcagta gcgtgaccc ctagcgggac 180
gagaaagggc gtaagctttaaat ctagcagtttg ctagccgctg ccaggggt 240
ttagcgggctt aagccaggt ccatggactg caagctgact tgaagtgcgag gaggtacggc 300
acacattgcc gcaggtacac gcgcggc 327

<210> SEQ ID NO 90
<211> LENGTH: 300
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU182

<400> SEQUENCE: 90

gatgaacgt gcgcgcgcgc gtaacacgct gaagtcgaac ggagcacccc tgactgagtt 60
gggcagcggc gtagaaactac ctaataaac gcggccgtag gtaaagcgtg agaaacctac 120
gagaaagggc gtaagctttaaat ctagcagtttg ctagccgctg ccaggggt 180
cctcagaggg ggaagcgcag gtaagcagta gcgtgaccc ctagcgggac 240
gagaaagggc gtaagctttaaat ctagcagtttg ctagccgctg ccaggggt 300

<210> SEQ ID NO 91
<211> LENGTH: 323
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU189

<400> SEQUENCE: 91

gatgaacgt gcgcgcgcgc gtaacacgct gaagtcgaac ggagcgtattt agatgaagtt 60

ttcggagcgg gtcgagcgtg ctagaggtgc gcggccgtag gtaaagcgtg agaaacctac 120
cctcagaggg ggaagcgcag gtaagcagta gcgtgaccc ctagcgggac 180
gagaaagggc gtaagctttaaat ctagcagtttg ctagccgctg ccaggggt 240
ggaagcgcgc gcgcgcgacta gcggcagctg ctagccgctg ccaggggt 300
agatgagttt ctagccgctg ccaggggt 323
<210> SEQ ID NO 92
<211> LENGTH: 322
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATU:
<223> OTHER INFORMATION: OTU196

<400> SEQUENCE: 92

gacgaacgt gcggcgcgtgc ttaacacagt caagtcgaac ggacgccccc tgaatgagt 60
ttcggccaaa ggaggggaat gcttagtggc ggacggtgtg gtaaccgctg aggaacctgc 120
ccttcagagg gcacacacgt ttggaacgcgt tcgctaatacc gcagacacata cgaatgggag 180
atccaatggt tgtcaaggt ttatacgtg aagatgagct cgggtcggtat tagtagtagtag 240
gccgggtcag ggccaccaaa gcggcgactg gtagacggta ctagaggggt aacggccac 300
attgggaact agataaagcc ca 322

<210> SEQ ID NO 93
<211> LENGTH: 307
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATU:
<223> OTHER INFORMATION: OTU199

<400> SEQUENCE: 93

gatgaacgct gcggcgcgtgc ttaacacagt caagtcgaac gcggacactt gtgctgagt 60
ggcgacacgt tgtgaatad ataataaact tgggcttgac aacggtagta actattggaa 120
cgatagctaa gcggccatag tgcggacac tgcgtgtgtga cggatatataa agtagccctaa 180
aagacacttg tagggagct ctgttgctcttg tggggttgg gatcccgggcact 240
ccggggcgc tgcgggtccag gccacgttgg aagtgccacgg ccacactggg actgagacac 300

ggcctcgag 307

<210> SEQ ID NO 94
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATU:
<223> OTHER INFORMATION: OTU202

<400> SEQUENCE: 94

gatgaacgct gcggcgcgtgc ttaacacagt caagtcgaac gcagcaattt aacgggaagt 60
ttcgggtgaa agtagtaaatg actgagtggt gcggcgggttg gtaaccgctg ggtacatgcgg 120
cctgtactgg gcggcacaga ttagataaact tggcaatact gcggcactgc gacagtccgtg 180
catgataagcg tggcmaacc tcggcggtta caagcagac cggcgtgctg ttatacgtgt 240
gggaggtttaa gcgcacccac gcagcaagac cagtagcggg cctgagaggg ggacacgccca 300
catggggaact gagacacgagc ctagaggggt tgaatgggag 324

<210> SEQ ID NO 95
<211> LENGTH: 323
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATU:
<223> OTHER INFORMATION: OTU204

<400> SEQUENCE: 95

gatgaacgct gcggcgcgtgc ttaacacagt caagtcgaac gcagcaattt aacgggatttc 60
ttcgggtgaa agtagtaaatg actgagtggt gcggcgggttg gtaaccgctg ggtacatgcgg 120
ctcatacagg ggataacag ttagaatga ctgctaataac cgataaagac cacagtaacgg 180
catgtagcgc cgggaacact ctcctggtta tgtcattgagc ccgcgctctga tttagcgttgt 240
gtagctaatc aggtagcagc caagttagcag cctgagagg gtcgagggca 300
cattgagcct gagacaaggg cc 323

<210> SEQ ID NO 96
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU211

<400> SEQUENCE: 96

gatgaaatgct ggccggctgc ttagacatag caagtctgagc gaacgggttt tgtcataaggt 60
ttcgagattg ttagaatcctg aacctagggc gcacaaggtga gtaacgctgt ggtaaacctgc 120
cctgacattg ggggaacagt cttgaacagc atcgtttaac ccgataaagc cacaagatgc 180
catgtagcgc cgggaacact ctcctggtta tgtcattgagc ccgcgctctga tttagcgttgt 240
gtagctaatc aggtagcagc caagttagcag cctgagagg gtcgagggca 300
cattgagcct gagacaaggg cc 324

<210> SEQ ID NO 97
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU214

<400> SEQUENCE: 97

gatgaaatgct ggccggctgc ttagacatag caagtctgagc gaacgggttt tgtcataaggt 60
ttcgagattg ttagaatcctg aacctagggc gcacaaggtga gtaacgctgt ggtaaacctgc 120
cctgacattg ggggaacagt cttgaacagc atcgtttaac ccgataaagc cacaagatgc 180
goatgaaatgct ggccggctgc ttagacatag caagtctgagc gaacgggttt tgtcataaggt 240
ttcgagattg ttagaatcctg aacctagggc gcacaaggtga gtaacgctgt ggtaaacctgc 300

<210> SEQ ID NO 98
<211> LENGTH: 123
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU224

<400> SEQUENCE: 98

gatgaaatgct ggccggctgc ttagacatag caagtctgagc gaacggacctt gcgcggatctc 60
ttcgagattg ttagaatcctg aacctagggc gcacaaggtga gtaacgctgt ggtaaacctgc 120
cctgacattg ggggaacagt cttgaacagc atcgtttaac ccgataaagc cacaagatgc 180
catgtagcgc cgggaacact ctcctggtta tgtcattgagc ccgcgctctga tttagcgttgt 240
gtagctaatc aggtagcagc caagttagcag cctgagagg gtcgagggca 300
cattgagcct gagacaaggg cc 324

<210> SEQ ID NO 99
<211> LENGTH: 324
<212> TYPE: DNA
US 9,649,345 B2
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143

<210> SEQ ID NO 100
<211> LENGTH: 322
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU237

<400> SEQUENCE: 100

gatgacagt gcggcgctgc ctaacacatg caagtctgaaac ggagatctag cagaggaagt 60
ttcggtgatg gaagtccgtg aatctattgg ggacgggttg agtaaaggtg gggaaccttg 120
cctgtacgc gcggcgaacc acttagatga aatcgctaata cggcataacc gcacaagctc 180
acagtggycga ggtagtggaa ctcgggttgt acagagattg cccgctctcg atagaggcag 240
tggccaggtta aggcgttacc aaagcgacga tcagtaacgc gcctgagagg ggtaacgcgc 300
agatgggac tcagacacgg ccac 324

<210> SEQ ID NO 101
<211> LENGTH: 323
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU246

<400> SEQUENCE: 101

gatgacagt gcggcgctgc ctaacacatg caagtctgaaac ggagatctag cagaggaagt 60
ttcggtgatg gaagtccgtg aatctattgg ggacgggttg agtaaaggtg gggaaccttg 120
cctgtacgc gcggcgaacc acttagatga aatcgctaata cggcataacc gcacaagctc 180
acagtggycga ggtagtggaa ctcgggttgt acagagattg cccgctctcg atagaggcag 240
tggccaggtta aggcgttacc aaagcgacga tcagtaacgc gcctgagagg ggtaacgcgc 300
agatgggac tcagacacgg ccac 323

<210> SEQ ID NO 102
<211> LENGTH: 145
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU262

<400> SEQUENCE: 102

gatgacagt gcggcgctgc ctaacacatg caagtctgaaac gaacatatttt agatggaagt 60
ttcggtgatg gaagtccgtg aatctattgg ggacgggttg agtaaaggtg gggaaccttg 120
cctgtacgc gcggcgaacc acttagatga aatcgctaata cggcataacc gcacaagctc 180
acagtggycga ggtagtggaa ctcgggttgt acagagattg cccgctctcg atagaggcag 240
<210> SEQ ID NO 103
<211> LENGTH: 326
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU268

<400> SEQUENCE: 103

gatgaacgct gcgcctggttc ctaacatcg caagtcgacg gaagcgtgtc tttcgaatc 60
ttccgcagaa gagacacgtg actgagcggc gcacggctga gtaacgcttg gcacaacgcg 120
cctccacaggg gggataacag ttgaaatag ta tgtaaatc gcctagacgc cacaggacg 180
catggtctag tgcagaaaaa ctcgctgggtga tggatgtgac cgcgtctgta ttgggtattt 240
ggtgcgggtt aaccctacca aaggagcgat cagtagcogc cctgagaagg gttacgcgg 300
cctgcgggct tcagacaccgg gccaacaa 326

<210> SEQ ID NO 104
<211> LENGTH: 329
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU269

<400> SEQUENCE: 104

gatgaacgct gcgcctggttc ctaacatcg caagtcgacg gaagcgtgtt agatgaasggtt 60
ttcgggtggt96 gtaatcgtgg gcagcggctga gtaacacgtg gtaacacgtgc 120
cctccacaggg gggataacac tggagaaaaa ctcgctgggtga tggatgtgac gcctagacgc 180
cctccacaggg gggataacat gcctagacgc cacaggacg 240
gttaggcgggg caaaacgcac gatcagtagc gcacagctgag gttgcgggg 300
cagacagtct gtcctgacac aacggccga 329

<210> SEQ ID NO 105
<211> LENGTH: 326
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU277

<400> SEQUENCE: 105

gatgaacgct gcgcctggttc ctaacatcg caagtcgacg gaagcactaa gaacggatacc 60
tttgcgttag ctgctttcgtgg gcagcggctga gtaacgcttg gttacactgc 120
cctccacaggg gggataacag tgcctaatag gcctacacag cacaggatgc 180
catggtctag ttgagaaaaa ctcgctgggtga tggatgtgac gcctagacgc 240
tgtagggggta aaccctacca aaggagcgat cagtagcogc gttcggagag gttcatgcgc 300
cagatgcgga cagtagcagc gcccag 326

<210> SEQ ID NO 106
<211> LENGTH: 323
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU279
<400> SEQUENCE: 106

gatgaacagt ggcggcgctgc ctacaacatgc caagtcgaac gaagttggag cacggaagtt
60
ttcggatgga atcgggtatata cttagtgagcg gaagggatgag taaacgtggtg gaaacctggcc
120
tgtgccggg gaataaacaatt agataaatgg tcttaatacc gcataacgag ccggacggcag
180
atgggtctgt gtgaagaact acggctggta cagagtgtgt gacgcggtcga ttacgcagtt
240
gcgcgggtta cgcgctacca aagcgacgat cagtagacgg cctggagagg tgaacgcca
300
cattgggct ggaacaaggg cca
323

<210> SEQ ID NO 107
<211> LENGTH: 315
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU280

<400> SEQUENCE: 107

gatgaacagt ggcggcgctgc ctacaacatgc caagtcgaac gccttggataa ggaagttgct
60
ttttaaaag gatcgggtgca cgggtgaata atcataaag gacgcgctggg
120
atcagatgt gcgcgaacctac tcataactgg ataggtgtgt ttcggcactc cgggacacta
180
taagggggt gcagtaacag gatgatgtcg ctctggtctg attagctagt agttaaggtga
240
acggccaccc tagggcatga taagctacgg accctgaggg gtaacgccc accatgggac
300
tgagacacc ggacaccc cca
315

<210> SEQ ID NO 108
<211> LENGTH: 325
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU286

<400> SEQUENCE: 108

gatgaacagt ggcggcgctgc ttaacaacatga caagtcgaac gacgcgcttgc gacggtttcc
60
ttcggatgga atcgggtatata cttagtgagcg gaagggatgag taaacgtggtg gaaacctggcc
120
tgtgccggg gaataaacaatt agataaatgg tcttaatacc gcataacgag ccggacgagcg
180
atgggtctgt gtgaagaact acggctggta cagagtgtgt gacgcggtcga ttacgcagtt
240
gcgcgggtta cgcgccaccc aagcgacgat cagtagacgg cctggagagg tgaacgcca
300
cattgggct ggaacaaggg cca
325

<210> SEQ ID NO 109
<211> LENGTH: 329
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU287

<400> SEQUENCE: 109

gacgcaacgc ggacgcgcgc ctaacaacagt caagtcgaac gcacacatcc gacggaatag
60
tttgtctagga agatgtgagta ttgtaggccc gaacggtgatg caataacgctg agcaaacctgc
120
tgctaggttt ggacaaactgt ttagaaaaa ctgctaatac gcacatacgg ggttggggga
180
catcctcctgc ccagagaagg ttatatacgc ctctgagatg ggatgcgctgt cggttagcta
240
gtgggctggt gatgacgagac cgacagaacc ccaagagca acagctagcct aggacgcaag
300
gcacaagct ggacgagcaca cgacgcccag
329
<210> SEQ ID NO 110
<211> LENGTH: 328
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU297

<400> SEQUENCE: 110

gatgaagct ggccgctgtgc ctaacacatg caagtcgaac gaagcatott ataggaagtt 60
ttccgatgga atatgggttg actgagtgc ggacgggtga gtaacgctgg gataaacctgc 120
ctcacacctg ggagtaaca gttgaasaat gtcgctaat aacaacctag ccgacacgtaac 180
cgcatgtac gggtgtgaaaa accaaagttgg ttggagatgg gtcgctgtct gattagccag 240
ttgccggggt aacccccaacg ccaacagcga cgcacagtag cccacctgag aaggtgtaccc 300
gccgacattg ggcctgagac acggcccc 328

<210> SEQ ID NO 111
<211> LENGTH: 326
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU303

<400> SEQUENCE: 111

gatgaagct ggccgctgtgc ctaacacatg caagtcgaac gaagcattttt agatgaagtt 60
ttccgatgga ttctgagatg actgagtgc ggacgggtga gtaacgctgg gataaacctgc 120
ctcacacctg ggacacacag ttagaaaatg cgctctatctg ccgacacgtaac 180
cgcatgtac gggtgtgaaaa accaaagttgg ttggagatgg gtcgctgtct gattagccag 240
gccgacattg ggcctgagac acggcccc 326

<210> SEQ ID NO 112
<211> LENGTH: 310
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU304

<400> SEQUENCE: 112

attgaacgct ggccgacagc ctaacacatg caagtcgaac gtaacacagga agcagctttgc 60
tgtttgtgc agaatgtgggc gcggggttag taatgcttgg gaaactgccc gatggaagggg 120
gataaactg ggaacagctg ctaacgctgg ccaacaccag gggggggacc 180
ttgccggtc tggcatgtgc aatgtgcccc agggattagg ctagttgtgg gggtaaaggg 240
tcaccctgg cgcagtcccc atgcgctgctg agaggttagc cagccacact ggaactgcac 300
cagcagctgc 310

<210> SEQ ID NO 113
<211> LENGTH: 255
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU307

<400> SEQUENCE: 113

gatgaacgct ggccgctgtgc ctaacacatg caagtcgaac gaagcatott ataggaagtt 60
ttcggatgga atatgggatg actgagtgcg gcacggtgta gtaacgcttg gatgaacctg 120
cctcacactg ggggataca gttagaaaat gtctgctaaa cccgataagg gcacgatgac 180
gcataatcg gtgtgaaaaa cccagttgtg tgtagatgga tcggctctcg attagccagt 240
tgycgggttaca cggcgagcca aagcgacgat cagtagcgcg cctgagggg tgacgacgac 300
gatgggacc tgagacaggg gcgca 325

<210> SEQ ID NO: 114
<211> LENGTH: 323
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU312
<400> SEQUENCE: 114
gatgaaagtgt gggcggtggtg ctaaacatgc caagtcgaaac gaagttatat cggaggactg 60
ttcggatgga atatgggatg actgagtgcg gcacggtgta gtaacgcttg gatgaacctg 120
cctcacactg ggggataca gttagaaaat gtctgctaaa cccgataagg gcacgatgac 180
gcataatcg gtgtgaaaaa cccagttgtg tgtagatgga tcggctctcg attagccagt 240
ggagggct ggcaccccaaa ggccagcgcc acgtacgccg cctgaggggt gaaacggcacc 300
gaatggacg acagacgccg cag 323

<210> SEQ ID NO: 115
<211> LENGTH: 301
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU313
<400> SEQUENCE: 115
gatgaaagtgt gggcggtggtg ctaaacatgc caagtcgaaac gcgcgcagca atgcccagctg 60
gggaacggg tggagtaatgc ataatgtaacc tgcccctttact ggggggataa ctattggaaa 120
cgatgctcaat gacggcctatg tgaataatgc ctgctgtaaa cttatatataa gttgcaaggg 180
actggtgacag gatggaacctt cgggctatata gctagtattgt gccagtaaag gcctacccag 240
cagagctgatccagcgcca cagaggttga cgggcacac cttgcgactg agcagcgcc 300
a 301

<210> SEQ ID NO: 116
<211> LENGTH: 122
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU319
<400> SEQUENCE: 116
gatgaaagtgt gggcggtggtg ctaaacatgc caagtcgaaac gaagttacagc agaggaacctt 60
ttcggatgga atatgggatg actgagtgcg gcacggtgta gtaacgcttg gatgaacctg 120
cctcacactg ggggataca gttagaaaat gtctgctaaa cccgataagg gcacgatgac 180
gcataatcg gtgtgaaaaa cccagttgtg tgtagatgga tcggctctcg attagccagt 240
gcgggcggt gccaccccaaa ggcagcagtc atgacgccgc cttgaggggt gacgccccaa 300
tgcggactct gcagacgccg aa 322

<210> SEQ ID NO: 117
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU326

<400> SEQUENCE: 117

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gatgaacgct ggccggttgcc ttaacacatg caaactcaac gaagaacatta aaatgaagtt 60
ttccgttgga ttttttagtt actaggtgct gcgaccggtga ttaacgcttg gataaacatgc 120
ttcacactcg ggataacag ttagaatgta tgcctaataac cgcataaagc cacacgttca 180
catgaagcg ttcgaaacac tttcggctgta cggcgagttc cccgctctga tttagccagtt 240
ggcgcgggtta cggcctacca aagcgaacag cagaacggcg cctgagaggg tgaaccggcca 300
cattggaact gacacacggc ccaa 324
```

<210> SEQ ID NO 118
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU328

<400> SEQUENCE: 119

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gatgaacgct ggccggttgcc ttaacacatg caaactcaac gaagaacatta aaatgaagga 60
ttccgtcacaag ttagaatgta tgcctaataac cgcataaagc aagacgcttg gataaacatgc 120
ttcacactcg ggataacag aacgaaagtt acctgcaaat tcgcataaat gcgcataatgc aagacgcttg 180
cattggaact gacacacggc ccaa 324
```

<210> SEQ ID NO 119
<211> LENGTH: 323
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU333

<400> SEQUENCE: 119

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gatgaacgct ggccggttgcc ttaacacatg caaactcaac gaagaacatta aaatgaagga 60
ttccgtcacaag ttagaatgta tgcctaataac cgcataaagc aagacgcttg gataaacatgc 120
ttcacactcg ggataacag aacgaaagtt acctgcaaat tcgcataaat gcgcataatgc aagacgcttg 180
cattggaact gacacacggc ccaa 324
```

<210> SEQ ID NO 120
<211> LENGTH: 323
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OTU339

<400> SEQUENCE: 120

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gatgaacgct ggccggttgcc ttaacacatg caaactcaac gaagaacatta aaatgaaggt 60
ttccgttgga ttttttagtt actaggtgct gcgaccggtga ttaacgcttg gataaacatgc 120
ttcacactcg ggataacag ttagaatgta tgcctaataac cgcataaagc cacacgttca 180
```
gacgaacgct ggccgacgc ccctaacaatg caagtcgaac gggagtgtgtg ttgaagcctg

tcggatgct ccctgagctg agtggatctgc ccctcattag
ggggatatc cagctcgctt gcgagtctctg ccctcattag
ttgacatgac cagtaaatc ctcgcaacct gcctcattag
tcggatgct ccctgagctg agtggatctgc ccctcattag
ggggatatc cagctcgctt gcgagtctctg ccctcattag
ttgacatgac cagtaaatc ctcgcaacct gcctcattag
tcggatgct ccctgagctg agtggatctgc ccctcattag
ggggatatc cagctcgctt gcgagtctctg ccctcattag
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tcggatgct ccctgagctg agtggatctgc ccctcattag
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ttgacatgac cagtaaatc ctcgcaacct gcctcattag
tcggatgct ccctgagctg agtggatctgc ccctcattag
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tcggatgct ccctgagctg agtggatctgc ccctcattag
ggggatatc cagctcgctt gcgagtctctg ccctcattag
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tcggatgct ccctgagctg agtggatctgc ccctcattag
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tcggatgct ccctgagctg agtggatctgc ccctcattag
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ttgacatgac cagtaaatc ctcgcaacct gcctcattag
tcggatgct ccctgagctg agtggatctgc ccctcattag
ggggatatc cagctcgctt gcgagtctctg ccctcattag
ttgacatgac cagtaaatc ctcgcaacct gcctcattag
tcggatgct ccctgagctg agtggatctgc ccctcattag
ggggatatc cagctcgctt gcgagtctctg ccctcattag
ttgacatgac cagtaaatc ctcgcaacct gcctcattag
tcggatgct ccctgagctg agtggatctgc ccctcattag
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tcggatgct ccctgagctg agtggatctgc ccctcattag
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tcggatgct ccctgagctg agtggatctgc ccctcattag
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tcggatgct ccctgagctg agtggatctgc ccctcattag
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ttgacatgac cagtaaatc ctcgcaacct gcctcattag
tcggatgct ccctgagctg agtggatctgc ccctcattag
ggggatatc cagctcgctt gcgagtctctg ccctcattag
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tcggatgct ccctgagctg agtggatctgc ccctcattag
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ttgacatgac cagtaaatc ctcgcaacct gcctcattag
tcggatgct ccctgagctg agtggatctgc ccctcattag
ggggatatc cagctcgctt gcgagtctctg ccctcattag
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ttgacatgac cagtaaatc ctcgcaacct gcctcattag
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What is claimed is:

1. A method of treating autoimmune disease in a subject, the method comprising
administering a pharmaceutical composition comprising a
purified bacterial mixture consisting of bacteria comprising 16S rDNA sequences of at least 95% homology
to SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21,
SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ
ID NO:26, SEQ ID NO:27, SEQ ID NO:30, SEQ ID
NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID
NO:34, SEQ ID NO:39, SEQ ID NO:40, SEQ ID
NO:41, and SEQ ID NO:42, to the subject in an amount
sufficient to treat autoimmune disease.

2. The method of claim 1, wherein the autoimmune
disease is organ transplant rejection, inflammatory bowel
disease (IBD), ulcerative colitis, pouchitis, Crohn’s disease,
sprue, rheumatoid arthritis, Type 1 diabetes, graft versus
host disease, or multiple sclerosis.

3. The method of claim 2, wherein the autoimmune
disease is inflammatory bowel disease (IBD), ulcerative
colitis, pouchitis, or Crohn’s disease.

4. The method of claim 1, wherein the bacteria are
human-derived bacteria.

5. The method of claim 1, wherein the bacteria are
isolated from a chloroform-treated fecal sample.

6. The method of claim 1, wherein the bacteria are
isolated from a heat-treated fecal sample.

7. The method of claim 1, wherein at least a portion of the
bacteria are in spore-form.

8. The method of claim 1, wherein the composition further
comprises a pharmaceutically acceptable excipient.

9. The method of claim 1, wherein the pharmaceutical
composition is formulated for oral administration.

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