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(54) Title: COMPOSITIONS COMPRISING BACTERIAL STRAINS

(57) Abstract: Provided are compositions comprising a bacterial strain of the genus *Blautia*, for use in a method of increasing the microbiota diversity and/or inducing stability of the microbiota of a subject.

COMPOSITIONS COMPRISING BACTERIAL STRAINS

TECHNICAL FIELD

This invention is in the field of compositions comprising bacterial strains isolated from the mammalian digestive tract and the use of such compositions in the treatment of disease.

BACKGROUND TO THE INVENTION

The human intestine is thought to be sterile *in utero*, but it is exposed to a large variety of maternal and environmental microbes immediately after birth. Thereafter, a dynamic period of microbial colonization and succession occurs, which is influenced by factors such as delivery mode, environment, diet and host genotype, all of which impact upon the composition of the gut microbiota, particularly during early life. Subsequently, the microbiota stabilizes and becomes adult-like [1]. The human gut microbiota contains more than 1500 different phylotypes dominated in abundance levels by two major bacterial divisions *(phyla*), the Bacteroidetes and the Firmicutes [2]. The successful symbiotic relationships arising from bacterial colonization of the human gut have yielded a wide variety of metabolic, structural, protective and other beneficial functions. The enhanced metabolic activities of the colonized gut ensure that otherwise indigestible dietary components are degraded with release of by-products providing an important nutrient source for the host and additional health benefits. Similarly, the immunological importance of the gut microbiota is well-recognized and is exemplified in germfree animals which have an impaired immune system that is functionally reconstituted following the introduction of commensal bacteria [3-5].

Dramatic changes in microbiota composition have been documented in gastrointestinal disorders such as inflammatory bowel disease (IBD). For example, the levels of *Clostridium* cluster XIVa bacteria are reduced in IBD subjects whilst numbers of *E. coli* are increased, suggesting a shift in the balance of symbionts and pathobionts within the gut [6-9, 18].

In recognition of the potential positive effect that certain bacterial strains may have on the animal gut, various strains have been proposed for use in the treatment of various diseases (see, for example, [10-13]). A number of strains, including mostly *Lactobacillus* and *Bifidobacterium* strains, have been proposed for use in treating various bowel disorders (see [14] for a review and see [15]). Reference [16] proposes the use of strains of the genus *Blautia* for use in modulating the microbial balance of the digestive ecosystem. In the context of reference 16, modulation refers to promoting the activity of the acetogenic bacterial flora to the detriment of methanogenic and sulfur reducing bacteria. This document therefore teaches only an increase in acetogenic bacteria. There is no discussion relating to the diversity of species belonging to a number of taxa. Reference [17] discusses the use of *Blautia* for improving survival in patients affected by graft versus host disease (GVDH). It mentions that increased bacterial diversity is associated with reduced GVDH-related mortality and that increased amounts of bacteria of the *Blautia* genus were associated with reduced GVDH. However, there is no suggestion that the administration of *Blautia* to a patient effects an increase in microbiota diversity and/or induces stability of the microbiota in a subject.

The relationship between different bacterial strains and different diseases, and the precise effects of particular bacterial strains on the gut and at a systemic level and on any particular types of diseases, are poorly characterised and results to date are variable and pose more questions than provide answers [18].

A hallmark of many human diseases linked to microbiota alteration is loss of microbiota diversity. As distinct from so-called *dysbiosis* which is simply an altered microbiota composition compared to the typical aggregate microbiota in healthy subjects, loss of microbiota diversity may be quantified by a measurable reduction in number of the sequence-based bacterial classifications or Operational Taxonomic Units (OTUs) in a sample, typically determined by 16S rRNA amplicon sequencing methods. Loss of diversity is also measured by reductions in the Shannon Diversity Index, or the Chao index. Reduced microbiota diversity is reported in recent studies of obesity, inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), type 2 diabetes and frailer older people [20]. Re-establishing the healthy microbiota can be difficult as the bacteria in the gut are resistant to colonisation. This poses a challenge when trying to treat the microbiota of unhealthy subjects by increasing the diversity of the microbiota [19]. The accompanying loss of microbial metabolic function is assumed to be a contributory factor to the symptoms of these pathophysiologies. In contrast to healthy adults in whom the microbiota is stable, the microbiota of unhealthy subjects such as those suffering IBD, IBS and frail elderly subjects is unstable [18, 20].

There is a requirement for the potential effects of gut bacteria to be characterised so that new therapies using gut bacteria can be developed.

SUMMARY OF THE INVENTION

The inventors have developed new therapies for treating and preventing diseases and disorders by increasing or maintaining the intestinal microbiota diversity in a subject. In particular, the inventors have identified that bacterial strains from the genus *Blautia* can be effective in increasing or maintaining the number and/or evenness of different types of bacteria in the distal gut of a subject. As described in the examples, oral administration of compositions comprising *Blautia hydrogenotrophica* increases the microbiota diversity in stool. This increase in diversity was seen in healthy and IBS subjects. However, IBS subjects receiving placebo had a statistically significant reduction in microbiome diversity during the course of the study. Additionally, the examples show that treatment with compositions comprising *Blautia hydrogenotrophica*, but not placebo, increased the stability of the microbiota in IBS subjects throughout the course of the study. The stability of the microbiota in subjects receiving the composition comprising *Blautia hydrogenotrophica* was comparable to that of healthy control subjects.

In one aspect, the present invention provides a method of treating or preventing a disease associated with a level of microbiota diversity that is reduced relative to the microbiota diversity of a healthy subject and/or has reduced stability of its microbiota compared to a healthy subject comprising administering a composition comprising a bacterial strain of the species *Blautia hydrogenotrophica* to a subject, wherein the disease is irritable bowel syndrome, one or more autoimmune diseases or one or more allergic diseases and wherein the method comprises increasing the microbiota diversity in the subject and/or inducing stability of the microbiota in the subject.

In another aspect, the present invention provides the use of a composition comprising a bacterial strain of the species *Blautia hydrogenotrophica* in the manufacture of a medicament for treating or preventing a disease associated with a level of microbiota diversity that is reduced relative to the microbiota diversity of a healthy subject and/or has reduced stability of its microbiota compared to a healthy subject in a subject, wherein the disease is irritable bowel syndrome, one or more autoimmune diseases or one or more allergic diseases and wherein the medicament increases the microbiota diversity in the subject and/or induces stability of the microbiota in the subject.

In another aspect, the present invention provides a food product comprising a composition comprising a bacterial strain of the species *Blautia hydrogenotrophica*, when used in a method according to the invention.

In another aspect, the present invention provides a vaccine composition comprising a composition comprising a bacterial strain of the species *Blautia hydrogenotrophica*, when used in a method according to the invention.

In another aspect, the present invention provides the use of:

a) a food product comprising a composition comprising a bacterial strain of the species *Blautia hydrogenotrophica*; or

b) a vaccine comprising a composition comprising a bacterial strain of the species *Blautia hydrogenotrophica;*

in the manufacture of a medicament for treating or preventing a disease associated with a level of microbiota diversity that is reduced relative to the microbiota diversity of a healthy subject and/or has reduced stability of its microbiota compared to a healthy subject in a subject, wherein the disease is irritable bowel syndrome, one or more autoimmune diseases or one or more allergic diseases and wherein the medicament increases the microbiota diversity in the subject and/or induces stability of the microbiota in the subject.

In another aspect, the present invention provides a method of treating or preventing IBS, an autoimmune disease or an allergic disease in a subject, wherein the method comprises diagnosing a subject as having a reduced level of intestinal microbiota diversity and/or reduced stability of its intestinal microbiota relative to the microbiota diversity of a healthy subject and then administering a composition comprising a bacterial strain of the species *Blautia hydrogenotrophica* to the subject.

The invention relates to a composition comprising a bacterial strain of the genus *Blautia*, for use in a method of increasing or maintaining the microbiota diversity. Similarly, there is also provided a method of increasing or maintaining the microbiota diversity in a subject comprising use of a bacterial strain of the genus *Blautia*.

The term "increasing or maintaining the microbiota diversity" is used herein to mean increasing or maintaining the number of different types of bacteria and/or the evenness of the different types of bacteria in the microbiota of a subject. In some embodiments, the microbiota diversity is increased. In some embodiments, the number of different genera of bacteria in the microbiota is increased. In some embodiments, the number of different species of bacteria in the microbiota is increased. In some embodiments, the number of different strains of bacteria in the microbiota is increased. In some embodiments, the microbiota diversity is maintained. In some embodiments, the number of different genera of bacteria in the microbiota is maintained. In some embodiments, the number of different species of bacteria in the microbiota is maintained. In some embodiments, the number of different strains of bacteria in the microbiota is maintained. In some embodiments, the number of genera, species and strains in the microbiota is increased or maintained.

The increase in microbiotia diversity may be for non-acetogenic bacteria. It may also be for both acetogenic and non-acetogenic bacteria. Such bacteria are well known in the art. Briefly, acetogenic bacteria produce acetate as an end product of anaerobic respiration or fermentation.

In some embodiments, loss, increase or maintenance of microbiota diversity may be quantified by a measurable reduction, increase or maintenance, respectively, in the number of the sequence-based bacterial classifications or Operational Taxonomic Units (OTUs) in a sample, typically determined by 16S rRNA amplicon sequencing methods. In some embodiments, loss of diversity may be measured by reductions in the Shannon Diversity Index, or the Chao index. Conversely, in some embodiments, an increase of diversity may be measured by an increase in the Shannon Diversity Index, or the Chao index. Similarly, in some embodiments, maintenance of diversity may be measured by the same result in the Shannon Diversity Index, or the Chao index.

In some embodiments, the evenness of the different types of bacteria is increased. In some embodiments, the relative abundance of the different types of bacteria in the microbiota becomes more even following treatment or prevention with a composition of the invention.

The inventors have also developed new therapies for treating and preventing diseases and disorders by inducing stability of the intestinal microbiota. In particular, the inventors have identified that bacterial strains from the genus *Blautia* induce stability of the intestinal microbiota. By “induce stability" is meant that the microbiota diversity remains stable and also the relative numbers of the different Genus in the microbiota remains stable. This is important as a number of diseases and disorders, including IBS and IBD, are characterised by reduced stability of the microbiota. As described in the examples, oral administration of compositions comprising *Blautia hydrogenotrophica* induces stability of the microbiota in stool. Therefore, in a further embodiment, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in a method of inducing stability of the microbiota in a subject. Similarly, there is also provided a method of inducing stability of the microbiota in a subject comprising use of a bacterial strain of the genus *Blautia*.

In some embodiments, the relative numbers of the different bacterial species in the microbiota of a subject becomes more stable following treatment or prevention with a composition of the invention, for example in a subject diagnosed with a disease or disorder characterised by a reduction in the diversity of microbiota. In some embodiments, the relative numbers of the different bacterial Genus in the microbiota of a subject becomes more stable following treatment or prevention with a composition of the invention, for example in a subject diagnosed with a disease or disorder characterised by a reduction in the diversity of microbiota. The stability of a subject's microbiota can be assessed by comparing the microbiome from the subject at two different time points. If there is a difference in the microbiome, this can be indicative of disease or of a disorder being present. In some embodiments, the two different time points are at least three days apart (e.g. at least 1 week, 2 weeks, 1 month, 3 months, 6 months, 1 year, 2 years apart). In some embodiments, the two different time points are 3-7 days apart, 1-2 weeks apart, 2-4 weeks apart, 4-8 weeks apart, 8-24 weeks apart, 24-40 weeks apart, 40-52 weeks apart or more than 52 weeks apart. In some embodiments, more than two different time points are used, e.g. three, four, five or more than five time points. Suitable intervals are chosen between the various time points, for example, as set out above.

In preferred embodiments of all aspects of the invention, the bacterial strain is of *Blautia hydrogenotrophica* and is preferably the bacterium deposited under accession number DSM 10507/14294.

In some embodiments, the microbiota diversity and/or the stability of the microbiota refers to the microbiota diversity and/or the stability in stool in the subject. In some embodiments, the microbiota diversity and/or the stability of the microbiota refers to the microbiota diversity and/or the stability in a stool sample from the subject. In some embodiments, the microbiota diversity and/or the stability of the microbiota refers to the microbiota diversity and/or the stability in the distal gut of the subject. In some embodiments, the microbiota diversity and/or the stability of the microbiota refers to the microbiota diversity and/or the stability in the gastrointestinal tract of the subject. In some embodiments, the microbiota diversity and/or the stability of the microbiota refers to the microbiota diversity and/or the stability in the caecum. In some embodiments, the microbiota diversity and/or the stability of the microbiota refers to the microbiota diversity and/or the stability in the colon.

In some embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in a method of treating or preventing a disease or disorder associated with a level of microbiota diversity that is reduced relative to the microbiota diversity of a healthy subject. In some embodiments, the treatment or prevention using a composition of the invention results in the microbiota diversity increasing to the levels present in a healthy individual. In some embodiments, treatment or prevention using a composition of the invention results in the microbiota diversity increasing to levels greater than those present in some healthy individuals. In some embodiments, the healthy individual is of a similar/same age to the subject and/or is of a similar/same race to the subject. Similarly, the invention also provides a method of treatment or prevention of a disease or disorder associated with a level of microbiota diversity that is reduced relative to the microbiota diversity of a healthy subject wherein the method comprises administering a composition comprising a bacterial strain of the genus *Blautia*. Examples of diseases or disorders associated with a reduced level of microbiota diversity include but are not limited to: IBS, IBD [21], obesity [22], type 2 diabetes, infectious diseases, allergic diseases, autoimmune diseases and metabolic diseases/disorders. Treatment or prevention of these diseases and disorders is encompassed by the invention. In some embodiments, the disease or disorder is IBS.

In some embodiments, the subject is an infant or child with a reduced microbiota diversity compared to a healthy infant or child, respectively. It has been observed that some children who develop an allergic disease later in life have a reduced diversity of faecal microbiota as 1 week old infants [23]. Thus, in some embodiments, the infant is less than 1 week old, is less than 2 weeks old, is less than one month old, is less than two months old or is less than four months old. In some embodiments, the subject is an infant who has not been delivered via a vaginal birth. For example, in some embodiments, the subject is an infant who has been delivered by Caesarean section. Reduced microbiota diversity has also been reported in frail elderly subjects. In some embodiments, therefore, the subject is an elderly subject, for example, a frail elderly subject. In some embodiments, the subject is 65 or more years in age (e.g. 70 or more, 75 or more, 80 or more, 85 or more or 90 or more years in age) [20].

It has been estimated that a single human individual has approximately 101 different bacterial species and 195 different strains in its microbiota [24]. Accordingly, in some embodiments, the composition is for use in treating a subject having less than 101 different bacterial species (e.g. less than 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 85, 80, 75 or 70 bacterial species) and/or less than 195 different strains (e.g. less than 194, 193, 192, 191, 190, 189, 188, 187, 186, 185, 183, 180, 175, 170, 165, 160, 150, 140 bacterial strains) in its microbiota. In some embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 80 bacterial species (e.g. more than 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 bacterial species) or to 101 bacterial species. For example, in some embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 90 bacterial species. For example, in some embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 95 bacterial species. For example, in some embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 97 bacterial species. For example, in some embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 99 bacterial species. In some embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 160 bacterial strains (e.g. more than 165, 170, 185, 186, 187, 188, 189, 190, 191, 192, 193 or 194 bacterial species) or to 195 bacterial strains. For example, in some embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 175 bacterial strains. For example, in some embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 185 bacterial strains. For example, in some embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 190 bacterial strains.

In some embodiments, the treatment or prevention results in the microbiota diversity increasing by at least one bacterial genus (e.g. by at least two, three, four, five, six, seven, eight, nine or ten bacterial genera). In some embodiments, the treatment or prevention results in the microbiota diversity increasing by at least one bacterial species (e.g. by at least two, three, four, five, six, seven, eight, nine, ten, 12, 15, 17 or 20 bacterial species). In some embodiments, the treatment or prevention results in the microbiota diversity increasing by at least one bacterial strain (e.g. by at least two, three, four, five, six, seven, eight, nine, ten, 12, 15, 17, 20 or 25 bacterial strains).

In some embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in a method of treating or preventing a disease or disorder associated with reduced stability of the microbiota compared to the stability of the microbiota in a healthy subject (or compared to a population of healthy subjects). By "reduced stability of the microbiota" is meant that the microbiota diversity does not remain as stable and also the relative numbers of the different Genus in the microbiota do not remain as stable as the stability observed in a healthy subject or in a population of healthy subjects. In some embodiments, inducing stability of the microbiota results in the stability being induced to a similar level as is present in a healthy subject, or in a population of healthy subjects. In some embodiments, inducing stability of the microbiota results in the stability being induced to the same level as is present in a healthy subject, or in a population of healthy subjects. Similarly, the invention provides a method of treating or preventing a disease or disorder associated with reduced stability of the microbiota wherein the method comprises administering a composition comprising a bacteria strain of the genus *Blautia*. For example, the pathogenesis of some diseases or disorders is characterised by reduced stability of the microbiota. Examples of such diseases and disorders are IBS, IBD, diabetes (e.g. type 2 diabetes), allergic diseases, autoimmune diseases and metabolic diseases/disorders. Accordingly, in some embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in a method of treating or preventing a disease or disorder associated with reduced stability of the microbiota, wherein the treatment or prevention comprises inducing stability of the microbiota. In some embodiments, the disease or disorder is selected from IBS, IBD, diabetes (e.g. type 2 diabetes), allergic diseases, autoimmune diseases and metabolic diseases/disorders. In some embodiments, the disease or disorder is IBS or IBD. In some embodiments, the disease or disorder is IBS. Accordingly, in some embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in a method of treating or preventing IBS or IBD, wherein the treatment or prevention comprises inducing stability of the microbiota.

In some embodiments, the invention provides a method of treatment or prevention of a disease or disorder associated with a level of microbiota diversity that is reduced relative to the microbiota diversity of a healthy subject wherein the method comprises diagnosing a subject as having a reduced level of microbiota diversity and then if a reduced level of diversity is found to be present, administering a composition comprising a bacterial strain of the genus *Blautia* to the subject.

In some embodiments, the invention provides a method of treatment or prevention of a disease or disorder associated with reduced stability of microbiota relative to the stability of microbiota in a healthy subject wherein the method comprises diagnosing a subject as having reduced stability of microbiota and then if reduced stability is found to be present, administering a composition comprising a bacterial strain of the genus *Blautia* to the subject.

In preferred embodiments of the invention, the bacterial strain in the composition is of *Blautia hydrogenotrophica*. Closely related strains may also be used, such as bacterial strains that have a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Blautia hydrogenotrophica*. Preferably, the bacterial strain has a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:5. Preferably, the bacterial strain has the 16s rRNA sequence of SEQ ID NO:5. Most preferably, the bacterial strain in the composition is the *Blautia hydrogenotrophica* strain deposited under accession number DSM 10507/14294.

In further embodiments of the invention, the bacterial strain in the composition is of *Blautia stercoris*. Closely related strains may also be used, such as bacterial strains that have a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Blautia stercoris*. Preferably, the bacterial strain has a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:1 or 3. Preferably, the sequence identity is to SEQ ID NO:3. Preferably, the bacterial strain for use in the invention has the 16s rRNA sequence represented by SEQ ID NO:3.

In further embodiments of the invention, the bacterial strain in the composition is of *Blautia wexlerae*. Closely related strains may also be used, such as bacterial strains that have a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Blautia wexlerae*. Preferably, the bacterial strain has a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:2 or 4. Preferably, the sequence identity is to SEQ ID NO:4. Preferably, the bacterial strain for use in the invention has the 16s rRNA sequence represented by SEQ ID NO:4.

In certain embodiments, the composition of the invention is for oral administration. Oral administration of the strains of the invention can be effective for increasing the microbiota diversity and/or inducing the stability of the microbiota. Also, oral administration is convenient for subjects and practitioners and allows delivery to and / or partial or total colonisation of the intestine.

In certain embodiments, the composition of the invention comprises one or more pharmaceutically acceptable excipients or carriers.

In certain embodiments, the composition of the invention comprises a bacterial strain that has been lyophilised. Lyophilisation is an effective and convenient technique for preparing stable compositions that allow delivery of bacteria, and is shown to provide effective compositions in the examples.

In certain embodiments, the invention provides a food product comprising the composition as described above.

In certain embodiments, the invention provides a vaccine composition comprising the composition as described above.

Additionally, the invention provides a method of increasing the microbiota diversity and/or inducing the stability of the microbiota and thereby treating or preventing diseases or disorders associated with a reduced microbiota diversity and/or with reduced stability of the microbiota, comprising administering a composition comprising a bacterial strain of the genus *Blautia*.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1: Comparison of microbiota in healthy and IBS patients.

Figure 2: Comparison of microbiota diversity between Day 16 and Day 1 for healthy and IBS patients after treatment with BlautiX or placebo.

Figure 3: A) Interconnectivity of the microbiome in healthy patients at Day 1, Day 16 and end of study after BlautiX treatment, B) Interconnectivity of the microbiome in IBS patients at Day 1, Day 16 and end of study after BlautiX treatment. The interconnectivity results seen on Day 1, Day 16 and at the End of Study for healthy individuals that are presented in Figure 3A are also shown in Figures 3A1, 3A2 and 3A3, respectively. The interconnectivity results seen on Day 1, Day 16 and at the End of Study for IBS patients that are presented in Figure 3B are also shown in Figures 3B1, 3B2 and 3B3, respectively.

Figure 4: A comparison of the instability in microbiota profiles in healthy and IBS patients after treatment with BlautiX or placebos at A) Day 16 and Day 1, B) end of study and Day 1 and C) end of study and Day 16.

Figure 5: Comparison of microbiota diversity at different time points for healthy and IBS patients after BlautiX treatment.

Figure 6: A) The mutual exclusion network in healthy patients at Day 1, Day 16 and end of study after BlautiX treatment, B) The mutual exclusion network in IBS patients at Day 1, Day 16 and end of study

after BlautiX treatment. The mutual exclusion results seen on Day 1, Day 16 and at the End of Study for healthy individuals that are presented in Figure 6A are also shown in Figures 6A1, 6A2 and 6A3, respectively. The mutual exclusion results seen on Day 1, Day 16 and at the End of Study for IBS patients that are presented in Figure 6B are also shown in Figures 6B1, 6B2 and 6B3, respectively.

Figure 7: Hierarchical clustering of microbiota.

Figure 8: Comparison of microbiota profiles before (D14) and after (D32) Blautix treatment based on Bray-Curtis dissimilarities.

Figure 9: (A) Visualization of microbiota profiles of different groups at D-14 using PCoA based on Bray-Curtis dissimilarities. (B) Visualization of microbiota profiles of the groups at D14 using PCoA based on Bray-Curtis dissimilarities. (C) Significant difference (p-value=0.002) in the microbiota profiles for the Blautix group was seen across the timepoints.

Figure 10: Comparison of microbiota profiles for Blautix treatment at study timepoints (D-14, D-1, D34) based on Bray-Curtis dissimilarities.

DISCLOSURE OF THE INVENTION

Bacterial strains

The compositions of the invention comprise a bacterial strain of the genus *Blautia*. The examples demonstrate that bacteria of this genus are useful for increasing the microbiota diversity and/or inducing the stability of the microbiota. The preferred bacterial strains are of the species *Blautia hydrogenotrophica, Blautia stercoris* and *Blautia wexlerae.* Most preferred is *Blautia hydrogenotrophica*, particularly the bacterium deposited under accession number DSM 10507/14294.

Examples of *Blautia* strains for use in the invention include *Blautia hydrogenotrophica*, *B. stercoris, B. faecis, B. coccoides*, *B. glucerasea*, *B. hansenii*, *B. luti*, *B. producta*, *B. schinkii* and *B. wexlerae.* The *Blautia* species are Gram-reaction-positive, non-motile bacteria that may be either coccoid or oval and all are obligate anaerobes that produce acetic acid as the major end product of glucose fermentation [25]. Blautia may be isolated from the human gut, although *B. producta* was isolated from a septicaemia sample.

Blautia hydrogenotrophica (previously known as *Ruminococcus hydrogenotrophicus*) has been isolated from the guts of mammals, is strictly anaerobic, and metabolises H_2/CO_2 to acetate, which may be important for human nutrition and health. The type strain of *Blautia hydrogenotrophica* is S5a33 = DSM 10507 = JCM 14656. The GenBank accession number for the 16S rRNA gene sequence of *Blautia hydrogenotrophica* strain S5a36 is X95624.1 (disclosed herein as SEQ ID NO:5). This exemplary *Blautia hydrogenotrophica* strain is described in [25] and [26]. The S5a33 strain and the

S5a36 strain correspond to two subclones of an acetogenic strain isolated from a faecal sample of a healthy subject. They show identical morphology, physiology and metabolism and have identical 16S rRNA sequences. Thus, in some embodiments, the *Blautia hydrogenotrophica* for use in the invention has the 16S rRNA sequence of SEQ ID NO:5.

The *Blautia hydrogenotrophica* bacterium deposited under accession number DSM 10507 and also under accession number DSM 14294 was tested in the examples and is also referred to herein as strain BH. Strain BH was deposited with the Deutsche Sammlung von Mikroorganismen [German Microorganism Collection] (Mascheroder Weg 1b, 38124 Braunschweig, Germany) on 10th May 2001 as "*Ruminococcus hydrogenotrophicus*" under accession number DSM 10507 and also under accession number DSM 14294. The depositor was INRA Laboratoire de Microbiologie CR de Clermont-Ferrand/Theix 63122 Saint Genès Champanelle, France. Ownership of the bacterium deposited as DSM 10507 and DSM 14294 has passed via assignment to 4D Pharma Plc.

The GenBank accession number for the 16S rRNA gene sequence of *Blautia stercoris* strain GAM6-1^T is HM626177 (disclosed herein as SEQ ID NO:1). An exemplary *Blautia stercoris* strain is described in [27]. The type strain of *Blautia wexlerae* is WAL 14507 = ATCC BAA-1564 = DSM 19850 [25]. The GenBank accession number for the 16S rRNA gene sequence of *Blautia wexlerae* strain WAL 14507 T is EF036467 (disclosed herein as SEQ ID NO:2). This exemplary *Blautia wexlerae* strain is described in [25].

A preferred *Blautia stercoris* strain is the strain deposited under accession number NCIMB 42381, which is also referred to herein as strain 830. A 16S rRNA sequence for the 830 strain is provided in SEQ ID NO:3. Strain 830 was deposited with the international depositary authority NCIMB, Ltd. (Ferguson Building, Aberdeen, AB21 9YA, Scotland) by GT Biologics Ltd. (Life Sciences Innovation Building, Aberdeen, AB25 2ZS, Scotland) on 12th March 2015 as "*Blautia stercoris* 830" and was assigned accession number NCIMB 42381. GT Biologics Ltd. subsequently changed its name to 4D Pharma Research Limited.

A preferred *Blautia wexlerae* strain is the strain deposited under accession number NCIMB 42486, which is also referred to herein as strain MRX008. A 16S rRNA sequence for the MRX008 strain is provided in SEQ ID NO:4. Strain MRX008 was deposited with the international depositary authority NCIMB, Ltd. (Ferguson Building, Aberdeen, AB21 9YA, Scotland) by 4D Pharma Research Ltd. (Life Sciences Innovation Building, Aberdeen, AB25 2ZS, Scotland) on 16th November 2015 as "*Blautia/Ruminococcus*" and was assigned accession number NCIMB 42486.

Bacterial strains closely related to the strain tested in the examples are also expected to be effective for increasing the microbiota diversity and/or inducing the stability of the microbiota. In certain embodiments, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Blautia hydrogenotrophica*. Preferably, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:5. Preferably, the bacterial strain for use in the invention has a 16s rRNA sequence that has the sequence of SEQ ID NO:5.

In certain embodiments, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Blautia stercoris*. Preferably, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:1 or SEQ ID NO:3. Preferably, the sequence identity is to SEQ ID NO:3. Preferably, the bacterial strain for use in the invention has the 16s rRNA sequence represented by SEQ ID NO:3. In certain embodiments, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Blautia wexlerae*. Preferably, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:2 or SEQ ID NO:4. Preferably, the sequence identity is to SEQ ID NO:4. Preferably, the bacterial strain for use in the invention has the 16s rRNA sequence represented by SEQ ID NO:4.

Bacterial strains that are biotypes of the bacterium deposited under accession number DSM 10507/14294 or biotypes of the bacteria deposited under accession numbers NCIMB 42381 and NCIMB 42486 are also expected to be effective for increasing the microbiota diversity and/or inducing the stability of the microbiota. A biotype is a closely related strain that has the same or very similar physiological and biochemical characteristics.

Strains that are biotypes of a bacterium deposited under accession number DSM 10507/14294, NCIMB 42381 or NCIMB 42486 and that are suitable for use in the invention may be identified by sequencing other nucleotide sequences for a bacterium deposited under accession number DSM 10507/14294, NCIMB 42381 or NCIMB 42486. For example, substantially the whole genome may be sequenced and a biotype strain for use in the invention may have at least 97%, 98%, 99%, 99.5% or 99.9% sequence identity across at least 80% of its whole genome (e.g. across at least 85%, 90%, 95% or 99%, or across its whole genome). For example, in some embodiments, a biotype strain has at least 98% sequence identity across at least 98% of its genome or at least 99% sequence identity across 99% of its genome. Other suitable sequences for use in identifying biotype strains may include hsp60 or repetitive sequences such as BOX, ERIC, $(GTG)_5$, or REP or [28]. Biotype strains may have sequences with at least 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of a bacterium deposited under accession number DSM 10507/14294, NCIMB 42381 or NCIMB 42486. In some embodiments, a biotype strain has a sequence with at least 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of the *Blautia hydrogenotrophica* strain deposited under accession number DSM 10507/14294 and comprises a 16S rRNA sequence that is at least 99% identical (e.g. at least 99.5% or at least 99.9% identical) to SEQ ID NO:5. In some embodiments, a biotype strain has a sequence with at least 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of the *Blautia hydrogenotrophica* strain deposited under accession number DSM 10507/14294 and has the 16S rRNA sequence of SEQ ID NO:5.

Alternatively, strains that are biotypes of a bacterium deposited under accession number DSM 10507/14294, NCIMB 42381 or NCIMB 42486 and that are suitable for use in the invention may be identified by using the accession number DSM 10507/14294 deposit, the accession number NCIMB 42381 deposit, or the accession number NCIMB 42486 deposit, and restriction fragment analysis and/or PCR analysis, for example by using fluorescent amplified fragment length polymorphism (FAFLP) and repetitive DNA element (rep)-PCR fingerprinting, or protein profiling, or partial 16S or 23s rDNA sequencing. In preferred embodiments, such techniques may be used to identify other *Blautia hydrogenotrophica, Blautia stercoris* or *Blautia wexlerae* strains.

In certain embodiments, strains that are biotypes of a bacterium deposited under accession number DSM 10507/14294, NCIMB 42381 or NCIMB 42486 and that are suitable for use in the invention are strains that provide the same pattern as a bacterium deposited under accession number DSM 10507/14294, NCIMB 42381 or NCIMB 42486 when analysed by amplified ribosomal DNA restriction analysis (ARDRA), for example when using Sau3AI restriction enzyme (for exemplary methods and guidance see, for example,[29]). Alternatively, biotype strains are identified as strains that have the same carbohydrate fermentation patterns as a bacterium deposited under accession number DSM 10507/14294, NCIMB 42381 or NCIMB 42486.

Other *Blautia* strains that are useful in the compositions and methods of the invention, such as biotypes of a bacterium deposited under accession number DSM 10507/14294, NCIMB 42381 or NCIMB 42486, may be identified using any appropriate method or strategy, including the assays described in the examples. For instance, strains for use in the invention may be identified by culturing bacteria and administering to rats to test in the distension assay. In particular, bacterial strains that have similar growth patterns, metabolic type and/or surface antigens to a bacterium deposited under accession number DSM 10507/14294, NCIMB 42381 or NCIMB 42486 may be useful in the invention. A useful strain will have comparable microbiota modulatory activity to the DSM 10507/14294, NCIMB 42381 or NCIMB 42486 strain. In particular, a biotype strain will elicit comparable effects on the microbiota to the effects shown in the Examples, which may be identified by using the culturing and administration protocols described in the Examples.

A particularly preferred strain of the invention is the *Blautia hydrogenotrophica* strain deposited under accession number DSM 10507/14294. This is the exemplary BH strain tested in the examples and shown to be effective for increasing the microbiota diversity and/or inducing the stability of the microbiota. Therefore, the invention provides a cell, such as an isolated cell, of the *Blautia hydrogenotrophica* strain deposited under accession number DSM 10507/14294, or a derivative thereof, for use in therapy, in particular for the diseases and disorders described herein.

A derivative of the strain deposited under accession number DSM 10507/14294, NCIMB 42381 or NCIMB 42486 may be a daughter strain (progeny) or a strain cultured (subcloned) from the original. A derivative of a strain of the invention may be modified, for example at the genetic level, without ablating the biological activity. In particular, a derivative strain of the invention is therapeutically active. A derivative strain will have comparable microbiota modulatory activity to the original DSM 10507/14294, NCIMB 42381 or NCIMB 42486 strain. In particular, a derivative strain will elicit comparable effects on the microbiota to the effects shown in the Examples, which may be identified by using the culturing and administration protocols described in the Examples. A derivative of the DSM 10507/14294 strain will generally be a biotype of the DSM 10507/14294 strain. A derivative of the NCIMB 42381 strain will generally be a biotype of the NCIMB 42381 strain. A derivative of the NCIMB 42486 strain will generally be a biotype of the NCIMB 42486 strain.

References to cells of the *Blautia hydrogenotrophica* strain deposited under accession number DSM 10507/14294 encompass any cells that have the same safety and therapeutic efficacy characteristics as the strains deposited under accession number DSM 10507/14294, and such cells are encompassed by the invention. References to cells of the *Blautia stercoris* strain deposited under accession number NCIMB 42381 encompass any cells that have the same safety and therapeutic efficacy characteristics as the strains deposited under accession number NCIMB 42381, and such cells are encompassed by the invention. References to cells of the *Blautia wexlerae* strain deposited under accession number NCIMB 42486 encompass any cells that have the same safety and therapeutic efficacy characteristics as the strains deposited under accession number NCIMB 42486, and such cells are encompassed by the invention.

In preferred embodiments, the bacterial strains in the compositions of the invention are viable and capable of partially or totally colonising the intestine.

Therapeutic uses

In certain embodiments, the compositions of the invention are for use in increasing the microbiota diversity and/or inducing the stability of the microbiota. Reduced diversity of the microbiota and/or reduced stability of the microbiota are associated with numerous pathological diseases and disorders, and the examples demonstrate that the compositions of the invention may be effective for increasing the microbiota diversity and/or inducing the stability of the microbiota. Accordingly, the disease or disorder to be treated or prevented using a composition of the invention is preferably a disease or disorder associated with a level of microbiota diversity that is reduced relative to the microbiota diversity of a healthy subject and/or a disease or disorder that is associated with reduced stability of the microbiota. Thus, in some embodiments, the disease or disorder may be associated with a level of microbiota diversity that is reduced relative to the microbiota diversity of a healthy subject and also be associated with reduced stability of the microbiota.

In certain embodiments, the compositions of the invention are for use in treating or preventing a disease or disorder selected from IBS, IBD, obesity, type 2 diabetes, one or more infectious diseases, one or more allergic diseases, one or more autoimmune diseases and one or more metabolic diseases/disorders. Treatment or prevention of other diseases and disorders is also envisaged. In certain embodiments, the compositions of the invention are for use in treating or preventing IBS or IBD. In certain embodiments, the compositions of the invention are for use in treating or preventing IBS. In certain embodiments, the compositions of the invention are for use in treating or preventing IBD. In certain embodiments, the compositions of the invention are for use in treating or preventing one or more allergic diseases. In certain embodiments, the compositions of the invention are for use in treating or preventing obesity. In certain embodiments, the compositions of the invention are for use in treating or preventing one or more infectious diseases. In certain embodiments, the compositions of the invention are for use in treating or preventing one or more autoimmune diseases. In certain embodiments, the compositions of the invention are for use in treating or preventing one or more metabolic diseases/disorders. Preferably, the treatment or prevention comprises increasing the microbiota diversity and/or inducing the stability of the microbiota in the subject.

In certain embodiments, the one or more infectious diseases is selected from a viral, bacterial or fungal disease. In certain embodiments, the one or more allergic diseases is asthma. In certain embodiments, the one or more metabolic diseases/disorders is selected from diabetes, e.g. type 2 diabetes, and obesity. In certain embodiments, the one or more autoimmune diseases is selected from multiple sclerosis and rheumatoid arthritis.

In certain embodiments, the compositions of the invention are for use in treating or preventing IBS, IBD, obesity, type 2 diabetes, one of more infectious diseases, one or more allergic diseases, one or more autoimmune diseases or one or more metabolic diseases/disorders by increasing the microbiota diversity in the microbiota. In certain embodiments, the compositions of the invention are for use in treating or preventing IBS or IBD by inducing the stability of the microbiota. In certain embodiments, the compositions of the invention are for use in treating or preventing IBS by inducing the stability of the microbiota

In preferred embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia*, for use in the treatment or prevention of IBD, IBS, obesity, type 2 diabetes, one or more infectious diseases, one or more allergic diseases, one or more autoimmune diseases or one or more metabolic diseases/disorders, wherein the treatment or prevention comprises increasing the microbiota diversity and/or inducing the stability of the microbiota in the subject.

In some embodiments, the invention provides a composition comprising a bacterial strain of the genus *Blautia* for use in treating or preventing a disease or disorder selected from IBS, IBD, obesity, type 2 diabetes, one or more infectious diseases, one or more allergic diseases, one or more autoimmune diseases and one or more metabolic diseases/disorders. In some embodiments, the invention provides a method of treating or preventing a disease or disorder selected from IBS, IBD, obesity, type 2 diabetes, one or more infectious diseases, one or more allergic diseases, one or more autoimmune diseases and one or more metabolic diseases/disorders, comprising administering a composition comprising a bacterial strain of the genus *Blautia*.

In preferred embodiments, the compositions of the invention comprise the bacterium deposited under accession number DSM 10507/14294 and are for use in increasing the microbiota diversity and/or inducing the stability of the microbiota in the subject in the treatment of IBD, IBS, obesity, type 2 diabetes, one or more infectious diseases, one or more allergic diseases, one or more autoimmune diseases or one or more metabolic diseases/disorders. In further preferred embodiments, the compositions of the invention comprise the bacterium deposited under accession number DSM 10507/14294 and are for use in treating or preventing IBD, IBS, obesity, type 2 diabetes, one or more infectious diseases, one or more allergic diseases, one or more autoimmune diseases or one or more metabolic diseases/disorders by increasing the microbiota diversity and/or inducing the stability of the microbiota.

In some embodiments, the pathogenesis of the disease or disorder affects the intestine. In some embodiments, the pathogenesis of the disease or disorder does not affect the intestine. In some embodiments, the pathogenesis of the disease or disorder is not localised at the intestine. In some embodiments, the treating or preventing occurs at a site other than at the intestine. In some embodiments, the treating or preventing occurs at the intestine and also at a site other than at the intestine. In certain embodiments, the disease or disorder is systemic.

In certain embodiments, the compositions are for use in subjects that exhibit, or are expected to exhibit, reduced levels of microbiota diversity, for example, when compared to a healthy subject, or a population of healthy subjects. For example, in some embodiments, the composition is for use in treating a subject having less than 101 different bacterial species (e.g. less than 100, 99, 98, 97, 96, 95, 93, 90, 85, 80, 75 or 70 bacterial species) and/or less than 195 different strains (e.g. less than 193, 190, 187, 185, 183, 180, 175, 170, 165, 160, 150, 140 bacterial strains) in its microbiota. For example, in some embodiments, the composition is for use in treating a subject that has at least one bacterial genus (e.g. at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 bacterial genera) fewer in its intestinal microbiota compared to a healthy subject or compared to a population of healthy subjects. In some embodiments, the treatment or prevention comprises a step of diagnosing a subject as having a reduced level of microbiota diversity and then if a reduced level of diversity is found to be present, the subject is then treated with a composition according to the invention.

In certain embodiments, the compositions are for use in subjects that exhibit, or are expected to exhibit, reduced stability of the microbiota. In some embodiments, the compositions are for use in subjects that exhibit, or are expected to exhibit, reduced stability in its microbiota, for example, when compared to a healthy subject, or a population of healthy subjects. In some embodiments, the treatment or prevention comprises a step of diagnosing a subject as having a reduced stability in its microbiota and then if reduced stability is found to be present, the subject is then treated with a composition according to the invention.

In certain embodiments, the subject is an infant. In certain embodiments, the subject is a child. In certain embodiments, the subject is an adult.

In certain embodiments, the subject is a healthy subject. For example, in some embodiments in which the composition is used for preventing a disease or disorder, the subject is a healthy subject, optionally one identified as being at risk of developing a disease or disorder characterised by a reduction in microbiota diversity.

In certain embodiments, the subject has previously received, is receiving, or will be receiving antibiotic treatment. Accordingly, in some embodiments, the treatment or prevention comprises administering the composition of the invention after, together with, or before antibiotic treatment. The composition of the invention and the one or more antibiotics may be for separate, simultaneous or sequential administration.

In some embodiments, the composition of the invention is for use in a method of increasing the microbiota diversity and/or inducing the stability of the microbiota in a subject having an increased level of hydrogen in their breath relative to a healthy subject. In some embodiments, the composition of the invention is for use in reducing the hydrogen level in the breath of a subject exhibiting or who is expected to exhibit a reduced level of diversity of its microbiota and/or reduced stability of the microbiota. The subject is preferably a subject diagnosed as having IBS, IBD, obesity, type 2 diabetes, one or more infectious diseases, one or more allergic diseases, one or more autoimmune diseases and/or one or more metabolic diseases/disorders. Treatment with a composition of the invention reduces the level of hydrogen detected in hydrogen breath tests. Accordingly, the hydrogen levels are preferably assessed using a hydrogen breath test. The hydrogen breath test is well known in the art and so the skilled person will know how to conduct such a test. In some embodiments, the subject is administered lactulose as the substrate for the test.

The hydrogen breath test is also a useful tool for monitoring the effectiveness or likely effectiveness of increasing the microbiota diversity and/or inducing the stability of the microbiota and of treatment or prevention using a composition of the invention. For example, a reduction in the level of hydrogen detected in a subject's breath following treatment with a composition of the invention may indicate that the treatment is having an increasing, stabilising, therapeutic or preventative effect. Accordingly, in some embodiments the methods and uses of the invention further comprise monitoring the hydrogen level in a subject's breath during and/or following treatment with a composition of the invention and thereby assessing the effectiveness or likely effectiveness of increasing, stabilising, treatment or prevention. For example, hydrogen levels may be monitored at one or more (e.g. 1, 2, 3, 4 or more than 4) times, for example, including before treatment, at the start of treatment, during treatment, at the end of treatment and/or following treatment, as desired. In some embodiments, the level of hydrogen in the subject's breath at the end and/or following the dosing period (during which the composition is administered to the subject) is compared to the level at the start and/or before the dosing period and a reduction in the level indicates the effectiveness or likely effectiveness of the increasing, stabilising, treatment or prevention. For example, in embodiments in which the dosing period is 16 days, it may be desirable to take measurements at day 1 and day 16, or for example at day 1, day 2, day 15 and day 16. In some embodiments, multiple measurements are taken and the mean of those measurements obtained (for example, the mean of day 1 and day 2 and the mean of day 15 and day 16). In some embodiments, a reduction in at least 40 ppm in the hydrogen level Cmax indicates that the increasing, stabilising, treatment or prevention is effective or likely to be effective. In some embodiments, the hydrogen level in the subject's breath is measured only once, for example, at the end of or following treatment, and the finding that the level is at or close to a predetermined level is indicative that the increasing stabilising, treatment or prevention is likely to have been effective. The hydrogen breath test is a standard assay and so predetermined levels are known in the art.

Treatment or prevention may refer to, for example, an alleviation of the severity of symptoms or a reduction in the frequency of exacerbations or the range of triggers that are a problem for the subject.

Bacteria in the microbiota may be detected in faeces from a subject, using standard techniques, such as the qPCR techniques used in the examples.

Modes of administration

Preferably, the compositions of the invention are to be administered to the gastrointestinal tract in order to enable delivery to and / or partial or total colonisation of the intestine with the bacterial strain of the invention. Generally, the compositions of the invention are administered orally, but they may be administered rectally, intranasally, or via buccal or sublingual routes.

In certain embodiments, the compositions of the invention may be administered as a foam, as a spray or a gel.

In certain embodiments, the compositions of the invention may be administered as a suppository, such as a rectal suppository, for example in the form of a theobroma oil (cocoa butter), synthetic hard fat (e.g. suppocire, witepsol), glycero-gelatin, polyethylene glycol, or soap glycerin composition.

In certain embodiments, the composition of the invention is administered to the gastrointestinal tract via a tube, such as a nasogastric tube, orogastric tube, gastric tube, jejunostomy tube (J tube), percutaneous endoscopic gastrostomy (PEG), or a port, such as a chest wall port that provides access to the stomach, jejunum and other suitable access ports.

The compositions of the invention may be administered once, or they may be administered sequentially as part of a treatment regimen. In certain embodiments, the compositions of the invention are to be administered daily. The examples demonstrate that daily administration provides successful delivery and clinical benefits.

In certain embodiments, the compositions of the invention are administered regularly, such as daily, every two days, or weekly, for an extended period of time, such as for at least one week, two weeks, one month, two months, six months, or one year. The examples demonstrate that *B. hydrogenotrophica* administration may not result in permanent colonisation of the intestines, so regular administration for extended periods of time may provide greater therapeutic and/or prophylactic benefits.

In certain embodiments of the invention, treatment according to the invention is accompanied by assessment of the subject's gut microbiota. Treatment may be repeated if delivery of and / or partial or total colonisation with the strain of the invention is not achieved such that efficacy is not observed, or treatment may be ceased if delivery and / or partial or total colonisation is successful and efficacy is observed.

In certain embodiments, the composition of the invention may be administered to a pregnant animal, for example a mammal such as a human in order to prevent reduced levels of diversity in the microbiota and/or reduced stability of the microbiota developing in her child *in utero* and / or after it is born.

The compositions of the invention may be administered to a subject that has been diagnosed with reduced microbiota diversity relative to a healthy subject and/or reduced stability of the microbiota or a disease or disorder associated with reduced microbiota diversity relative to a healthy subject and/or reduced stability of the microbiota, or that has been identified as being at risk of reduced microbiota diversity relative to a healthy subject and/or reduced stability of the microbiota. The compositions may also be administered as a prophylactic measure to prevent the development of reduced microbiota diversity relative to a healthy subject and/or reduced stability of the microbiota in a healthy subject.

The compositions of the invention may be administered to a subject that has been identified as having an abnormal gut microbiota. For example, the subject may have reduced or absent colonisation by *Blautia*, and in particular *Blautia hydrogenotrophica*, *Blautia stercoris* or *Blautia wexlerae*.

The compositions of the invention may be administered as a food product, such as a nutritional supplement.

Generally, the compositions of the invention are for the treatment of humans, although they may be used to treat animals including monogastric mammals such as poultry, pigs, cats, dogs, horses or rabbits. The compositions of the invention may be useful for enhancing the growth and performance of animals. If administered to animals, oral gavage may be used.

Compositions

Generally, the composition of the invention comprises bacteria. In preferred embodiments of the invention, the composition is formulated in freeze-dried form. For example, the composition of the invention may comprise granules or gelatin capsules, for example hard gelatin capsules, comprising a bacterial strain of the invention.

Preferably, the composition of the invention comprises lyophilised bacteria. Lyophilisation of bacteria is a well-established procedure and relevant guidance is available in, for example, references [30-32]. The examples demonstrate that lyophilisate compositions are particularly effective.

Alternatively, the composition of the invention may comprise a live, active bacterial culture.

In some embodiments, the bacterial strain in the composition of the invention has not been inactivated, for example, has not been heat-inactivated. In some embodiments, the bacterial strain in the composition of the invention has not been killed, for example, has not been heat-killed. In some embodiments, the bacterial strain in the composition of the invention has not been attenuated, for example, has not been heat-attenuated. For example, in some embodiments, the bacterial strain in the composition of the invention has not been killed, inactivated and/or attenuated. For example, in some embodiments, the bacterial strain in the composition of the invention is live. For example, in some embodiments, the bacterial strain in the composition of the invention is viable. For example, in some embodiments, the bacterial strain in the composition of the invention is capable of partially or totally colonising the intestine. For example, in some embodiments, the bacterial strain in the composition of the invention is viable and capable of partially or totally colonising the intestine.

In some embodiments, the composition comprises a mixture of live bacterial strains and bacterial strains that have been killed.

In preferred embodiments, the composition of the invention is encapsulated to enable delivery of the bacterial strain to the intestine. Encapsulation protects the composition from degradation until delivery at the target location through, for example, rupturing with chemical or physical stimuli such as pressure, enzymatic activity, or physical disintegration, which may be triggered by changes in pH. Any appropriate encapsulation method may be used. Exemplary encapsulation techniques include entrapment within a porous matrix, attachment or adsorption on solid carrier surfaces, self-aggregation by flocculation or with cross-linking agents, and mechanical containment behind a microporous membrane or a microcapsule. Guidance on encapsulation that may be useful for preparing compositions of the invention is available in, for example, references [33] and [34].

The composition may be administered orally and may be in the form of a tablet, capsule or powder. Encapsulated products are preferred because *Blautia* are anaerobes. Other ingredients (such as vitamin C, for example), may be included as oxygen scavengers and prebiotic substrates to improve the delivery and / or partial or total colonisation and survival *in vivo*. Alternatively, the probiotic composition of the invention may be administered orally as a food or nutritional product, such as milk or whey based fermented dairy product, or as a pharmaceutical product.

The composition may be formulated as a probiotic.

A composition of the invention includes a therapeutically effective amount of a bacterial strain of the invention. A therapeutically effective amount of a bacterial strain is sufficient to exert a beneficial effect upon a subject. A therapeutically effective amount of a bacterial strain may be sufficient to result in delivery to and / or partial or total colonisation of the subject’s intestine.

A suitable daily dose of the bacteria, for example for an adult human, may be from about 1 x 10^3 to about 1 x 10^{11} colony forming units (CFU); for example, from about 1 x 10^7 to about 1 x 10^{10} CFU; in another example from about 1 x 10^7 to about 1 x 10^{11} CFU; in another example from about 1 x 10^8 to about 1 x 10^{10} CFU; in another example from about 1 x 10^8 to about 1 x 10^{11} CFU; in another example from about 1 x 10^6 to about 1 x 10^{10} CFU.

In certain embodiments, the dose of the bacteria is at least 10^9 cells per day, such as at least 10^{10}, at least 10^{11}, or at least 10^{12} cells per day.

In certain embodiments, the composition contains the bacterial strain in an amount of from about 1 x 10^6 to about 1 x 10^{11} CFU/g, respect to the weight of the composition; for example, from about 1 x 10^8 to about 1 x 10^{10} CFU/g. The dose may be, for example, 1 g, 3g, 5g, and 10g.

Typically, a probiotic, such as the composition of the invention, is optionally combined with at least one suitable prebiotic compound. A prebiotic compound is usually a non-digestible carbohydrate such as an oligo- or polysaccharide, or a sugar alcohol, which is not degraded or absorbed in the upper digestive tract. Known prebiotics include commercial products such as inulin and transgalacto-oligosaccharides.

In certain embodiments, the probiotic composition of the present invention includes a prebiotic compound in an amount of from about 1 to about 30% by weight, respect to the total weight composition, (e.g. from 5 to 20% by weight). Carbohydrates may be selected from the group consisting of: fructo- oligosaccharides (or FOS), short-chain fructo-oligosaccharides, inulin, isomalt-oligosaccharides, pectins, xylo-oligosaccharides (or XOS), chitosan-oligosaccharides (or COS), beta-glucans, arable gum modified and resistant starches, polydextrose, D-tagatose, acacia fibers, carob, oats, and citrus fibers. In one aspect, the prebiotics are the short-chain fructo-oligosaccharides (for simplicity shown herein below as FOSs-c.c); said FOSs-c.c. are not digestible carbohydrates, generally obtained by the conversion of the beet sugar and including a saccharose molecule to which three glucose molecules are bonded.

The compositions of the invention may comprise pharmaceutically acceptable excipients or carriers. Examples of such suitable excipients may be found in the reference [35]. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art and are described, for example, in reference [36]. Examples of suitable carriers include lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol and the like. Examples of suitable diluents include ethanol, glycerol and water. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as, or in addition to, the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s). Examples of suitable binders include starch, gelatin, natural sugars such as glucose, anhydrous lactose, free-flow lactose, beta-lactose, corn sweeteners, natural and synthetic gums, such as acacia, tragacanth or sodium alginate, carboxymethyl cellulose and polyethylene glycol. Examples of suitable lubricants include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Preservatives, stabilizers, dyes and even flavouring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid, cysteine and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used. A further example of a suitable carrier is saccharose. A further example of a preservative is cysteine.

The compositions of the invention may be formulated as a food product. For example, a food product may provide nutritional benefit in addition to the therapeutic effect of the invention, such as in a nutritional supplement. Similarly, a food product may be formulated to enhance the taste of the composition of the invention or to make the composition more attractive to consume by being more similar to a common food item, rather than to a pharmaceutical composition. In certain embodiments, the composition of the invention is formulated as a milk-based product. The term "milk-based product" means any liquid or semi-solid milk- or whey- based product having a varying fat content. The milk-based product can be, e.g., cow's milk, goat's milk, sheep's milk, skimmed milk, whole milk, milk recombined from powdered milk and whey without any processing, or a processed product, such as yoghurt, curdled milk, curd, sour milk, sour whole milk, butter milk and other sour milk products. Another important group includes milk beverages, such as whey beverages, fermented milks, condensed milks, infant or baby milks; flavoured milks, ice cream; milk-containing food such as sweets.

In certain embodiments, the compositions of the invention contain a single bacterial strain or species and do not contain any other bacterial strains or species. Such compositions may comprise only *de minimis* or biologically irrelevant amounts of other bacterial strains or species. Such compositions may be a culture or lyophilisate that is substantially free from other species of organism.

In certain embodiments, the compositions of the invention comprise one or more bacterial strains of the genus *Blautia,* for example, a *Blautia hydrogenotrophica,* and do not contain any other bacterial genus, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another genus. In certain embodiments, the compositions of the invention comprise a single species of *Blautia,* for example, a *Blautia hydrogenotrophica,* and do not contain any other bacterial species, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another species. In certain embodiments, the compositions of the invention comprise a single strain of *Blautia,* for example, of *Blautia hydrogenotrophica,* and do not contain any other bacterial strains or species, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another strain or species.

In some embodiments, the compositions of the invention comprise more than one bacterial strain or species. For example, in some embodiments, the compositions of the invention comprise more than one strain from within the same species (e.g. more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40 or 45 strains), and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise less than 50 strains from within the same species (e.g. less than 45, 40, 35, 30, 25, 20, 15, 12, 10, 9, 8, 7, 6, 5, 4 or 3 strains), and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise 1-40, 1-30, 1-20, 1-19, 1-18, 1-15, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-50, 2-40, 2-30, 2-20, 2-15, 2-10, 2-5, 6-30, 6-15, 16-25, or 31-50 strains from within the same species and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise more than one species from within the same genus (e.g. more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, 23, 25, 30, 35 or 40 species), and, optionally, do not contain bacteria from any other genus. In some embodiments, the compositions of the invention comprise less than 50 species from within the same genus (e.g. less than 50, 45, 40, 35, 30, 25, 20, 15, 12, 10, 8, 7, 6, 5, 4 or 3 species), and, optionally, do not contain bacteria from any other genus. In some embodiments, the compositions of the invention comprise 1-50, 1-40, 1-30, 1-20, 1-15, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-50, 2-40, 2-30, 2-20, 2-15, 2-10, 2-5, 6-30, 6-15, 16-25, or 31-50 species from within the same genus and, optionally, do not contain bacteria from any other genus. The invention comprises any combination of the foregoing.

In some embodiments, the composition comprises a microbial consortium. For example, in some embodiments, the composition comprises the *Blautia* bacterial strain, for example, a *Blautia hydrogenotrophica* bacterial strain as part of a microbial consortium. For example, in some embodiments, the *Blautia* bacterial strain is present in combination with one or more (e.g. at least 2, 3, 4, 5, 10, 15 or 20) other bacterial strains from other genera with which it can live symbiotically *in vivo* in the intestine. For example, in some embodiments, the composition comprises a bacterial strain of *Blautia hydrogenotrophica* in combination with a bacterial strain from a different genus. In some embodiments, the microbial consortium comprises two or more bacterial strains obtained from a faeces sample of a single organism, e.g. a human. In some embodiments, the microbial consortium is not found together in nature. For example, in some embodiments, the microbial consortium comprises bacterial strains obtained from faeces samples of at least two different organisms. In some embodiments, the two different organisms are from the same species, e.g. two different humans. In some embodiments, the two different organisms are an infant human and an adult human. In some embodiments, the two different organisms are a human and a non-human mammal.

In some embodiments, the composition of the invention additionally comprises a bacterial strain that has the same safety and therapeutic efficacy characteristics as the *Blautia hydrogenotrophica* strain deposited under accession number DSM 10507/14294, but which is not the *Blautia hydrogenotrophica* strain deposited under accession number DSM 10507/14294, or which is not a *Blautia hydrogenotrophica* or which is not a *Blautia*.

In some embodiments in which the composition of the invention comprises more than one bacterial strain, species or genus, the individual bacterial strains, species or genera may be for separate, simultaneous or sequential administration. For example, the composition may comprise all of the more than one bacterial strain, species or genera, or the bacterial strains, species or genera may be stored separately and be administered separately, simultaneously or sequentially. In some embodiments, the more than one bacterial strains, species or genera are stored separately but are mixed together prior to use.

In some embodiments, the bacterial strain for use in the invention is obtained from human adult faeces. In some embodiments in which the composition of the invention comprises more than one bacterial strain, all of the bacterial strains are obtained from human adult faeces or if other bacterial strains are present they are present only in *de minimis* amounts. In some embodiments, the bacteria may have been cultured subsequent to being obtained from the human adult faeces and being used in a composition of the invention.

In some embodiments, the one or more *Blautia* bacterial strains is/are the only therapeutically active agent(s) in a composition of the invention. In some embodiments, the bacterial strain(s) in the composition is/are the only therapeutically active agent(s) in a composition of the invention.

The compositions for use in accordance with the invention may or may not require marketing approval.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is lyophilised. In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is spray dried. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is live. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is viable. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is capable of partially or totally colonising the intestine. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is viable and capable of partially or totally colonising the intestine.

In some cases, the lyophilised or spray dried bacterial strain is reconstituted prior to administration. In some cases, the reconstitution is by use of a diluent described herein.

The compositions of the invention can comprise pharmaceutically acceptable excipients, diluents or carriers.

In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain as used in the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to increase the microbiota diversity in a subject and/or induce stability of the microbiota and/or treat a disorder associated with reduced microbiota diversity and/or reduced stability of the microbiota when administered to a subject in need thereof, microbiota diversity, for example, a disease or disorder such as IBS, IBD, obesity, type 2 diabetes, one or more infectious diseases, one or more allergic diseases, one or more autoimmune diseases or one or more metabolic diseases/disorders.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the amount of the bacterial strain is from about 1×10^3 to about 1×10^{11} colony forming units per gram with respect to a weight of the composition.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the composition is administered at a dose of 1 g, 3 g, 5 g or 10 g.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the composition is administered by a method selected from the group consisting of oral, rectal, subcutaneous, nasal, buccal, and sublingual.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a carrier selected from the group consisting of lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol and sorbitol.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a diluent selected from the group consisting of ethanol, glycerol and water.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising an excipient selected from the group consisting of starch, gelatin, glucose, anhydrous lactose, free-flow lactose, beta-lactose, corn sweetener, acacia, tragacanth, sodium alginate, carboxymethyl cellulose, polyethylene glycol, sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate and sodium chloride.

In certain embodiments, the invention provides the above pharmaceutical composition, further comprising at least one of a preservative, an antioxidant and a stabilizer.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a preservative selected from the group consisting of sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is lyophilised.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein when the composition is stored in a sealed container at about 4°C or about 25°C and the container is placed in an atmosphere having 50% relative humidity, at least 80% of the bacterial strain as measured in colony forming units, remains after a period of at least about: 1 month, 3 months, 6 months, 1 year, 1.5 years, 2 years, 2.5 years or 3 years.

In some embodiments, the composition of the invention is provided in a sealed container comprising a composition as described herein. In some embodiments, the sealed container is a sachet or bottle. In some embodiments, the composition of the invention is provided in a syringe comprising a composition as described herein.

The composition of the present invention may, in some embodiments, be provided as a pharmaceutical formulation. For example, the composition may be provided as a tablet or capsule. In some embodiments, the capsule is a gelatine capsule ("gel-cap").

In some embodiments, the compositions of the invention are administered orally. Oral administration may involve swallowing, so that the compound enters the gastrointestinal tract, and/or buccal, lingual, or sublingual administration by which the compound enters the blood stream directly from the mouth.

Pharmaceutical formulations suitable for oral administration include solid plugs, solid microparticulates, semi-solid and liquid (including multiple phases or dispersed systems) such as tablets; soft or hard capsules containing multi- or nano-particulates, liquids (e.g. aqueous solutions), emulsions or powders; lozenges (including liquid-filled); chews; gels; fast dispersing dosage forms; films; ovules; sprays; and buccal/mucoadhesive patches.

In some embodiments the pharmaceutical formulation is an enteric formulation, i.e. a gastro-resistant formulation (for example, resistant to gastric pH) that is suitable for delivery of the composition of the invention to the intestine by oral administration. Enteric formulations may be particularly useful when the bacteria or another component of the composition is acid-sensitive, e.g. prone to degradation under gastric conditions.

In some embodiments, the enteric formulation comprises an enteric coating. In some embodiments, the formulation is an enteric-coated dosage form. For example, the formulation may be an enteric-coated tablet or an enteric-coated capsule, or the like. The enteric coating may be a conventional enteric coating, for example, a conventional coating for a tablet, capsule, or the like for oral delivery. The formulation may comprise a film coating, for example, a thin film layer of an enteric polymer, e.g. an acid-insoluble polymer.

In some embodiments, the enteric formulation is intrinsically enteric, for example, gastro-resistant without the need for an enteric coating. Thus, in some embodiments, the formulation is an enteric formulation that does not comprise an enteric coating. In some embodiments, the formulation is a capsule made from a thermogelling material. In some embodiments, the thermogelling material is a cellulosic material, such as methylcellulose, hydroxymethylcellulose or hydroxypropylmethylcellulose (HPMC). In some embodiments, the capsule comprises a shell that does not contain any film forming polymer. In some embodiments, the capsule comprises a shell and the shell comprises hydroxypropylmethylcellulose and does not comprise any film forming polymer (e.g. see [37]). In some embodiments, the formulation is an intrinsically enteric capsule (for example, Vcaps® from Capsugel).

In some embodiments, the formulation is a soft capsule. Soft capsules are capsules which may, owing to additions of softeners, such as, for example, glycerol, sorbitol, maltitol and polyethylene glycols, present in the capsule shell, have a certain elasticity and softness. Soft capsules can be produced, for example, on the basis of gelatine or starch. Gelatine-based soft capsules are commercially available from various suppliers. Depending on the method of administration, such as, for example, orally or rectally, soft capsules can have various shapes, they can be, for example, round, oval, oblong or torpedo-shaped. Soft capsules can be produced by conventional processes, such as, for example, by the Scherer process, the Accogel process or the droplet or blowing process.

Culturing methods

The bacterial strains for use in the present invention can be cultured using standard microbiology techniques as detailed in, for example, references [38-40].

The solid or liquid medium used for culture may be YCFA agar or YCFA medium. YCFA medium may include (per 100ml, approximate values): Casitone (1.0 g), yeast extract (0.25 g), $NaHCO_3$ (0.4 g), cysteine (0.1 g), K_2HPO_4 (0.045 g), KH_2PO_4 (0.045 g), NaCl (0.09 g), $(NH_4)_2SO_4$ (0.09 g), $MgSO_4 \cdot 7H_2O$ (0.009 g), $CaCl_2$ (0.009 g), resazurin (0.1 mg), hemin (1 mg), biotin (1 μg), cobalamin (1 μg), *p*-aminobenzoic acid (3 μg), folic acid (5 μg), and pyridoxamine (15 μg).

Bacterial strains for use in vaccine compositions

The inventors have identified that the bacterial strains of the invention are useful for treating or preventing diseases or disorders associated with a level of microbiota diversity that is reduced relative to the microbiota diversity of a healthy subject (or relative to the microbiota diversity of a population of healthy subjects) and/or diseases or disorders that are associated with reduced stability of the microbiota compared to a healthy subject (or compared to a population of healthy subjects). This is likely to be a result of the effect that the bacterial strains of the invention have on the host immune system. Therefore, the compositions of the invention may also be useful for preventing such diseases or disorders when administered as vaccine compositions. In certain such embodiments, the bacterial strains of the invention are viable. In certain such embodiments, the bacterial strains of the invention are capable of partially or totally colonising the intestine. In certain such embodiments, the bacterial strains of the invention are viable and capable of partially or totally colonising the intestine. In other certain such embodiments, the bacterial strains of the invention may be killed, inactivated or attenuated. In certain such embodiments, the compositions may comprise a vaccine adjuvant. In certain embodiments, the compositions are for administration via injection, such as via subcutaneous injection.

General

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, references [41] and [42-48], *etc.*

The term "comprising" encompasses "including" as well as "consisting" e.g. a composition "comprising" X may consist exclusively of X or may include something additional e.g. X + Y.

The term "about" in relation to a numerical value x is optional and means, for example, x+10%.

The word "substantially" does not exclude "completely" e.g. a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

References to a percentage sequence identity between two nucleotide sequences means that, when aligned, that percentage of nucleotides are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of ref. [49]. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in ref. [50].

Unless specifically stated, a process or method comprising numerous steps may comprise additional steps at the beginning or end of the method, or may comprise additional intervening steps. Also, steps may be combined, omitted or performed in an alternative order, if appropriate.

Various embodiments of the invention are described herein. It will be appreciated that the features specified in each embodiment may be combined with other specified features, to provide further embodiments. In particular, embodiments highlighted herein as being suitable, typical or preferred may be combined with each other (except when they are mutually exclusive).

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each claim of this application.

MODES FOR CARRYING OUT THE INVENTION

Example 1 – Changes in patient microbiota after Blautia hydrogenotrophica treatment

Summary

The effect of Blautia hydrogenotrophica on the diversity and stability of patient microbiota was tested in healthy and IBS patients.

Methodology

Study Design

A Phase I clinical trial was conducted in which Blautia hydrogenotrophica ("Blautix", strain deposited under accession number DSM 10507 and also under accession number DSM 14294) was administered to human patients having IBS or healthy human patients. Patients were administered Blautix during a dosing period (days 1-16) with the washout period being day 19-23. Faecal samples were collected from IBS & healthy subjects, placebo or Blautix treated, at: baseline, day 1 (D1) prior to treatment;

end of treatment day 16 (D-16); and at end of study (EOS), which was 2-4 weeks (wash-out) post-treatment.

16S Amplicon sequencing

A Qiagen DNeasy Blood & Tissue Kit was used following the manufacturer's instructions, to extract microbial DNA from 0.2g of frozen faecal samples from IBS & Healthy subjects, placebo or Blautix treated, at: baseline, day 1 (D1) prior to treatment; end of treatment day 16 (D-16); and at end of study (EOS), which was 2-4 weeks (wash-out) post -treatment.

Preparation and sequencing of the 16S rRNA gene amplicons was carried out using the 16S Sequencing Library Preparation Nextera protocol developed by Illumina (San Diego, California, USA). 50 ng of each of the DNA faecal extracts was amplified using PCR and primers targeting the V3/V4 variable region of the 16S rRNA gene. The products were purified and forward and reverse barcodes were attached by a second round of adapter PCR. The resulting PCR products were purified, quantified and equimolar amounts of each amplicon were then pooled before being sent for sequencing to the commercial Supplier GATC Gmbh., on either the MiSeq (2×250 bp chemistry) or HiSeq (2 x 300 bp chemistry) platforms.

Data Analysis (Post-sequencing)

The raw sequence data was merge trimmed using flash methodology. This filters out low quality reads. The USEARCH pipeline methodology (version 8.1.1861_i86_linux64) was used to identify singletons and hide them from the OTU (Operational Taxonomic Unit) generating step. The UPARSE algorithm was used to cluster the sequences into OTUs. Chimeric sequences generated in the amplification step were removed using the UCHIME chimera removal algorithm with the Chimeraslayer reference database (downloaded: 9th September 2016). The USEARCH global alignment algorithm was then used to map all reads, including singletons onto the remaining OTU sequences. In-house scripts were used to then group the sequences into OTUs as classified by the USEARCH global alignment algorithm. Individual sequences were grouped into OTUs to give microbiome compositional information (abundance and diversity).

High-level Data analysis

The Bray-Curtis dissimilarity matrix was generated for each sample pairing using the Vegan library in R 3.3.1. Dataset was visualised using Principal Coordinate analysis with the Bray-Curtis dissimilarity matrix.

An in-house heatplot R function was used to generate a heatmap visualisation with hierarchical clustering based on the Bray-Curtis dissimilarity and ward linkage.

Shannon and Simpson diversity indexes were generated using the phyloseq library in R.

DESeq2 methodology was used to identify taxonomic variables that were significant for chosen comparisons.

Permutational MANOVA was performed on the dissimilarity matrix using the Adonis function in R.

Results

Samples from all time points were pooled for both groups (71 IBS patients and 67 healthy controls including both the Blautix treated and placebo groups). Analysis was performed using a distance measure generated on the full microbiome dataset. Figure 1 reports that the microbiota of IBS subjects is significantly different from that of healthy subjects.

Diversity analysis was carried out using Observed number of predicted Taxa (OTUs), Shannon diversity index and Simpson Diversity index. Both treatment groups showed an increase in diversity at Day 16 timepoint which was significant for the observed OTUs and showed a trend for the Simpson (Raw P-value: < 0.1) (Figure 2). This increase in diversity was not observed with patients treated with the placebo. A significant decrease in microbiota diversity was observed in the untreated IBS placebo group between End of study and Day 1.

Figure 3 reports that Blautix treatment increased the microbiota network connectivity of certain health-associated taxa. In healthy patients a substantial increase in inter-microbe connections was observed from Day 1 to Day 16 (after Blautix treatment), which suggests an increase in cooperation and microbiota structure (Figure 3A). Connectivity is correlated with diversity and stability. After the wash period the network structure reverted to a network similar to that observed on Day 1. Blautix treatment was, therefore, able to increase interconnectivity in healthy patients but the effect was lost post wash out. In IBS patients the network remained similar in terms of connectivity between Day 1 and Day 16, but an increase in connectivity was observed by the end of the study suggesting an increased microbiota structure post washout period in Blautix-treated IBS patients (Figure 3B). The effect of Blautix on microbiome connectivity was, therefore, delayed in IBS patients compared to healthy patients.

Instability/change in the microbiota profiles were represented by Bray Curtis distances between time-points of the same subject. Bray-Curtis shows dissimilarity between species abundance profiles limited between 0-1 (0 = same; 1 = do not share any species). Treatment of IBS patients with Blautix reduced the magnitude of microbiota changes during the treatment (Fig. 4A) and after the treatment (Figures 4B-4C). This shows that Blautix increased the stability of the microbiota in IBS patients and that the change continues after the intervention. This increased stability was not observed when IBS patients were administered the placebo (Figures 4 A-C).

Figure 5 reports that there was a significant increase in microbiota diversity at the genus level for IBS patients treated with Blautix at Day 16 compared to Day 1. The diversity analysis was carried out using the Shannon diversity index applied to the Genus level (Raw *p*-value:0.04, Day 1 versus Day 16).

Figure 6A and Figure 6B show the changes in the mutual exclusion networks in healthy and IBS patients after Blautix treatment. In healthy individuals the mutual exclusion network becomes more dense and interconnected at Day 16, which is suggestive of increased competition and inhibition. This effect was lost, however, by the end of the study as the network structure reverted back to the initial time point during the washout period (Figure 6A). In IBS patients the effect of Blautix on mutual exclusion connectivity was to increase the network diameter over the treatment period and the washout period. This was opposite to the effect seen in the healthy individuals where the network become denser. During the washout phase for the IBS patients, multiple independent interactions were observed that were not seen previously. Multiple independent interactions represent pairs of taxa that are interacting in a manner that is independent of the rest of the network, i.e. they do not have any interactions to the rest of the network.

Visualisation of microbiota shows that after Blautix treatment there was an increased network connectivity for certain health-associated taxa (Figure 7). The health associated taxa include Clostridium cluster IV, Bifidobacterium and Prevotella. Oscillibacter is also potentially a health associated genera. These health-associated taxa are implicated in the response to treatment.

Example 2 - Protective effect in models of neurodevelopmental disorders

The BTBR mouse model

The BTBR mouse model uses inbred, genetically modified mice that display a robust autistic-like phenotype. Deficits in social behaviours, increased repetitive behaviours and increased anxiety-related behaviours have been reported in this strain [51]. Due to this robust behavioural phenotype, the BTBR mouse is an ideal animal model to assess the efficacy of novel therapeutic agents for the treatment of autistic-related behaviours. Alleviation of such symptoms by a live biotherapeutic can also be indicative of efficacy of the biotherapeutic in the treatment of other psychiatric or neurological diseases.

Mice

Male BTBR mice were bred in house. The animals were housed in a temperature- and humidity-controlled room on a 12hr dark cycle (lights on from 7:00-19:00 hr). All experiments were conducted in accordance with the European Directive 2010/63/EEC, the requirements of S.I. No 543 of 2012, and approved by the Animal Experimentation Ethics Committee of University College Cork.

Strain

Blautia hydrogenotrophica bacterium deposited under accession number DSM 10507 and also under accession number DSM 14294.

Biotherapeutic was provided in glycerol stock. Live biotherapeutics were grown in the facility in anaerobic conditions.

Live biotherapeutic administration

Dosing with *Blautia hydrogenotrophica* commenced when the mice were 8 weeks old. These mice were treated once daily for 3 weeks via oral gavage.

Administration schedule

The vehicle for oral administration is PBS. Daily oral administration occurs via oral gavage.

Fecal collection

Fresh fecal samples were collected from individual mice before and after administration of *Blautia hydrogenotrophica*. At least 20 mg of fresh faeces were placed in a microcentrifuge tube, placed immediately on ice and then stored at -80°C.

Results

The effect of Blautix treatment on microbiota between timepoints (D14, D32) is shown in Figure 8. Significant temporal variation in the microbiota profiles was observed (p-value = 0.001) between the before treatment (D14) and after treatment (D32) study timepoints.

Differential analysis using DESeq2 yielded 25 significant (adjusted p-value< 0.05) differentially abundant taxa for the Blautix treatment between the D14 and D32 Autism study timepoints. The taxa are listed in **Table 1** below.

OTU_ID	Lowest Level	Classification	log2 fold change	st error	adjusted p-value
2440650	Genus	Clostridium XIVa	19.706	3.008	6.9E-10
307526	Species	Bacteroides acidifaciens	11.275	0.912	5.0E-33
39008	Species	Bacteroides acidifaciens	10.501	1.345	1.0E-13
277773	Species	Alistipes finegoldii	9.954	0.906	2.8E-26
1105465	Genus	Barnesiella	9.255	0.923	2.8E-22
943687	Family	Porphyromonadaceae	9.200	0.850	1.1E-25
47662	Species	Barnesiella intestinihominis	8.844	0.988	7.0E-18
181003	Genus	Alistipes	8.370	2.069	4.2E-04
1282905	Species	Barnesiella intestinihominis	7.373	1.004	2.8E-12

1370810	Species	Barnesiella intestinihominis	6.633	1.986	0.006
1203483	Species	Bacteroides acidifaciens	6.599	1.584	2.7E-04
74179	Species	Alistipes massiliensis	6.318	1.899	0.006
1640334	Species	Barnesiella intestinihominis	6.258	2.066	0.013
76239	Family	Lachnospiraceae	6.202	1.229	4.6E-06
308030	Species	Barnesiella intestinihominis	6.196	1.451	1.8E-04
1156020	Family	Erysipelotrichaceae	5.827	1.607	0.002
712755	Species	Barnesiella intestinihominis	5.614	1.749	0.008
11297	Family	Porphyromonadaceae	5.450	1.021	1.0E-06
2218722	Genus	Clostridium IV	3.983	1.017	0.001
594012	Species	Clostridium lactatifermentans	2.900	0.952	0.013
453043	Species	Eubacterium ventriosum	-3.675	1.260	0.018
451019	Species	Barnesiella intestinihominis	-4.055	1.540	0.041
466087	Species	Akkermansia muciniphila	-6.727	0.876	2.5E-13
2153421	Genus	Blautia XIVa	-8.051	2.577	0.010
866478	Species	Barnesiella intestinihominis	-8.961	0.846	9.3E-25

Table 1. Significant differentially abundant taxa between D14 and D32 time points in the autism study. A positive fold change is interpreted as increased at D32 when compared to D14.

Summary

In a mouse model of autism in which animals were administered Blautix, a significant variation on their microbiome was observed, including a substantial net increase in bacterial diversity.

Example 3 –Effect in models of cerebral ischemia

Summary

The protective effect of *Blautia hydrogenotrophica* was tested in mouse models of cerebral ischemia. To this end three groups of 5-17 mice were tested. Only normally behaving animals were included in the study. The first dosing day was Day -14. One group received freeze dried bacteria daily from the first dosing day until termination. The control groups received either vehicle or lyobuffer.

On Day 1, all mice were anesthetized. A midline incision was created in the ventral side of the neck to expose the right and left common carotid-arteries. A cerebral ischemia-reperfusion I/R model was then induced by bilateral common carotid artery occlusion (BCCAO) using vascular clips for 15 minutes. At the end of each occlusion, the clips were removed.

Strain

Blautia hydrogenotrophica bacterium deposited under accession number DSM 10507 and also under accession number DSM 14294.

Administration Schedule

No. of Animals	Treatment	Dose Level (mg/kg)	Dose Volumes (ml/kg or ml/animal)
12	PBS (negative control)	n/a	10
17	Freeze-dried Powder	7.8 mg in 100 μl	100 μl per animal
13	Freeze-dried Bacteria	15.6 mg in 100μl (bacteria dose = $2x10^8$)	100 μl per animal

Study design

Days -14 to 14: Daily dose of PBS control (lyobuffer), freeze dried powder control (vehicle) or freeze dried bacteria (Blautix).

Day 1: Cerebral ischemia-reperfusion I/R model induced by surgery.

Day 14: Half of the mice in each group were terminated.

Day 14 to 28: Daily dose of PBS control (lyobuffer), freeze dried powder control (vehicle) or freeze dried bacteria (Blautix) for the remaining mice in each group.

Day 28: Termination of remaining mice.

Faecal pellets were collected at three time points: Day -14, Day 14 and Day 28. Each take was carried out in a sterile environment (fully aseptic = cleaned between animals), with every mouse being taken out of the cage and placed separately into a new sterile box for individual pellet harvesting. As many pellets as possible were collected in order to reach a minimum of 80mg and preferably 100mg of material per mouse.

Results

No significant differences in microbiota profiles between the Blautix treatment, Vehicle and Lyobuffer groups were detected at D-14 (p-value= 0.177) before administration of Blautix (see Figure 9A). However, significant differences were observed in microbiota profiles between the different treatment groups at Day 14 (see Figure 9B) with a p-value of 0.011 observed. The inventors further assessed the temporal variation in the microbiota of the Blautix treated group and found a significant difference (see Figure 9C) with a p-value of 0.002 observed.

Differential analysis using DESeq2 yielded significant (adjusted p-value <0.05) differentially abundant taxa for the Vehicle, Lyobuffer and Blautix treatment between timepoints in the Stroke study, as shown in Table 2, demonstrating a longer term impact on bacterial diversity imparted by Blautix. The taxa for the Blautix treatment are listed in Table 3, Table 4 and Table 5.

	D-14->D14	**D14->D28**	**D-14->D28**
Vehicle	4	0	2
Lyobuffer	17	2	0
Blautix	7	14	12

Table 2. Significant differentially abundant taxa between time points in the Stroke study.

OTU_ID	Lowest Level	Classification	log2 fold change	st error	adjusted p-value
321825	Family	Ruminococcaceae	1.647	0.470	0.027
74771	Species	Alistipes massiliensis	1.530	0.442	0.027
567799	Genus	Alistipes	-1.215	0.308	0.008
77091	Genus	Clostridium	-1.634	0.489	0.036
472737	Family	Lachnospiraceae	-2.585	0.667	0.008
615246	Family	Lachnospiraceae	-3.003	0.711	0.007
166882	Family	Lachnospiraceae	-5.547	1.406	0.008

Table 3. Significant differentially abundant taxa between D-14 and D14 timepoints for the Blautix treatment in the Stroke study. A positive fold change is interpreted as increased at D14 when compared to D-14.

OTU_ID	Lowest Level	Classification	log2 fold change	st error	adjusted p-value
1101936	Order	Clostridiales	3.275	0.709	0.001
218505	Species	Roseburia faecis	2.568	0.630	0.002

948888	Genus	Barnesiella	2.499	0.575	0.001
612631	Genus	Clostridium XIVa	2.473	0.723	0.011
201398	Phylum	Bacteroidetes	2.045	0.605	0.011
1370810	Species	Barnesiella intestinihominis	1.878	0.579	0.016
770554	Species	Alistipes putredinis	1.868	0.626	0.033
558330	Genus	Prevotella	1.795	0.453	0.002
943687	Family	Porphyromonadaceae	1.586	0.546	0.039
308030	Species	Barnesiella intestinihominis	1.324	0.361	0.005
176124	Phylum	Bacteroidetes	1.163	0.294	0.002
565518	Species	Oscillospira guilliermondii	-1.571	0.488	0.016
544582	Species	Flavonifractor plautii	-1.599	0.569	0.050
25678	Species	Mucispirillum schaedleri	-2.751	0.640	0.001

Table 4. Significant differentially abundant taxa between D14 and D28 timepoints for the Blautix treatment in the Stroke study. A positive fold change is interpreted as increased at D28 when compared to D14.

OTU_ID	Lowest Level	Classification	log2 fold change	st error	adjusted p-value
688867	Genus	Clostridium XIVa	8.296	1.136	3.8E-11
612631	Genus	Clostridium XIVa	7.814	1.348	3.1E-07
560658	Family	Lachnospiraceae	5.241	1.243	0.001
929749	Species	Eubacterium ruminantium	3.190	0.829	0.003
518034	Species	Desulfovibrio fairfieldensis	3.098	0.982	0.024
74771	Species	Alistipes massiliensis	2.548	0.714	0.007
23310	Species	Odoribacter laneus	1.621	0.475	0.011
117624	Order	Clostridiales	-1.748	0.612	0.049
411272	Genus	Clostridium XIVa	-2.923	1.019	0.049
39008	Species	Bacteroides acidifaciens	-2.953	0.816	0.007
331352	Genus	Clostridium XIVa	-3.969	0.626	1.6E-08
77091	Genus	Clostridium	-4.247	1.426	0.039

Table 5. Significant differentially abundant taxa between D-14 and D28 timepoints for the Blautix treatment in the Stroke study. A positive fold change is interpreted as increased at D28 when compared to D-14.

Differential analysis using DESeq2 yielded significant (adjusted p-value <0.05) differentially abundant taxa for the Blautix treatment vs. Vehicle as well as Blautix treatment vs. Lyobuffer for the Stroke study timepoints, as shown in Table 6. The taxa are listed in Table 7, Table 8 and Table 9.

	D-14	**D14**	**D28**
Blautix vs. Vehicle	0	10	0
Blautix vs. Lyobuffer	2	13	0

Table 6. Significant differentially abundant taxa for the Blautix treatment in the Stroke study.

OTU_ID	Lowest Level	Classification	log2 fold change	st error	adjusted p-value
25678	Species	Mucispirillum schaedleri	2.604	0.688	0.014
3119687	Family	Lachnospiraceae	2.445	0.642	0.014
321825	Family	Ruminococcaceae	2.174	0.564	0.014
627	Genus	Clostridium XlVa	1.915	0.601	0.043
308030	Species	Barnesiella intestinihominis	-1.324	0.419	0.043
1370810	Species	Barnesiella intestinihominis	-1.540	0.425	0.019
187271	Species	Ruminococcus flavefaciens	-3.475	1.065	0.042
277773	Species	Alistipes finegoldii	-3.751	1.178	0.043
940566	Species	Staphylococcus lentus	-5.228	1.519	0.026
930972	Genus	Staphylococcus	-5.418	1.536	0.023

Table 7. Significant differentially abundant taxa for the Blautix treatment vs. Vehicle at D14 in the Stroke study.

OTU_ID	Lowest Level	Classification	log2 fold change	st error	adjusted p-value
1161472	Family	Lachnospiraceae	6.511	1.403	0.001
392940	Kingdom	Bacteria	-5.169	1.346	0.022

Table 8. Significant differentially abundant taxa for the Blautix treatment vs. Lyobuffer at D-14 in the Stroke study

OTU_ID	Lowest Level	Classification	log2 fold change	st error	adjusted p-value
25678	Species	Mucispirillum schaedleri	2.704	0.753	0.012
1379349	Genus	Clostridium XIVa	2.517	0.771	0.027
742656	Species	Oscillibacter valericigenes	1.738	0.459	0.009
558330	Genus	Prevotella	-1.634	0.406	0.006
1370810	Species	Barnesiella intestinihominis	-1.780	0.390	0.001
712755	Species	Barnesiella intestinihominis	-1.827	0.464	0.006
47662	Species	Barnesiella intestinihominis	-2.109	0.606	0.014
1640334	Species	Barnesiella intestinihominis	-2.260	0.693	0.027
1105465	Genus	Barnesiella	-2.306	0.627	0.010
161658	Family	Lachnospiraceae	-2.565	0.816	0.037
277773	Species	Alistipes finegoldii	-3.619	1.034	0.014
187271	Species	Ruminococcus flavefaciens	-3.924	1.057	0.010
459041	Species	Lactobacillus johnsonii	-4.029	0.981	0.006

Table 9. Significant differentially abundant taxa for the Blautix treatment vs. Lyobuffer at D14 in the Stroke study

Summary

Blautix effects a significant increase in microbiota diversity throughout the period of the study in a mouse model of stroke, when compared to lyobuffer or vehicle control.

Example 4 - Protective effect in models of neuroinflammatory conditions

Experimental Autoimmune Encephalomyelitis (EAE) is a mouse model of CNS inflammation that mirrors many aspects of the human disease MS and EAE is the most commonly used experimental model for human MS. EAE is also used more generally as a model for CNS-specific autoimmune disorders [52] and for other specific conditions, including acute disseminated encephalomyelitis. EAE is induced using immunisation with myelin peptides and adjuvants to elicit an immune and inflammatory response that closely corresponds to the mechanisms underlying many autoimmune and inflammatory disorders of the CNS, and in particular MS. Many therapies showing efficacy in EAE have also shown efficacy in treatment of MS in human patients [52]. Most importantly, EAE reproduces key features of MS, including inflammation, demyelination, axonal loss and gliosis. The effects of demyelination are mainly restricted to the spinal cord in EAE, with little alteration of the brain stem and the cerebellum. In EAE the CD4+ T cells are the dominant cell population found in the CNS.

Methodology

Blautia hydrogenotrophica ("Blautix", strain deposited under accession number DSM 10507 and also under accession number DSM 14294) was used as a freeze-dried powder and reconstituted as required.

12 adult female C57BL/6J mice were used.

On Day 0 and Day 7, animals were administered with an emulsion containing MOG35-55 and complete Freund's adjuvant (CFA) supplemented with Mycobacterium Tuberculosis H37Ra by subcutaneous injections under gas (isoflurane) anaesthesia. On Day 0, two subcutaneous injections were performed in the flanks; one in each of the lower quadrant of the back. On Day 7, two subcutaneous injections were performed in the flanks, one in each of the upper quadrant of the back.

On Day 0 and Day 2, animals were administered with pertussis toxin (PTx) in phosphate buffered saline (PBS) by intra-peritoneal injections. On Day 0, PTx administration was performed after MOG injections.

Treatments with Blautix or controls were administered from Day -14 according to the following schedule:

Day 0: MOG/CFA, once, SC
Day 0: PTx, once, IP
Day 2, PTx, once, IP
Day 7: MOG/CFA, once, SC

Treatments were administered within 15 minutes of their preparation. Blautix was administered at a dose of 2 x 10^8; 100μl/mouse.

From Day 0 until the end of the experiment, animals were scored daily for clinical signs of EAE, including paresis and paralysis of the tail and/or limbs.

On Day -14, Day -1 and Day 34, faecal pellets were collected from each animal, immediately snap-frozen and stored at -80°C.

Results

The effect of Blautix treatment on microbiota between timepoints (D-14, D-1, D34) for the MS model is shown in Figure 10. Significant temporal variation in the microbiota profiles was observed (p-value = 0.001) for the study timepoints.

Differential analysis using DESeq2 yielded significant (adjusted p-value< 0.05) differentially abundant taxa for the Blautix treatment between study timepoints, as shown in Table 10. The taxa are listed in Table 11, Table 12 and Table 13.

	D-14->D-1	**D-1->D34**	**D-14->D34**
MS (Blautix)	42	30	58

Table 10. Significant differentially abundant taxa between timepoints in the MS study.

OTU_ID	Lowest Level	Classification	log2 fold change	st error	adjusted p-value
1105465	Genus	Barnesiella	8.076	0.702	2.2E-28
48633	Genus	Clostridium XlVa	7.304	0.825	7.0E-17
490405	Species	Turicibacter sanguinis	6.824	0.778	1.0E-16
491106	Species	Flavonifractor plautii	5.116	0.923	4.3E-07
43241	Genus	Clostridium XlVa	5.041	0.739	2.2E-10
948888	Genus	Barnesiella	4.649	0.605	4.3E-13
47662	Species	Barnesiella intestinihominis	4.276	0.501	5.1E-16
1288839	Family	Lachnospiraceae	4.117	1.170	0.003
11297	Family	Porphyromonadaceae	4.081	0.600	2.2E-10
198591	Family	Lachnospiraceae	3.757	0.788	2.3E-05
49543	Family	Lachnospiraceae	3.275	0.897	0.002
1009304	Species	Oscillospira guilliermondii	3.140	1.043	0.015
930464	Species	Insolitispirillum peregrinum	2.804	1.033	0.029
1793164	Genus	Parasutterella	2.720	0.576	2.6E-05
1260915	Kingdom	Bacteria	2.678	0.804	0.006
36112	Species	Clostridium leptum	2.584	0.887	0.018
181003	Genus	Alistipes	2.581	0.555	3.3E-05
149837	Family	Lachnospiraceae	2.434	0.678	0.002
1056232	Genus	Clostridium XlVa	2.308	0.856	0.030
770554	Species	Alistipes putredinis	2.223	0.556	0.001
1176501	Family	Lachnospiraceae	2.079	0.758	0.028
33530	Species	Acetatifactor muris	1.965	0.569	0.004
43033	Genus	Alistipes	1.788	0.379	2.6E-05
576748	Family	Ruminococcaceae	1.740	0.603	0.019
50759	Species	Oscillospira guilliermondii	1.570	0.409	0.001
592877	Species	Pseudoflavonifractor capillosus	1.512	0.418	0.002
712755	Species	Barnesiella intestinihominis	1.509	0.502	0.015
375558	Species	Barnesiella intestinihominis	1.505	0.554	0.029
307526	Species	Bacteroides acidifaciens	1.499	0.492	0.014
74641	Species	Bacteroides acidifaciens	1.418	0.532	0.032
943687	Family	Porphyromonadaceae	1.162	0.397	0.018
791734	Genus	Clostridium XlVa	-1.064	0.377	0.023
19031	Species	Anaerotruncus colihominis	-1.391	0.516	0.030
74179	Species	Alistipes massiliensis	-1.810	0.305	4.7E-08
211238	Species	Anaeroplasma abactoclasticum	-2.662	0.859	0.012
76239	Family	Lachnospiraceae	-2.721	0.668	4.2E-04

743544	Genus	Clostridium XIVa	-3.014	0.672	7.0E-05
993522	Genus	Clostridium XIVa	-3.394	0.708	2.2E-05
76325	Genus	Lactobacillus	-3.621	0.575	5.2E-09
209309	Family	Lachnospiraceae	-3.857	1.295	0.016
567799	Genus	Alistipes	-5.435	0.634	4.3E-16
77091	Genus	Clostridium	-6.877	1.048	1.0E-09

Table 11. Significant differentially abundant taxa between D-14 and D-1 timepoints in the MS study. A positive fold change is interpreted as increased at D-1 when compared to D-14.

OTU_ID	Lowest Level	Classification	log2 fold change	st error	adjusted p-value
1370810	Species	Barnesiella intestinihominis	4.794	1.196	0.001
1684470	Species	Parasutterella excrementihominis	4.434	1.167	0.002
1070245	Species	Eubacterium plexicaudatum	3.870	0.961	0.001
518034	Species	Desulfovibrio fairfieldensis	3.867	0.962	0.001
1482481	Species	Clostridium disporicum	3.228	1.112	0.029
567799	Genus	Alistipes	3.218	0.864	0.002
1404432	Species	Bacteroides acidifaciens	2.978	0.835	0.004
1067514	Genus	Barnesiella	2.967	0.921	0.011
76325	Genus	Lactobacillus	2.893	0.683	0.001
307526	Species	Bacteroides acidifaciens	2.218	0.351	1.8E-08
1288839	Family	Lachnospiraceae	2.084	0.746	0.035
866478	Species	Barnesiella intestinihominis	1.936	0.647	0.022
23133	Family	Ruminococcaceae	1.840	0.544	0.007
472737	Family	Lachnospiraceae	1.697	0.524	0.011
842401	Order	Clostridiales	1.601	0.535	0.022
39008	Species	Bacteroides acidifaciens	1.494	0.390	0.002
74179	Species	Alistipes massiliensis	1.426	0.328	3.9E-04
277773	Species	Alistipes finegoldii	1.323	0.461	0.029
76234	Family	Lachnospiraceae	-1.183	0.333	0.004
948888	Genus	Barnesiella	-1.453	0.520	0.035
150155	Family	Lachnospiraceae	-1.609	0.421	0.002
783115	Family	Desulfovibrionaceae	-2.262	0.608	0.002
773427	Species	Anaerotruncus colihominis	-2.443	0.661	0.003
201157	Family	Lachnospiraceae	-2.587	0.754	0.006
596894	Genus	Clostridium XIVa	-2.616	0.909	0.029
43033	Genus	Alistipes	-3.236	0.718	2.2E-04
1793164	Genus	Parasutterella	-3.758	0.632	1.4E-07
49543	Family	Lachnospiraceae	-4.849	0.920	5.5E-06
490405	Species	Turicibacter sanguinis	-5.152	0.704	2.5E-11
48282	Family	Lachnospiraceae	-5.460	0.666	4.7E-14

Table 12. Significant differentially abundant taxa between D-1 and D34 timepoints in the MS study. A positive fold change is interpreted as increased at D34 when compared to D-1.

OTU_ID	Lowest Level	Classification	log2 fold change	st error	adjusted p-value
1105465	Genus	Barnesiella	7.221	0.754	2.1E-19
48633	Genus	Clostridium XIVa	6.734	0.959	8.7E-11
1288839	Family	Lachnospiraceae	5.820	0.811	3.5E-11
518034	Species	Desulfovibrio fairfieldensis	5.459	0.999	8.3E-07
1684470	Species	Parasutterella excrementihominis	5.289	1.408	0.001
1482481	Species	Clostridium disporicum	4.947	1.295	0.001
1370810	Species	Barnesiella intestinihominis	4.734	1.263	0.001
1070245	Species	Eubacterium plexicaudatum	4.620	0.794	1.3E-07
1067514	Genus	Barnesiella	4.544	1.103	2.8E-04
1575843	Species	Clostridium ruminantium	4.393	1.743	0.040
43241	Genus	Clostridium XIVa	4.284	0.817	2.4E-06
47662	Species	Barnesiella intestinihominis	4.273	0.478	3.8E-17
1728285	Genus	Lachnospiracea incertae sedis	4.204	1.221	0.003
11297	Family	Porphyromonadaceae	3.921	0.577	3.4E-10
307526	Species	Bacteroides acidifaciens	3.539	0.534	9.9E-10
198591	Family	Lachnospiraceae	3.273	0.762	1.4E-04
236126	Species	Oscillospira guilliermondii	3.175	1.086	0.015
930464	Species	Insolitispirillum peregrinum	3.152	0.848	0.001
948888	Genus	Barnesiella	3.040	0.629	1.6E-05
491106	Species	Flavonifractor plautii	3.039	1.170	0.034
563211	Family	Lachnospiraceae	2.564	0.965	0.030
149837	Family	Lachnospiraceae	2.562	0.890	0.016
770554	Species	Alistipes putredinis	2.520	0.455	6.2E-07
1260915	Kingdom	Bacteria	2.505	0.677	0.001
36112	Species	Clostridium leptum	2.483	0.916	0.026
1056232	Genus	Clostridium XIVa	2.319	0.664	0.002
39008	Species	Bacteroides acidifaciens	2.107	0.274	9.9E-13
23133	Family	Ruminococcaceae	2.049	0.614	0.004
74641	Species	Bacteroides acidifaciens	2.002	0.457	1.0E-04
712755	Species	Barnesiella intestinihominis	1.977	0.470	2.0E-04
277773	Species	Alistipes finegoldii	1.881	0.388	1.6E-05
1404432	Species	Bacteroides acidifaciens	1.805	0.563	0.006
1176501	Family	Lachnospiraceae	1.654	0.630	0.032
544582	Species	Flavonifractor plautii	1.418	0.540	0.032
76234	Family	Lachnospiraceae	-0.991	0.389	0.038
80190	Family	Lachnospiraceae	-1.113	0.348	0.006
182471	Order	Clostridiales	-1.315	0.472	0.021
494032	Species	Clostridium oroticum	-1.502	0.580	0.034
2367602	Order	Clostridiales	-1.518	0.472	0.006
74771	Species	Alistipes massiliensis	-1.617	0.408	0.001
172154	Genus	Clostridium XIVa	-1.628	0.442	0.001
993522	Genus	Clostridium XIVa	-1.799	0.594	0.011
791734	Genus	Clostridium XIVa	-1.842	0.387	2.2E-05
150155	Family	Lachnospiraceae	-1.859	0.532	0.002
743544	Genus	Clostridium XIVa	-2.196	0.558	0.001
567799	Genus	Alistipes	-2.378	0.513	3.9E-05

96345	Genus	Clostridium XIVa	-2.528	0.667	0.001
19031	Species	Anaerotruncus colihominis	-2.575	0.610	1.9E-04
201157	Family	Lachnospiraceae	-2.615	0.866	0.011
578360	Family	Lachnospiraceae	-2.870	0.586	1.4E-05
76239	Family	Lachnospiraceae	-3.325	0.631	2.3E-06
1165458	Family	Lachnospiraceae	-3.346	0.754	8.1E-05
773427	Species	Anaerotruncus colihominis	-3.475	0.776	7.0E-05
209309	Family	Lachnospiraceae	-3.639	1.042	0.002
320120	Genus	Clostridium XIVa	-3.670	0.811	5.9E-05
1628488	Species	Vallitalea guaymasensis	-4.144	1.538	0.027
48282	Family	Lachnospiraceae	-4.653	1.012	4.5E-05
77091	Genus	Clostridium	-7.493	1.192	7.9E-09

Table 13. Significant differentially abundant taxa between D-14 and D34 timepoints in the MS study. A positive fold change is interpreted as increased at D34 when compared to D-14.

Summary

Blautix effects a significant increase in microbiota diversity and results in significant temporal variation during treatment in an animal model for multiple sclerosis.

The invention has been described above by way of example only and it will be understood that further modifications may be made which fall within the scope of the claims.

Sequences

SEQ ID NO:1 (*Blautia stercoris* strain GAM6-1 16S ribosomal RNA gene, partial sequence - HM626177)

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  1 tgcaagtcga gcgaagcgct tacgacagaa ccttcggggg aagatgtaag ggactgagcg
 61 gcggacgggt gagtaacgcg tgggtaacct gcctcataca gggggataac agttggaaac
121 ggctgctaat accgcataag cgcacggtat cgcatgatac agtgtgaaaa actccggtgg
181 tatgagatgg acccgcgtct gattagctag ttggaggggt aacggcccac caaggcgacg
241 atcagtagcc ggcctgagag ggtgaacggc cacattggga ctgagacacg gcccagactc
301 ctacgggagg cagcagtggg gaatattgca caatggggga aaccctgatg cagcgacgcc
361 gcgtgaagga agaagtatct cggtatgtaa acttctatca gcagggaaga aaatgacggt
421 acctgactaa gaagccccgg ctaactacgt gccagcagcc gcggtaatac gtagggggca
481 agcgttatcc ggatttactg ggtgtaaagg gagcgtagac ggaagagcaa gtctgatgtg
541 aaaggctggg gcttaacccc aggactgcat tggaaactgt ttttcttgag tgccggagag
601 gtaagcggaa ttcctagtgt agcggtgaaa tgcgtagata ttaggaggaa caccagtggc
661 gaaggcggct tactggacgg taactgacgt tgaggctcga aagcgtgggg agcaaacagg
721 attagatacc ctggtagtcc acgccgtaaa cgatgaatac taggtgttgg ggagcaaagc
781 tcttcggtgc cgcagcaaac gcaataagta ttccacctgg ggagtacgtt cgcaagaatg
841 aaactcaaag gaattgacgg ggacccgcac aagcggtgga gcatgtggtt taattcgaag
901 caacgcgaag aaccttacca agtcttgaca tcgatctgac cggttcgtaa tggaaccttt
```

```
 961 ccttcgggac agagaagaca ggtggtgcat ggttgtcgtc agctcgtgtc gtgagatgtt
1021 gggttaagtc ccgcaacgag cgcaacccct atcctcagta gccagcaggt gaagctgggc
1081 actctgtgga gactgccagg gataacctgg aggaaggcgg ggacgacgtc aaatcatcat
1141 gccccttatg atttgggcta cacacgtgct acaatggcgt aaacaaaggg aagcgagccc
1201 gcgagggggga gcaaatccca aaaataacgt cccagttcgg actgcagtct gcaactcgac
1261 tgcacgaagc tggaatcgct agtaatcgcg aatcagaatg tcgcggtgaa tacgttcccg
1321 ggtcttgtac acaccgcccg tcacaccatg ggagtcagta acgcccgaag tc
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SEQ ID NO:2 (*Blautia wexlerae* strain WAL 14507 16S ribosomal RNA gene, partial sequence - EF036467)

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   1 caagtcgaac gggaattant ttattgaaac ttcggtcgat ttaatttaat tctagtggcg
  61 gacgggtgag taacgcgtgg gtaacctgcc ttatacaggg ggataacagt cagaaatggc
 121 tgctaatacc gcataagcgc acagagctgc atggctcagt gtgaaaaact ccggtggtat
 181 aagatggacc cgcgttggat tagcttgttg gtggggtaac ggcccaccaa ggcgacgatc
 241 catagccggc ctgagagggt gaacggccac attgggactg agacacggcc cagactccta
 301 cgggaggcag cagtggggaa tattgcacaa tgggggaaac cctgatgcag cgacgccgcg
 361 tgaaggaaga agtatctcgg tatgtaaact tctatcagca gggaagatag tgacggtacc
 421 tgactaagaa gccccggcta actacgtgcc agcagccgcg gtaatacgta gggggcaagc
 481 gttatccgga tttactgggt gtaaagggag cgtagacggt gtggcaagtc tgatgtgaaa
 541 ggcatgggct caacctgtgg actgcattgg aaactgtcat acttgagtgc cggaggggta
 601 agcggaattc ctagtgtagc ggtgaaatgc gtagatatta ggaggaacac cagtggcgaa
 661 ggcggcttac tggacggtaa ctgacgttga ggctcgaaag cgtggggagc aaacaggatt
 721 agataccctg gtagtccacg ccgtaaacga tgaataacta ggtgtcgggt ggcaaagcca
 781 ttcggtgccg tcgcaaacgc agtaagtatt ccacctgggg agtacgttcg caagaatgaa
 841 actcaaagga attgacgggg acccgcacaa gcggtggagc atgtggttta attcgaagca
 901 acgcgaagaa ccttaccaag tcttgacatc cgcctgaccg atccttaacc ggatctttcc
 961 ttcgggacag gcgagacagg tggtgcatgg ttgtcgtcag ctcgtgtcgt gagatgttgg
1021 gttaagtccc gcaacgagcg caacccctat cctcagtagc cagcatttaa ggtgggcact
1081 ctggggagac tgccagggat aacctggagg aaggcgggga tgacgtcaaa tcatcatgcc
1141 ccttatgatt tgggctacac acgtgctaca atggcgtaaa caaagggaag cgagattgtg
1201 agatggagca aatcccaaaa ataacgtccc agttcggact gtagtctgca acccgactac
1261 acgaagctgg aatcgctagt aatcgcggat cagaatgccg cggtgaatac gttcccgggt
1321 cttgtacaca ccgcccgtca caccatggga gtcagtaacg cccgaagtca gtgacctaac
1381 tgcaaagaag gagctgccga aggcgggacc gatgactggg gtgaagtcgt aacaaggt
```

SEQ ID NO:3 (consensus 16S rRNA sequence for *Blautia stercoris* strain 830)

```
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GTTGGAAACGGCTGCTAATACCGCATAAGCGCACAGTATCGCATGATACAGTGTGAAAAACTCCGGTGGTATGAGAT
GGACCCGCGTCTGATTAGCTAGTTGGAGGGGTAACGGCCCACCAAGGCGACGATCAGTAGCCGGCCTGAGAGGGTGA
ACGGCCACATTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAAA
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TACCTGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTATCCGGATTT
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ACCATGGGAGTCAGTAACGCCCGAAGTCAGTGACCCAACCTTAGGGAGGGAGCTGCCGAAGGCGGGATTGATAACTG
GGGTGAAGTCTAGGGGGT

SEQ ID NO:4 (consensus 16S rRNA sequence for *Blautia wexlerae* strain MRX008)

TTCATTGAGACTTCGGTGGATTTAGATTCTATTTCTAGTGGCGGACGGGTGAGTAACGCGTGGGTAACCTGCCTTAT
ACAGGGGGATAACAGTCAGAAATGGCTGCTAATACCGCATAAGCGCACAGAGCTGCATGGCTCAGTGTGAAAAACTC
CGGTGGTATAAGATGGACCCGCGTTGGATTAGCTTGTTGGTGGGGTAACGGCCCACCAAGGCGACGATCCATAGCCG
GCCTGAGAGGGTGAACGGCCACATTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
CACAATGGGGGAAACCCTGATGCAGCGACGCCGCGTGAAGGAAGAAGTATCTCGGTATGTAAACTTCTATCAGCAGG
GAAGATAGTGACGGTACCTGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAG
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GTGGACTGCATTGGAAACTGTCATACTTGAGTGCCGGAGGGGTAAGCGGAATTCCTAGTGTAGCGGTGAAATGCGTA
GATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTACTGGACGGTAACTGACGTTGAGGCTCGAAAGCGTGGGGAGC
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CCGTCGCAAACGCAGTAAGTATTCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGGGACCC
GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAGTCTTGACATCCGCCTGACCGA
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GGGTTAAGTCCCGCAACGAGCGCAACCCCTATCCTCAGTAGCCAGCATTTAAGGTGGGCACTCTGGGGAGACTGCCA
GGGATAACCTGGAGGAAGGCGGGGATGACGTCAAATCATCATGCCCCTTATGATTTGGGCTACACACGTGCTACAAT
GGCGTAAACAAAGGGAAGCGAGATCGTGAGATGGAGCAAATCCCAAAAATAACGTCCCAGTTCGGACTGTAGTCTGC
AACCCGACTACACGAAGCTGGAATCGCTAGTAATCGCGGATCAGAATGCCGCGGTGAATACGTTCCCGGGTCTTGTA
CACACCGCCCGTCACACCATGGGAGTCAGTAACGCCCGAAGTCAGTGACCTAACTGCAAAGAAGGAGCTGCCGAA

SEQ ID NO:5 (*Blautia hydrogenotrophica* strain S5a36 16S ribosomal RNA gene, partial sequence - X95624.1)

```
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 421 acttctatca gcagggaaga aagtgacggt acctgactaa gaagccccgg ctaattacgt
 481 gccagcagcc gcggtaatac gtaaggggca agcgttatcc ggatttactg ggtgtaaagg
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 601 tggaaactgt cagacttgag tgccggagag gcaagcggaa ttcctagtgt agcggtgaaa
 661 tgcgtagata ttaggaggaa caccagtggc gaaggcggcc tgctggacgg taactgacgt
 721 tgaggctcga aagcgtgggg agcaaacagg attagatacc ctggtagtcc acgctgtaaa
 781 cgatgaatac taggtgtcgg gtggcaaagc cattcggtgc cgcagcaaac gcaataagta
 841 ttcccacctg gggagtacgt tcgcaagaat gaaactcaaa ggaattgacg gggacccgca
 901 caagcggtgg agcatgtggt ttaattcgaa gcaacgcgaa gaaccttacc aaatcttgac
 961 atccctctga ccgggaagta atgttccctt ttcttcggaa cagaggagac aggtggtgca
1021 tggttgtcgt cagctcgtgt cgtgagatgt tgggttaagt cccgcaacga gcgcaaccct
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1321 gaatcagaat gtcgcggtga atacgttccc gggtcttgta cacaccgccc gtcacaccat
1381 gggagtcagt aacgcccgaa gtcagtgacc caaccnaaag gagggagctg ccgaaggtgg
1441 gactgataac tggggtga
```

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CLAIMS

1. A method of treating or preventing a disease associated with a level of microbiota diversity that is reduced relative to the microbiota diversity of a healthy subject and/or has reduced stability of its microbiota compared to a healthy subject comprising administering a composition comprising a bacterial strain of the species *Blautia hydrogenotrophica* to a subject, wherein the disease is irritable bowel syndrome, one or more autoimmune diseases or one or more allergic diseases and wherein the method comprises increasing the microbiota diversity in the subject and/or inducing stability of the microbiota in the subject.

2. Use of a composition comprising a bacterial strain of the species *Blautia hydrogenotrophica* in the manufacture of a medicament for treating or preventing a disease associated with a level of microbiota diversity that is reduced relative to the microbiota diversity of a healthy subject and/or has reduced stability of its microbiota compared to a healthy subject in a subject, wherein the disease is irritable bowel syndrome, one or more autoimmune diseases or one or more allergic diseases and wherein the medicament increases the microbiota diversity in the subject and/or induces stability of the microbiota in the subject.

3. The method according to claim 1, or the use according to claim 2, wherein the subject has less than 99 different bacterial species and/or less than 190 different bacterial strains in its microbiota.

4. The method according to claim 1 or claim 3, or the use according to claim 2 or claim 3, wherein the increase in microbiota diversity and/or induction of stability of microbiota is for non-acetogenic bacteria.

5. The method according to any one of claims 1, 3 or 4, or the use according to any one of claims 2-4, wherein the increase in microbiota diversity and/or induction of stability of microbiota is for both acetogenic and non-acetogenic bacteria.

6. The method according to any one of claims 1 or 3-5, wherein the method is for treating or preventing one or more allergic diseases, for example, asthma; or the use according to any one of claims 2-5, wherein the medicament is for treating or preventing one or more allergic diseases, for example, asthma.

7. The method according to any one of claims 1 or 3-5, wherein the method is for treating or preventing one or more autoimmune diseases, for example, rheumatoid arthritis or multiple sclerosis; or the use according to any one of claims 2-5, wherein the medicament is for treating or preventing one or more autoimmune diseases, for example, rheumatoid arthritis or multiple sclerosis.

8. The method according to any one of claims 1 or 3-7, or the use according to any one of claims 2-7, wherein the subject is a frail elderly subject.

9. The method according to any one of claims 1 or 3-7, or the use according to any one of claims 2-7, wherein the subject is an infant who has been delivered by Caesarean section.

10. The method according to any one of claims 1 or 3-9, or the use according to any one of claims 2-9, wherein the microbiota diversity is increased and/or stability of the microbiota is induced in the intestine of the subject.

11. The method according to any one of claims 1 or 3-10, or the use according to any one of claims 2-10, wherein the microbiota diversity is increased and/or stability of the microbiota is induced in the distal gut of the subject.

12. The method according to any one of claims 1 or 3-11, or the use according to any one of claims 2-11, wherein the bacterial strain is the *Blautia hydrogenotrophica* bacterium deposited under accession number DSM 10507 and also under accession number DSM 14294.

13. The method according to any one of claims 1 or 3-12, wherein the composition is administered orally; or the use according to any one of claims 2-12, wherein the medicament is provided for oral administration.

14. The method according to any one of claims 1 or 3-13, or the use according to any one of claims 2-13, wherein the composition comprises one or more pharmaceutically acceptable excipients or carriers.

15. The method according to any one of claims 1 or 3-14, or the use according to any one of claims 2-14, wherein the bacterial strain is lyophilised.

16. The method according to any one of claims 1 or 3-15, or the use according to any one of claims 2-15, wherein the bacterial strain is viable.

17. The method according to any one of claims 1 or 3-16, or the use according to any one of claims 2-16, wherein the bacterial strain is capable of partially or totally colonising the intestine.

18. The method according to any one of claims 1 or 3-17, wherein the composition comprises a single strain of *Blautia hydrogenotrophica*, or the use according to any one of claims 2-17, wherein the medicament comprises a single strain of *Blautia hydrogenotrophica*.

19. The method according to any one of claims 1 or 3-18, wherein the composition comprises *Blautia hydrogenotrophica* and does not comprise bacteria from any other genus or comprises such other bacteria only in *de minimis* amounts; or the use according to any one of claims 2-18, wherein the medicament comprises *Blautia hydrogenotrophica* and does not comprise bacteria from any other genus or comprises such other bacteria only in *de minimis* amounts.

20. The method according to any one of claims 1 or 3-18, wherein the composition comprises *Blautia hydrogenotrophica* as part of a microbial consortium; or the use according to any one of claims 2-18, wherein the medicament comprises *Blautia hydrogenotrophica* as part of a microbial consortium.

21. A food product comprising a composition comprising a bacterial strain of the species *Blautia hydrogenotrophica*, when used in a method according to any one of claims 1 or 3-20.

22. A vaccine composition comprising a composition comprising a bacterial strain of the species *Blautia hydrogenotrophica*, when used in a method according to any one of claims 1 or 3-20.

23. Use of:

a) a food product comprising a composition comprising a bacterial strain of the species *Blautia hydrogenotrophica*; or

b) a vaccine comprising a composition comprising a bacterial strain of the species *Blautia hydrogenotrophica;*

in the manufacture of a medicament for treating or preventing a disease associated with a level of microbiota diversity that is reduced relative to the microbiota diversity of a healthy subject and/or has reduced stability of its microbiota compared to a healthy subject in a subject, wherein the disease is irritable bowel syndrome, one or more autoimmune diseases or one or more allergic diseases and wherein the medicament increases the microbiota diversity in the subject and/or induces stability of the microbiota in the subject.

24. A method of treating or preventing IBS, an autoimmune disease or an allergic disease in a subject, wherein the method comprises diagnosing the subject as having a reduced level of intestinal microbiota diversity and/or reduced stability of its intestinal microbiota relative to the microbiota diversity of a healthy subject and then administering a composition comprising a bacterial strain of the species *Blautia hydrogenotrophica* to the subject.

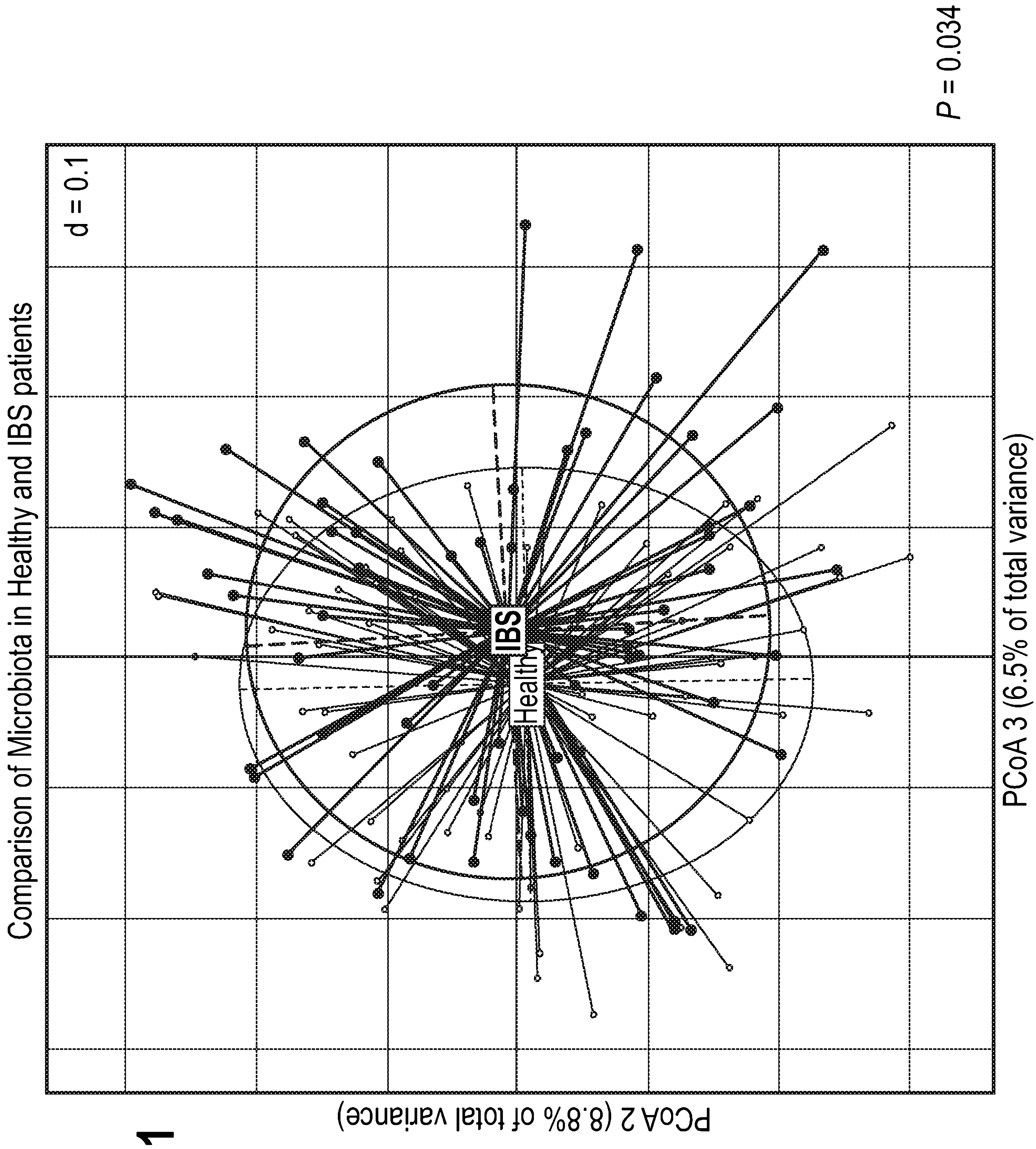

Comparison of Microbiota in Healthy and IBS patients
d = 0.1
IBS
Healthy
PCoA 3 (6.5% of total variance)
PCoA 2 (8.8% of total variance)
P = 0.034

FIG. 1

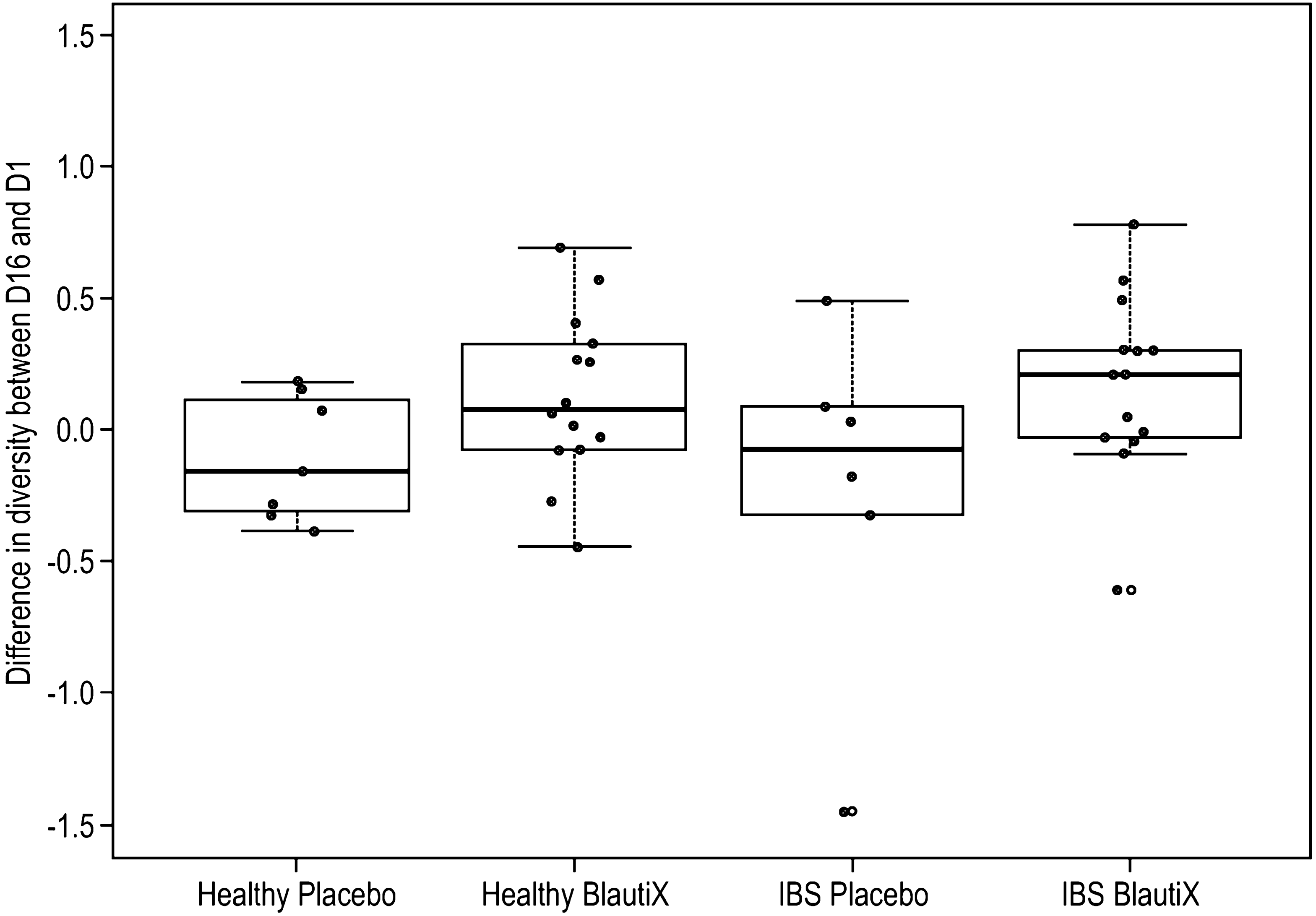


FIG. 2

Shannon Diversity
Day 16 Versus Day 1

FIG. 3A
Healthy individuals

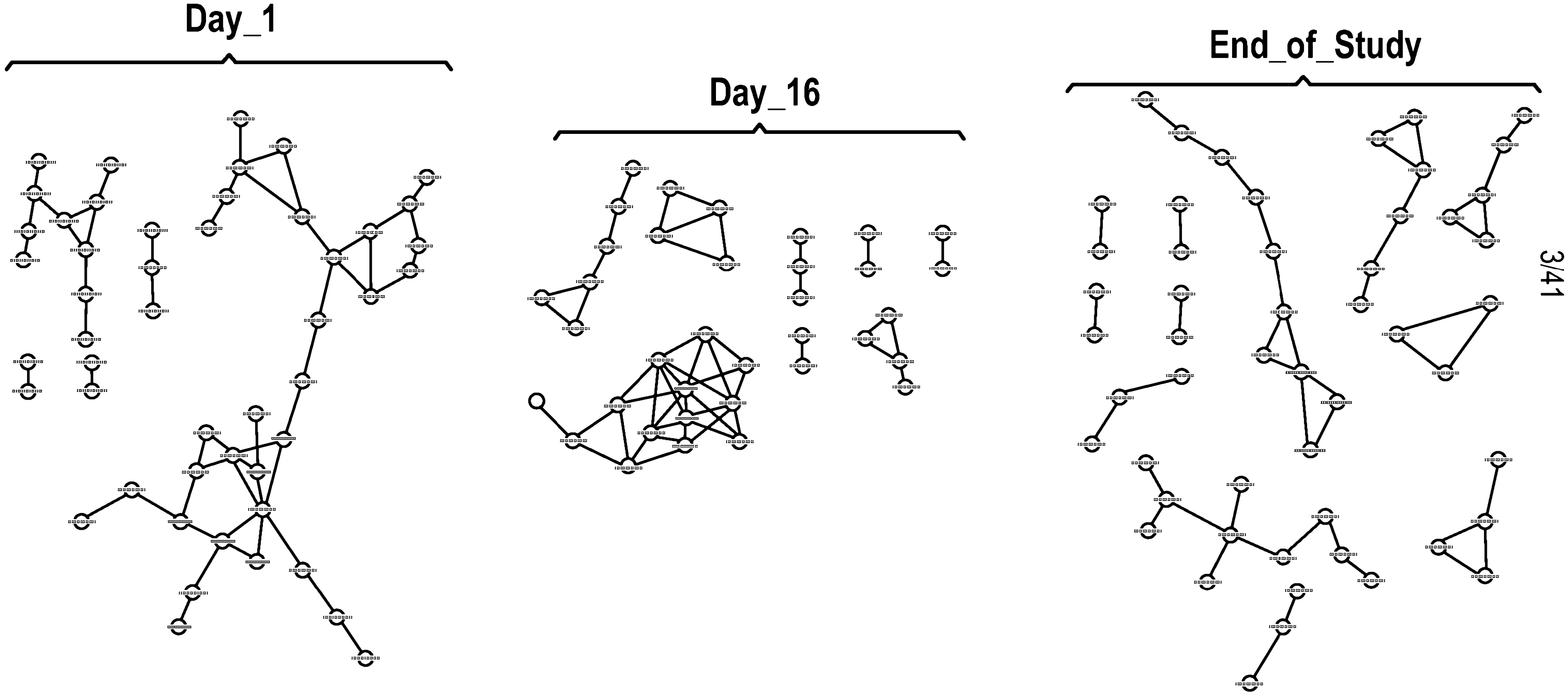
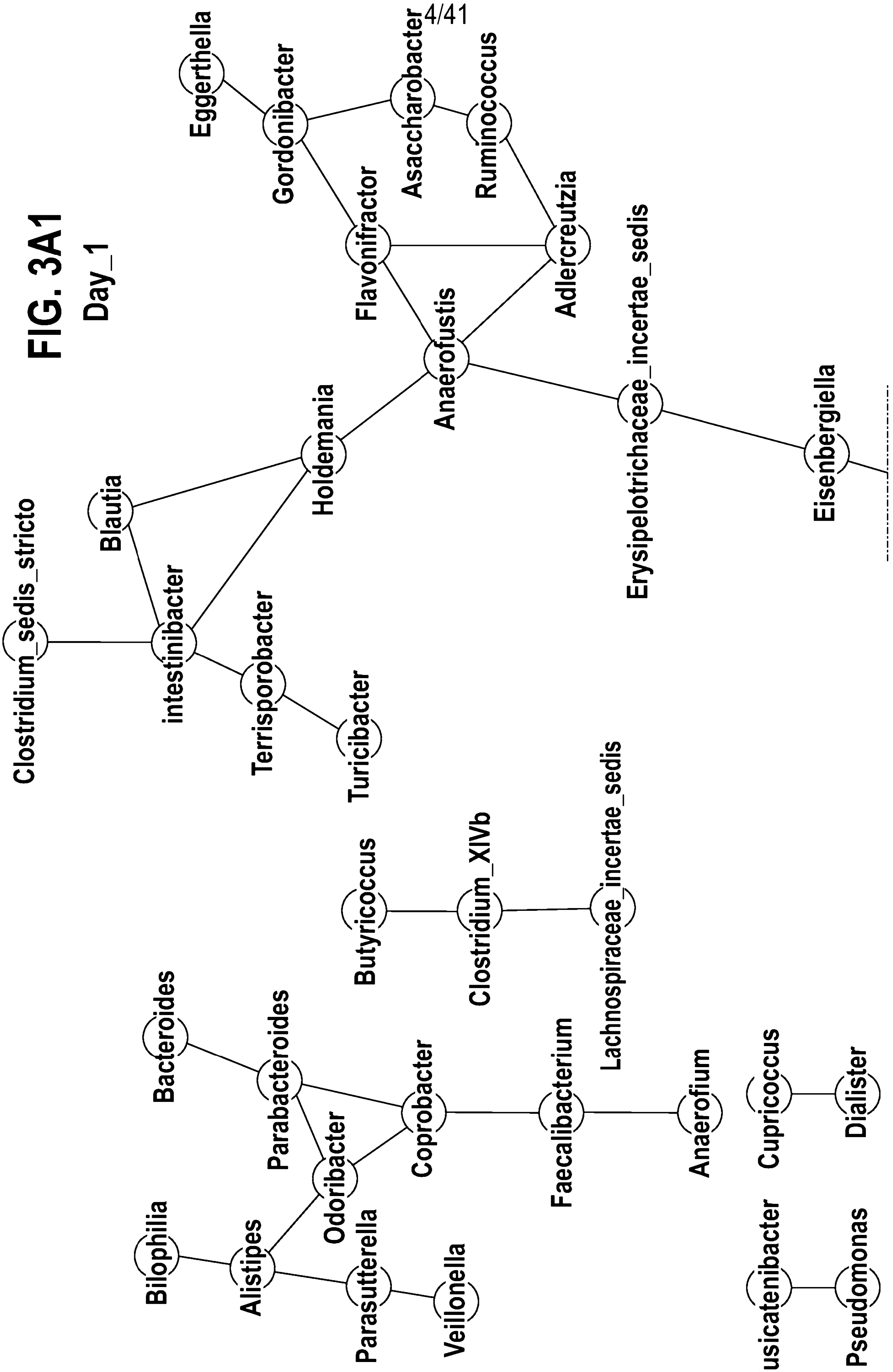


FIG. 3A1

Day_1


Eggerthella
Gordonibacter
Asaccharobacter
Ruminococcus
Flavonifractor
Adlercreutzia
Anaerofustis
Erysipelotrichaceae_incertae_sedis
Eisenbergiella
Holdemania
Blautia
Clostridium_sedis_stricto
intestinibacter
Terrisporobacter
Turicibacter
Butyricoccus
Clostridium_XlVb
Lachnospiraceae_incertae_sedis
Bacteroides
Parabacteroides
Coprobacter
Faecalibacterium
Anaerofium
Cupricoccus
Dialister
Bilophilia
Alistipes
Odoribacter
Parasutterella
Veillonella
Fusicatenibacter
Pseudomonas

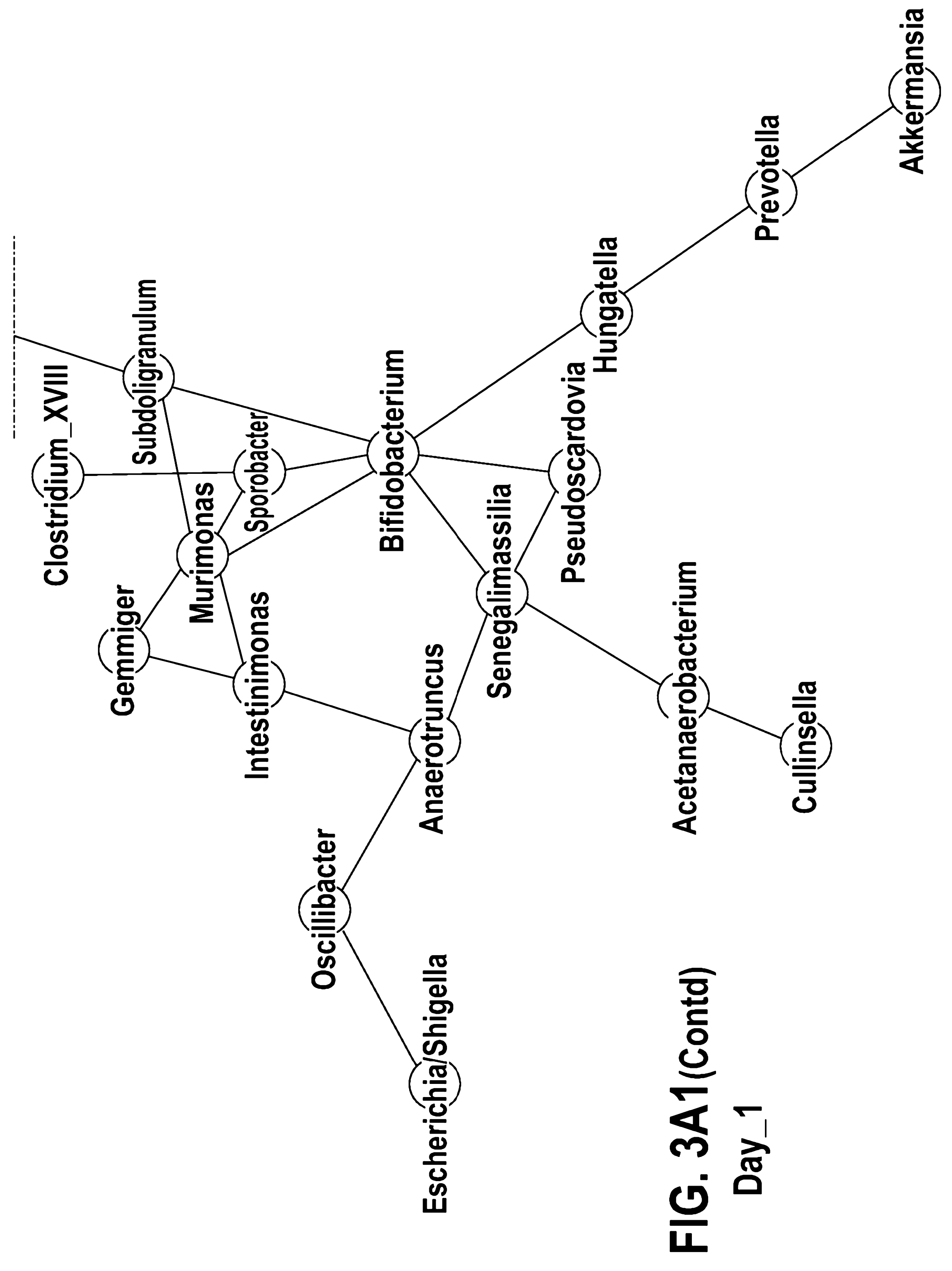


FIG. 3A1(Contd)
Day_1

FIG. 3A2

Day_16

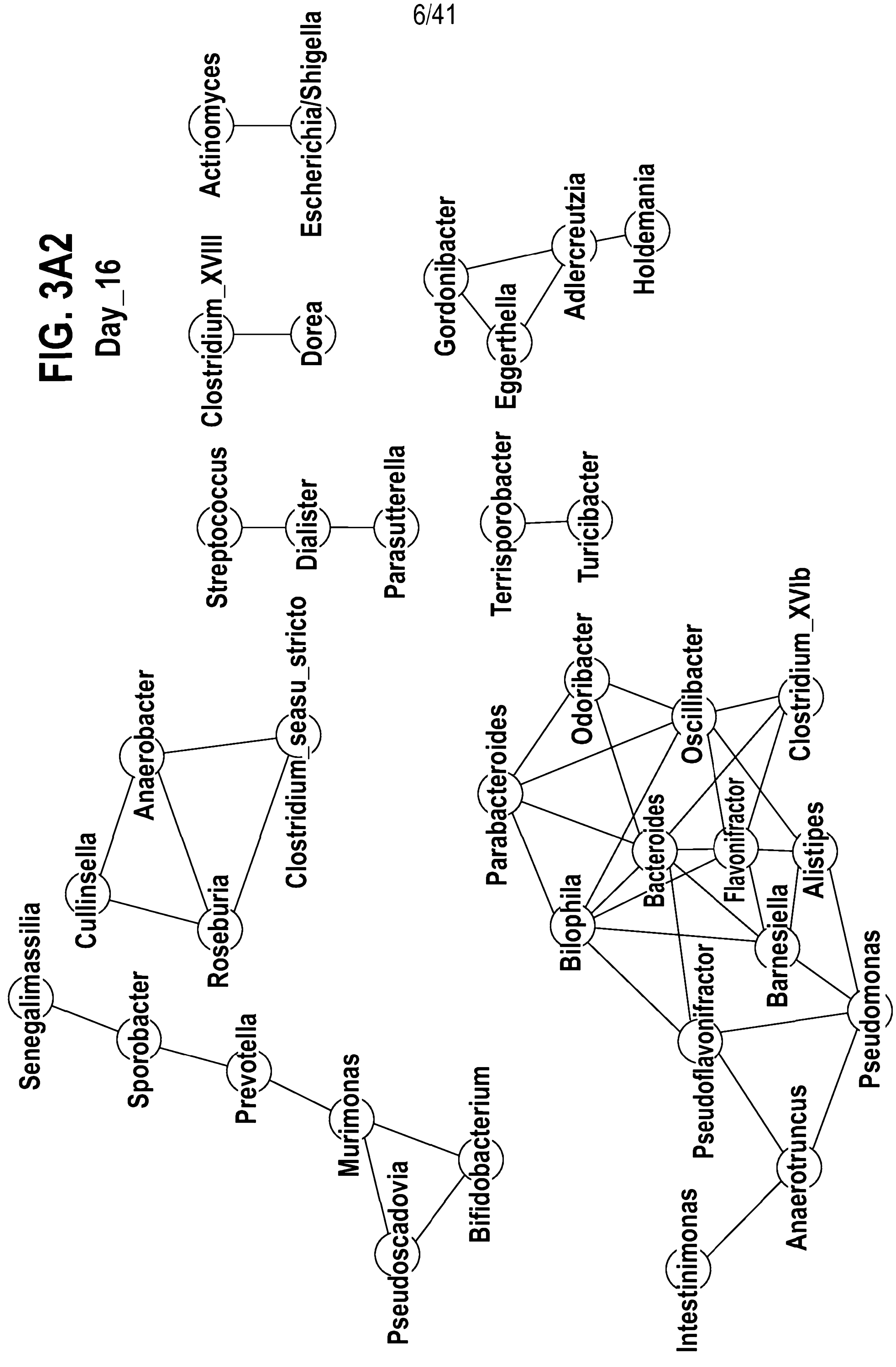
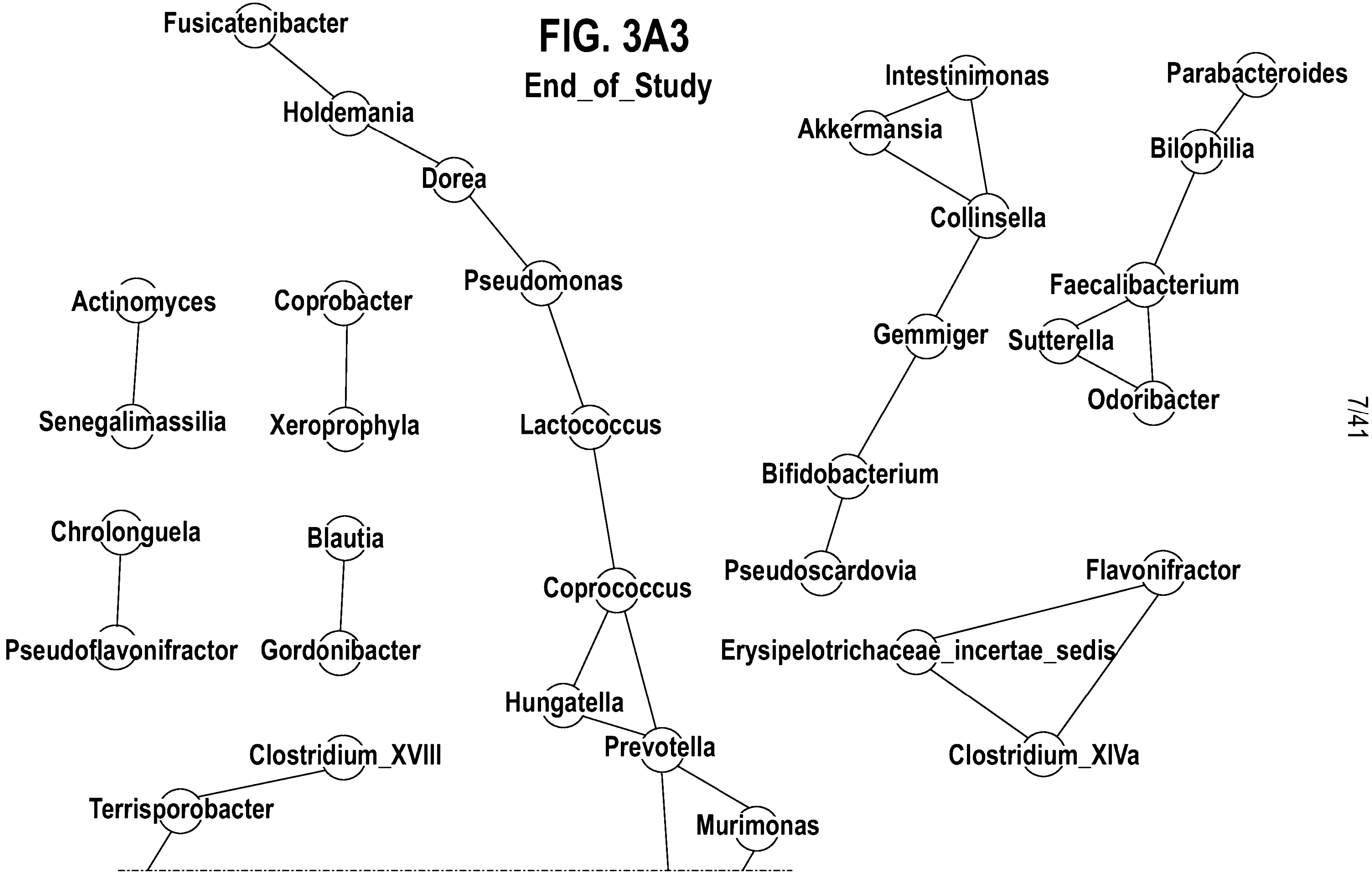


FIG. 3A3

End_of_Study



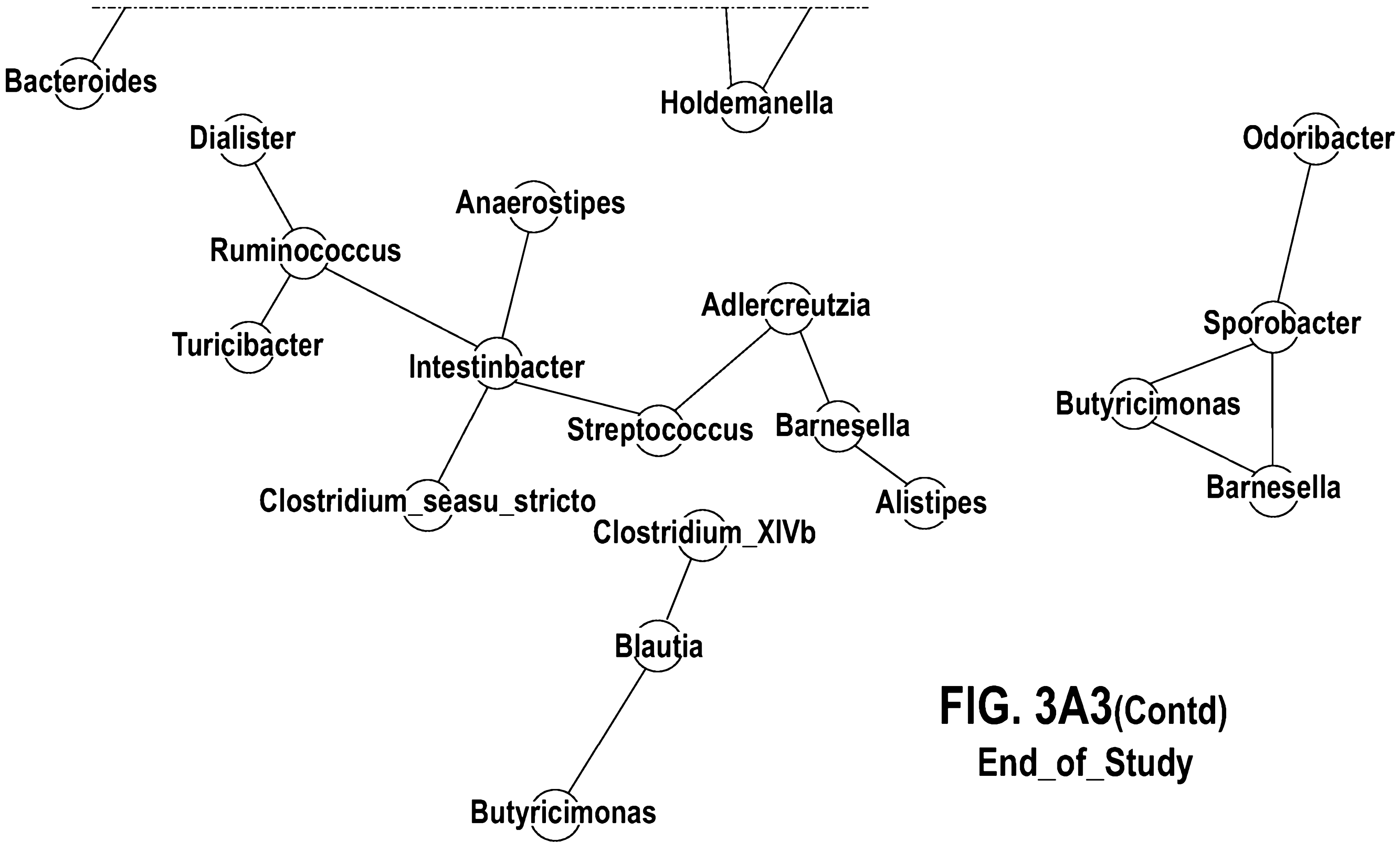


FIG. 3A3(Contd)
End_of_Study

FIG. 3B

IBS patients

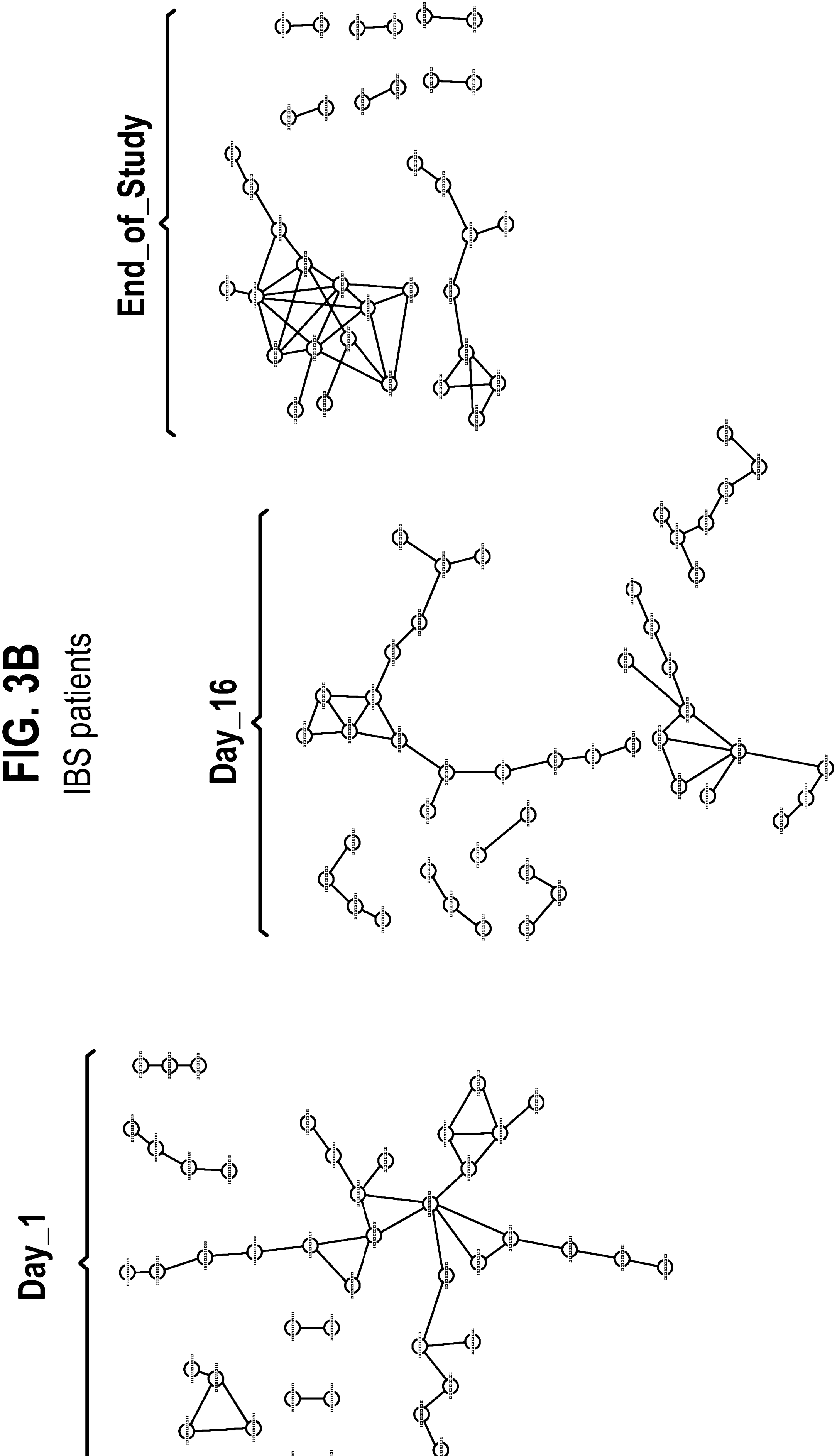
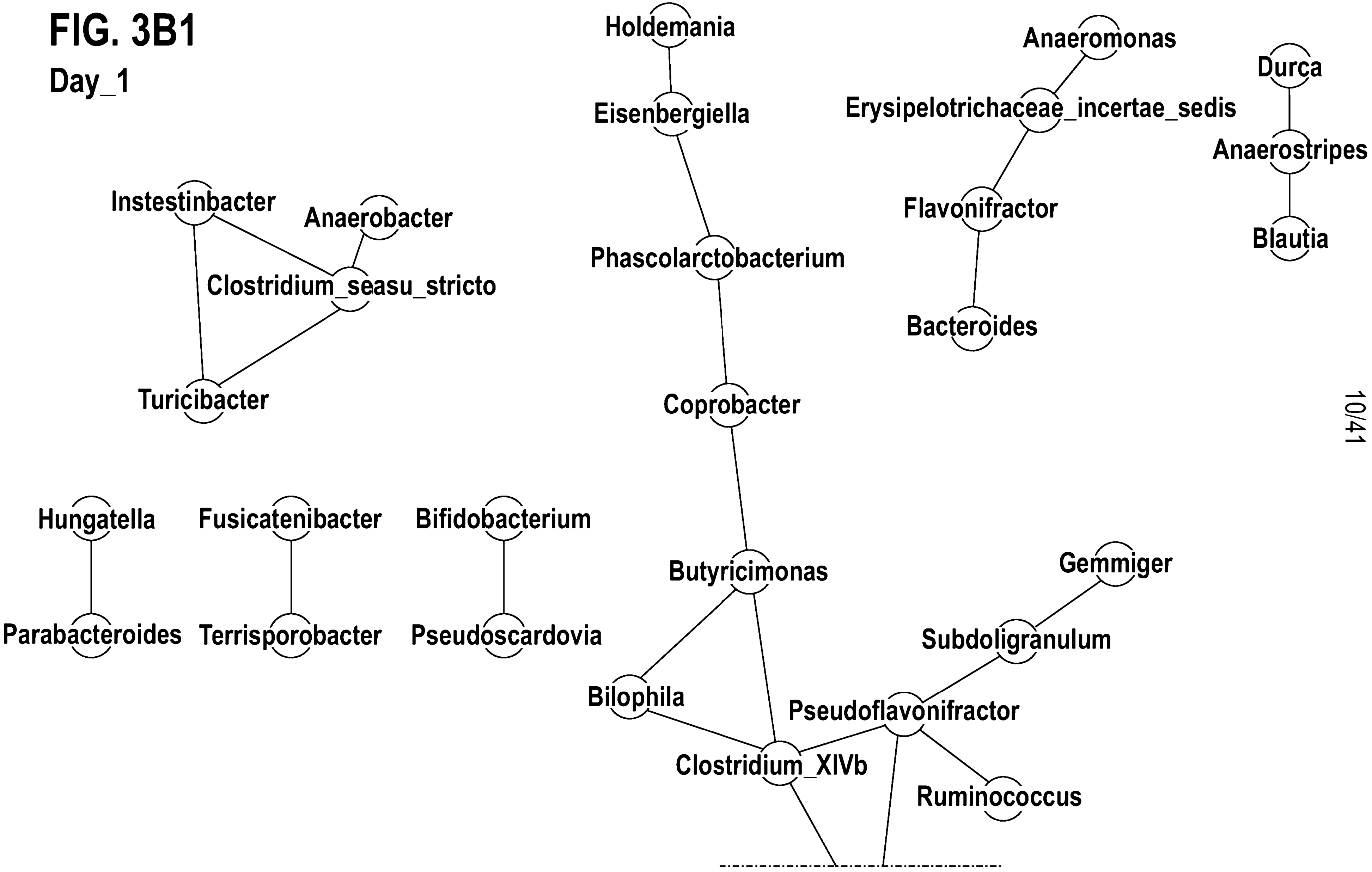


FIG. 3B1

Day_1


Holdemania
Eisenbergiella
Phascolarctobacterium
Coprobacter
Butyricimonas
Bilophila
Clostridium_XIVb
Pseudoflavonifractor
Subdoligranulum
Gemmiger
Ruminococcus
Anaeromonas
Erysipelotrichaceae_incertae_sedis
Flavonifractor
Bacteroides
Durca
Anaerostripes
Blautia
Instestinbacter
Anaerobacter
Clostridium_seasu_stricto
Turicibacter
Hungatella
Parabacteroides
Fusicatenibacter
Terrisporobacter
Bifidobacterium
Pseudoscardovia

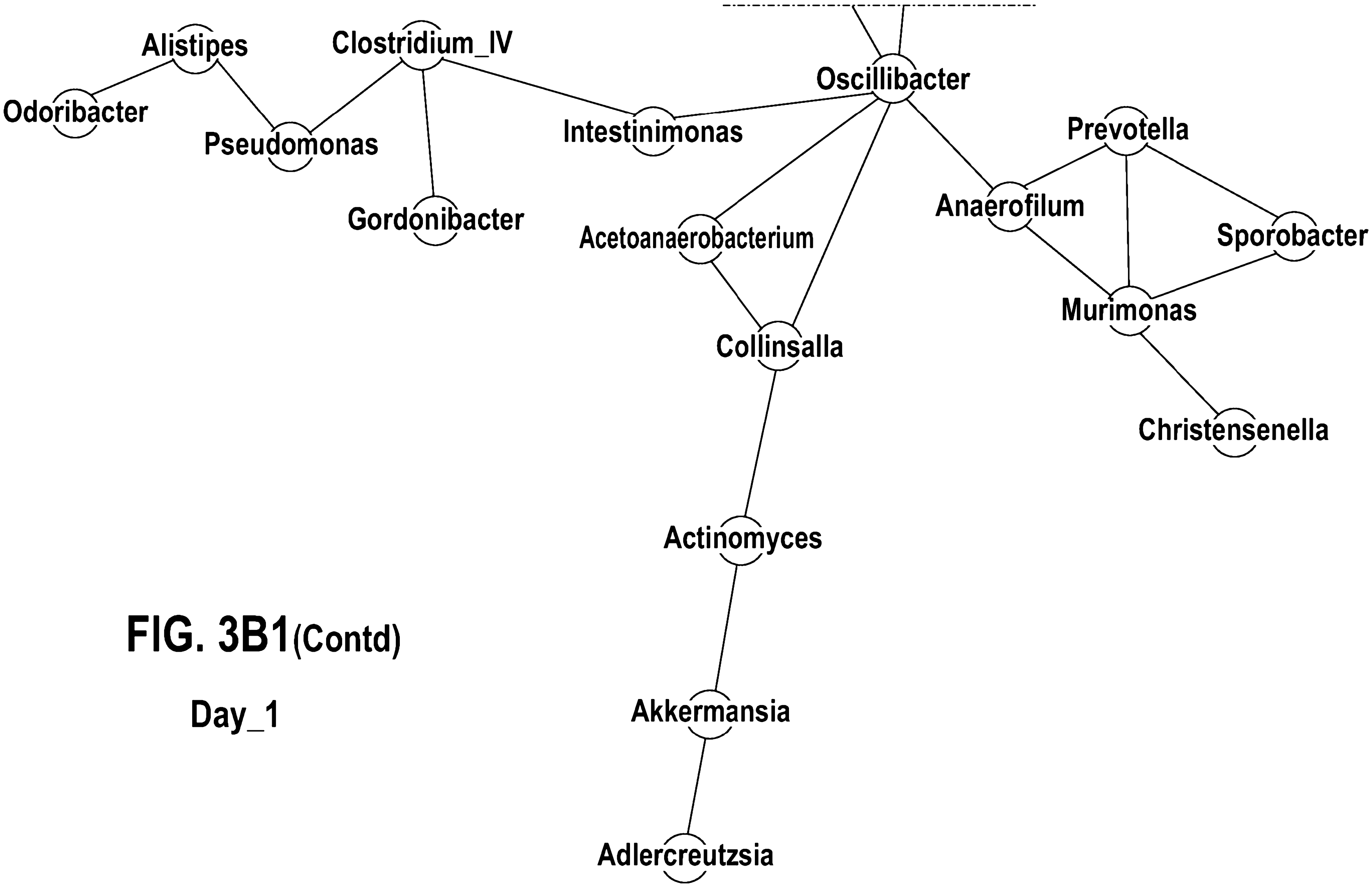
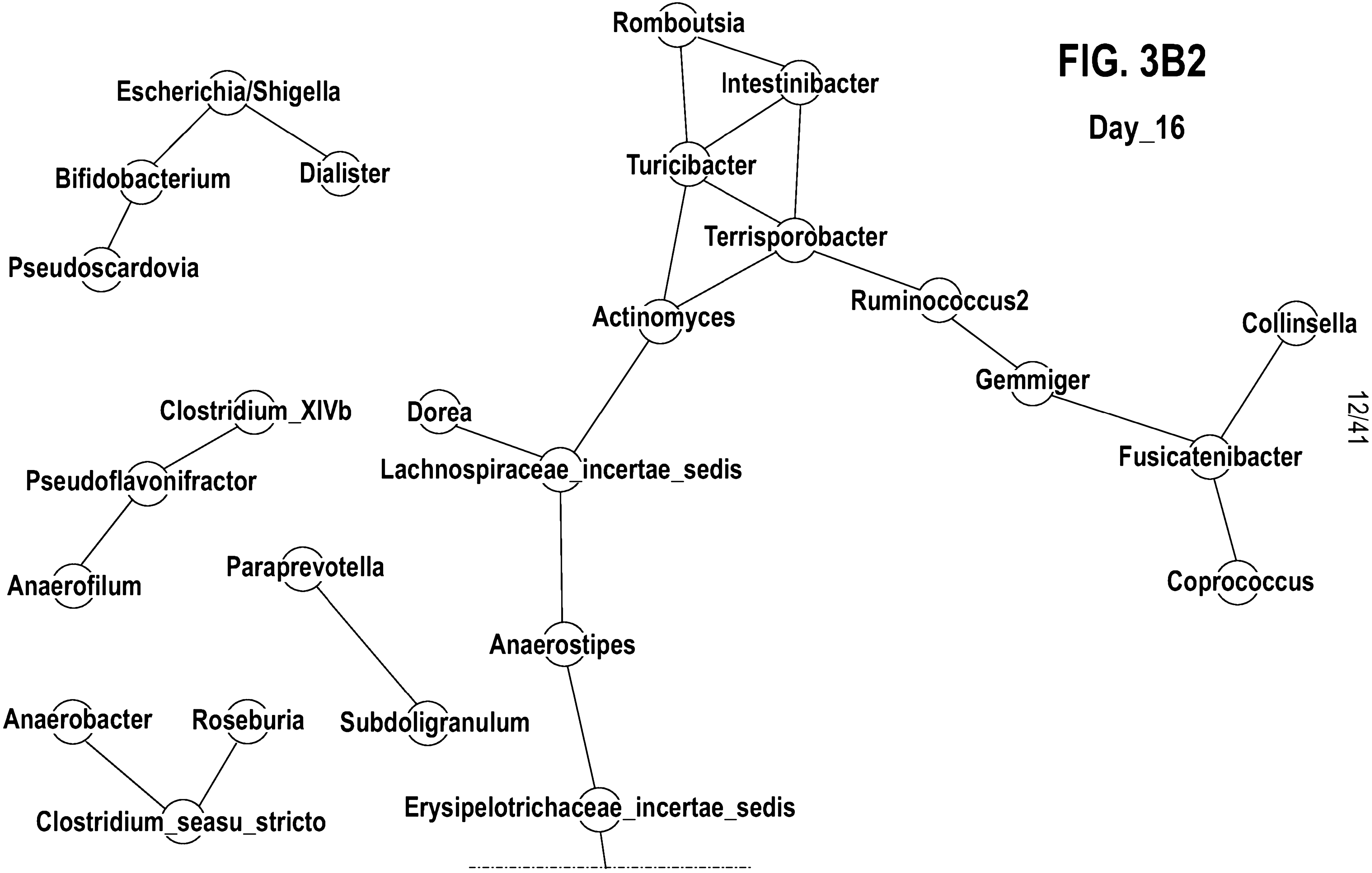


FIG. 3B1(Contd)

Day_1

FIG. 3B2

Day_16



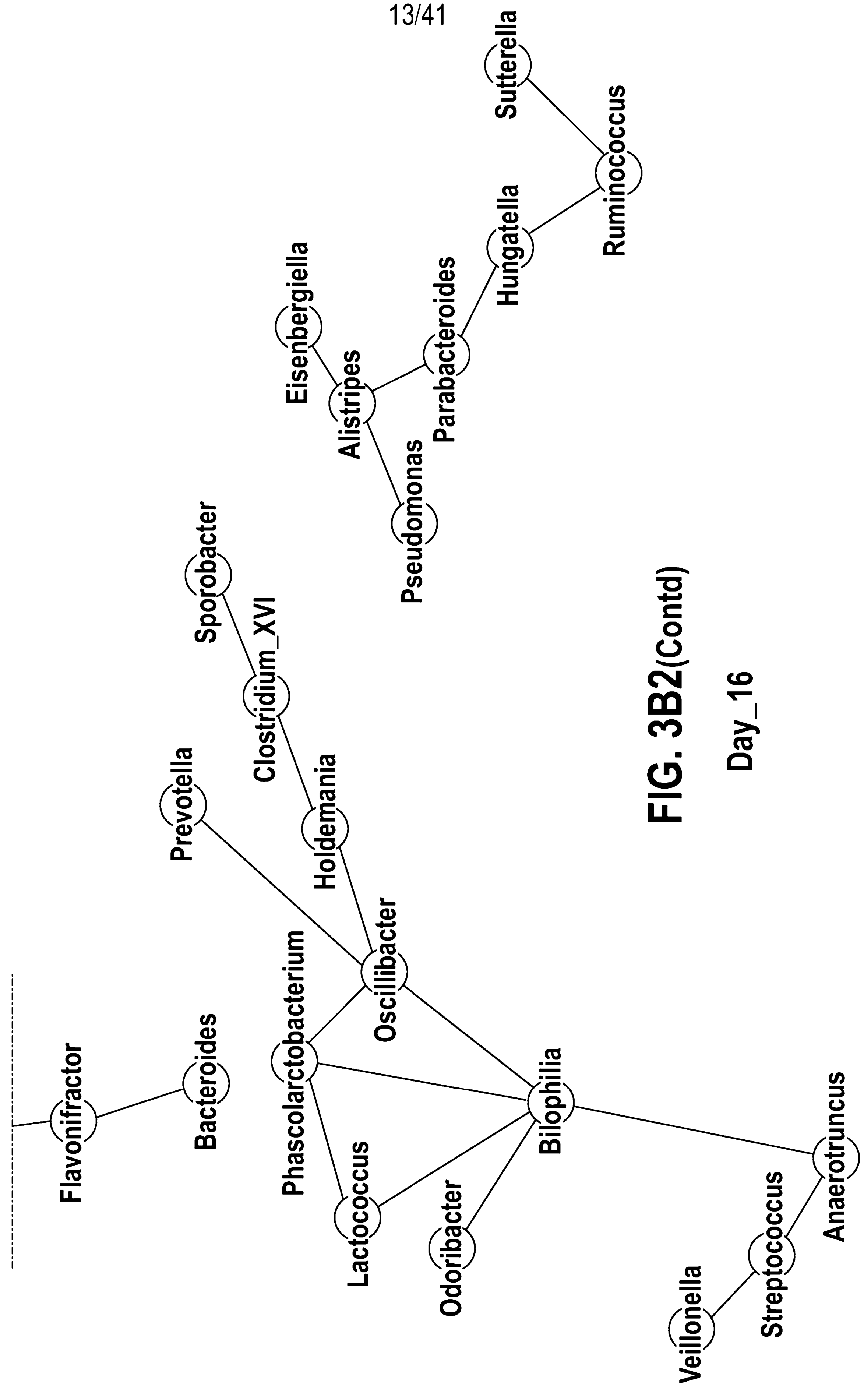


FIG. 3B2(Contd)

Day_16

FIG. 3B3

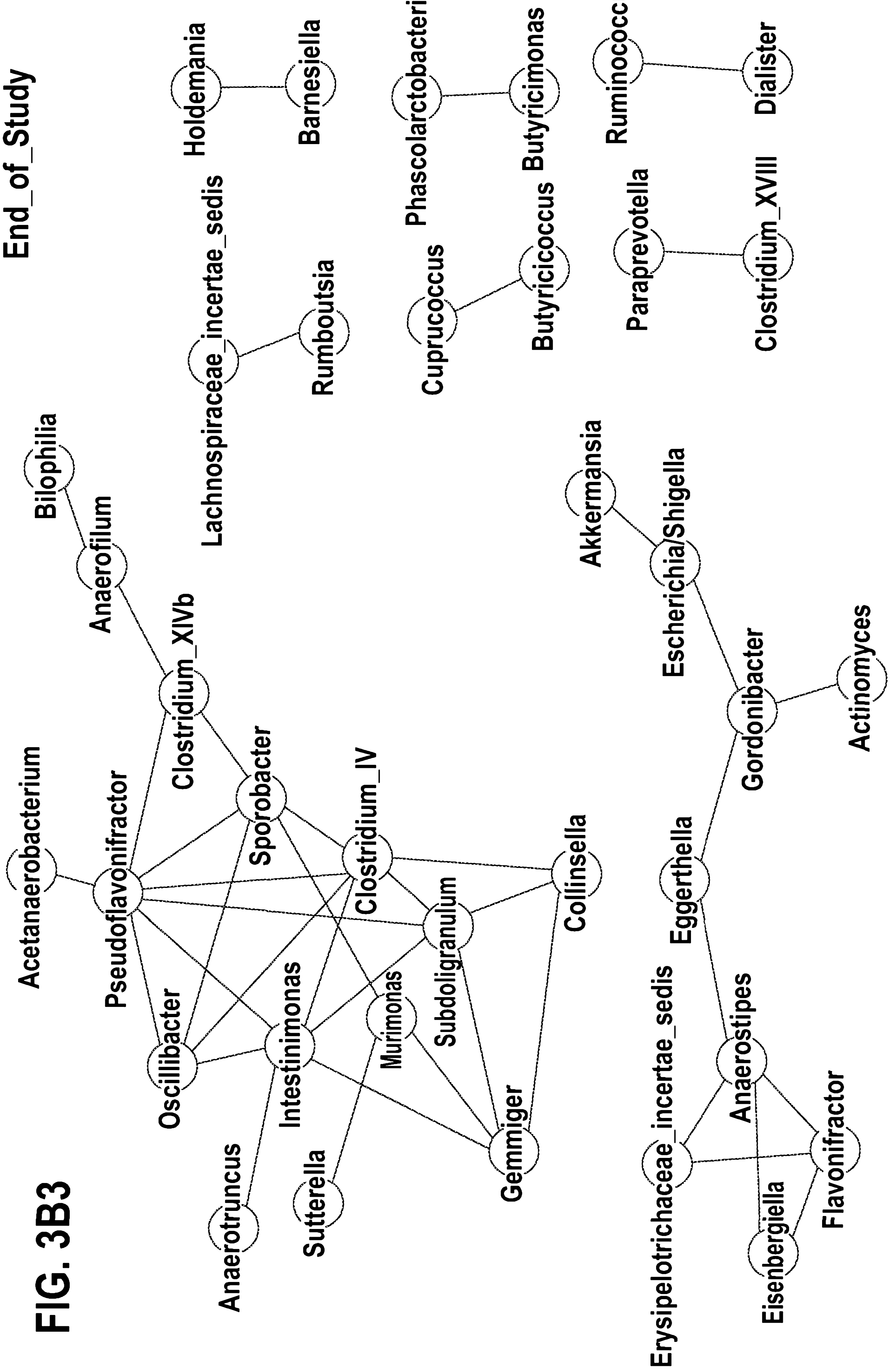

End_of_Study
Holdemania
Barnesiella
Lachnospiraceae_incertae_sedis
Rumboutsia
Phascolarctobacterium
Butyricimonas
Cuprucoccus
Butyricicoccus
Ruminococcus
Dialister
Paraprevotella
Clostridium_XVIII
Bilophilia
Anaerofilum
Clostridium_XIVb
Akkermansia
Escherichia/Shigella
Gordonibacter
Actinomyces
Acetanaerobacterium
Pseudoflavonifractor
Sporobacter
Clostridium_IV
Collinsella
Subdoligranulum
Eggerthella
Oscillibacter
Intestinimonas
Murimonas
Gemmiger
Anaerotruncus
Sutterella
Erysipelotrichaceae_incertae_sedis
Anaerostipes
Flavonifractor
Eisenbergiella

FIG. 4A

Bray-Curtis Dissimilarity between
Day 16 Versus Day 1

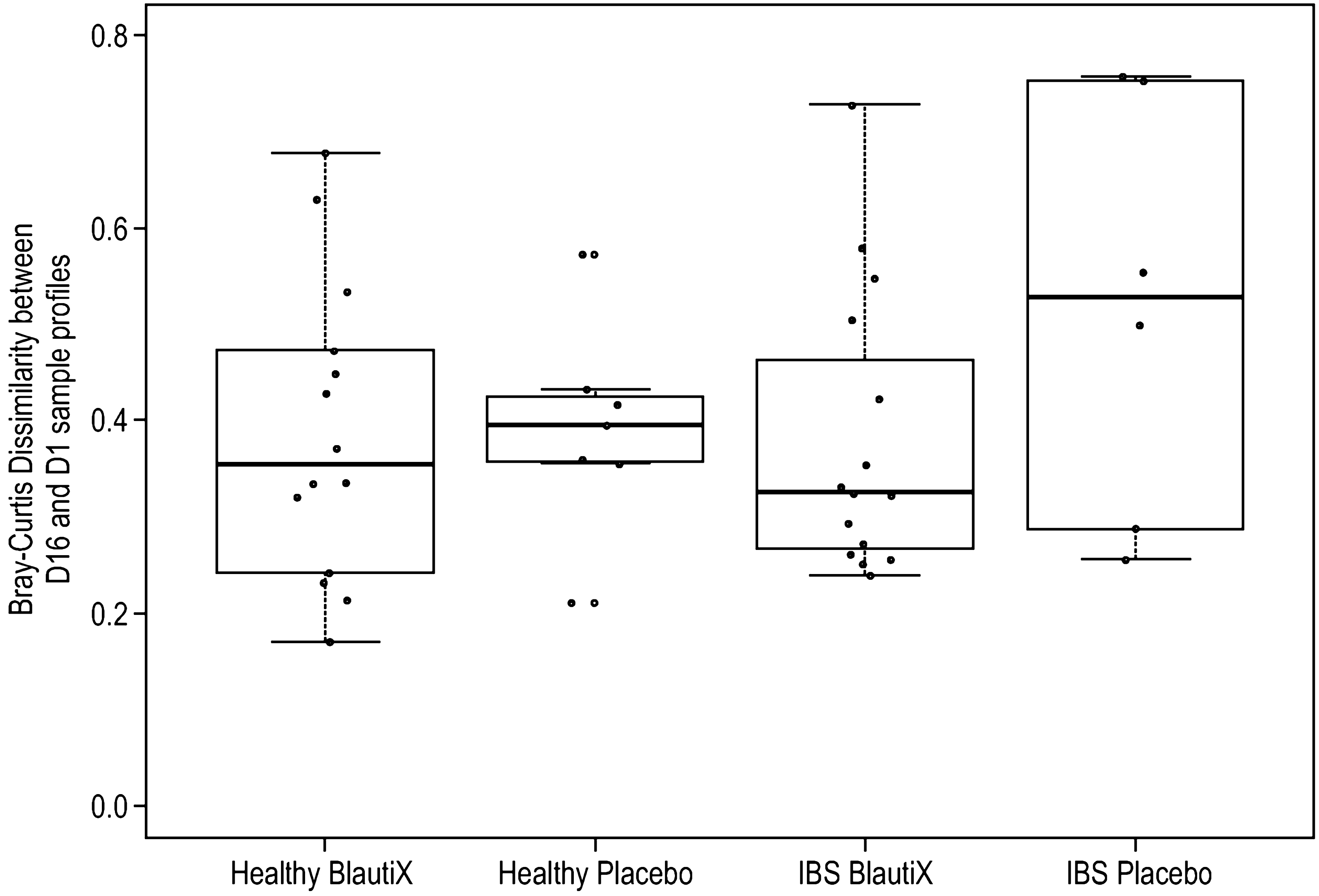
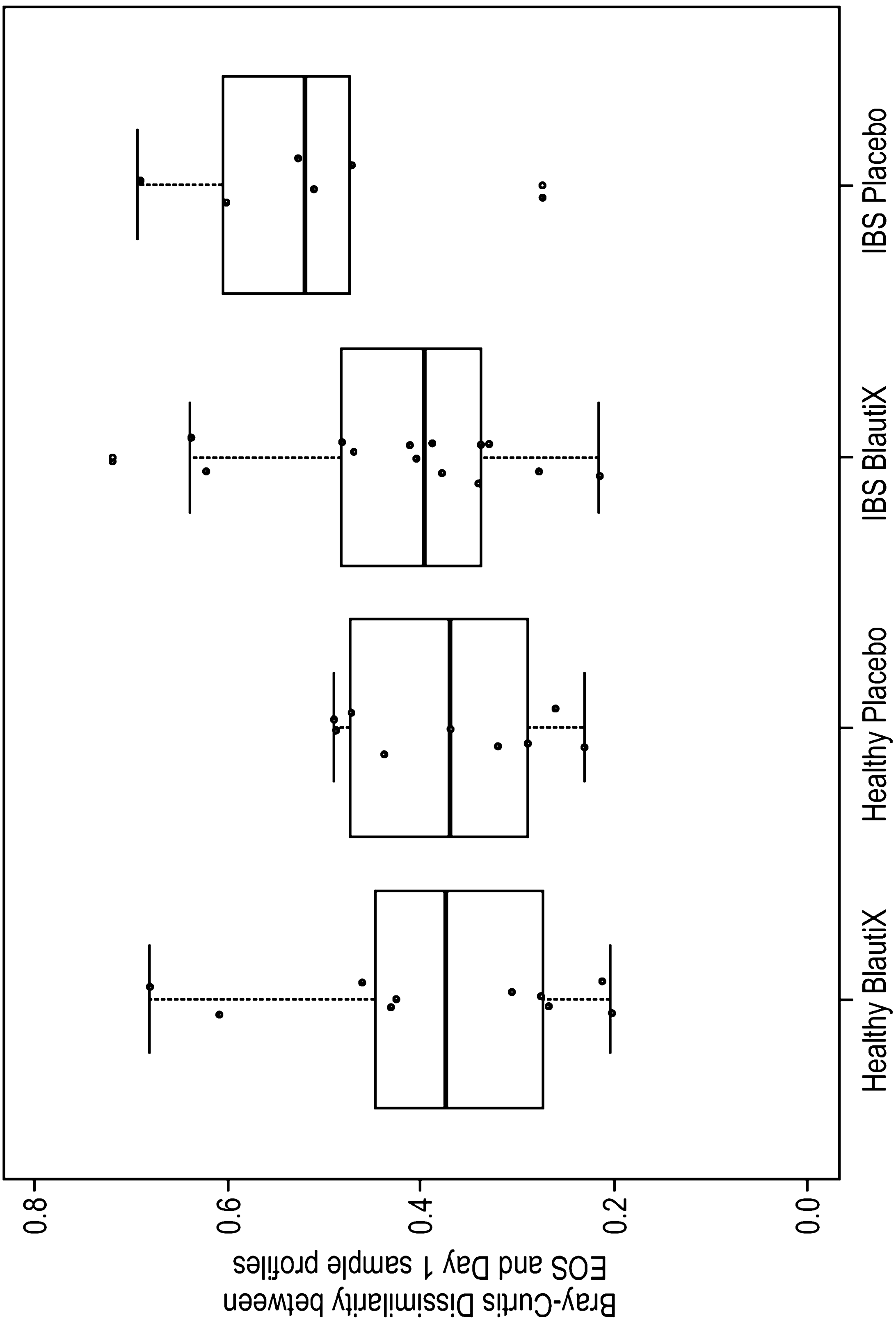


FIG. 4B

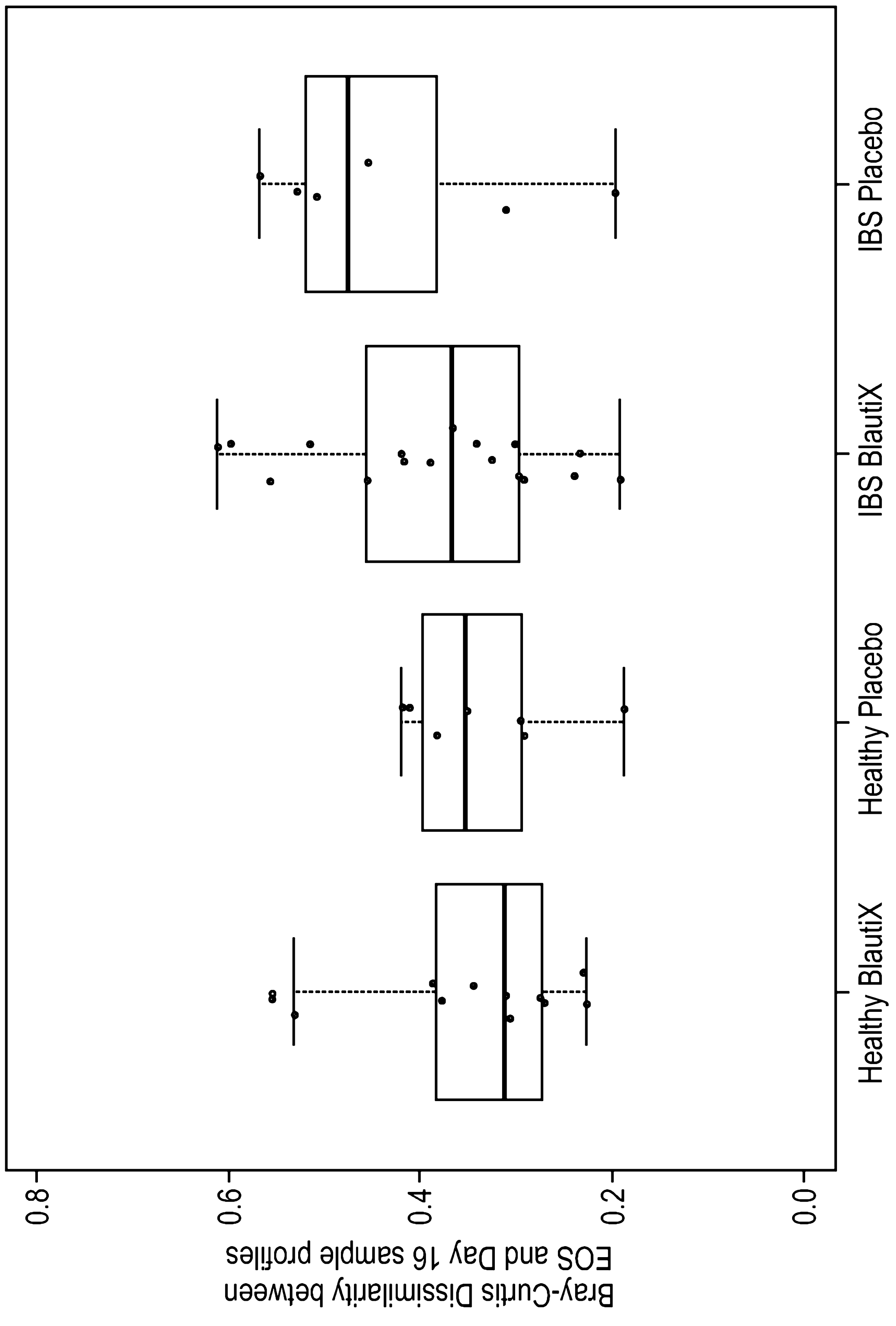
Bray-Curtis Dissimilarity
End of Study Versus Day 1



]

FIG. 4C

Bray-Curtis Dissimilarity
Day 16 Versus End of Study



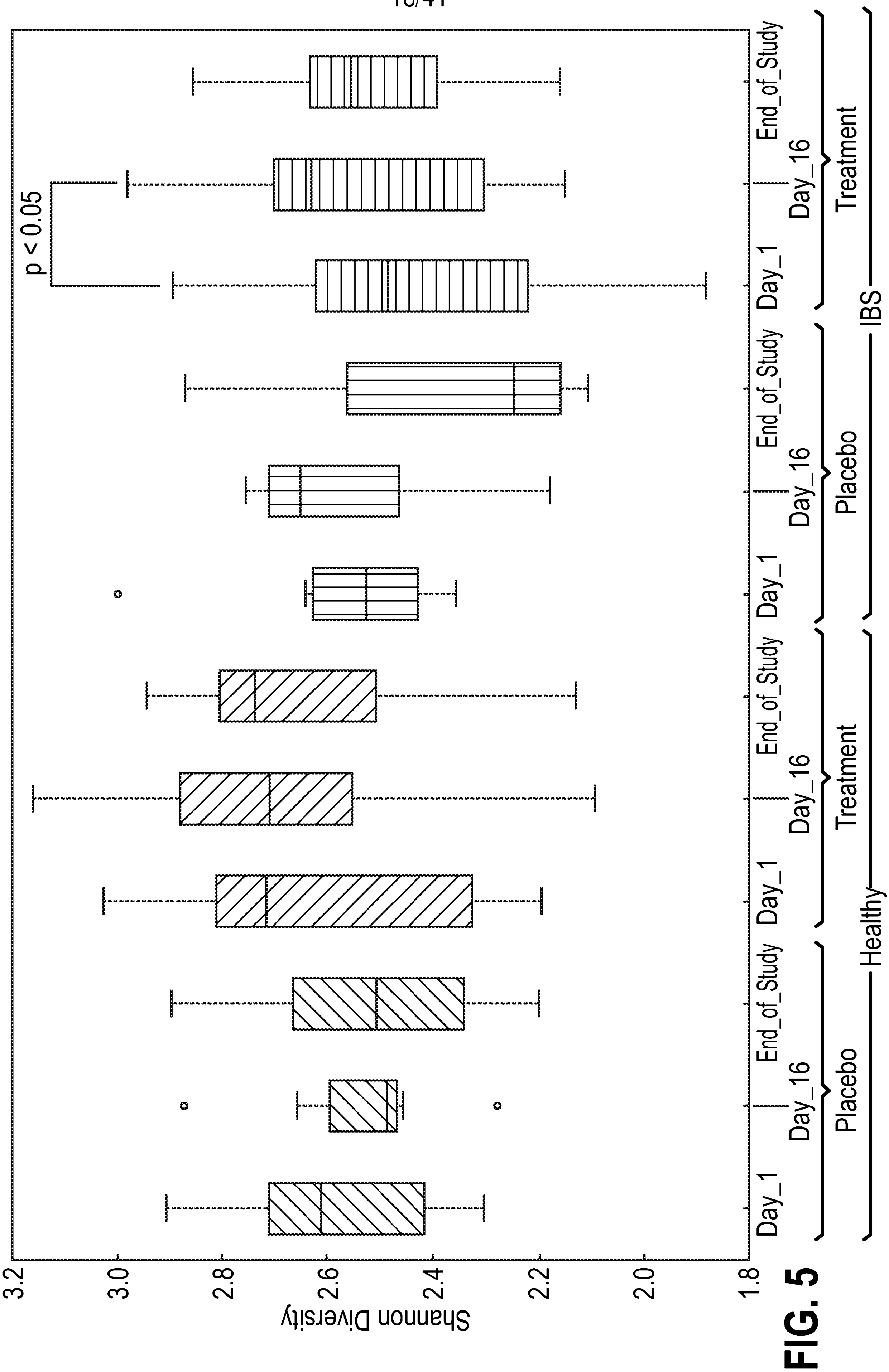
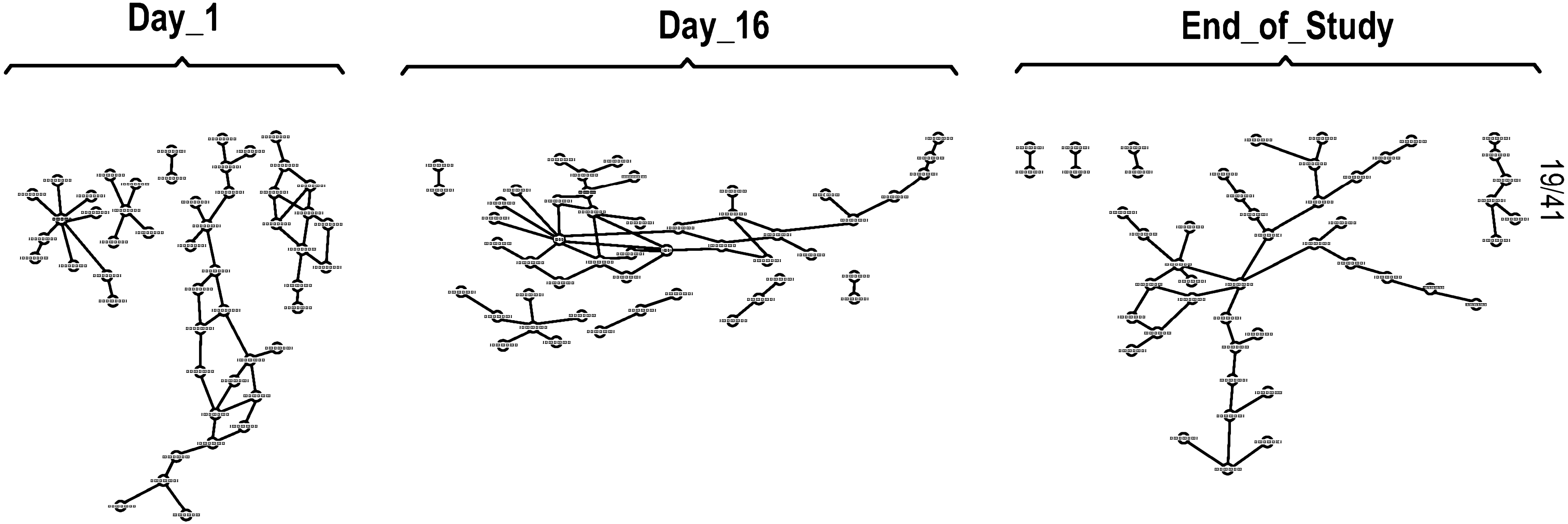

Shannon Diversity
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
p < 0.05
Day_1
Day_16
End_of_Study
Day_1
Day_16
End_of_Study
Day_1
Day_16
End_of_Study
Day_1
Day_16
End_of_Study
Placebo
Treatment
Placebo
Treatment
Healthy
IBS

FIG. 5

FIG. 6A

Healthy individuals



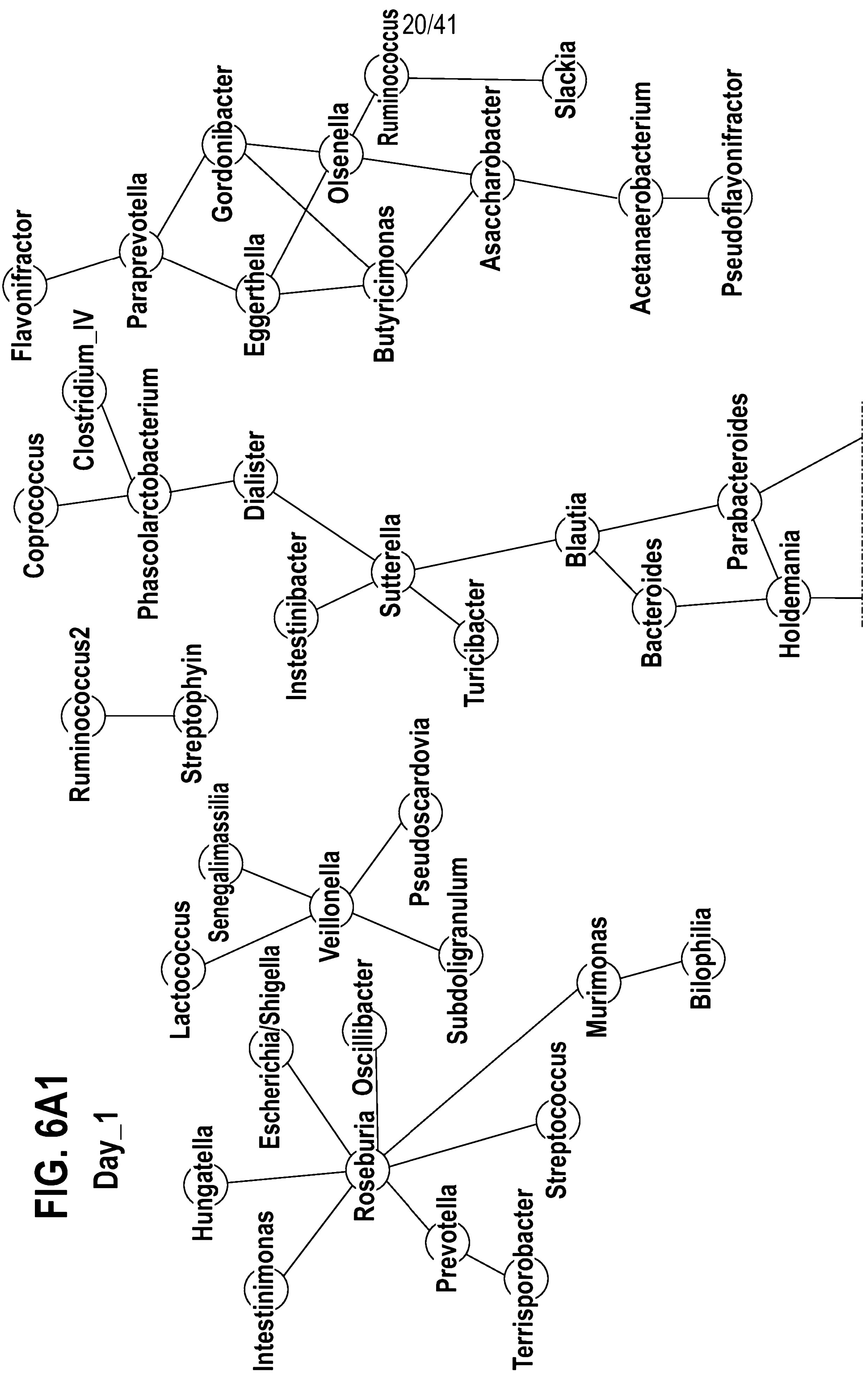


FIG. 6A1

Day_1

Flavonifractor
Paraprevotella
Gordonibacter
Olsenella
Ruminococcus
Slackia
Eggerthella
Butyricimonas
Asaccharobacter
Acetanaerobacterium
Pseudoflavonifractor
Coprococcus
Clostridium_IV
Phascolarctobacterium
Dialister
Instestinibacter
Sutterella
Turicibacter
Blautia
Bacteroides
Parabacteroides
Holdemania
Ruminococcus2
Streptophyin
Lactococcus
Senegalimassilia
Veillonella
Pseudoscardovia
Subdoligranulum
Hungatella
Intestinimonas
Escherichia/Shigella
Roseburia
Oscillibacter
Prevotella
Terrisporobacter
Streptococcus
Murimonas
Bilophilia

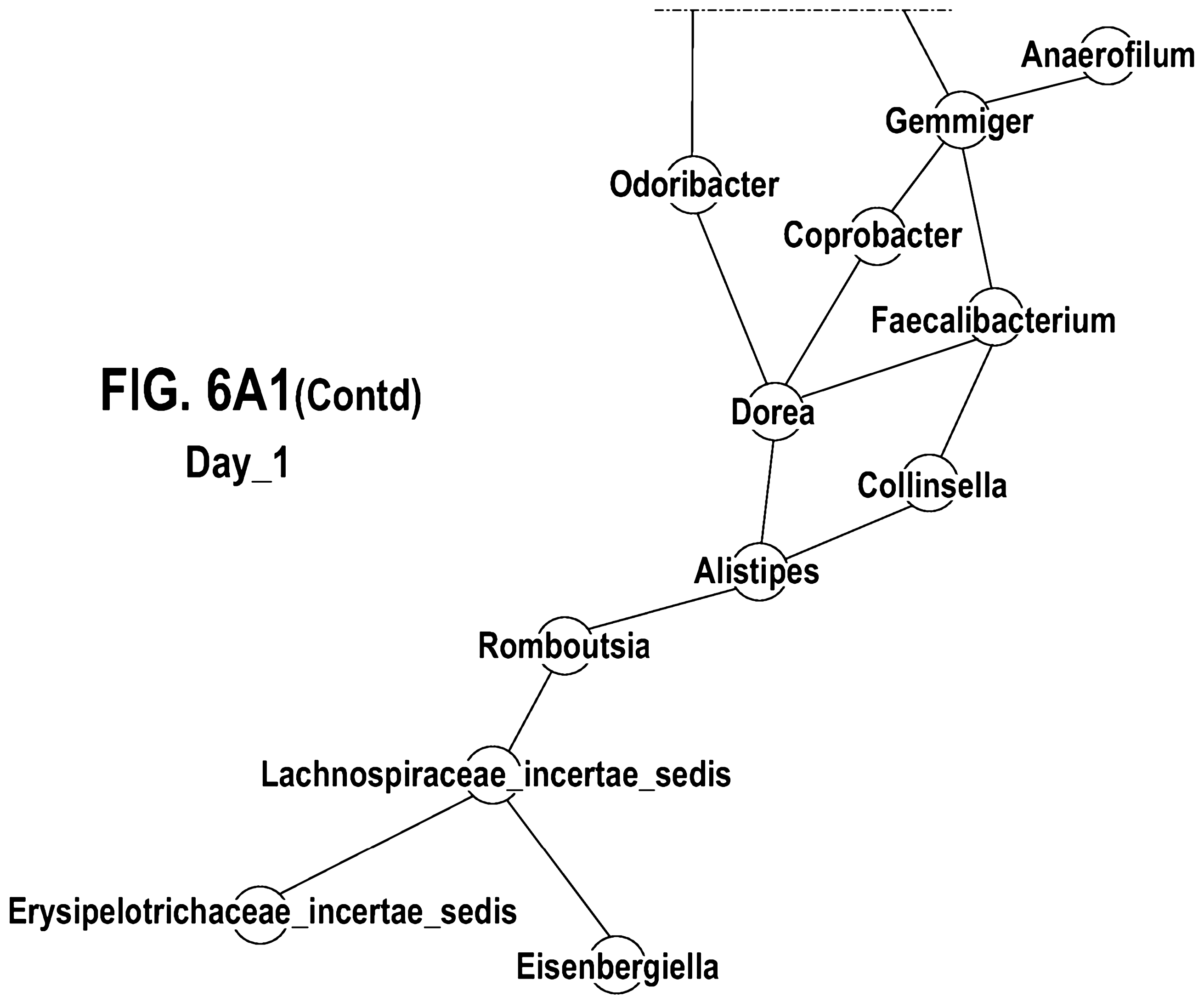


FIG. 6A1(Contd)

Day_1

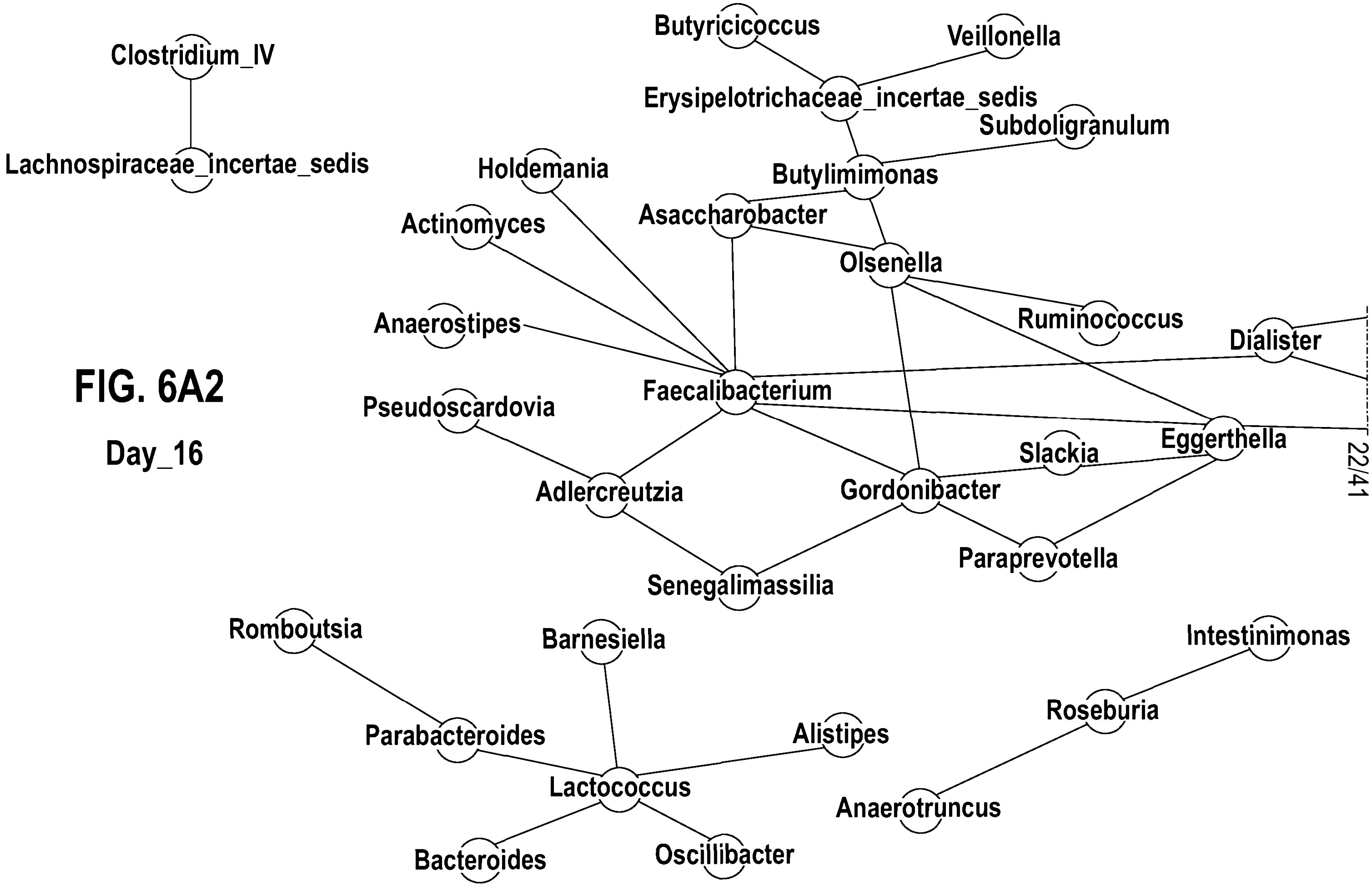


FIG. 6A2

Day_16

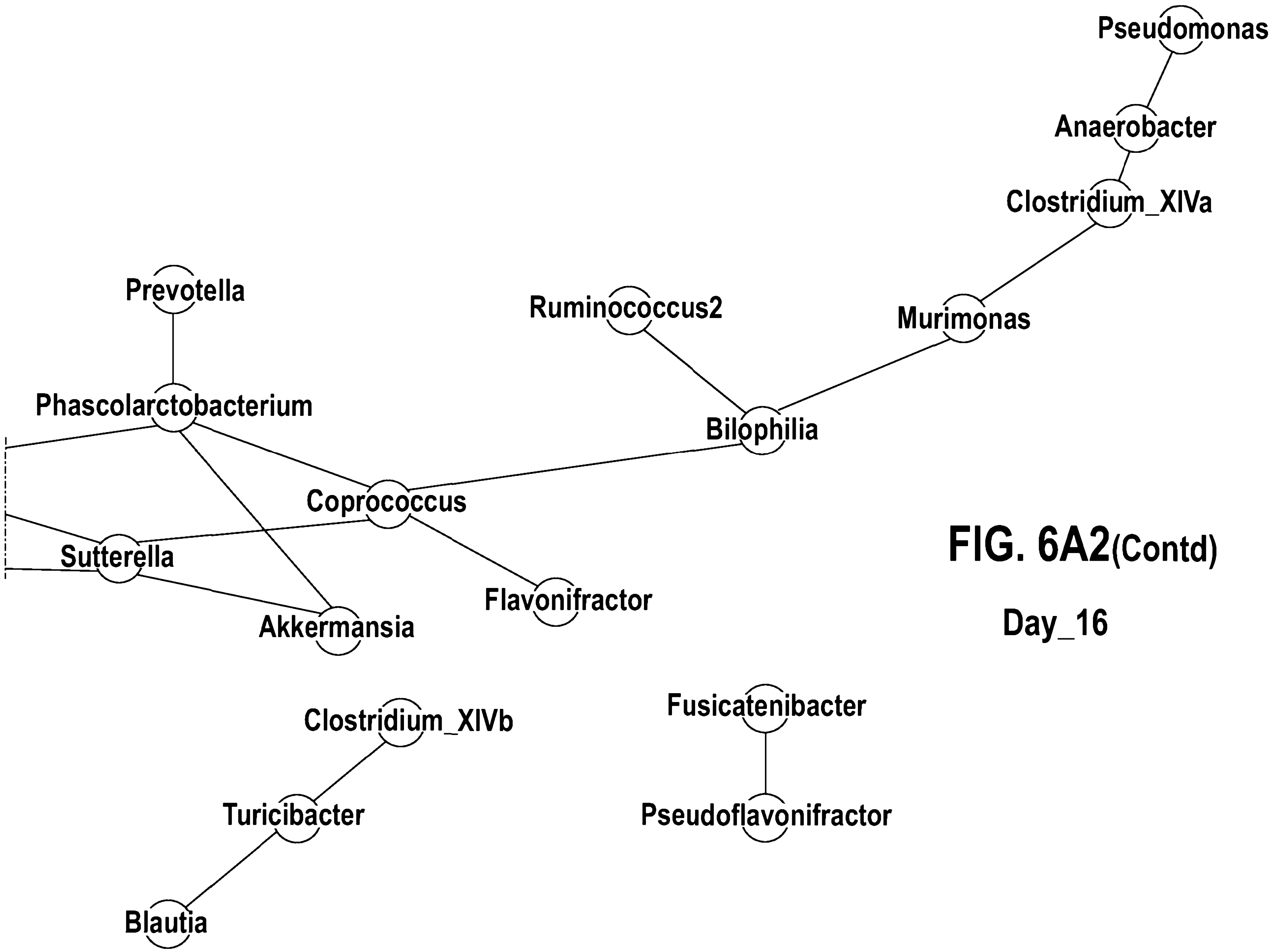


FIG. 6A2(Contd)

Day_16

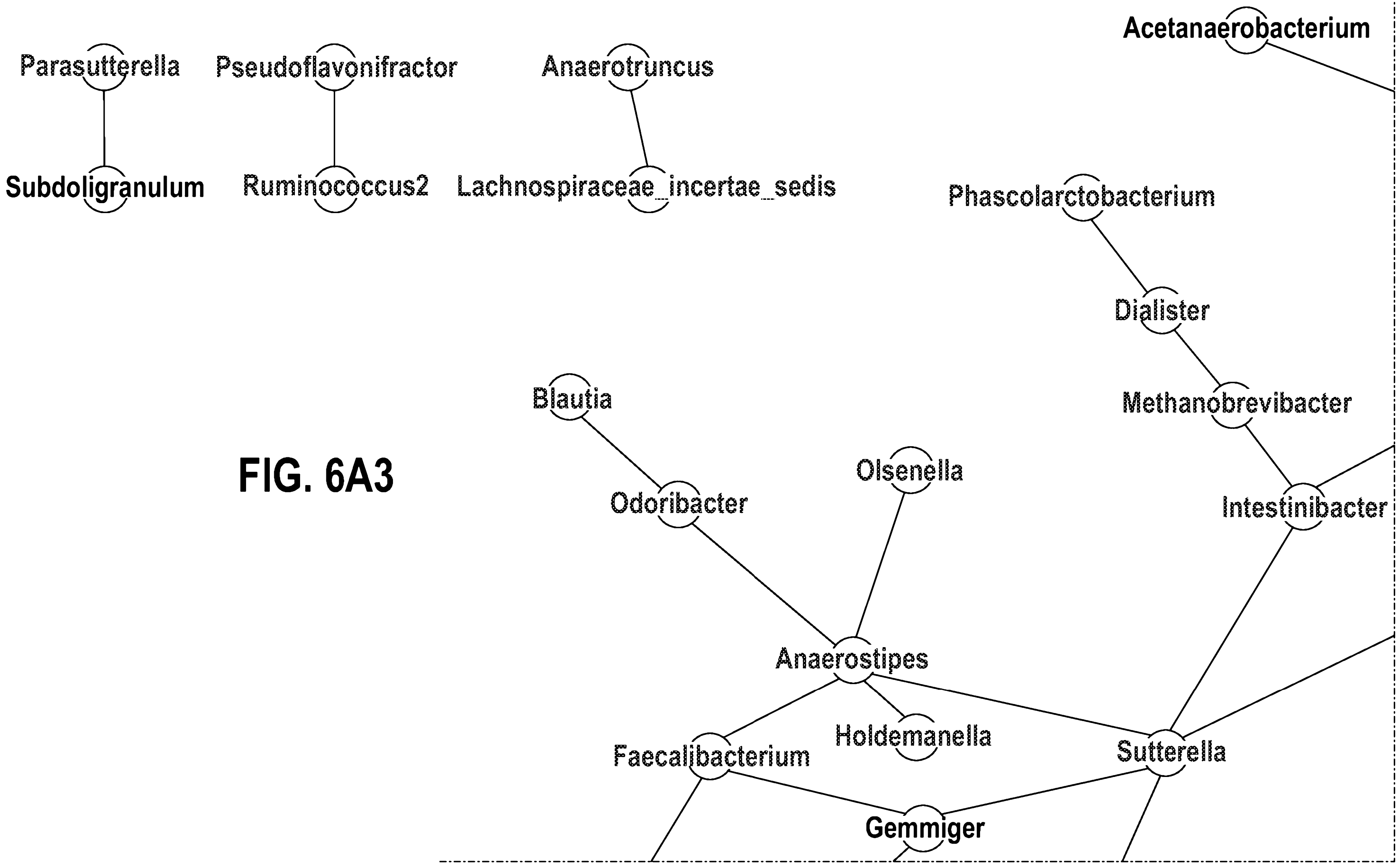

Acetanaerobacterium
Parasutterella
Subdoligranulum
Pseudoflavonifractor
Ruminococcus2
Anaerotruncus
Lachnospiraceae_incertae_sedis
Phascolarctobacterium
Dialister
Methanobrevibacter
Intestinibacter
Blautia
Odoribacter
Olsenella
Anaerostipes
Holdemanella
Faecalibacterium
Sutterella
Gemmiger

FIG. 6A3

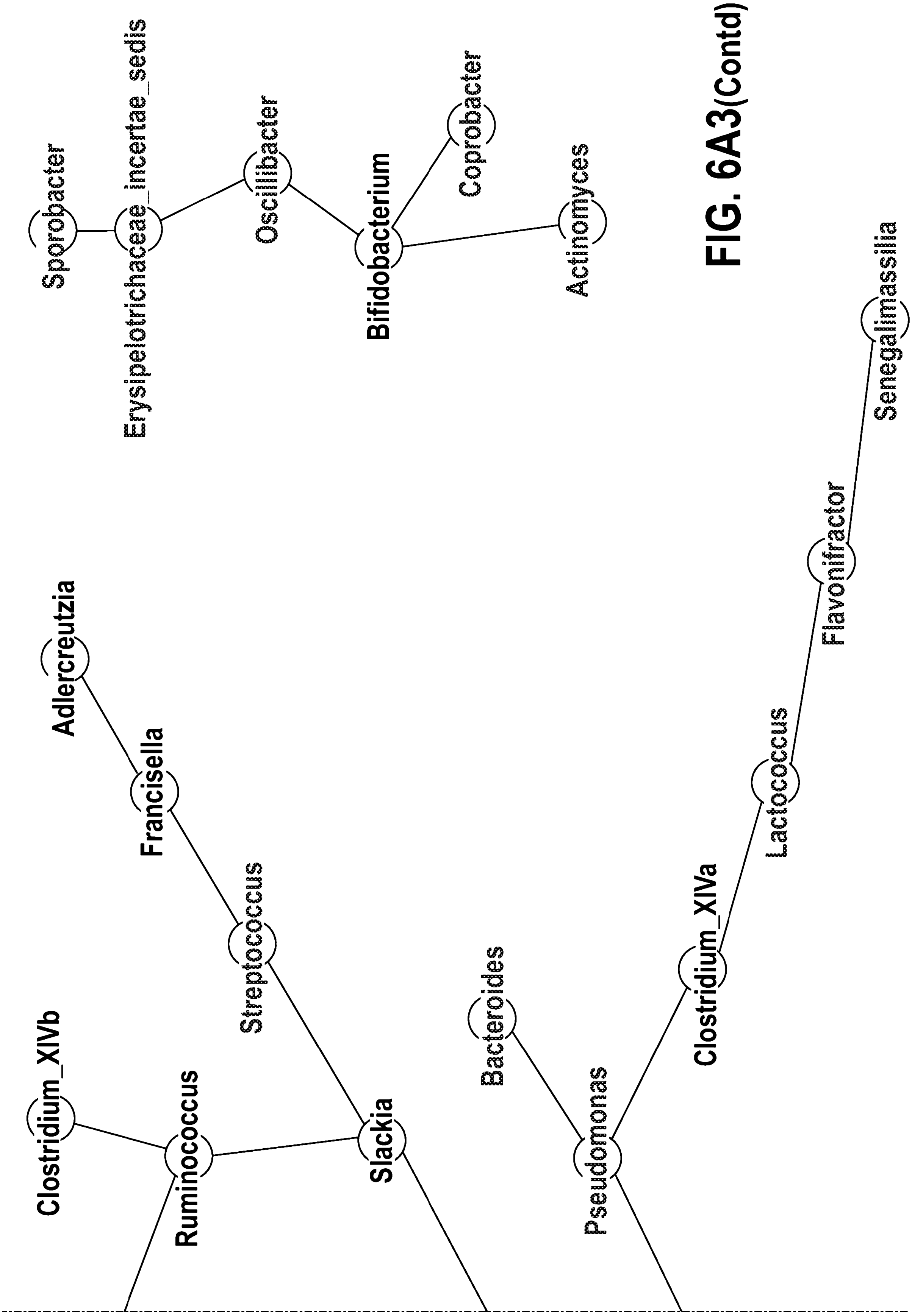


FIG. 6A3(Contd)

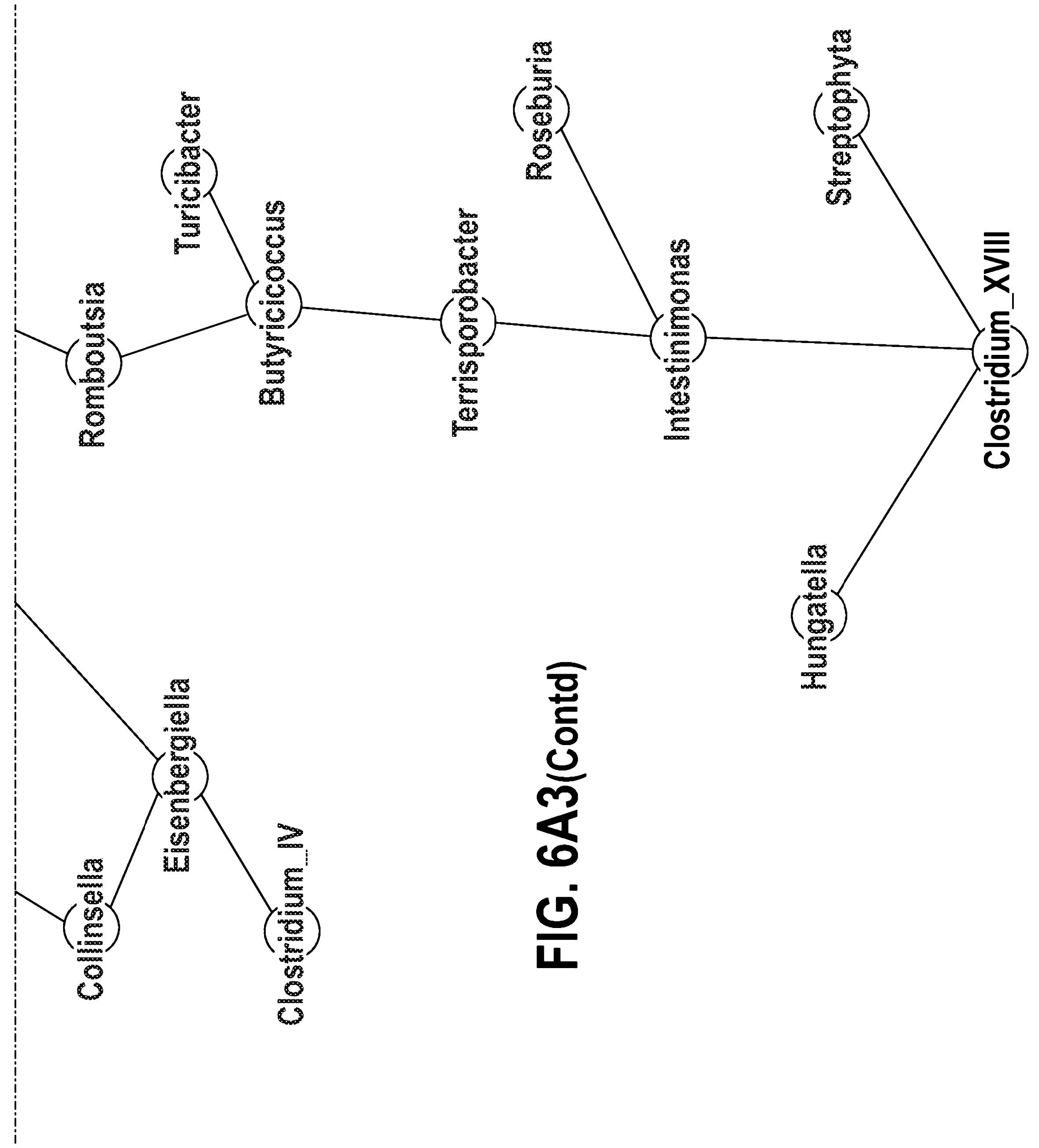


FIG. 6A3(Contd)

FIG. 6B

IBS individuals

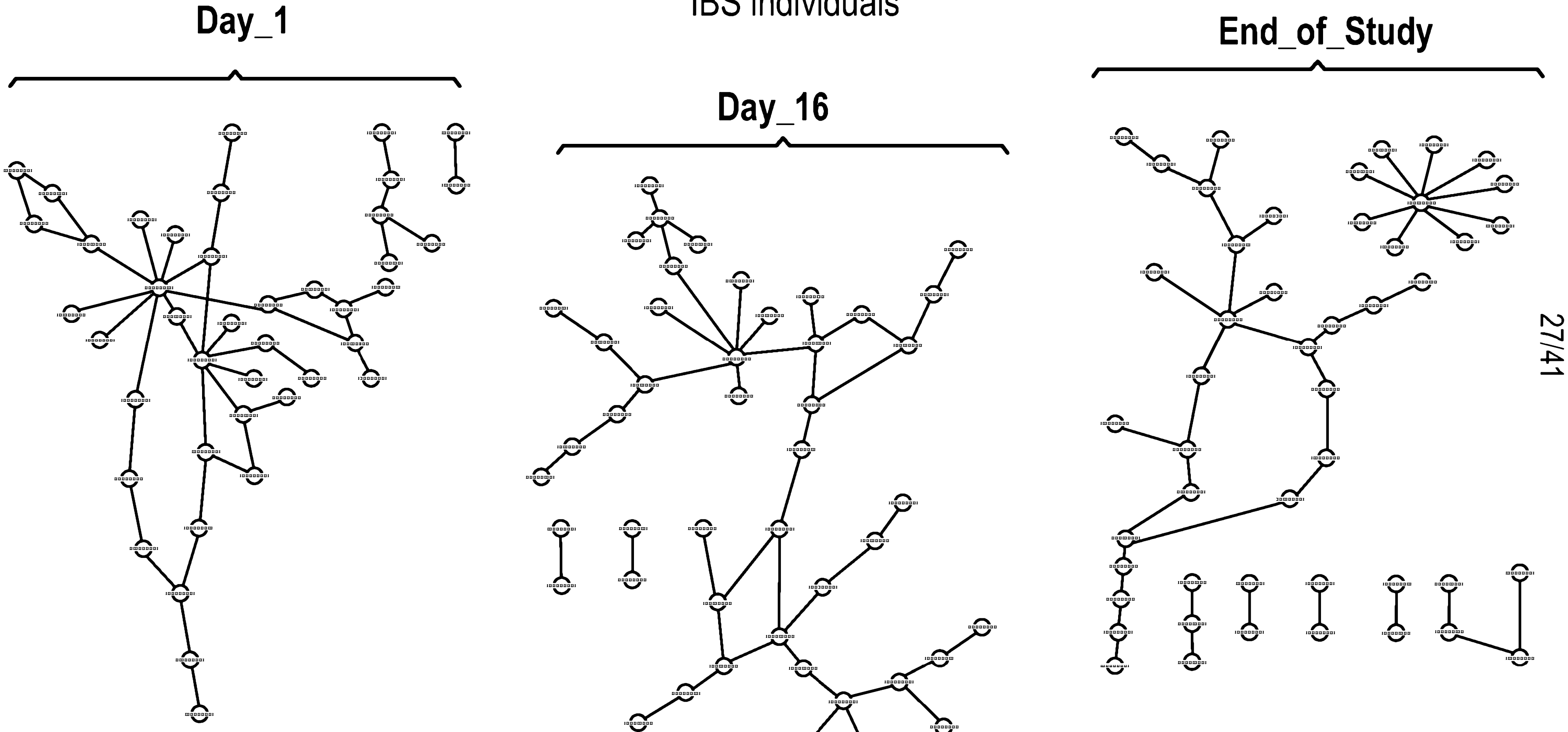
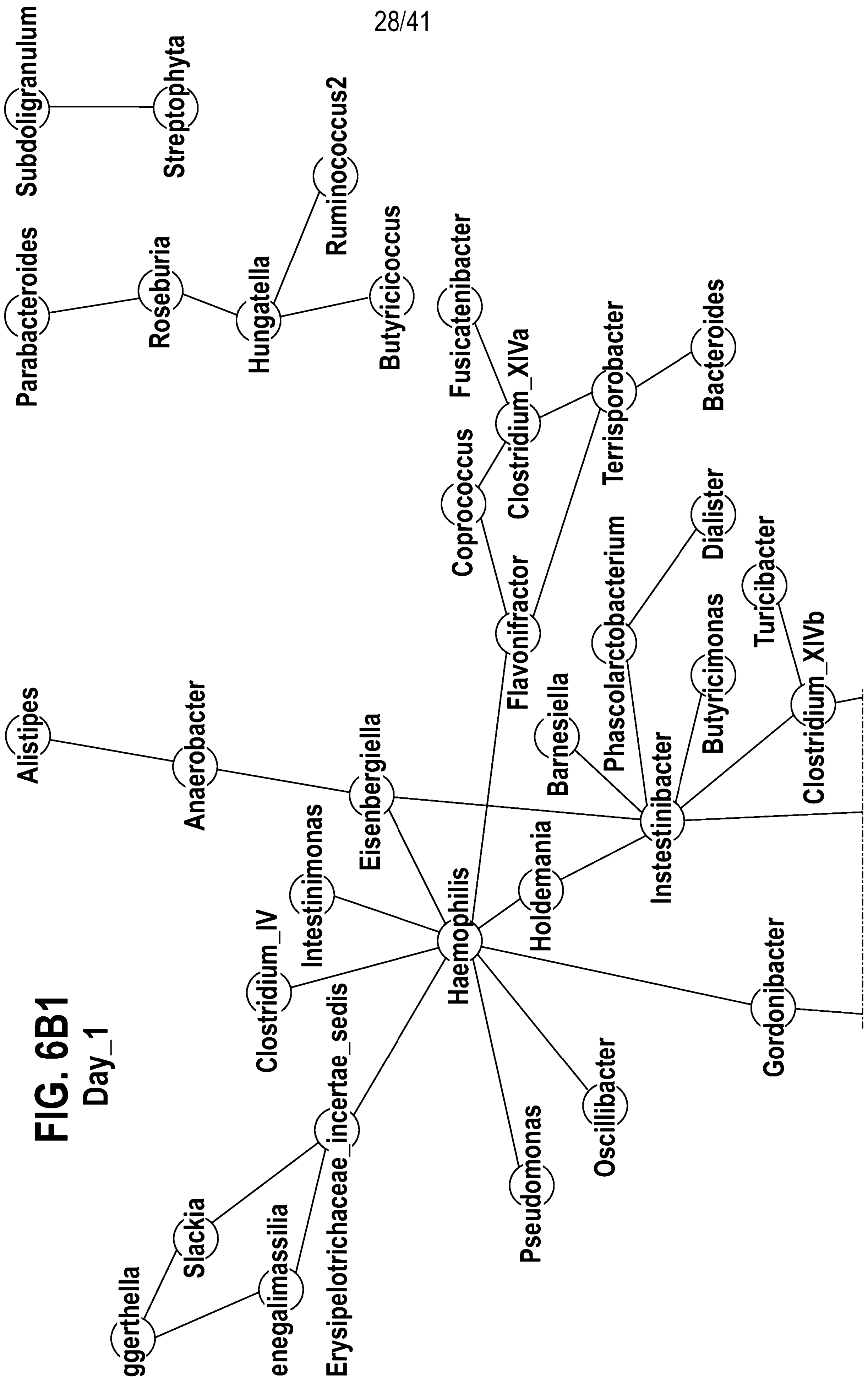
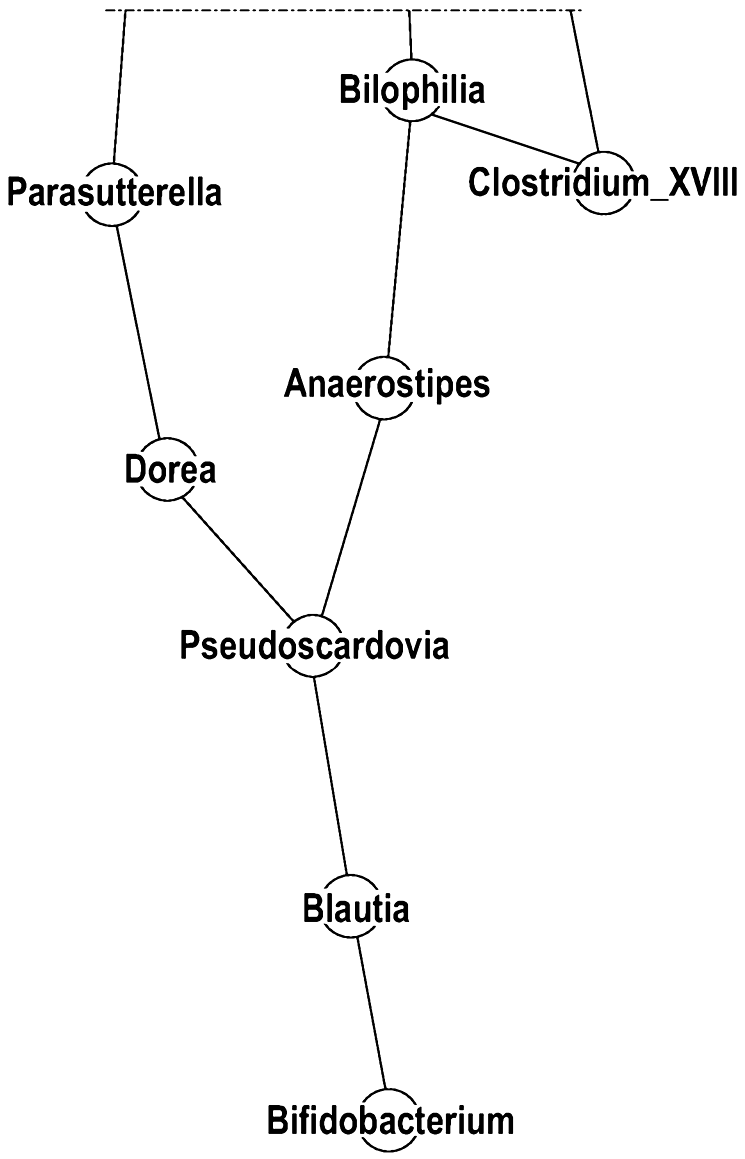


FIG. 6B1

Day_1


Subdoligranulum
Streptophyta
Parabacteroides
Roseburia
Hungatella
Ruminococcus2
Butyricicoccus
Fusicatenibacter
Coprococcus
Clostridium_XIVa
Terrisporobacter
Bacteroides
Flavonifractor
Dialister
Phascolarctobacterium
Turicibacter
Butyricimonas
Clostridium_XIVb
Alistipes
Anaerobacter
Eisenbergiella
Barnesiella
Instestinibacter
Intestinimonas
Holdemania
Haemophilis
Clostridium_IV
Gordonibacter
Erysipelotrichaceae_incertae_sedis
Oscillibacter
Pseudomonas
Slackia
Senegalimassilia
Eggerthella

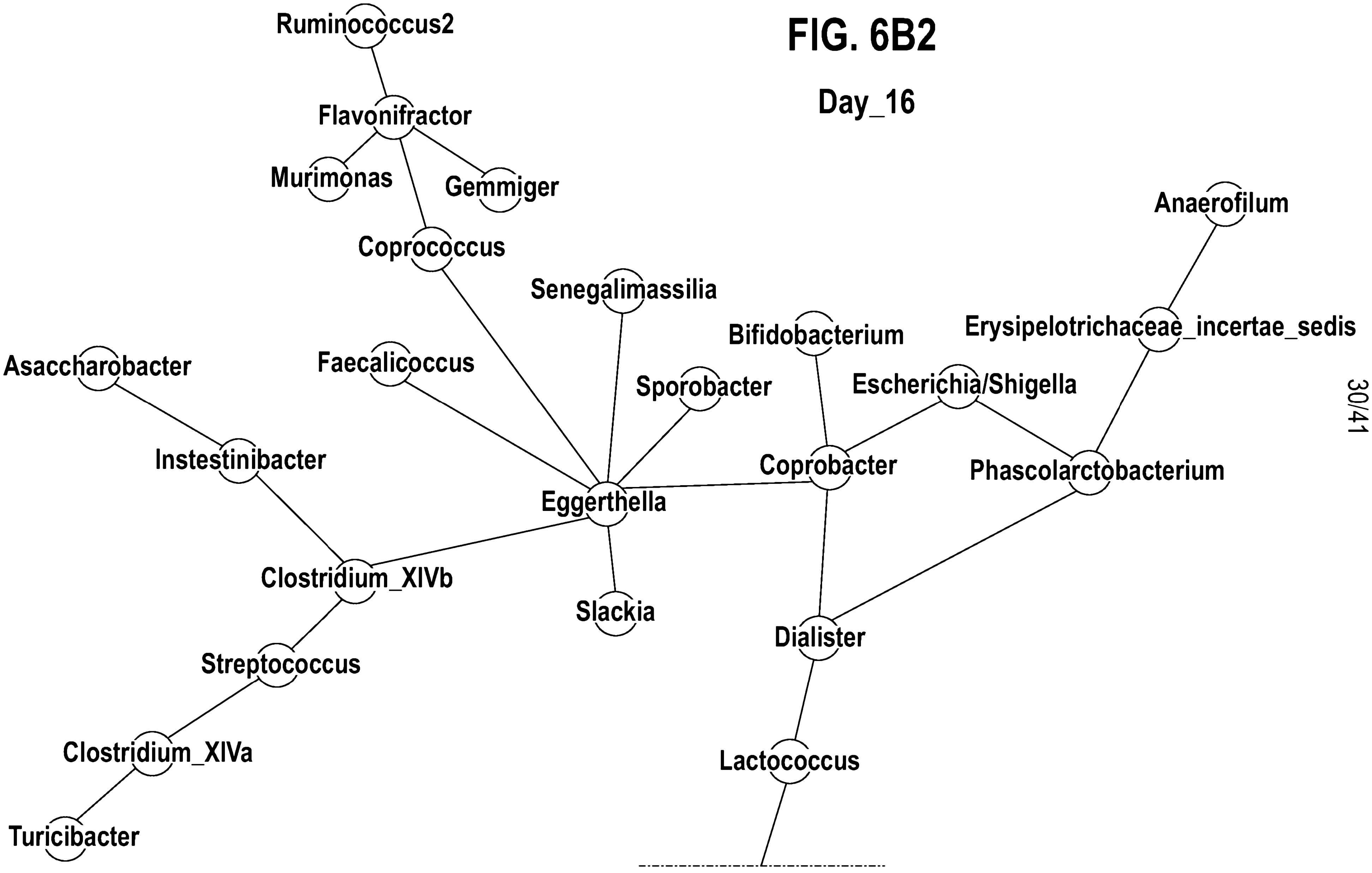


Day_1

FIG. 6B1(Contd)

FIG. 6B2

Day_16



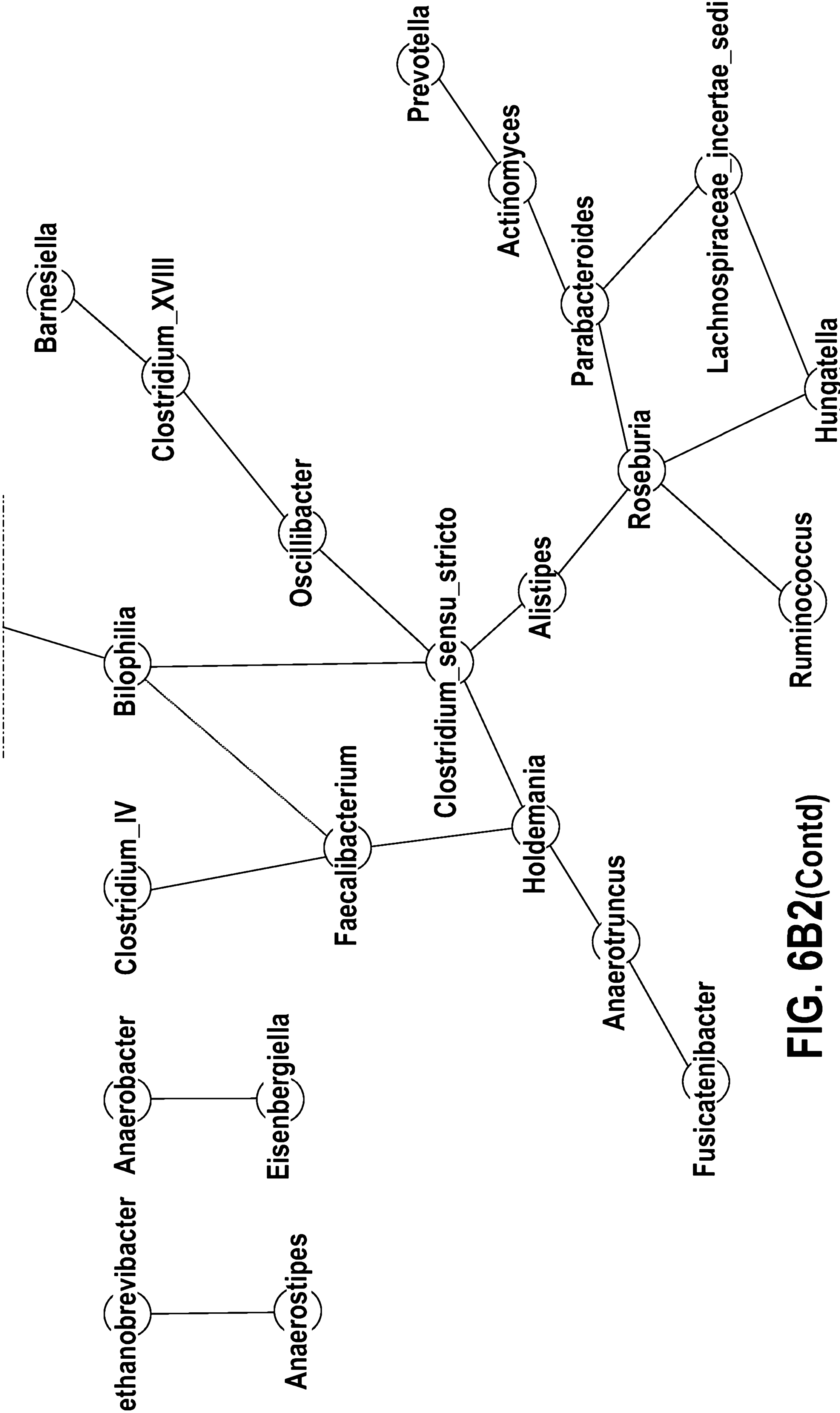


FIG. 6B2(Contd)

Day_16

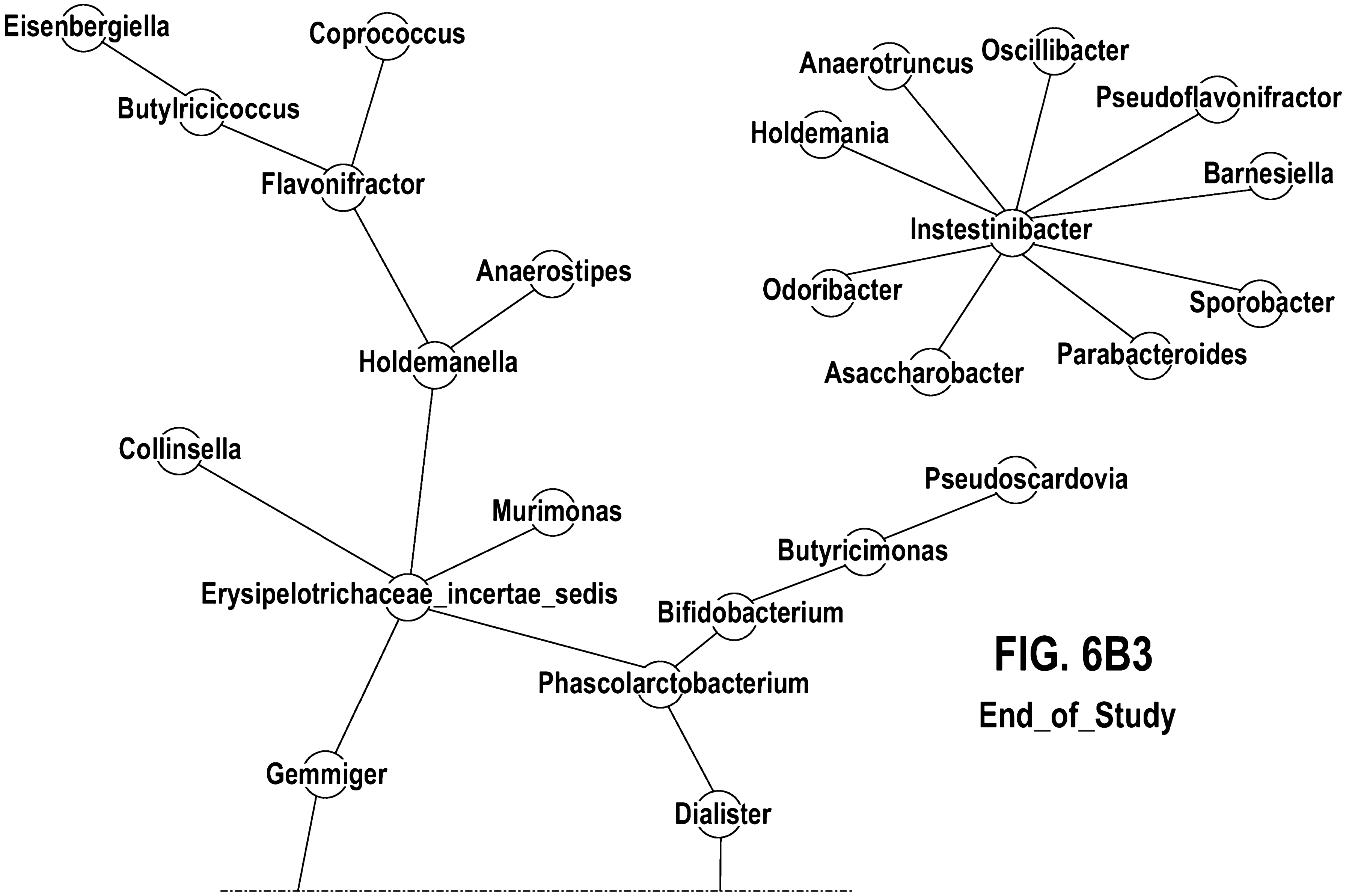


FIG. 6B3

End_of_Study

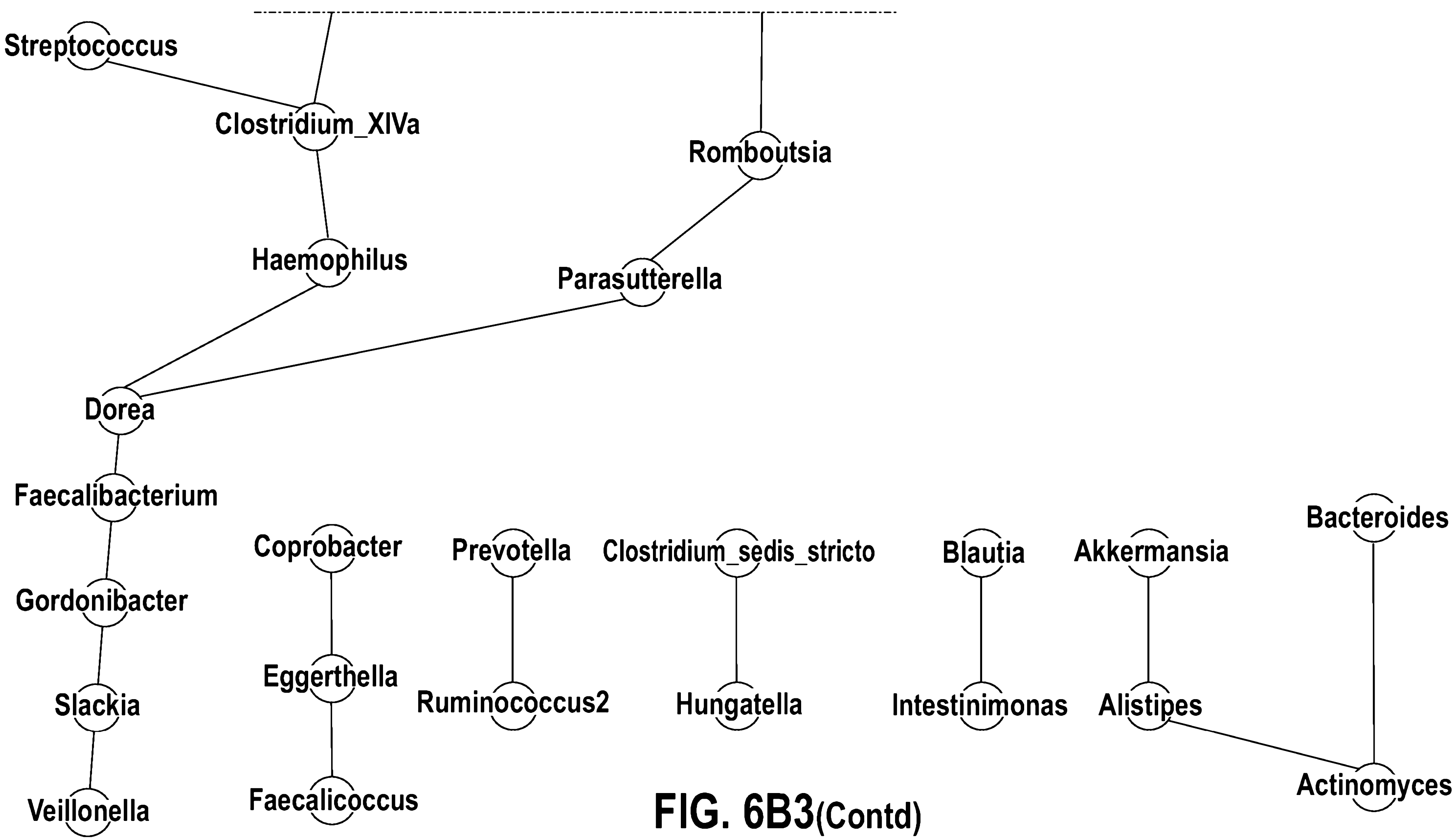


FIG. 6B3(Contd)
End_of_Study

FIG. 7

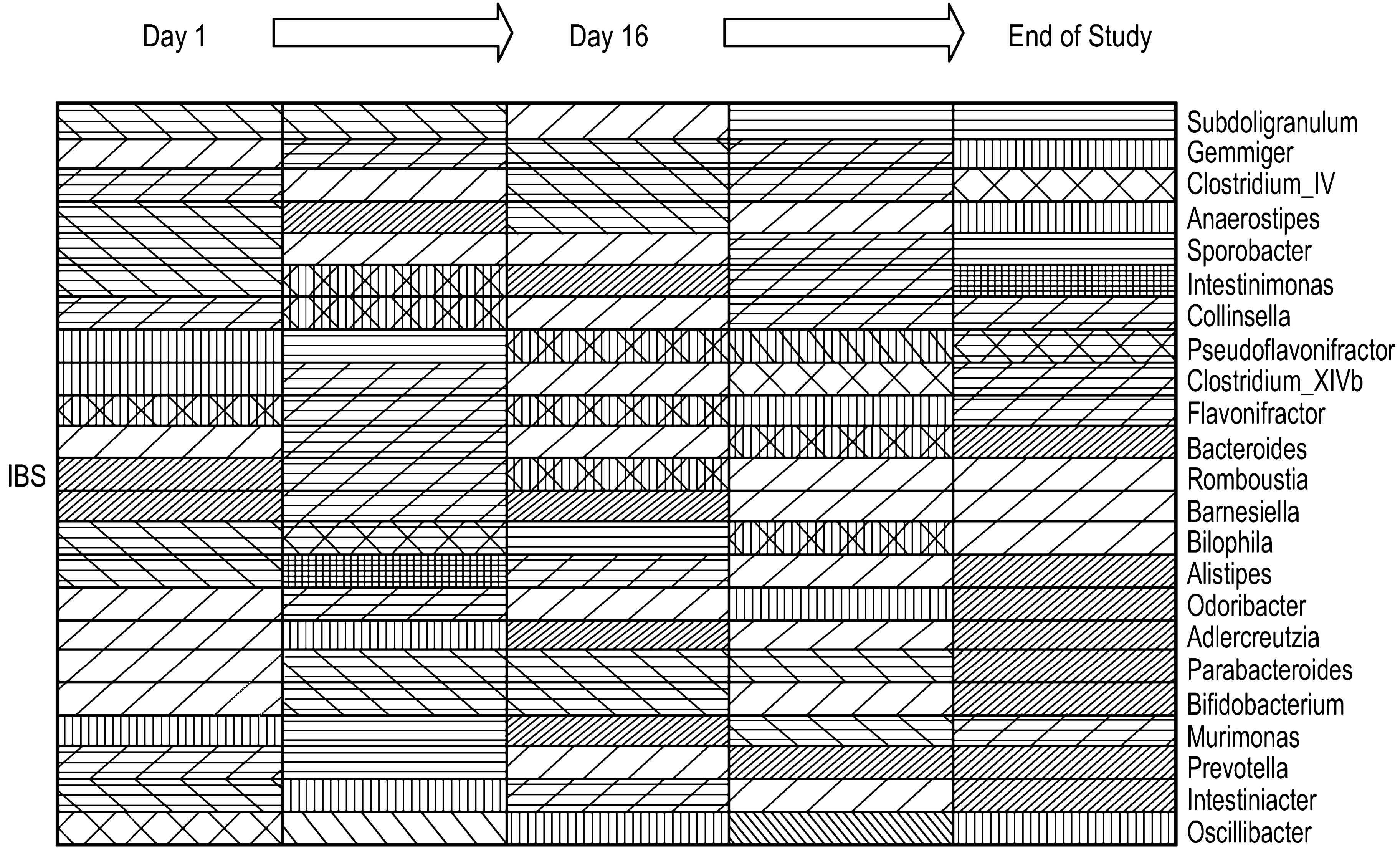
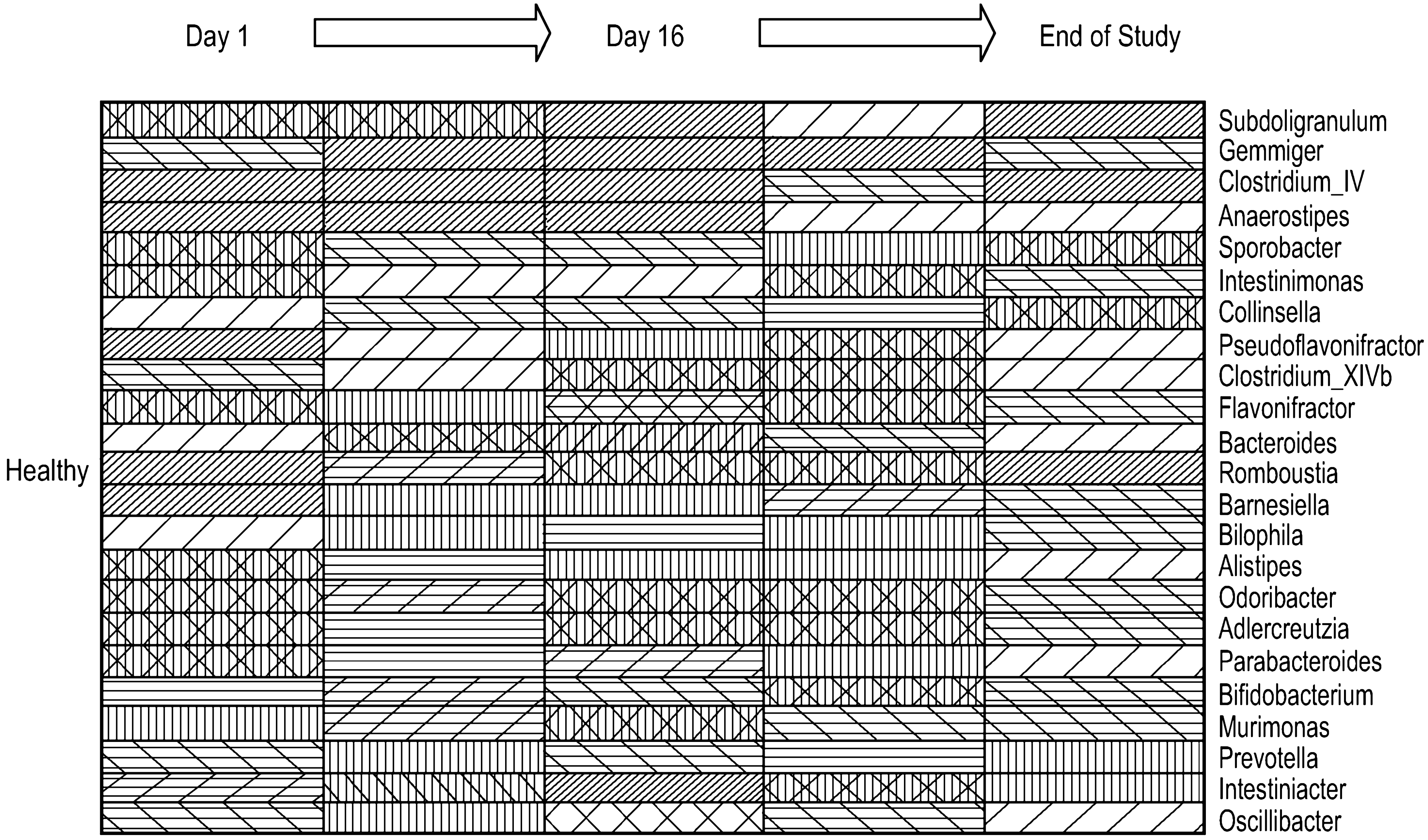

Day 1
Day 16
End of Study
IBS
Subdoligranulum
Gemmiger
Clostridium_IV
Anaerostipes
Sporobacter
Intestinimonas
Collinsella
Pseudoflavonifractor
Clostridium_XIVb
Flavonifractor
Bacteroides
Romboustia
Barnesiella
Bilophila
Alistipes
Odoribacter
Adlercreutzia
Parabacteroides
Bifidobacterium
Murimonas
Prevotella
Intestiniacter
Oscillibacter

FIG. 7(Contd)


Day 1
Day 16
End of Study
Healthy
Subdoligranulum
Gemmiger
Clostridium_IV
Anaerostipes
Sporobacter
Intestinimonas
Collinsella
Pseudoflavonifractor
Clostridium_XIVb
Flavonifractor
Bacteroides
Romboustia
Barnesiella
Bilophila
Alistipes
Odoribacter
Adlercreutzia
Parabacteroides
Bifidobacterium
Murimonas
Prevotella
Intestiniacter
Oscillibacter

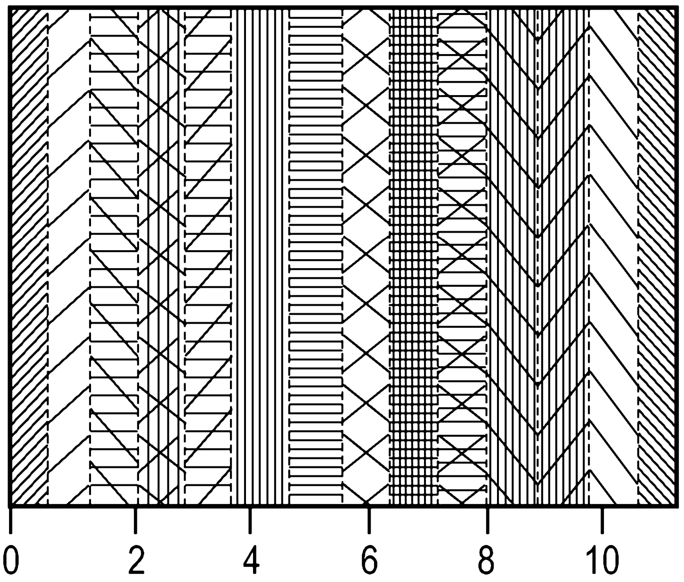


FIG. 7(Contd)

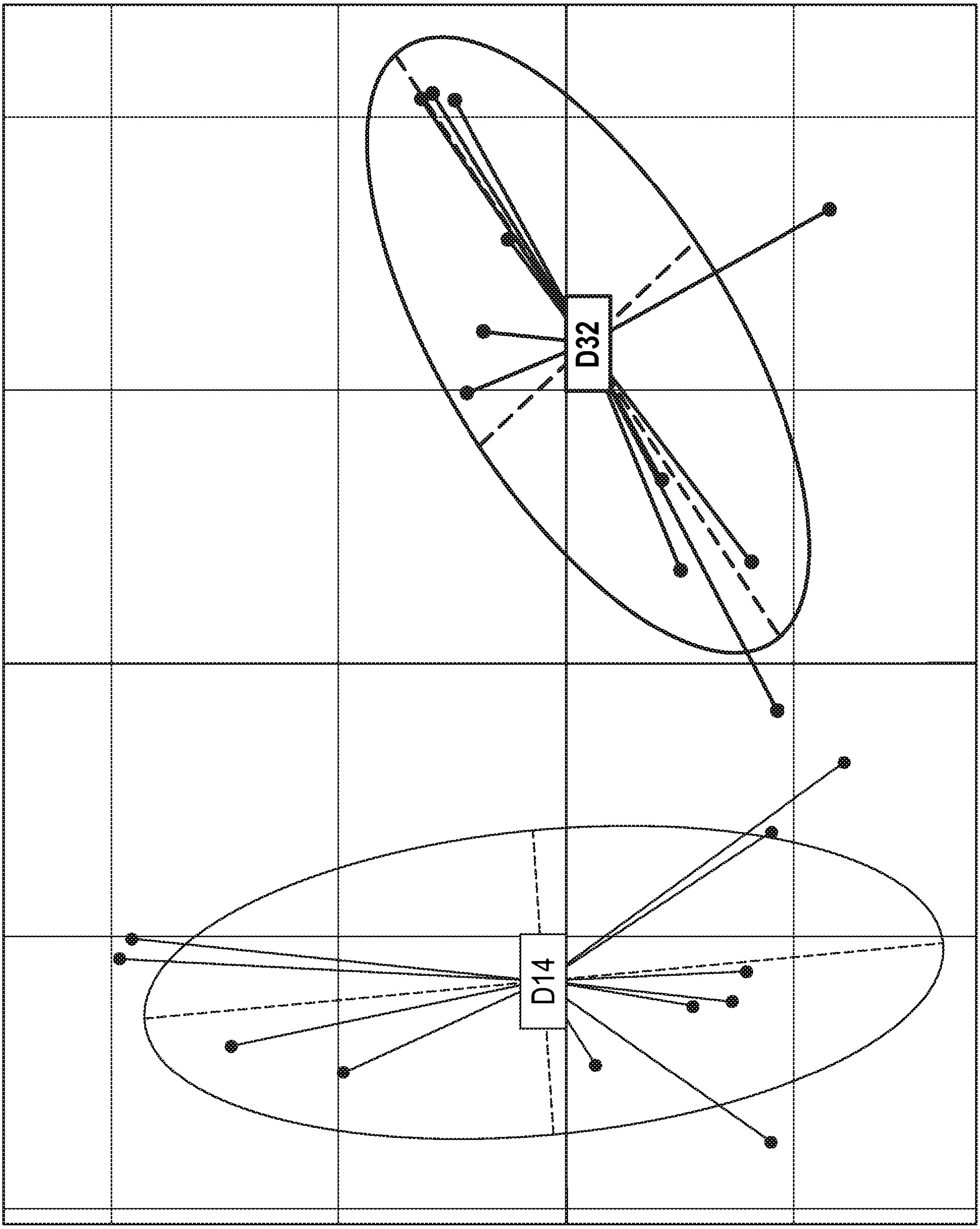


FIG. 8

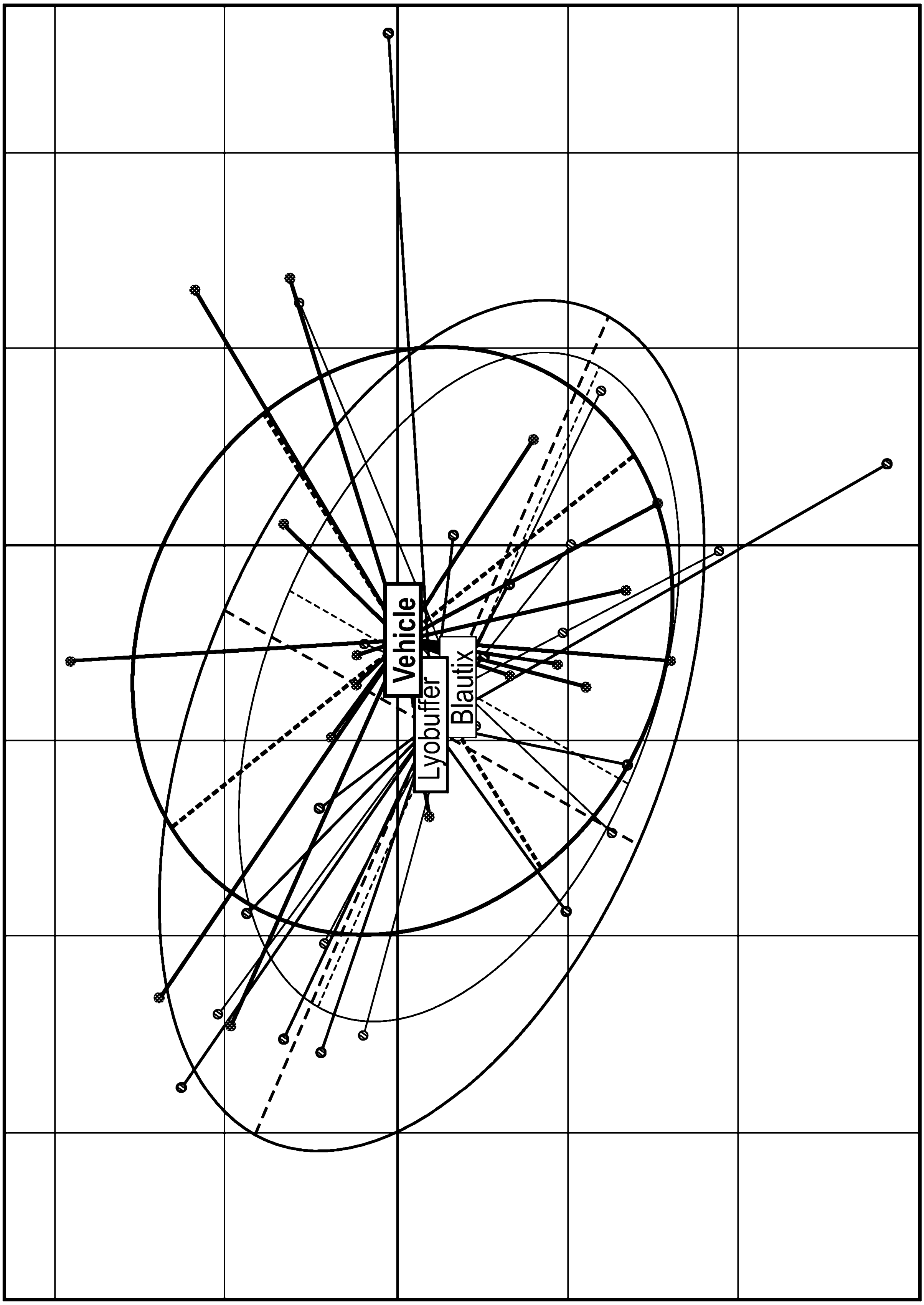


FIG. 9A

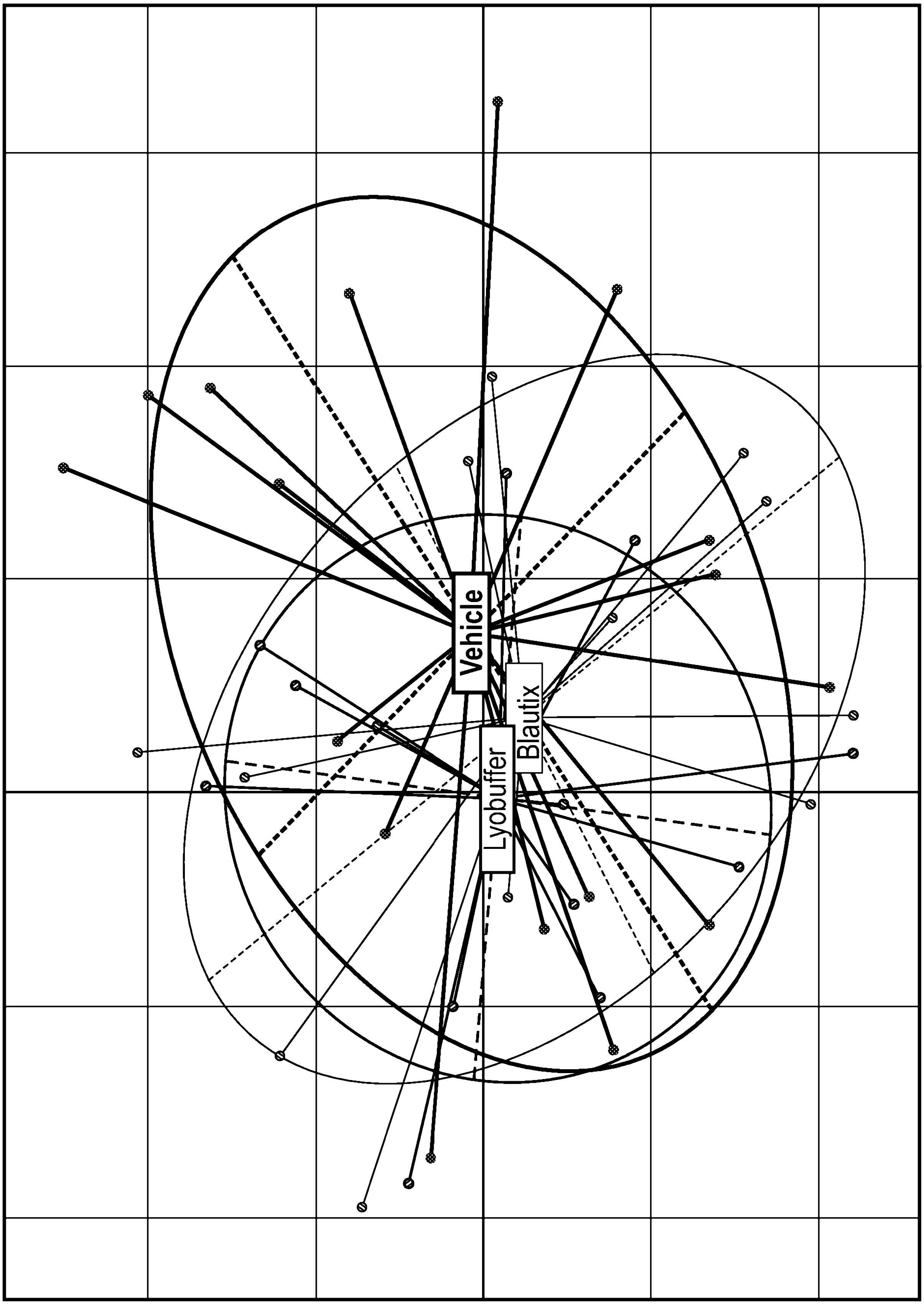


FIG. 9B

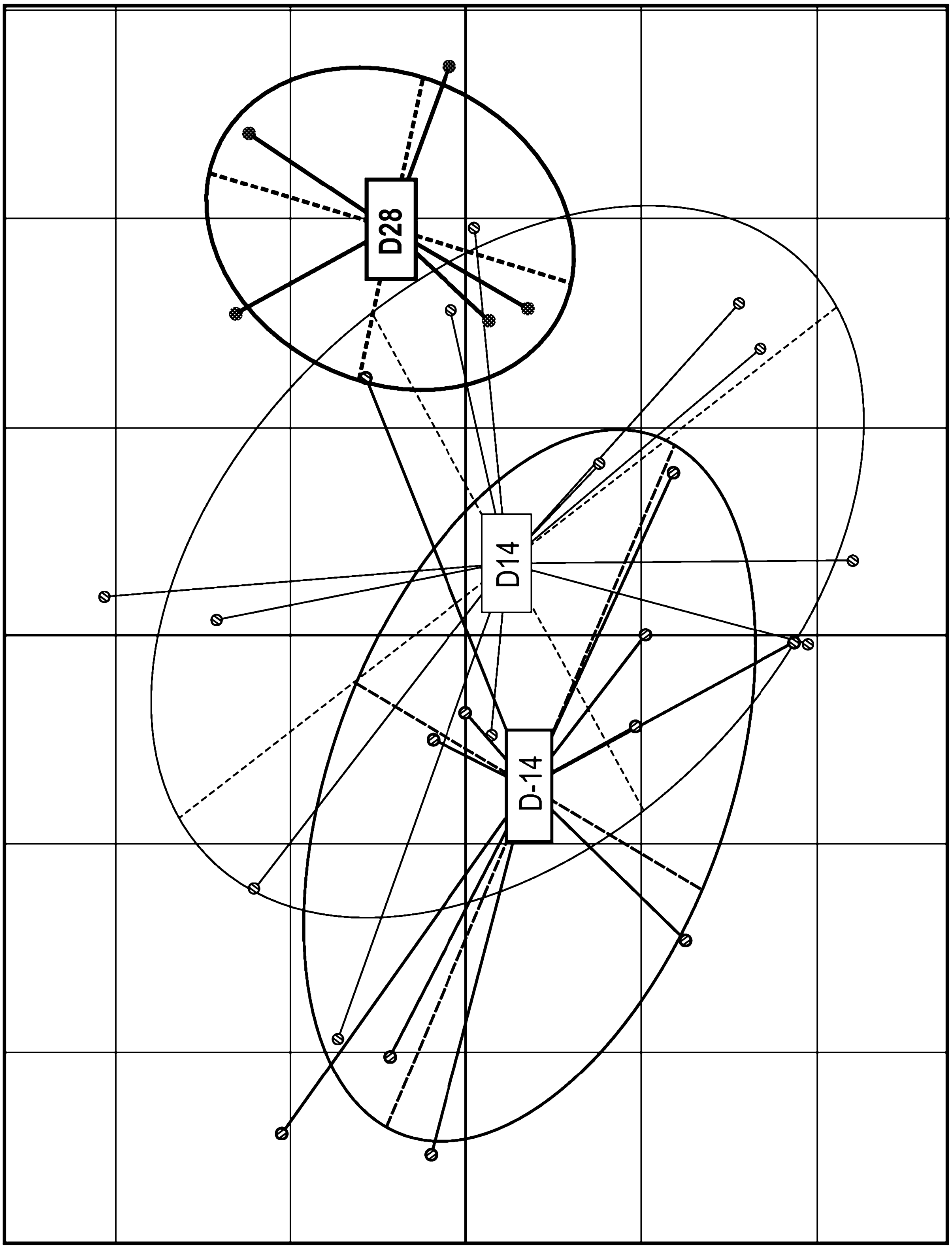


FIG. 9C

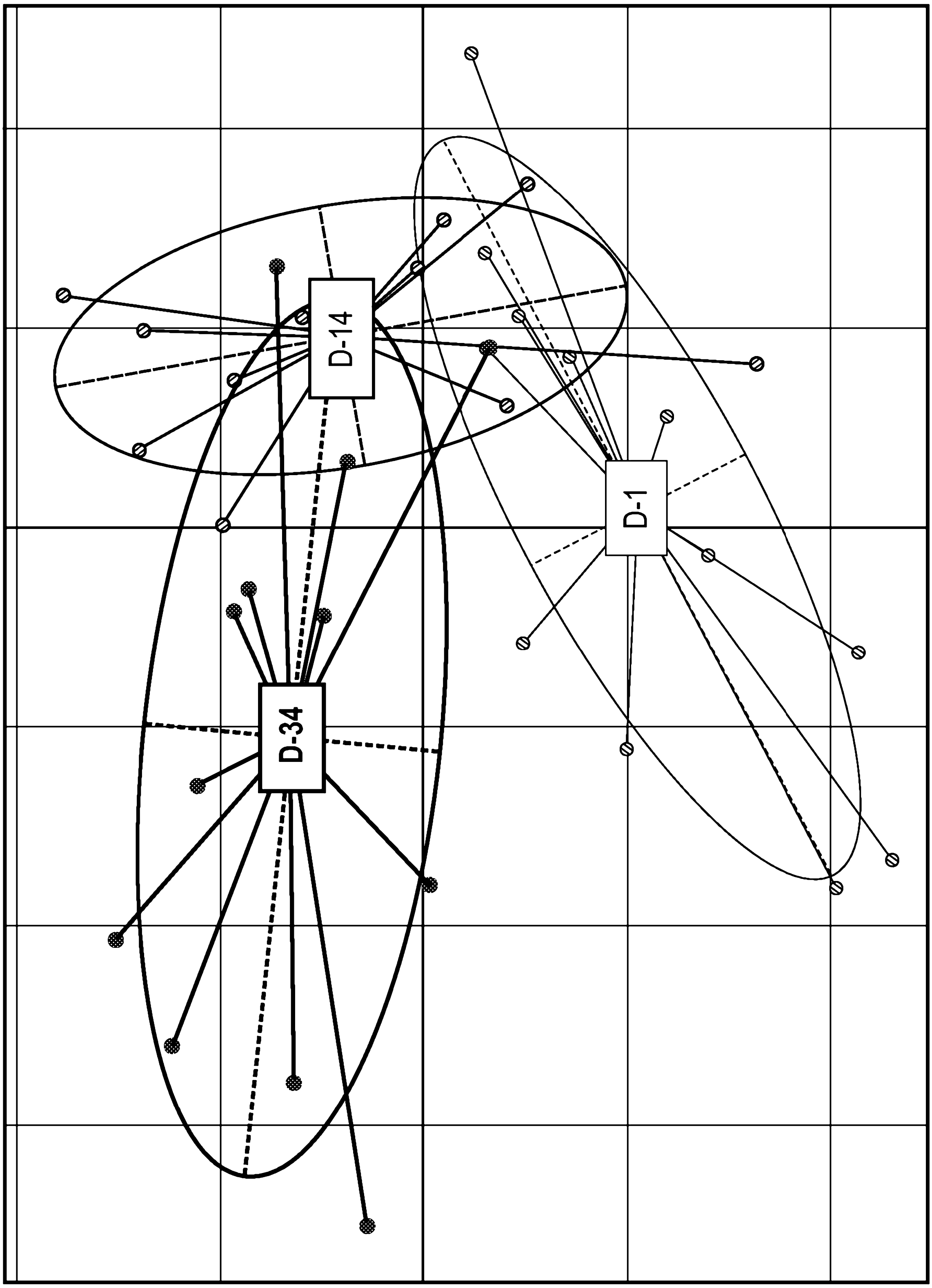


FIG. 10

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<170>   SeqWin2010, version 1.0

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gcgtgaagga agaagtatct cggtatgtaa acttctatca gcagggaaga aaatgacggt  420
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gtaagcggaa ttcctagtgt agcggtgaaa tgcgtagata ttaggaggaa caccagtggc  660
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attagatacc ctggtagtcc acgccgtaaa cgatgaatac taggtgttgg ggagcaaagc  780
tcttcggtgc cgcagcaaac gcaataagta ttccacctgg ggagtacgtt cgcaagaatg  840
aaactcaaag gaattgacgg ggacccgcac aagcggtgga gcatgtggtt taattcgaag  900
caacgcgaag aaccttacca agtcttgaca tcgatctgac cggttcgtaa tggaaccttt  960
ccttcgggac agagaagaca ggtggtgcat ggttgtcgtc agctcgtgtc gtgagatgtt  1020
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actctgtgga gactgccagg gataacctgg aggaaggcgg ggacgacgtc aaatcatcat  1140
gccccttatg atttgggcta cacacgtgct acaatggcgt aaacaaaggg aagcgagccc  1200
gcgaggggga gcaaatccca aaaataacgt cccagttcgg actgcagtct gcaactcgac  1260
tgcacgaagc tggaatcgct agtaatcgcg aatcagaatg tcgcggtgaa tacgttcccg  1320
ggtcttgtac acaccgcccg tcacaccatg ggagtcagta acgcccgaag tc          1372

<210>   2
<211>   1438
<212>   DNA
<213>   Blautia wexlerae

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<223>   'n' is a, c, g or t

<220>
<221>   misc_feature
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ttcgggacag gcgagacagg tggtgcatgg ttgtcgtcag ctcgtgtcgt gagatgttgg 1020
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ccttatgatt tgggctacac acgtgctaca atggcgtaaa caaagggaag cgagattgtg 1200
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cttgtacaca ccgcccgtca caccatggga gtcagtaacg cccgaagtca gtgacctaac 1380
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<213> Artificial Sequence

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gagggtgaac ggccacattg ggactgagac acggcccaga ctcctacggg aggcagcagt 360
ggggaatatt gcacaatggg ggaaaccctg atgcagcgac gccgcgtgaa ggaagaagta 420
tctcggtatg taaacttcta tcagcaggga agaaaatgac ggtacctgac taagaagccc 480
cggctaacta cgtgccagca gccgcggtaa tacgtagggg gcaagcgtta tccggattta 540
ctgggtgtaa agggagcgta gacggaagag caagtctgat gtgaaaggct ggggcttaac 600
cccaggactg cattggaaac tgtttttctt gagtgccgga gaggtaagcg gaattcctag 660
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<220>
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cgcacagagc tgcatggctc agtgtgaaaa actccggtgg tataagatgg acccgcgttg  180
gattagcttg ttggtggggt aacggcccac caaggcgacg atccatagcc ggcctgagag  240
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gccctataca gggggataac agttagaaat gactgctaat accgcataag cgcacagctt  180
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