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(54) **Title**: MODULATION OF THE GUT MICROBIOME TO TREAT MENTAL DISORDERS OR DISEASES OF THE CENTRAL NERVOUS SYSTEM

(57) **Abstract**: The present disclosure relates to methods of treating at least one symptom of a mental disorder or disease of the central nervous system in a subject by modulating the amount of GABA produced in the subject's gut. The present disclosure also relates to methods of culturing the bacterial strain new bacterial strains. Also disclosed are methods of identifying bacterial strains capable of producing GABA, and engineering strains to produce GABA.

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MODULATION OF THE GUT MICROBIOME TO TREAT MENTAL DISORDERS OR DISEASES OF THE CENTRAL NERVOUS SYSTEM

Cross Reference to Related Applications

[0001] This application claims priority to, and benefit of, US Provisional Application No. 62/307,991, filed March 14, 2016, the contents of which is incorporated by reference in its entirety.

Incorporation of Sequence Listing

[0002] The contents of the text file named "HOBE001001WOSeqList.txt," which was created on March 13, 2017 and is 7.6 MB in size, are hereby incorporated by reference in their entirety.

Government Funding

[0003] This invention was made with government support under 3R01HG005824-02S1 awarded by the National Institute of Health. The government has certain rights in the invention.

Field of the Disclosure

[0004] The present disclosure relates to compositions and methods for treating at least one symptom of a disease in a subject. In some cases, the disease is a mental disorder or a disease of the central nervous system. The present disclosure teaches treatment of the disease by modulating (e.g., increasing) the amount of endogenous GABA in a subject's body. In some embodiments, the present disclosure teaches modulating (e.g., increasing) the amount of GABA produced in the subject's gut by bacteria in the gut. For example, the present disclosure teaches administration to a subject in need thereof bacteria that are capable of producing GABA (e.g., inside a human gut).

[0005] The present disclosure also relates to methods of culturing previously uncultured bacterial strains. For instance, the present disclosure teaches the previously uncultured bacterial strain *Evtepia gabavorous* KLE1738. As set forth herein, newly uncultured bacterial strains such as *Evtepia gabavorous* KLE1738 can be cultured by providing growth factors necessary for the bacteria's growth and reproduction.

[0006] Also disclosed are methods of identifying bacterial strains capable of producing certain growth factors. For instance, described herein are methods of identifying bacterial strains capable of producing GABA, for instance under physiologically relevant conditions such as at a physiologically relevant pH.

Background

[0007] The gut microbiome affects certain gastrointestinal and metabolic disorders, such as irritable bowel syndrome (IBS), Crohn's disease, ulcerative colitis, celiac disease, obesity, heart disease, type I and II diabetes, and colon cancer.

[0008] Microbiological studies have so far been limited, by necessity, to cultivable microorganisms. By some estimates, in external environments, 99% of bacteria are thought to be uncultured. The development of new techniques for culturing previously uncultured or unculturable bacteria can thus help to expand the scope of microbiology research.

Summary of the Invention

[0009] The present disclosure provides compositions and methods for treating diseases such as mental illness or diseases of the central nervous system. In some embodiments, the present disclosure teaches therapeutic compositions comprising one or more bacteria (e.g., purified bacteria) that are capable of producing GABA. The bacteria can be capable of producing GABA under physiologically relevant conditions, including within a human gut. The present disclosure also provides methods of treating a subject in need thereof comprising administering to a subject a therapeutic composition comprising GABA-producing bacteria. As set forth herein, the GABA-producing bacteria can produce GABA in the subject's gut. The GABA can diffuse into other systems of the subject's body (e.g., the circulatory and nervous systems). There, the endogenous GABA can act as a neurotransmitter. In some embodiments, increased levels of GABA (e.g., in the nervous system) can improve the symptoms of the mental illness or disease of the central nervous system.

[0010] In some embodiments, the present disclosure also provides methods for identifying bacteria that produce GABA in humans at a physiologically relevant pH range and uses for these bacteria to modulate GABA levels in humans to treat mental illness.

[0011] The present disclosure also relates to a method of culturing previously uncultivated bacterial species. For instance the present disclosure teaches the isolation and characterization of a bacterial species KLE1738, provisionally named *Evtepia gabavorous*. Growth of *E. gabavorous* requires the presence of the growth factor GABA, which can be supplied by GABA-producing bacteria such as *Bacteroides fragilis* KLE1758.

[0012] In one aspect, the present disclosure provides a therapeutic composition comprising at least one purified bacterial population consisting of bacteria capable of producing GABA in a subject in need thereof.

[0013] In some embodiments, the at least one bacterial population consists of a bacteria comprising a 16S rDNA sequence at least about 95% identical to a 16S rDNA sequence selected from one of Seq. ID. Nos. 1-31 set forth in Table 1. In some embodiments, the at least one purified bacterial population consists of bacteria selected from the group consisting of: *Bacteroides caccae* KLE1911; *Bacteroides clarus* KLE1930; *Bacteroides dorei* KLE1912; *Bacteroides finegoldii* KLE1931; *Bacteroides fragilis* KLE1958; *Bacteroides massiliensis* KLE1932; *Bacteroides ovatus* KLE1770; *Bacteroides stercoris* KLE1933; *Bacteroides thetaiotaomicron* KLE1934; *Bacteroides uniformis* KLE1913; *Bacteroides vulgatus* KLE1910; *Bacteroides xylanisolvens* KLE1935; *Bifidobacterium adolescentis* KLE 1879; *Blautia obeum* KLE1914; *Blautia wexlerae* KLE1916; *Butyricimonas virosa* KLE1938; *Clostridium perfringens* KLE1937; *Clostridium sordellii* KLE1939; *Clostridium sp.* KLE1862; *Clostridium sp.* KLE1918; *Coprobacillus sp.* KLE1779; *Coprococcus sp.* KLE1880; *Dorea longicatena* KLE1917; *Eggerthella lenta* KLE1926; *Eubacterium rectale* KLE1922; *Gordonibacter pamelaeae* KLE1915; *Oscillibacter sp.* KLE1928; *Parabacteroides distasonis* KLE2020; *Parabacteroides merdae* KLE1863; *Ruminococcus gnavus* KLE1940; *Turicibacter sanguinis* KLE1941, and combinations thereof.

[0014] In some embodiments, the at least one purified bacterial population consists of a bacteria comprising a 16S rDNA sequence at least about 95% identical to a 16S rDNA sequence selected from one of Seq. ID. Nos. 32-274 set forth in Table 2. In some embodiments, the at least one purified bacterial population consists of bacteria

comprising a 16S rDNA sequence having at least 95% similarity to the 16S rDNA sequence selected from one of Seq. ID. Nos. 305-2217 set forth in Table 10. In some embodiments, the at least one purified bacterial population consists of bacteria comprising a DNA sequence which encodes an enzyme selected from: glutamate decarboxylase; putrescine aminotransferase; gamma-aminobutyraldehyde dehydrogenase; arginine decarboxylase; agmatinase; ornithine decarboxylase; or a combination thereof. In some embodiments, the glutamate decarboxylase; putrescine aminotransferase; gamma-aminobutyraldehyde dehydrogenase; arginine decarboxylase; agmatinase; ornithine decarboxylase; or a combination thereof, is encoded by a DNA sequence at least 70% similar in DNA sequence to any one of Seq. ID. Nos. 275-304 set forth in Table 3.

[0015] In some embodiments, the glutamate decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 4. In some embodiments, the putrescine aminotransferase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 5. In some embodiments, the gamma-aminobutyraldehyde dehydrogenase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 6. In some embodiments, the arginine decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 7. In some embodiments, the agmatinase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 8. In some embodiments, the ornithine decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 9.

[0016] In some embodiments, the at least one purified bacterial population consists of bacteria comprising a 16S rDNA sequence having at least 95% similarity to a reference bacterium selected from the group consisting of: *Escherichia coli* MG1655; *Escherichia coli* Nissle 1917; or a combination thereof.

[0017] In some embodiments, the bacterial population consists of bacteria capable of producing GABA at a physiologically relevant pH. In some embodiments, the bacterial

population consists of bacteria capable of producing GABA at a pH range between about 4.5 and about 7.5. In some embodiments, the bacterial population consists of bacteria capable of producing GABA inside the human gut.

[0018] In some embodiments, the composition is in the form of a probiotic, prebiotic, a capsule, a tablet, a caplet, a pill, a troche, a lozenge, a powders, a granule, a medical food, or a combination thereof. In some embodiments, the composition is administered as a fecal transplant.

[0019] In some embodiments, the bacteria are capable of producing GABA via expression of any combination of glutamate decarboxylase, putrescine aminotransferase, gamma-aminobutyraldehyde dehydrogenase, arginine decarboxylase, agmatinase, and/or ornithine decarboxylase.

[0020] In some embodiments, the therapeutic composition further comprises a purified bacterial strain that is cytotoxic or cytostatic to a GABA-consuming bacteria. In some embodiments, the GABA-consuming bacteria is *Evtepia gabavorous* or *Firmicutes bacterium* MGS:114.

[0021] In some embodiments, the therapeutic composition further comprises a prebiotic capable of stimulating the growth or GABA-production levels of a GABA-producing bacteria.

[0022] In one aspect, the present disclosure provides a method of treating a disease or disorder in a subject in need thereof, the method comprising administering to the subject a therapeutic composition comprising at least one purified bacterial population consisting of bacteria capable of producing GABA in a subject in need thereof.

[0023] In one aspect, the present disclosure provides the use of a therapeutic composition comprising at least one purified bacterial population consisting of bacteria capable of producing GABA in the manufacture of a medicament for the treatment of a disease.

[0024] In one aspect, the present disclosure provides the use of a therapeutic composition comprising at least one purified bacterial population consisting of bacteria capable of producing GABA for the treatment of a disease.

[0025] In some embodiments, the disease or disorder is a mental disease or disorder. In some embodiments, the mental disease or disorder is selected from the group consisting of depression, bipolar disorder, schizophrenia, anxiety, anxiety disorders, addiction, social phobia, treatment-resistant major depressive disorder (TR-MDD), major depressive disorder and its subtypes (melancholic depression, atypical depression, catatonic depression, postpartum depression, and seasonal affective disorder), Neurodegenerative amyloid disorders (Parkinson's, Alzheimer's, and Huntington's diseases) orthostatic tremor, Lafora disease, restless leg syndrome, neuropathic pain, pain disorders, dementia, epilepsy, stiff-person syndrome, premenstrual dysphoric disorder, autism spectrum disorder, sleep disorders, and attention deficit hyperactivity disorder (ADHD), and combinations thereof. In some embodiments, treating a disease or disorder comprises decreasing at least one symptom of the disease or disorder, such as fatigue, insomnia, motor dysfunction, stress, persistent anxiety, persistent sadness, social withdrawal, substance withdrawal, irritability, thoughts of suicide, thoughts of self-harm, restlessness, low sex drive, lack of focus, seizures, memory loss, anger, bouts of emotional reactivity, confusion, pain, and muscle spasms, loss of appetite, altered intestine motility, and combinations thereof.

[0026] In some embodiments, the at least one bacterial population consists of a bacteria comprising a 16S rDNA sequence at least about 95% identical to a 16S rDNA sequence selected from one of Seq. ID. Nos. of 1-31 set forth in Table 1. In some embodiments, the at least one purified bacterial population consists of bacteria selected from the group consisting of: *Bacteroides caccae* KLE1911; *Bacteroides clarus* KLE1930; *Bacteroides dorei* KLE1912; *Bacteroides finegoldii* KLE1931; *Bacteroides fragilis* KLE1958; *Bacteroides massiliensis* KLE1932; *Bacteroides ovatus* KLE1770; *Bacteroides stercoris* KLE1933; *Bacteroides thetaiotaomicron* KLE1934; *Bacteroides uniformis* KLE1913; *Bacteroides vulgatus* KLE1910; *Bacteroides xylanisolvens* KLE1935; *Bifidobacterium adolescentis* KLE 1879; *Blautia obeum* KLE1914; *Blautia wexlerae* KLE1916; *Butyricimonas virosa* KLE1938; *Clostridium perfringens* KLE1937; *Clostridium sordellii* KLE1939; *Clostridium sp.* KLE1862; *Clostridium sp.* KLE1918; *Coprobacillus sp.* KLE1779; *Coprococcus sp.* KLE1880; *Dorea longicatena* KLE1917; *Eggerthella lenta* KLE1926; *Eubacterium rectale* KLE1922; *Gordonibacter pamelaeae* KLE1915;

Oscillibacter sp. KLE1928; *Parabacteroides distasonis* KLE2020; *Parabacteroides merdae* KLE1863; *Ruminococcus gnavus* KLE1940; *Turicibacter sanguinis* KLE1941, and combinations thereof.

[0027] In some embodiments, the at least one purified bacterial population consists of a bacteria comprising a 16S rDNA sequence at least about 95% identical to a 16S rDNA sequence selected from one of Seq. ID. Nos. of 32-274 set forth in Table 2.

[0028] In some embodiments, the at least one bacterial population consists of a bacteria comprising a 16S rDNA sequence at least about 95% identical to any one of Seq ID Nos. 305-2217 set forth in Table 10.

[0029] In some embodiments, the at least one purified bacterial population consists of bacteria comprising a DNA sequence which encodes an enzyme selected from: glutamate decarboxylase; putrescine aminotransferase; gamma-aminobutyraldehyde dehydrogenase; arginine decarboxylase; agmatinase; ornithine decarboxylase; or a combination thereof.

[0030] In some embodiments, the glutamate decarboxylase; putrescine aminotransferase; gamma-aminobutyraldehyde dehydrogenase; arginine decarboxylase; agmatinase; ornithine decarboxylase; or a combination thereof, is encoded by a DNA sequence at least 70% similar to a DNA sequence selected from one of Seq. ID. Nos. 275-304 set forth in Table 3.

[0031] In some embodiments, the glutamate decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 4. In some embodiments, the putrescine aminotransferase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 5. In some embodiments, the gamma-aminobutyraldehyde dehydrogenase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 6. In some embodiments, the arginine decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 7. In some embodiments, the agmatinase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 8. In some embodiments, the ornithine

decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 9.

[0032] In some embodiments, the bacteria is genetically engineered to produce GABA. In some embodiments, the bacteria is engineered to produce GABA via expression of glutamate decarboxylase; putrescine aminotransferase; gamma-aminobutyraldehyde dehydrogenase; arginine decarboxylase; agmatinase; ornithine decarboxylase; or a combination thereof.

[0033] In some embodiments, wherein the glutamate decarboxylase; putrescine aminotransferase; gamma-aminobutyraldehyde dehydrogenase; arginine decarboxylase; agmatinase; ornithine decarboxylase; or a combination thereof, is encoded by a DNA sequence at least 70% similar in DNA sequence selected from one of Seq. ID. Nos. of 275-304 set forth in Table 3.

[0034] In some embodiments, the glutamate decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 4. In some embodiments, the putrescine aminotransferase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 5. In some embodiments, the gamma-aminobutyraldehyde dehydrogenase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 6. In some embodiments, the arginine decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 7. In some embodiments, the agmatinase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 8. In some embodiments, the ornithine decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 9.

[0035] In some embodiments, the at least one purified bacterial population consists of bacteria comprising a 16S rDNA sequence having at least 95% similarity to a reference bacterium selected from the group consisting of: *Escherichia coli* MG1655; *Escherichia coli* Nissle 1917; or a combination thereof.

[0036] In some embodiments, the bacterial population consists of bacteria capable of producing GABA at a physiologically relevant pH. In some embodiments, the bacterial population consists of bacteria capable of producing GABA at a pH range between about 4.5 and about 7.5. In some embodiments, bacterial population consists of bacteria capable of producing GABA inside the human gut. In some embodiments, the composition is administered as a fecal transplant. In some embodiments, the composition is administered as a probiotic. In some embodiments, the bacteria are capable of producing GABA via expression any combination of glutamate decarboxylase, putrescine aminotransferase, gamma-aminobutyraldehyde dehydrogenase, arginine decarboxylase, agmatinase, ornithine decarboxylase, and combinations thereof.

[0037] In some embodiments, the at least one bacterial strain is cytotoxic or cytostatic to a GABA-consuming bacteria. In some embodiments, the GABA-consuming bacteria is *Evtepia gabavorous* or *Firmicutes bacterium* MGS:114.

[0038] In some embodiments, the method of treating a subject further comprises identifying a subject in need of treatment by determining whether the subject would benefit from an increase in endogenous GABA by measuring an initial amount of GABA in the subject's stool. In some embodiments, the initial amount of GABA in the subject's stool is below about 8 μg per gram of wet or dry stool. In some embodiments, the amount of GABA in the subject's stool is increased relative to the initial amount after administering the therapeutic composition.

[0039] In some embodiments, the method of treating a subject further comprises identifying a subject in need of treatment by determining whether the subject would benefit from an increase in endogenous GABA by measuring an initial amount of GABA-producing bacteria in the subject's stool. In some embodiments, the initial amount of GABA-producing bacteria in the subject's stool is less than about 10% of total bacteria as measured by 16S sequence mapping. In some embodiments, at least one GABA-producing bacteria is increased in the subject's stool relative to the initial amount of GABA-producing bacteria in the subject's stool after administering the therapeutic composition.

[0040] In some embodiments, the method of treating a subject further comprises identifying a subject in need of treatment by determining whether the subject would benefit from an increase in endogenous GABA by measuring an initial amount of GABA in the subject's blood or serum. In some embodiments, the amount of GABA in the subject's blood or serum is below about 10 μg per liter of blood. In some embodiments, the amount of GABA in the subject's blood or serum is increased relative to the initial amount after administering the therapeutic composition.

[0041] In some embodiments, the method of treating a subject further comprises identifying a subject in need of treatment by determining whether the subject would benefit from an increase in endogenous GABA by measuring an amount of GABA in the subject's brain. In some embodiments, the amount of GABA in the subject's brain is below about 1.0 mM/kg. In some embodiments, the amount of GABA in the subject's brain is increased relative to the initial amount after administering the therapeutic composition.

[0042] In some embodiments, the method of treating a subject further comprises identifying a subject in need of treatment by determining whether the subject would benefit from an increase in endogenous GABA by measuring an initial amount of expression of GABA-producing enzymes in the subject's stool. In some embodiments, the GABA-producing enzymes are selected from glutamate decarboxylase, putrescine aminotransferase, gamma-aminobutyraldehyde dehydrogenase, arginine decarboxylase, agmatinase, ornithine decarboxylase, and combinations thereof. In some embodiments, the initial amount of enzyme expression is measured by qPCR. In some embodiments, the expression of enzymes is increased relative to the initial amount of enzyme expression after administering the therapeutic composition.

[0043] In some embodiments, the method of treating a subject further comprises identifying a subject in need of treatment by determining whether the subject would benefit from an increase in endogenous GABA by measuring an initial amount of GABAergic response in the subject's brain. In some embodiments, the amount of the GABAergic response in the subject's brain is increased relative to the initial amount after administering the therapeutic composition. In some embodiments, the therapeutic

composition comprises a prebiotic capable of stimulating the growth or GABA production of GABA-producing bacteria.

[0044] In one aspect, the present disclosure provides a method of culturing a GABA-dependent bacteria, comprising disposing at least one live GABA-dependent bacterial cell on a suitable substrate, and providing a source of GABA.

[0045] In some embodiments, the suitable substrate is agar. In some embodiments, providing a source of GABA comprises co-culturing with another bacterial strain, said strain is capable of producing GABA. In some embodiments, GABA is added to the substrate. In some embodiments, the GABA-dependent bacteria is *E. gabavorous*.

[0046] In one aspect, the present disclosure provides a method of identifying a bacterial strain or strains capable of producing GABA at a physiologically relevant pH of the human intestinal tract, comprising:

(a) dispersing a sample believed to contain GABA-producing bacteria within a substrate, the substrate being at least partially permeable to GABA,

(b) contacting the substrate loaded with potential GABA-producing bacteria with a GABA-dependent bacterium; and

(c) identifying a GABA-producing bacteria by observing the formation of a colonies of the GABA-dependent bacteria around potential GABA-producing bacteria in the substrate.

[0047] In some embodiments, the substrate is being buffered to maintain the pH at a physiologically range found in the human gastrointestinal tract. In some embodiments, the GABA-dependent bacteria is *E.gabavorous*. In some embodiments, the pH range is between about 4.5 and about 7.5.

[0048] The present disclosure provides compositions and methods for treating mental illness or disease of the central nervous system in a subject and therapeutic compositions for the same. The method can comprise administering to the subject a bacterium and/or multiple bacteria capable of producing endogenous GABA in the subject's gut at a physiologically relevant pH. The present technology can have the advantage of alleviating the symptoms of a mental illness or disease of the central nervous system without the aid of synthetic medications (e.g., antidepressants), which can have unwanted side-effects, or in

combination with existing medications. Additionally, the present technology can have the advantage of further improving the digestive health of the subject, such as improving intestinal motility and reducing gastrointestinal pain. Additional features and advantages of the present technology will be apparent to one of skill in the art upon reading the Detailed Description of the Invention, below.

Brief Description of the Figures

[0049] Figure 1A shows a co-culture assay to grow and identify previously unculturable bacteria.

[0050] Figure 1B shows the growth of *Evtepia gabavorous* KLE1738 in the presence of the helper bacteria *Bacteroides fragilis* KLE1758.

[0051] Figure 2A shows the growth of *Evtepia gabavorous* KLE1738 in the presence of the supernatant from *Bacteroides fragilis*.

[0052] Figure 2B shows a lack of growth of *Evtepia gabavorous* KLE1738 in the presence of the empty vehicle.

[0053] Figure 2C shows the growth of *Evtepia gabavorous* in the presence of the most polar fraction of the supernatant from *Bacteroides fragilis* KLE1758.

[0054] Figure 2D shows a close-view of the lack of growth of *Evtepia gabavorous* KLE1738 in the presence of the empty vehicle.

[0055] Figure 2E shows a close-view of the growth of *Evtepia gabavorous* KLE1738 in the presence of the supernatant from *Bacteroides fragilis* KLE1758.

[0056] Figure 2F shows a close-view of the lack of growth of *Evtepia gabavorous* KLE1738 in the presence of the empty vehicle (bacterial media).

[0057] Figure 2G shows a table of potential growth factors for *E. gabavorous* KLE1738, with green indicating induction of growth.

[0058] Figure 3 shows a proposed GABA metabolism of *E. gabavorous* KLE1738.

[0059] Figure 4a shows GABA production of *B. fragilis* KLE1758 is observed only at a pH of 5.5 and below, highlighting the importance of acidic pH on GABA production for certain strains or species of bacteria.

[0060] Figure 4B shows an assay for the identification of GABA-producing bacteria, capable of producing GABA at a pH between 4.5 and 7.5, using the GABA-requirement of *E. gabavorous* KLE1738. The medium was heavily buffered to maintain a pH of the desired range and identify strains of bacteria capable of producing GABA at a pH physiologically relevant to the human gut.

[0061] Figure 4C shows a phylogenetic tree of GABA producers, capable of producing GABA at a pH of about 4.5 to about 7.5, identified by the methods herein.

[0062] Figure 5 shows GABA production capabilities of several strains identified using the screen described in Example 7. The ability of organisms to produce GABA at a range of pH conditions is highlighted.

[0063] Figure 6A shows the growth of *E. gabavorous* KLE1738 in the presence of *E. coli* that has been engineered to express glutamate decarboxylase gadA.

[0064] Figure 6B shows the growth of *E. gabavorous* KLE1738 in the presence of *E. coli* that has been engineered to express glutamate decarboxylase gadB.

[0065] Figure 6C shows the growth of *E. gabavorous* KLE1738 in the presence of *B. fragilis* KLE 1758 that is known to produce GABA.

[0066] Figure 6D shows the absence of growth of *E. gabavorous* KLE1738 in the presence of *E. coli* that has been engineered to express glutamate decarboxylase gadC.

[0067] Figure 6E shows the absence of growth of *E. gabavorous* KLE1738 in the presence empty vector.

Detailed Description of the Invention

[0068] The present disclosure relates to compositions and methods for treating or decreasing a symptom of a disease in a subject. The disease can be a mental illness or a disease of the central nervous system. After identifying a subject with a mental illness or disease of the central nervous system, the method can comprise determining whether the subject would benefit from an increase in endogenous GABA, for instance by measuring the amount of GABA in the subject's stool, blood serum, or other bodily fluids, measuring

levels of GABA in different regions of the brain, measuring the GABAergic response in different regions in the brain, measuring activity of GABA producing enzymes in stool, or by measuring the amount of GABA-producing bacteria in the subject's stool. The method can further comprise administering to the subject a GABA-producing bacterium or bacteria that can be capable of producing GABA in the subject's gut, (e.g., at a physiologically relevant pH of the gut).

[0069] Some bacteria produce GABA from gamma-aminobutyrate to maintain intracellular pH homoeostasis in order to overcome acid stress. As set forth herein, the production of GABA by microbes (e.g., bacteria) in the human gut can impact the health of a subject. For instance, GABA produced by bacteria in the human gut can act as a neurotransmitter to treat a mental illness, a disease of the central nervous system, or improve gastrointestinal health in a subject.

Definitions

[0070] As used herein "administer" and "administration" encompasses embodiments in which one person directs another to consume a bacteria or a bacterial composition in a certain manner and/or for a certain purpose, and also situations in which a user uses bacteria or a bacterial composition in a certain manner and/or for a certain purpose independently of or in variance to any instructions received from a second person. Non-limiting examples of embodiments in which one person directs another to consume a bacteria or bacterial composition in a certain manner and/or for a certain purpose include when a physician prescribes a course of conduct and/or treatment to a patient, when a parent commands a minor user (such as a child) to consume bacteria or a bacterial composition, when a trainer advises a user (such as an athlete) to follow a particular course of conduct and/or treatment, and when a manufacturer, distributer, or marketer recommends conditions of use to an end user, for example through advertisements or labeling on packaging or on other materials provided in association with the sale or marketing of a product.

[0071] The term "isolated" encompasses a bacterium or other entity or substance that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature, such as human stool, or in an experimental setting, such as a Petri plate consisting of artificial growth medium), and/or (2) produced,

prepared, purified, and/or manufactured by the hand of man. Isolated bacteria may be separated from at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more of the other components with which they were initially associated. In some embodiments, isolated bacteria are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components (such as other bacterial species). The terms "purify," "purifying" and "purified" refer to a bacterium or other material that has been separated from at least some of the components with which it was associated either when initially produced or generated (e.g., whether in nature or in an experimental setting), or during any time after its initial production, as recognized by those skilled in the art of bacterial cultivation. A bacterium or a bacterial population may be considered purified if it is isolated at or after production, such as from a material or environment containing the bacterium or bacterial population, and a purified bacterium or bacterial population may contain other materials up to about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or above about 90% and still be considered "isolated." In some embodiments, purified bacteria and bacterial populations are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. In the instance of bacterial compositions provided herein, the one or more bacterial types present in the composition can be independently purified from one or more other bacteria produced and/or present in the material or environment containing the bacterial type. Bacterial compositions and the bacterial components thereof are generally purified from residual habitat products.

[0072] As used herein, "probiotic" is understood to mean "Live microorganisms which when administered in adequate amounts confer a health benefit on the host", as currently defined by the World Health Organization.

[0073] As used herein, "prebiotic" is understood to mean an ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that may (or may not) confer benefits upon the host.

[0074] As used herein, "medical food" is understood to mean "a food which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation", as defined by 5(b) of the Orphan Drug Act (21 U.S.C. 360ee (b) (3)).

[0075] As used herein "initial amount" is understood to mean the amount of a substance, e.g., GABA in an aliquot of sample prior to administration of GABA-producing bacteria to the subject. Initial amount can be measured in terms of concentration. For instance, an initial amount can be measured in terms of micrograms of substance per milliliter of sample, e.g., micrograms of GABA per milliliter of blood or serum (μg GABA/mL blood or serum). The initial amount of can also be measured, for instance, as the amount of GABA in regions of the brain, such as the prefrontal cortex prior to administration of the GABA-producing bacteria. The amount of GABA can be represented in terms of millimoles of GABA per kg tissue (mmol GABA per kg of brain tissue). The initial amount can also be measured, for instance, as the amount of GABA in a subject's stool sample prior to administration of GABA-producing bacteria to the subject. The amount of GABA can be represented in terms of micrograms of GABA per gram of stool (μg GABA/g stool). The initial amount can also be the level of expression of GABA producing enzymes in the stool (log change of reads), as measured by qPCR or other appropriate method. Unless otherwise defined herein, stool is weighed when wet or dry, i.e., without active drying, and within one hour of production of the stool. For instance, the stool can be weighed within 45 minutes, 30 minutes, 15 minutes, 10 minutes, or within 5 minutes of production of the stool.

[0076] As used herein, a "GABAergic response" means the response of a given organ (e.g., the brain or vagus nerve) to differences in the concentrations of GABA, GABA producing bacteria, or prebiotics to which it is exposed. A GABAergic response can include a change in concentrations of GABA as well as expression levels and/or activity of different $GABA_A$, $GABA_B$, and/or $GABA_C$ receptors.

[0077] "GABA-producing bacteria" is understood to mean bacteria that can produce measurable quantities of GABA, as detected by LC/MS, ELISA, or other appropriate analytical assays. In some embodiments, GABA-producing bacteria can produce GABA under the physiological conditions in a human, e.g., under the pH, and temperature of the human gut.

[0078] "Physiologically relevant pH" of the human intestinal tract is understood to mean a pH range that exists in the body. For instance, a pH range that is physiologically relevant to the human gut can be in the range of about 4.5 to about 7.5.

[0079] The term "gut" is understood to refer to the human gastrointestinal tract, also known as the alimentary canal. The gut includes the mouth, pharynx, oesophagus, stomach, small intestine (duodenum, jejunum, ileum), large intestines (cecum and colon) and rectum.

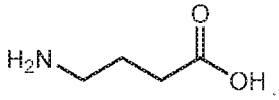
[0080] As used herein, "bacteria" or "bacterial strain" is understood to mean a species of bacteria. A "bacterium" is understood as a single bacterial cell of a given species.

[0081] The term "treating" with regard to a subject, refers to improving at least one symptom of the subject's disorder. Treating includes curing, improving, or at least partially ameliorating the disorder.

[0082] The term "EMBL/GenBank/DDBJ ID" refers to an accession number, which when used as in input for databases such as the European Molecular Biology Laboratory (EMBL), GenBank, or DNA Data Bank of Japan (DDBJ), via their respective internet websites, enables access to information, such as nucleotide sequence of a gene, and the bacterium encoding that sequence in its genome. The EMBL/GenBank/DDBJ ID is used in this application as a conveninent means to access sequence information.

Gamma-Aminobutyric Acid (GABA)

[0083] The term "GABA" is understood to mean *gamma*-aminobutyric acid (γ-aminobutyric acid). GABA has the chemical structure:



[0084] GABA is a major inhibitory neurotransmitter in the mammalian central nervous system. It plays a principal role in reducing neuronal excitability throughout the nervous system. GABA can be a difficult compound to deliver therapeutically due to efflux and half-life limitations. For example, in rodents, the brain efflux rate of GABA was found to be 17 times higher than the influx rate. Additionally, GABA only has a half-life of only 17 minutes in mice. Accordingly, because of the short half-life of GABA in vivo, oral GABA supplementation can be ineffective, as it may require frequent dosing, even with slow release capsules.

[0085] The present disclosure provides for delivering a therapeutic composition of one or more bacteria that can produce GABA within the intestinal tract, in order to consistently deliver GABA into systemic circulation (e.g., into the nervous system). The endogenously produced GABA can also activate vagal nerve receptors directly. This can mitigate the inherent half-life of GABA.

[0086] In some embodiments, the microbiome can influence GABA levels and the GABAergic response in the brain. For instance, germ-free animals can have substantially reduced luminal and serum levels of GABA. Without wishing to be bound by theory, this suggests the microbiome is important in regulating levels of this important neurotransmitter. As set forth herein, GABAergic modulation by microbiome intervention (e.g., using methods and compositions described herein) can have therapeutic potential.

[0087] In some embodiments, GABA can play a role in mental illness or disease of the central nervous system. For instance, in some embodiments, low levels of GABA can be associated with depression, bipolar disorder, schizophrenia, anxiety, anxiety disorders, addiction, social phobia, treatment-resistant major depressive disorder (TR-MDD), major depressive disorder and its subtypes (melancholic depression, atypical depression, catatonic depression, postpartum depression, and seasonal affective disorder), Neurodegenerative amyloid disorders (Parkinson's, Alzheimer's, and Huntington's diseases) orthostatic tremor, Lafora disease, restless leg syndrome, neuropathic pain, pain disorders, dementia, epilepsy, stiff-person syndrome, premenstrual dysphoric disorder, autism spectrum disorder, sleep disorders, and attention deficit hyperactivity disorder (ADHD). As set forth herein, the

present disclosure provides increasing the amount of endogenous GABA in a subject can decrease levels of mental illness or disease of the central nervous system in the subject.

[0088] In some embodiments, GABA produced by gut bacteria can play a role in mental illness or disease of the central nervous system via the vagus nerve, connecting the intestinal tract to the peripheral and central nervous systems.

[0089] In some embodiments, GABA produced by gut bacteria can play a role in mental illness or disease of the central nervous system via affecting circulating levels of systemic GABA in the host, which can influence the peripheral and central nervous systems.

Means of GABA Production by Microbes

[0090] Bacteria can produce GABA using a variety of different pathways. Set forth below are exemplary pathways that bacteria and other microbes can use to produce GABA (e.g., *in vivo*). As set forth below, any of the GABA production pathways described herein can be naturally occurring in a given bacterium. Alternatively, a necessary enzyme or grouping of enzymes can be added to the DNA sequence of a bacteria to enable the bacteria to produce GABA.

Glutamate Pathway

[0091] In some embodiments, microbes can produce GABA using the glutamate decarboxylase enzyme (e.g., glutamate decarboxylase EC 4.1.1.15). In some embodiments, glutamate decarboxylase is capable of directly converting glutamate to GABA.

Putrescine to 4-Aminobutanal Pathway

[0092] In some embodiments, microbes can produce GABA using the putrescine to 4-aminobutanal pathway. The microbes can then convert 4-aminobutanal to GABA. In some embodiments, putrescine aminotransferase (for instance, putrescine aminotransferase EC 2.6.1.82) can be used to convert putrescine to 4-aminobutanal. The 4-aminobutanal can then be converted in the presence of gamma-aminobutyraldehyde dehydrogenase (e.g., gamma-aminobutyraldehyde dehydrogenase (EC 1.2.1.19)) to GABA.

Arginine to Agmatine to Putrescine Pathway

[0093] In some embodiments, microbes can produce GABA using the arginine to agmatine to putrescine Pathway. Once the putrescine is produced, it can be converted as

described above (e.g., using the putrescine to 4-aminobutanal pathway) to GABA. In some embodiments, arginine decarboxylase (e.g., arginine decarboxylase (EC 4.1.1.19)) can convert arginine to agmatine. Agmatine can then be converted to putrescine using agmatinase (e.g., agmatinase (EC 3.5.3.11)).

L-ornithine to Putrescine pathway

In some embodiments, ornithine decarboxylase (e.g., ornithine decarboxylase (EC 4.1.1.17)) can be used to convert ornithine to putrescine. Once the putrescine is produced, it can be converted as described above (e.g., using the putrescine to 4-aminobutanal pathway) to GABA.

Bacterial Strains

[0094] The present disclosure provides bacterial strains (e.g., purified strains) and therapeutic compositions comprising the same for administration to a subject in need thereof. The bacteria can be naturally occurring, or can be engineered (e.g., through strain engineering or selection) to produce GABA. In some embodiments, one strain of GABA-producing bacteria can be administered to a subject. In some embodiments, multiple strains of GABA-producing bacteria can be administered to a subject in need thereof. In some embodiments, the one or more bacteria (e.g., purified bacteria) can act synergistically. For instance, the multiple bacteria can act synergistically to produce high levels of GABA. In some embodiments, the one or more bacteria can also help to reduce the number of GABA-consuming bacteria in a human gut. Accordingly, any one, or any combination of the GABA-producing bacteria taught herein can be administered to a subject in need thereof.

[0095] In some embodiments, the bacteria taught herein can produce GABA at physiologically relevant conditions, such as under the conditions of the human gut. In some embodiments, the GABA-producing bacteria taught herein can produce GABA a pH relevant to the human gut is between about 4.5 and about 7.5. For instance, the pH can be about 4.5, 5.0, 5.5., 6.0, 6.5, 7.0 7.5, or any value between about 4.5 and 7.5.

[0096] The ability to produce GABA at physiologically relevant pH is important in view of the pH restrictions of GABA production in many bacteria. For example, *E. coli* is unable to produce GABA above a pH of 4.5. Instead, without wishing to be bound by

theory, it can use conversion of glutamate to GABA coupled with GABA export, for example, as a means to neutralize the intracellular environment. Accordingly the present disclosure bacteria capable of producing GABA at a pH relevant to the host environment, such as the more neutral large intestines (e.g., between about pH 4.5 and about pH 7.5). In some embodiments, the bacteria taught herein can also produce GABA under other relevant conditions that exist within the human gut. That is, the GABA-producing bacteria taught herein can produce GABA in the absence of oxygen, in a nutrient-competitive and fluctuating environment, and in the absence of light.

Natural Strains

[0097] In some embodiments, the GABA-producing bacteria can be identified by having a 16S nucleic acid sequence substantially similar to the 16S sequences of reference bacteria listed in Table 1, with a Seq. ID. No. of 1-31. In some embodiments, the GABA-producing bacteria can have at least 90% 16S sequence similarity to the 16S sequences given in Table 1 (e.g., at least 91% similarity, at least 92% similarity, at least 93% similarity, at least 94% similarity, at least 95% similarity, at least 96% similarity, at least 97% similarity, at least 98% similarity, at least 99% similarity, at least 99.5% similarity, at least 99.9% similarity, or 100% similarity).

[0098] In some embodiments, the GABA-producing bacteria can be identified by NCBI Taxon ID of the organism they are most related to by 16S sequence.These bacteria include those belonging to the same NCBI Taxon assignment as *Bacteroides caccae* KLE1911; *Bacteroides clarus* KLE1930; *Bacteroides dorei* KLE1912; *Bacteroides finegoldii* KLE1931; *Bacteroides fragilis* KLE1958; *Bacteroides massiliensis* KLE1932; *Bacteroides ovatus* KLE1770; *Bacteroides stercoris* KLE1933; *Bacteroides thetaiotaomicron* KLE1934; *Bacteroides uniformis* KLE1913; *Bacteroides vulgatus* KLE1910; *Bacteroides xylanisolvens* KLE1935; *Bifidobacterium adolescentis* KLE 1879; *Blautia obeum* KLE1914; *Blautia wexlerae* KLE1916; *Butyricimonas virosa* KLE1938; *Clostridium perfringens* KLE1937; *Clostridium sordellii* KLE1939; *Clostridium sp.* KLE1862; *Clostridium sp.* KLE1918; *Coprobacillus sp.* KLE1779; *Coprococcus sp.* KLE1880; *Dorea longicatena* KLE1917; *Eggerthella lenta* KLE1926; *Eubacterium rectale* KLE1922; *Gordonibacter pamelaeae* KLE1915; *Oscillibacter sp.* KLE1928;

Parabacteroides distasonis KLE2020; *Parabacteroides merdae* KLE1863; *Ruminococcus gnavus* KLE1940; *Turicibacter sanguinis* KLE1941, and combinations thereof.

Table 1. Strain name of GABA producing bacteria, and their cooresponding Seq. ID No.

Strain	**Seq. ID No.**
Bacteroides caccae KLE1911	1
Bacteroides clarus KLE1930	2
Bacteroides dorei KLE1912	3
Bacteroides finegoldii KLE1931	4
Bacteroides fragilis KLE1958	5
Bacteroides massiliensis KLE1932	6
Bacteroides ovatus KLE1770	7
Bacteroides stercoris KLE1933	8
Bacteroides thetaiotaomicron KLE1934	9
Bacteroides uniformis KLE1913	10
Bacteroides vulgatus KLE1910	11
Bacteroides xylanisolvens KLE1935	12
Bifidobacterium adolescentis KLE 1879	13
Blautia obeum KLE1914	14
Blautia wexlerae KLE1916	15
Butyricimonas virosa KLE1938	16
Clostridium perfringens KLE1937	17
Clostridium sordellii KLE1939	18
Clostridium sp. KLE1862	19
Clostridium sp. KLE1918	20
Coprobacillus sp. KLE1779	21
Coprococcus sp. KLE1880	22
Dorea longicatena KLE1917	23
Eggerthella lenta KLE1926	24
Eubacterium rectale KLE1922	25
Gordonibacter pamelaeae KLE1915	26
Oscillibacter sp. KLE1928	27
Parabacteroides distasonis KLE2020	28
Parabacteroides merdae KLE1863	29
Ruminococcus gnavus KLE1940	30
Turicibacter sanguinis KLE1941	31

[0099] Also disclosed herein are bacteria that are predicted to be capable of producing GABA (e.g., under physiologically relevant conditions and/or in the human gut). Bacteria are identified as being candidate GABA producing bacteria if they have encoded in their

genome enzymes involved in GABA biosynthesis. In some embodiments, the bacteria that are predicted to be capable of producing GABA can be identified by having a 16S nucleic acid sequence substantially similar to the 16S sequences of reference bacteria listed in Table 2, with a Seq. ID. No. of 32-274. In some embodiments, the predicted GABA-producing bacteria can have at least 90% 16S sequence similarity to the 16S sequences given in Table 2, with a Seq. ID. No. of 32-274 (e.g., at least 91% similarity, at least 92% similarity, at least 93% similarity, at least 94% similarity, at least 95% similarity, at least 96% similarity, at least 97% similarity, at least 98% similarity, at least 99% similarity, at least 99.5% similarity, at least 99.9% similarity, or 100% similarity).

Table 2. Predicted GABA producing bacteria and their corresponding Seq. ID No..

Species	**Seq. ID No.**
Abiotrophia defective	32
Acetobacter okinawensis	33
Achromobacter arsenitoxydans	34
Achromobacter xylosoxidans	35
Acidovorax sp.	36
Acidovorax sp.	37
Acidovorax sp.	38
Acidovorax sp.	39
Acidovorax sp.	40
Actinoplanes friuliensis	41
Aeromonas enteropelogenes	42
Aeromonas hydrophila	43
Afipia birgiae	44
Afipia clevelandensis	45
Afipia sp.	46
Agrobacterium albertimagni	47
Agrobacterium sp.	48
Agrobacterium tumefaciens	49
Akkermansia muciniphila	50
Alcaligenes faecalis	51
Alicycliphilus denitrificans	52
Alistipes finegoldii	53
Alistipes indistinctus	54
Alistipes onderdonkii	55
Alistipes putredinis	56

Alistipes shahii	57
Aquamicrobium defluvii	58
Arenimonas donghaensis	59
Arthrobacter sp.	60
Azospirillum sp.	61
Bacillus bataviensis	62
Bacillus cereus	63
Bacillus cereus	64
Bacillus endophyticus	65
Bacillus weihenstephanensis	66
Bacteroidaceae bacterium	67
Bacteroides acidifaciens	68
Bacteroides caccae	69
Bacteroides cellulosilyticus	70
Bacteroides dorei	71
Bacteroides eggerthii	72
Bacteroides finegoldii	73
Bacteroides fragilis	74
Bacteroides gallinarum	75
Bacteroides intestinalis	76
Bacteroides massiliensis	77
Bacteroides oleiciplenus	78
Bacteroides ovatus	79
Bacteroides rodentium	80
Bacteroides salyersiae	81
Bacteroides sartorii	82
Bacteroides sp.	83
Bacteroides sp.	84
Bacteroides sp.	85
Bacteroides sp.	86
Bacteroides stercoris	87
Bacteroides thetaiotaomicron	88
Bacteroides uniformis	89
Bacteroides vulgatus	90
Bacteroides xylanisolvens	91
Barnesiella intestinihominis	92
Bhargavaea cecembensis	93
Bifidobacterium adolescentis	94
Bifidobacterium angulatum	95
Bifidobacterium dentium	96
Bifidobacterium ruminantium	97

Blastococcus sp.	98
Bordetella bronchiseptica	99
Bordetella trematum	100
Bosea sp.	101
Bradyrhizobium sp.	102
Brevibacillus borstelensis	103
Brevundimonas diminuta	104
Brevundimonas naejangsanensis	105
Brucella abortus	106
Brucella melitensis	107
Brucella neotomae	108
Burkholderia mallei	109
Burkholderia multivorans	110
Carnobacterium gallinarum	111
Caulobacter crescentus	112
Caulobacter sp.	113
Cellulomonas flavigena	114
Cellulomonas sp.	115
Cellulosimicrobium cellulans	116
Cetobacterium somerae	117
Citrobacter amalonaticus	118
Cloacibacillus evryensis	119
Clostridium acetobutylicum	120
Clostridium perfringens	121
Comamonas granuli	122
Corynebacterium variabile	123
Cupriavidus basilensis	124
Cupriavidus sp.	125
Dechloromonas agitata	126
Deinococcus geothermalis	127
Delftia tsuruhatensis	128
Desulfovibrio desulfuricans	129
Desulfovibrio sp.	130
Devosia riboflavina	131
Eggerthella sp.	132
Ensifer adhaerens	133
Enterococcus casseliflavus	134
Enterococcus flavescens	135
Escherichia coli	136
Eubacterium limosum	137
Eubacterium nodatum	138

Eubacterium saphenum	139
Fusobacterium periodonticum	140
Gordonia sputi	141
Gordonia terrae	142
Gordonibacter pamelaeae	143
Halomonas stevensii	144
Halomonas titanicae	145
Hoeflea sp.	146
Intrasporangium calvum	147
Janibacter hoylei	148
Kaistia granuli	149
Kineococcus radiotolerans	150
Lactobacillus coleohominis	151
Lactobacillus plantarum	152
Lactobacillus reuteri	153
Lactococcus garvieae	154
Lactococcus lactis	155
Lautropia mirabilis	156
Leucobacter salsicius	157
Luteimonas huabeiensis	158
Magnetospirillum magnetotacticum	159
Marinobacter lipolyticus	160
Marmoricola sp.	161
Megasphaera micronuciformis	162
Megasphaera sp.	163
Mesorhizobium sp.	164
Methanobrevibacter arboriphilus	165
Methylobacterium radiotolerans	166
Methylobacterium sp.	167
Microbacterium sp.	168
Micromonospora aurantiaca	169
Mogibacterium Mogibacterium	170
Morganella morganii	171
Mycobacterium smegmatis	172
Mycobacterium sp.	173
Mycobacterium sp.	174
Mycobacterium vanbaalenii	175
Neisseria sicca	176
Neorhizobium galegae	177
Nocardia rhamnosiphila	178
Nocardiopsis alkaliphila	179

Nocardiopsis ganjiahuensis	180
Nocardiopsis synnemataformans	181
Nocardiopsis valliformis	182
Novosphingobium nitrogenifigens	183
Ochrobactrum intermedium	184
Odoribacter laneus	185
Odoribacter splanchnicus	186
Oerskovia turbata	187
Pannonibacter phragmitetus	188
Pantoea vagans	189
Parabacteroides distasonis	190
Parabacteroides goldsteinii	191
Parabacteroides johnsonii	192
Parabacteroides merdae	193
Parabacteroides sp.	194
Parabacteroides sp.	195
Paracoccus denitrificans	196
Paracoccus sp.	197
Paracoccus yeei	198
Parvimonas Parvimonas	199
Pectobacterium carotovorum	200
Phyllobacterium sp.	201
Polaromonas sp.	202
Porphyromonas bennonis	203
Proteus mirabilis	204
Providencia alcalifaciens	205
Providencia burhodogranariea	206
Providencia rettgeri	207
Pseudacidovorax intermedius	208
Pseudoalteromonas sp.	209
Pseudochrobactrum sp.	210
Pseudomonas aeruginosa	211
Pseudomonas alcaligenes	212
Pseudomonas chloritidismutans	213
Pseudomonas chlororaphis	214
Pseudomonas japonica	215
Pseudomonas knackmussii	216
Pseudomonas mendocina	217
Pseudomonas monteilii	218
Pseudomonas oleovorans	219
Pseudomonas putida	220

Pseudomonas savastanoi	221
Pseudomonas sp.	222
Pseudomonas sp.	223
Pseudomonas sp.	224
Pseudomonas sp.	225
Pseudomonas sp.	226
Pseudomonas sp.	227
Pseudomonas sp.	228
Pseudomonas sp.	229
Pseudomonas sp.	230
Pseudomonas stutzeri	231
Pseudomonas synxantha	232
Pseudomonas syringae	233
Pseudonocardia sp.	234
Ralstonia solanacearum	235
Raoultella planticola	236
Rhizobium leguminosarum	237
Rhizobium sp.	238
Rhodococcus defluvii	239
Rhodococcus pyridinivorans	240
Rikenella microfusus	241
Robinsoniella sp.	242
Roseomonas cervicalis	243
Roseomonas sp.	244
Salmonella enterica	245
Sanguibacter keddieii	246
Shewanella baltica	247
Shewanella sp.	248
Shinella zoogloeoides	249
Sphingopyxis alaskensis	250
Starkeya novella	251
Stenotrophomonas maltophilia	252
Stenotrophomonas rhizophila	253
Streptococcus thermophilus	254
Streptomyces atroolivaceus	255
Streptomyces coelicoflavus	256
Streptomyces olindensis	257
Streptomyces rimosus	258
Streptomyces roseoverticillatus	259
Streptomyces sp.	260
Streptomyces sp.	261

Streptomyces sp.	262
Streptomyces sp.	263
Streptomyces sp.	264
Streptomyces sp.	265
Streptomyces sp.	266
Streptomyces toyocaensis	267
Streptomyces turgidiscabies	268
Synergistes sp.	269
Tannerella sp.	270
Thauera terpenica	271
Variovorax paradoxus	272
Variovorax sp.	273
Xanthomonas axonopodis	274

Engineered Strains

[00100] In some embodiments, bacteria can be engineered to produce GABA (e.g., under the conditions of the human gut). The bacteria can be engineered using techniques of molecular biology, or can be evolved using the process of selection to produce GABA in the human gut.

[00101] As set forth above, GABA can be produced by multiple pathways within a microbial cell. For example, GABA can be produced by the glutamate pathway, the putrescine to 4-aminobutanal pathway, the arginine to agmatine to putrescine pathway, the L-ornithine to putrescine pathway, or by a combination of pathways. In some embodiments, bacteria can be engineered to contain one or more enzymes in any one of the above pathways that can enable the bacteria to produce GABA or a necessary precursor to GABA.

[00102] A variety of different host bacteria can be engineered to produce GABA. For instance, in some embodiments, *Escherichia coli* Nissle 1917, can be genetically modified or selected through evolution to produce GABA. In some embodiments, the bacteria (e.g., *Escherichia coli* Nissle 1917) can be modified to express or overexpress glutamate decarboxylase A or glutamate decarboxylase B. The bacteria can also be made to produce GABA by one or more of the other pathways described herein.

[00103] Accordingly, in some embodiments, an engineered GABA-producing strain can be identified as having a specific enzyme encoded in its genomes. For example, the enzyme can be glutamate decarboxylase (EC 4.1.1.15); putrescine aminotransferase (EC 2.6.1.82); gamma-aminobutyraldehyde dehydrogenase (EC 1.2.1.19); arginine decarboxylase (EC 4.1.1.19); agmatinase (EC 3.5.3.11); ornithine decarboxylase (EC 4.1.1.17); or a combination thereof. In some embodiments, the GABA-producing strain can be engineered to contain an enzyme that has at least 50% similarity with with the representative sequences listed in Table 3 (e.g., at least 60% similarity, at least 70% similarity, at least 80% similarity, at least 90% similarity, at least 91% similarity, at least 92% similarity, at least 93% similarity, at least 94% similarity, at least 95% similarity, at least 96% similarity, at least 97% similarity, at least 98% similarity, at least 99% similarity, at least 99.5% similarity, at least 99.9% similarity, or 100% similarity). The enzyme classes, as identified by their Enzyme Commission (EC) numbers, are listed in Table 3.

Table 3. Enzymes involved in GABA-Production Pathways, and Seq. ID No. for representative sequences for each enzyme class

Enzyme	**Seq ID No.**
Glutamate decarboxylase (EC 4.1.1.15)	275-279
Putrescine aminotransferase (EC 2.6.1.82)	280-284
Gamma-aminobutyraldehyde dehydrogenase (EC 1.2.1.19)	285-289
Arginine decarboxylase (EC 4.1.1.19)	290-294
Agmatinase (EC 3.5.3.11)	295-299
Ornithine decarboxylase (EC 4.1.1.17)	300-304

[00104] Representative examples of glutamate decarboxylase (EC 4.1.1.15) are given below in Table 4 and identified by their EMBL/GENBANK/DDBJ ID numbers. Any of the bacteria given in Table 10 can be engineered with any version of the glutamate decarboxylase set forth in Table 3 or Table 4. For instance, the bacteria can be engineered with a version of the glutamate decarboxylase enzyme that has at least 50% nucleotide similarity with any of the versions of glutamate decarboxylase given in Table 4 (e.g., at least nucleotide 60% similarity, at least 70% nucleotide similarity, at least 80% nucleotide similarity, at least 90% nucleotide similarity, at least 91% nucleotide similarity, at least 92% nucleotide similarity, at least 93% nucleotide similarity, at least 94% nucleotide similarity,

at least 95% nucleotide similarity, at least 96% nucleotide similarity, at least 97% nucleotide similarity, at least 98% nucleotide similarity, at least 99% nucleotide similarity, at least 99.5% nucleotide similarity, at least 99.9% nucleotide similarity, or 100% nucleotide similarity).

Table 4. Representative Examples of Glutamate Decarboxylase by EMBL/GENBANK/DDBJ ID Number

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[00105] Representative examples of putrescine aminotransferase (EC 2.6.1.82) are given below in Table 5 and identified by their EMBL/GenBank/DDBJ ID numbers. Any of the bacteria given in Table 10 can be engineered with any version of the putrescine aminotransferase (EC 2.6.1.82) set forth in Table 3 and Table 5. For instance, the bacteria can be engineered with a version of the putrescine aminotransferase enzyme that has at least 50% nucleotide similarity with any of the versions of putrescine aminotransferase given in Table 5 (e.g., at least nucleotide 60% similarity, at least 70% nucleotide similarity, at least 80% nucleotide similarity, at least 90% nucleotide similarity, at least 91% nucleotide similarity, at least 92% nucleotide similarity, at least 93% nucleotide similarity, at least 94% nucleotide similarity, at least 95% nucleotide similarity, at least 96% nucleotide similarity, at least 97% nucleotide similarity, at least 98% nucleotide similarity, at least 99% nucleotide similarity, at least 99.5% nucleotide similarity, at least 99.9% nucleotide similarity, or 100% nucleotide similarity).

Table 5. Representative Examples of Putrescine Aminotransferase by EMBL/GenBank/DDBJ ID Number

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OCV69030.1
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OEH16092.1
OEN36946.1
OFD09909.1
SAE79353.1
SBL80466.1

SBZ09436.1

[00106] Representative examples of gamma-aminobutyraldehyde dehydrogenase (EC 1.2.1.19) are given below in Table 6 and identified by their EMBL/GENBANK/DDBJ ID numbers. Any of the bacteria given in Table 10 can be engineered with any version of the gamma-aminobutyraldehyde dehydrogenase (EC 1.2.1.19) set forth in Table 3 and Table 6. For instance, the bacteria can be engineered with a version of the gamma-aminobutyraldehyde dehydrogenase enzyme that has at least 50% nucleotide similarity with any of the versions of gamma-aminobutyraldehyde dehydrogenase given in Table 6 (e.g., at least nucleotide 60% similarity, at least 70% nucleotide similarity, at least 80% nucleotide similarity, at least 90% nucleotide similarity, at least 91% nucleotide similarity, at least 92% nucleotide similarity, at least 93% nucleotide similarity, at least 94% nucleotide similarity, at least 95% nucleotide similarity, at least 96% nucleotide similarity, at least 97% nucleotide similarity, at least 98% nucleotide similarity, at least 99% nucleotide similarity, at least 99.5% nucleotide similarity, at least 99.9% nucleotide similarity, or 100% nucleotide similarity).

Table 6. Representative Examples of Gamma-Aminobutyraldehyde Dehydrogenase by EMBL/GENBANK/DDBJ ID Number

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AAG56331.1	ABA81134.1	ABJ00886.1	ABX21278.1
AAL20515.1	ABB06719.1	ABO56067.1	ABX67082.1
AAN43345.1	ABB09933.1	ABP60799.1	ACA77853.1
AAN54343.1	ABB61850.1	ABP83011.1	ACB02660.1
AAN68409.1	ABC35115.1	ABQ62162.1	ACB16069.1
AAN80331.1	ABE07141.1	ABQ79255.1	ACB17411.1
AAO69141.1	ABE33447.1	ABQ81306.1	ACD08717.1
AAP17225.1	ABE35009.1	ABR77355.1	ACF65188.1
AAV77220.1	ABE58543.1	ABR80823.1	ACH48735.1
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AAY91615.1	ABF08506.1	ABV12611.1	ACI11654.1
AAZ88386.1	ABF77276.1	ABV19407.1	ACI38546.1

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[00107] Representative examples of arginine decarboxylase (EC 4.1.1.19) are given below in Table 7 and identified by their EMBL/GENBANK/DDBJ ID numbers. Any of the bacteria given in Table 10 can be engineered with any version of the arginine decarboxylase (EC 4.1.1.19) set forth in Table 3 and Table 7. For instance, the bacteria can be engineered with a version of the arginine decarboxylase enzyme that has at least 50% nucleotide similarity with any of the versions of arginine decarboxylase given in Table 7 (e.g., at least nucleotide 60% similarity, at least 70% nucleotide similarity, at least 80% nucleotide similarity, at least 90% nucleotide similarity, at least 91% nucleotide similarity, at least 92% nucleotide similarity, at least 93% nucleotide similarity, at least 94% nucleotide similarity, at least 95% nucleotide similarity, at least 96% nucleotide similarity, at least 97% nucleotide similarity, at least 98% nucleotide similarity, at least 99% nucleotide similarity, at least 99.5% nucleotide similarity, at least 99.9% nucleotide similarity, or 100% nucleotide similarity).

Table 7. Representative Examples of Arginine Decarboxylase by EMBL/GENBANK/DDBJ ID Number

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AAF09826.1	AAN83546.1	AAS94900.1	AAZ58866.1
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KXG92416.1	KZJ59532.1	OCW73841.1	SBZ90867.1

[00108] Representative examples of agmatinase (EC 3.5.3.11) are given below in Table 8 and identified by their EMBL/GENBANK/DDBJ ID numbers. Any of the bacteria given in Table 10 can be engineered with any version of the agmatinase (EC 3.5.3.11) set forth in Table 3 and Table 8. For instance, the bacteria can be engineered with a version of the agmatinase enzyme that has at least 50% nucleotide similarity with any of the versions of agmatinase given in Table 8 (e.g., at least nucleotide 60% similarity, at least 70% nucleotide similarity, at least 80% nucleotide similarity, at least 90% nucleotide similarity, at least 91% nucleotide similarity, at least 92% nucleotide similarity, at least 93% nucleotide similarity, at least 94% nucleotide similarity, at least 95% nucleotide similarity, at least 96% nucleotide similarity, at least 97% nucleotide similarity, at least 98% nucleotide similarity, at least 99% nucleotide similarity, at least 99.5% nucleotide similarity, at least 99.9% nucleotide similarity, or 100% nucleotide similarity).

Table 8. Representative Examples of Agmatinase by EMBL/GENBANK/DDBJ ID Number

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[00109] Representative examples of ornithine decarboxylase (EC 4.1.1.17) are given below in Table 9 and identified by their EMBL/GENBANK/DDBJ ID numbers. Any of the bacteria given in Table 10 can be engineered with any version of the ornithine decarboxylase (EC 4.1.1.17) set forth in Table 3 and 9. For instance, the bacteria can be engineered with a version of the ornithine decarboxylase enzyme that has at least 50% nucleotide similarity with any of the

versions of ornithine decarboxylase given in Table 9 (e.g., at least nucleotide 60% similarity, at least 70% nucleotide similarity, at least 80% nucleotide similarity, at least 90% nucleotide similarity, at least 91% nucleotide similarity, at least 92% nucleotide similarity, at least 93% nucleotide similarity, at least 94% nucleotide similarity, at least 95% nucleotide similarity, at least 96% nucleotide similarity, at least 97% nucleotide similarity, at least 98% nucleotide similarity, at least 99% nucleotide similarity, at least 99.5% nucleotide similarity, at least 99.9% nucleotide similarity, or 100% nucleotide similarity).

Table 9. Representative Examples of Ornithine Decarboxylase by EMBL/GENBANK/DDBJ ID Number

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CCJ89861.1	CDK97758.1	CDX31971.1	CRO16551.1
CCJ91600.1	CDK98655.1	CDX32761.1	CRP80377.1
CCJ97363.1	CDL07277.1	CDX44074.1	CRY55295.1

CRY56151.1	CTQ48990.1	CUH97680.1	EEJ73250.1
CRY73398.1	CTQ51582.1	CUI46350.1	EEN80026.1
CRZ77491.1	CTQ64509.1	CUI65295.1	EEO01571.1
CSA30444.1	CTQ70679.1	CUJ95194.1	EEO17726.1
CSB23271.1	CTQ77678.1	CUK08178.1	EEQ24327.1
CSB44423.1	CTR81841.1	EAA24580.1	EEV22915.1
CSB97832.1	CTR87483.1	EDL69175.1	EEW41165.1
CSC29054.1	CTS06036.1	EDM60163.1	EEX49266.1
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CSC55030.1	CTS78438.1	EDQ31943.2	EEY99726.1
CSC90483.1	CTT62185.1	EDQ34501.1	EFC91687.1
CSC91956.1	CTT62210.1	EDR33413.1	EFE96168.1
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CSI94697.1	CTT83136.1	EDU62245.1	EFE97489.1
CSK70247.1	CTT84900.1	EDU64911.1	EFG55431.1
CSL03024.1	CTT94762.1	EDU91579.1	EFM19932.1
CSL07641.1	CTU09480.1	EDU92556.1	EFM60159.1
CSM63721.1	CTU43474.1	EDX33853.1	EFP71802.1
CSM98897.1	CTU46798.1	EDX36097.1	EFQ23396.1
CSN54919.1	CTU67775.1	EDX47281.1	EFQ65259.1
CSP82502.1	CTV17999.1	EDX48079.1	EFR18105.1
CSP97222.1	CTV22603.1	EDY22541.1	EFR18206.1
CSR81748.1	CTV49713.1	EDY26086.1	EFW49207.1
CSS46262.1	CTV56172.1	EDZ45245.1	EFW49866.1
CSS69716.1	CTV59366.1	EDZ48441.1	EFW55944.1
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CTQ07290.1	CTX56302.1	EEB72505.1	EFY04546.1
CTQ10899.1	CTZ36862.1	EEH90224.1	EFZ35827.1
CTQ21239.1	CTZ54946.1	EEI47014.1	EGC73182.1
CTQ33367.1	CTZ68478.1	EEI67673.1	EGD26721.1
CTQ42919.1	CTZ90267.1	EEJ60488.1	EGF12882.1
CTQ43401.1	CUH38863.1	EEJ72107.1	EGF12883.1

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EGI92040.1
EGI93253.1
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EGL98455.1
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EGP08625.1
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EHC94859.1
EHC94874.1
EHD22909.1
EHE87725.1
EHE90319.1
EHP88849.1
EHP90237.1
EHU02113.1
EHU20119.1
EHU26736.1

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EIQ26095.1
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ELW31551.1
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EMU59716.1

EMU68355.1
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ENH01203.1
ENH07634.1
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EPA96228.1
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EPC38567.1
EPF66737.1
EPX75972.1
EPX79893.1
EPX85529.1
EPX86372.1
EQC66601.1
EQC67119.1
ERJ07090.1
ERJ12441.1
ERL16034.1
ERL56578.1
ESN64144.1

ESN64681.1
ESR23196.1
ESR27061.1
ESS57441.1
ESU78166.1
ETA69456.1
ETS92400.1
EUB20316.1
EUB71374.1
EUB75197.1
EUB84084.1
EUB97023.1
EUC12442.1
EWC58917.1
EWC60810.1
EWG73717.1
EWG77348.1
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EYD71269.1
EYD77132.1
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KDM66230.1

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KDM68717.1
KDR44432.1
KDU29207.1
KDW29935.1
KDX21151.1
KDX45737.1
KEA62619.1
KEC54001.1
KEJ45027.1
KEJ59156.1
KEJ73445.1
KEK27105.1
KEK29881.1
KEL65139.1
KEN53788.1
KEN65519.1
KEN98059.1
KEO08191.1
KEO30074.1
KEQ53465.1
KER02947.1
KEY57491.1
KEZ18534.1
KFC03280.1
KFC05486.1
KFC63691.1
KFC69417.1
KFC77644.1
KFC79295.1
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KFC88765.1
KFC94984.1

KFD06768.1
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KFD17151.1
KFE39747.1
KFE40588.1
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KGM71386.1
KGM77872.1
KGM80580.1
KGM83330.1
KGM84983.1
KGM87536.1
KGM88313.1
KHJ19976.1
KHN90703.1
KHQ55244.1
KID27997.1
KIH04277.1
KIQ45687.1
KIQ47241.1
KIQ68453.1
KIQ68551.1
KIR02360.1
KIR22223.1
KIS02883.1
KIT17643.1
KIV66814.1
KIV70876.1

KJL37417.1
KJL45196.1
KJW30930.1
KJX87011.1
KLU03251.1
KLY03824.1
KMK68540.1
KMM41085.1
KMM41943.1
KMO67617.1
KMO73993.1
KMO74679.1
KMQ72910.1
KMY85028.1
KMZ12404.1
KND54388.1
KND59806.1
KNE76189.1
KNF62494.1
KNF81913.1
KNF82765.1
KNH02424.1
KNH10085.1
KNX39995.1
KNX42257.1
KOA19269.1
KON65543.1
KPA20904.1
KPA88263.1
KPO11674.1
KPO18606.1
KPO45157.1
KPO46605.1

KPP86129.1
KPQ30708.1
KPW02487.1
KPZ58476.1
KPZ73698.1
KQJ13961.1
KQJ37165.1
KRK54316.1
KRK64371.1
KSH09715.1
KST27486.1
KTZ13476.1
KXL65724.1
KXQ42753.1
KYR53760.1
KYV67923.1
KYV72148.1
OAC43139.1
OAH24264.1
OAJ89982.1
OCS73051.1
OCV53530.1
ODH24445.1
OEI63490.1
OEI65510.1
OFJ34340.1
OIR53362.1
OJF06953.1
SAY77725.1
SEF59356.1

[00110] A variety of microbes (e.g., bacteria) can be engineered to produce GABA (e.g., by engineering one or more of the enzymes set forth in Table 2). For instance, any of the bacteria set forth in Table 10 can be engineered to produce GABA. In other words, a bacteria having a 16S rDNA nucleotide sequence that is at least 50% similar to that set forth in Table 10 below can be engineered (e.g., with one of the enzymes in Tables 3-9) to produce GABA. The bacteria may have a 16S rDNA sequence that is at least 60% similar, at least 70% similar, at least 80% similar, at least 90% similar, at least 91% similar, at least 92% similar, at least 93% similar, at least 94% similar, at least 95% similar, at least 96% similar, at least 97% similar, at least 98% similar, at least 99% similar, at least 99.5% similar, or 100% similar to the 16S rDNA nucleotide sequence given in Table 10.

[00111] As set forth in Example 8, *E. coli* was engineered to overexpress glutamate decarboxylase, which without wishing to be bound by theory, led to the expression of GABA by the engineered *E. coli*. As shown in Figure 6, the engineered E. coli was able to induce the growth of *E. gabavorous* KLE1738. GABA can be produced by intestinal epithelial cells and by some bacteria, such as *Escherichia coli* and *Listeria monocytogenes*, by decarboxylation of glutamate. In *E. coli*, the decarboxylation of glutamate can serve as a mechanism to decrease intracellular pH, and therefore in some embodiments GABA production generally occurs at a low pH.

[00112] As set forth in Example 8, Expression, e.g., overexpression of glutamate decarboxylase in *E. coli* (e.g., gadA or gadB), resulted in induction of *E. gabavorous* KLE1738 growth to levels seen with *B. fragilis* KLE 1758. For instance, Figure 6A shows induction of *E. gabavorous* KLE1738 in the presence of *E. coli* engineered to express glutamate decarboxylase gadA. Similarly, Figure 6B shows induction of *E. gabavorous* KLE1738 in the presence of *E. coli* engineered to express glutamate decarboxylase gadB. As shown in Figure 6C, the growth of *E. gabavorous* KLE1738 was qualitatively similar to that seen in the presence of *B. fragilis* KLE 1758. In contrast, as shown in Figure 6D, when *E. coli* was engineered to express gadC, a GABA antiporter, no growth of *E. gabavorous* KLE1738 was observed. Similarly, no growth of *E. gabavorous* KLE1738 was observed in the presence of empty vehicle (Figure 6E). Without wishing to be bound by theory the results of Example 8 demonstrate that bacteria can be

engineered to produce GABA (e.g., via the expression or overexpression of glutamate decarboxylase). In some embodiments, the bacteria (e.g., *E. coli*) can be engineered to produce GABA inside the human gut.

[00113] In some embodiments, the present disclosure also provides compromising one or more repressors of GABA production, (e.g., gadX or gadW). In some embodiments, these repressors can regulate the pH restrictions of GABA production in *E. coli*, is a way to increase native GABA production. This can be achieved, for instance, via gene deletions, insertions, or substitutions, as known by those skilled in the art of molecular biology.

[00114] Altering the pH of growth media for KLE1738 did not change the GABA-dependency phenotype. Without wishing to be bound by theory, this suggests that engineering bacteria to overexpress glutamate decarboxylase is an effective way to produce GABA, as well as induce the growth of *E. gabavorous*.

[00115] In addition to *E. coli*, other bacteria can be engineered to produce GABA (e.g., at a physiologically relevant pH, such as between 4.5 and 7.5). For instance, any of the bacteria set forth in Table 10 can be engineered to produce GABA (e.g., at a physiologically relevant pH, such as between 4.5 and 7.5). For example, the bacteria can be engineered to contain DNA that codes for one or more enzymes set forth in Table 3-9. Set forth in Table 10 is also a sequence ID number for the 16S nucleotide sequence of the listed bacteria. In some embodiments, the bacteria that are engineered to produce GABA can have at least 90% 16S sequence similarity to the 16S sequences given in Table 10 (e.g., at least 91% similarity, at least 92% similarity, at least 93% similarity, at least 94% similarity, at least 95% similarity, at least 96% similarity, at least 97% similarity, at least 98% similarity, at least 99% similarity, at least 99.5% similarity, at least 99.9% similarity, or 100% similarity).

Table 10. Bacteria Capable of Being Engineered to Produce GABA

Species	**Seq. ID No.**
Abiotrophia defectiva	305
Abiotrophia para-adiacens	306
Abiotrophia sp.	307
Acetanaerobacterium elongatum	308
Acetivibrio cellulolyticus	309
Acetivibrio ethanolgignens	310
Acetobacter aceti	311
Acetobacter fabarum	312
Acetobacter lovaniensis	313
Acetobacter malorum	314
Acetobacter orientalis	315
Acetobacter pasteurianus	316

Acetobacter pomorum	317
Acetobacter syzygii	318
Acetobacter tropicalis	319
Acetobacteraceae bacterium	320
Acholeplasma laidlawii	321
Achromobacter denitrificans	322
Achromobacter piechaudii	323
Achromobacter xylosoxidans	324
Acidaminococcus fermentans	325
Acidaminococcus intestini	326
Acidaminococcus sp.	327
Acidilobus saccharovorans	328
Acidithiobacillus ferrivorans	329
Acidovorax sp.	330
Acidovorax sp.	331
Acinetobacter baumannii	332
Acinetobacter calcoaceticus	333
Acinetobacter genomosp.	334
Acinetobacter haemolyticus	335
Acinetobacter johnsonii	336
Acinetobacter junii	337
Acinetobacter lwoffii	338
Acinetobacter parvus	339
Acinetobacter radioresistens	340
Acinetobacter schindleri	341
Acinetobacter sp.	342
Acinetobacter sp.	343
Acinetobacter sp.	344
Acinetobacter sp.	345
Acinetobacter sp.	346
Acinetobacter sp.	347
Acinetobacter sp.	348
Actinobacillus minor	349
Actinobacillus pleuropneumoniae	350
Actinobacillus succinogenes	351
Actinobacillus succinogenes	352
Actinobacillus ureae	353
Actinobaculum massiliae	354
Actinobaculum schaalii	355
Actinobaculum sp.	356

Actinobaculum sp.	357
Actinomyces cardiffensis	358
Actinomyces europaeus	359
Actinomyces funkei	360
Actinomyces genomosp.	361
Actinomyces genomosp.	362
Actinomyces genomosp.	363
Actinomyces georgiae	364
Actinomyces israelii	365
Actinomyces massiliensis	366
Actinomyces meyeri	367
Actinomyces naeslundii	368
Actinomyces nasicola	369
Actinomyces neuii	370
Actinomyces odontolyticus	371
Actinomyces oricola	372
Actinomyces orihominis	373
Actinomyces oris	374
Actinomyces sp.	375
Actinomyces sp.	376
Actinomyces sp.	377
Actinomyces sp.	378
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Actinomyces sp.	389
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Actinomyces sp.	391
Actinomyces sp.	392
Actinomyces sp.	393
Actinomyces sp.	394
Actinomyces sp.	395
Actinomyces sp.	396
Actinomyces sp.	397

Actinomyces sp.	398
Actinomyces sp.	399
Actinomyces urogenitalis	400
Actinomyces viscosus	401
Adlercreutzia equolifaciens	402
Aerococcus sanguinicola	403
Aerococcus urinae	404
Aerococcus urinaeequi	405
Aerococcus viridans	406
Aeromicrobium marinum	407
Aeromicrobium sp.	408
Aeromonas allosaccharophila	409
Aeromonas enteropelogenes	410
Aeromonas hydrophila	411
Aeromonas jandaei	412
Aeromonas salmonicida	413
Aeromonas trota	414
Aeromonas veronii	415
Afipia genomosp.	416
Aggregatibacter actinomycetemcomitans	417
Aggregatibacter aphrophilus	418
Aggregatibacter segnis	419
Agrobacterium radiobacter	420
Agrobacterium tumefaciens	421
Agrococcus jenensis	422
Akkermansia muciniphila	423
Alcaligenes faecalis	424
Alcaligenes sp.	425
Alcaligenes sp.	426
Alicyclobacillus acidocaldarius	427
Alicyclobacillus acidoterrestris	428
Alicyclobacillus contaminans	429
Alicyclobacillus cycloheptanicus	430
Alicyclobacillus herbarius	431
Alicyclobacillus pomorum	432
Alicyclobacillus sp.	433
Alistipes finegoldii	434
Alistipes indistinctus	435
Alistipes onderdonkii	436

Alistipes putredinis	437
Alistipes shahii	438
Alistipes sp.	439
Alistipes sp.	440
Alistipes sp.	441
Alkaliphilus metalliredigenes	442
Alkaliphilus oremlandii	443
Alloscardovia omnicolens	444
Alloscardovia sp.	445
Anaerobaculum hydrogeniformans	446
Anaerobiospirillum succiniciproducens	447
Anaerobiospirillum thomasii	448
Anaerococcus hydrogenalis	449
Anaerococcus lactolyticus	450
Anaerococcus octavius	451
Anaerococcus prevotii	452
Anaerococcus sp.	453
Anaerococcus sp.	454
Anaerococcus sp.	455
Anaerococcus sp.	456
Anaerococcus sp.	457
Anaerococcus sp.	458
Anaerococcus sp.	459
Anaerococcus sp.	460
Anaerococcus sp.	461
Anaerococcus tetradius	462
Anaerococcus vaginalis	463
Anaerofustis stercorihominis	464
Anaeroglobus geminatus	465
Anaerosporobacter mobilis	466
Anaerostipes caccae	467
Anaerostipes sp.	468
Anaerotruncus colihominis	469
Anaplasma marginale	470
Anaplasma phagocytophilum	471
Aneurinibacillus aneurinilyticus	472
Aneurinibacillus danicus	473
Aneurinibacillus migulanus	474
Aneurinibacillus terranovensis	475

Aneurinibacillus thermoaerophilus	476
Anoxybacillus contaminans	477
Anoxybacillus flavithermus	478
Arcanobacterium haemolyticum	479
Arcanobacterium pyogenes	480
Arcobacter butzleri	481
Arcobacter cryaerophilus	482
Arthrobacter agilis	483
Arthrobacter arilaitensis	484
Arthrobacter bergerei	485
Arthrobacter globiformis	486
Arthrobacter nicotianae	487
Atopobium minutum	488
Atopobium parvulum	489
Atopobium rimae	490
Atopobium sp.	491
Atopobium sp.	492
Atopobium sp.	493
Atopobium sp.	494
Atopobium vaginae	495
Aurantimonas coralicida	496
Aureimonas altamirensis	497
Auritibacter ignavus	498
Averyella dalhousiensis	499
Bacillus aeolius	500
Bacillus aerophilus	501
Bacillus aestuarii	502
Bacillus alcalophilus	503
Bacillus amyloliquefaciens	504
Bacillus atrophaeus	505
Bacillus badius	506
Bacillus cereus	507
Bacillus circulans	508
Bacillus clausii	509
Bacillus coagulans	510
Bacillus firmus	511
Bacillus flexus	512
Bacillus fordii	513
Bacillus gelatini	514
Bacillus halmapalus	515

Bacillus halodurans	516
Bacillus herbersteinensis	517
Bacillus horti	518
Bacillus idriensis	519
Bacillus lentus	520
Bacillus licheniformis	521
Bacillus megaterium	522
Bacillus nealsonii	523
Bacillus niabensis	524
Bacillus niacini	525
Bacillus pocheonensis	526
Bacillus pumilus	527
Bacillus safensis	528
Bacillus simple	529
Bacillus sonorensis	530
Bacillus sp.	531
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Bacillus sphaericus	555
Bacillus sporothermodurans	556

Bacillus subtilis	557
Bacillus thermoamylovorans	558
Bacillus thuringiensis	559
Bacillus weihenstephanensis	560
Bacteroidales bacterium	561
Bacteroidales genomosp.	562
Bacteroidales genomosp.	563
Bacteroidales genomosp.	564
Bacteroidales genomosp.	565
Bacteroidales genomosp.	566
Bacteroidales genomosp.	567
Bacteroidales genomosp.	568
Bacteroidales genomosp.	569
Bacteroides acidifaciens	570
Bacteroides barnesiae	571
Bacteroides caccae	572
Bacteroides cellulosilyticus	573
Bacteroides clarus	574
Bacteroides coagulans	575
Bacteroides coprocola	576
Bacteroides coprophilus	577
Bacteroides dorei	578
Bacteroides eggerthii	579
Bacteroides faecis	580
Bacteroides finegoldii	581
Bacteroides fluxus	582
Bacteroides fragilis	583
Bacteroides galacturonicus	584
Bacteroides helcogenes	585
Bacteroides heparinolyticus	586
Bacteroides intestinalis	587
Bacteroides massiliensis	588
Bacteroides nordii	589
Bacteroides oleiciplenus	590
Bacteroides ovatus	591
Bacteroides pectinophilus	592
Bacteroides plebeius	593
Bacteroides pyogenes	594
Bacteroides salanitronis	595
Bacteroides salyersiae	596
Bacteroides sp.	597

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Bacteroides ureolyticus	635
Bacteroides vulgatus	636
Bacteroides xylanisolvens	637
Bacteroidetes bacterium	638

Bacteroidetes bacterium	639
Bacteroidetes bacterium	640
Barnesiella intestinihominis	641
Barnesiella viscericola	642
Bartonella bacilliformis	643
Bartonella grahamii	644
Bartonella henselae	645
Bartonella quintana	646
Bartonella tamiae	647
Bartonella washoensis	648
Bdellovibrio sp.	649
Bifidobacteriaceae genomosp.	650
Bifidobacterium adolescentis	651
Bifidobacterium angulatum	652
Bifidobacterium animalis	653
Bifidobacterium bifidum	654
Bifidobacterium breve	655
Bifidobacterium catenulatum	656
Bifidobacterium gallicum	657
Bifidobacterium infantis	658
Bifidobacterium kashiwanohense	659
Bifidobacterium longum	660
Bifidobacterium pseudocatenulatum	661
Bifidobacterium pseudolongum	662
Bifidobacterium scardovii	663
Bifidobacterium sp.	664
Bifidobacterium sp.	665
Bifidobacterium sp.	666
Bifidobacterium sp.	667
Bifidobacterium sp.	668
Bifidobacterium thermophilum	669
Bifidobacterium urinalis	670
Bilophila wadsworthia	671
Bisgaard Taxon	672
Bisgaard Taxon	673
Bisgaard Taxon	674
Bisgaard Taxon	675
Blastomonas natatoria	676
Blautia coccoides	677

Blautia glucerasea	678
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Macrococcus caseolyticus	1407
Mannheimia haemolytica	1408
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Massilia sp.	1410
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Methanobrevibacter gottschalkii	1425
Methanobrevibacter millerae	1426
Methanobrevibacter olleyae	1427
Methanobrevibacter oralis	1428
Methanobrevibacter ruminantium	1429
Methanobrevibacter smithii	1430
Methanobrevibacter thaueri	1431
Methanobrevibacter woesei	1432

Methanobrevibacter wolinii	1433
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Mycoplasma amphoriforme	1497
Mycoplasma arthritidis	1498
Mycoplasma bovoculi	1499
Mycoplasma faucium	1500
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Mycoplasma genitalium	1503
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Mycoplasma orale	1505
Mycoplasma ovipneumoniae	1506
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Prevotella nigrescens	1694
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Pseudomonas poae	1782
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Pseudomonas stutzeri	1788
Pseudomonas tolaasii	1789
Pseudomonas viridiflava	1790
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Psychrobacter cryohalolentis	1794
Psychrobacter faecalis	1795
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Rhodococcus equi	1808
Rhodococcus erythropolis	1809
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Rhodopseudomonas palustris	1811
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Roseburia cecicola	1813
Roseburia faecalis	1814
Roseburia faecis	1815
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Roseomonas sp.	1824
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Scardovia wiggsiae	1853
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Segniliparus rugosus	1855
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Selenomonas sputigena	1881
Serratia fonticola	1882
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Serratia marcescens	1884
Serratia odorifera	1885
Serratia proteamaculans	1886
Shewanella putrefaciens	1887
Shuttleworthia satelles	1888
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Simonsiella muelleri	1891
Slackia equolifaciens	1892
Slackia exigua	1893
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Slackia isoflavoniconvertens	1896
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Solobacterium moorei	1899
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Staphylococcus equorum	1925
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Staphylococcus haemolyticus	1927
Staphylococcus hominis	1928
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Staphylococcus warneri	1940
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Streptococcus agalactiae	1945
Streptococcus alactolyticus	1946
Streptococcus anginosus	1947
Streptococcus australis	1948
Streptococcus bovis	1949
Streptococcus canis	1950
Streptococcus constellatus	1951
Streptococcus cristatus	1952
Streptococcus downei	1953

Streptococcus dysgalactiae	1954
Streptococcus equi	1955
Streptococcus equinus	1956
Streptococcus gallolyticus	1957
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Streptococcus milleri	1972
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Streptococcus mutans	1974
Streptococcus oligofermentans	1975
Streptococcus oralis	1976
Streptococcus parasanguinis	1977
Streptococcus pasteurianus	1978
Streptococcus peroris	1979
Streptococcus pneumoniae	1980
Streptococcus porcinus	1981
Streptococcus pseudopneumoniae	1982
Streptococcus pseudoporcinus	1983
Streptococcus ratti	1984
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Veillonella sp.	2144
Veillonellaceae bacterium	2145
Veillonellaceae bacterium	2146
Victivallaceae bacterium	2147
Victivallis vadensis	2148
Virgibacillus proomii	2149
Weissella beninensis	2150
Weissella cibaria	2151
Weissella confusa	2152
Weissella hellenica	2153
Weissella kandleri	2154
Weissella koreensis	2155

Weissella paramesenteroides	2156
Weissella sp.	2157
Wolinella succinogenes	2158
Xanthomonadaceae bacterium	2159
Xanthomonas campestris	2160
Xanthomonas sp.	2161
Xenophilus aerolatus	2162
Yokenella regensburgei	2163
Zimmermannella bifida	2164
Zymomonas mobilis	2165
Alistipes shahii	2166
Bacteroides caccae	2167
Bacteroides eggerthii	2168
Bacteroides sp.	2169
Bacteroides sp.	2170
Bacteroides sp.	2171
Bacteroides uniformis	2172
Bacteroides vulgatus	2173
Bacteroides vulgatus	2174
Bifidobacterium adolescentis	2175
Bifidobacterium pseudocatenulatum	2176
Blautia producta	2177
Blautia producta	2178
Blautia schinkii	2179
Clostridium bolteae	2180
Clostridium butyricum	2181
Clostridium disporicum	2182
Clostridium hathewayi	2183
Clostridium hylemonae	2184
Clostridium innocuum	2185
Clostridium innocuum	2186
Clostridium mayombei	2187
Clostridium nexile	2188
Clostridium orbiscindens	2189
Clostridium symbiosum	2190
Clostridium tertium	2191
Collinsella aerofaciens	2192
Coprobacillus sp.	2193
Coprococcus catus	2194
Coprococcus comes	2195

Dorea formicigenerans	2196
Dorea longicatena	2197
Enterococcus faecalis	2198
Erysipelotrichaceae bacterium	2199
Escherichia coli	2200
Escherichia coli	2201
Eubacterium eligens	2202
Eubacterium rectale	2203
Eubacterium rectale	2204
Faecalibacterium prausnitzii	2205
Faecalibacterium prausnitzii	2206
Lachnospiraceae bacterium	2207
Odoribacter splanchnicus	2208
Odoribacter splanchnicus	2209
Parabacteroides merdae	2210
Roseburia intestinalis	2211
Ruminococcus bromii	2212
Ruminococcus gnavus	2213
Ruminococcus obeum	2214
Ruminococcus torques	2215
Streptococcus thermophilus	2216
Escherichia coli	2217
Streptococcus thermophilus	2218
Escherichia coli	2219

Therapeutic Compositions

[00116] Any of the GABA-producing bacteria described herein (e.g., natural bacteria or engineered bacteria), or any combination thereof (including combinations of natural and engineered bacteria) can be incorporated into a therapeutic composition. For instance, the therapeutic compositions can be administered to a patient in need thereof to treat or alleviate the symptom of a mental illness or central nervous system disease.

Purification of Strains

[00117] In some embodiments, bacteria are purified prior to incorporation into a therapeutic composition. For instance, bacteria can be purified so that the population of bacteria is substantially free of other bacteria (e.g., contains at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, or at least 98%, at least 99% of the specific bacterial strain or strains desired in the composition).

[00118] In some embodiments, the therapeutic composition is a probiotic or a medical food comprising at least one GABA-producing bacterial strain. The strain can be administered, for instance, as a probiotic, as capsules, tablets, caplets, pills, troches, lozenges, powders, and/or granules. The strain can also be formulated as a medical food. The GABA-producing bacteria can also be administered as a fecal transplant or suppository.

[00119] In some embodiments, the dose of the therapeutic can contain 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8, 1×10^9, 1×10^{10}, 1×10^{11} or greater than 1×10^{11} colony forming units (CFUs) of the desired bacterial species. For instance, the desired bacterial species can be GABA-producing bacteria, bacteria that are capable of inhibiting the growth of GABA-consuming bacteria, or a combination thereof.

[00120] In some embodiments, the therapeutic composition or dose unit comprises a pharmaceutically acceptable formulation, including an enteric coating or similar to survive the acidity of the stomach and enabled delivery into the small or large intestines, prebiotics (such as, but not limited to, amino acids (including arginine, glutamate, and ornithine), biotin, fructooligosaccharide, galactooligosaccharides, hemicelluloses (e.g., arabinoxylan, xylan, xyloglucan, and glucomannan), inulin, chitin, lactulose, mannan oligosaccharides,

oligofructose-enriched inulin, gums (e.g., guar gum, gum arabic and carregenaan), oligofructose, oligodextrose, tagatose, resistant maltodextrins (e.g., resistant starch), trans-galactooligosaccharide, pectins (e.g., xylogalactouronan, citrus pectin, apple pectin, and rhamnogalacturonan-I), dietary fibers (e.g., soy fiber, sugarbeet fiber, pea fiber, corn bran, and oat fiber) and xylooligosaccharides, polyamines (such as but not limited to spermidine and putrescine), an effective amount of an anti-bacterial agent, anti-fungal agent, anti-viral agent, or anti-parasitic agent, or any combinations of the above. For instance, the therapeutic composition can also be in the form of a yogurt containing one or more purified strains of GABA-producing bacteria.

Disease Indications

[00121] In one or more embodiments of any of the above-aspects, the mental illness or disease of the central nervous system that can be treated by administration of a therapeutic composition described herein is selected from depression, bipolar disorder, schizophrenia, anxiety, anxiety disorders, addiction, social phobia, major depressive disorder, treatment-resistant major depressive disorder (TR-MDD), major depressive disorder and its subtypes (melancholic depression, atypical depression, catatonic depression, postpartum depression, and seasonal affective disorder), Neurodegenerative amyloid disorders (Parkinson's, Alzheimer's, and Huntington's diseases) orthostatic tremor, Lafora disease, restless leg syndrome, neuropathic pain, pain disorders, dementia, epilepsy, stiff-person syndrome, premenstrual dysphoric disorder, autism spectrum disorder, sleep disorders, and attention deficit hyperactivity disorder (ADHD).

[00122] In some embodiments, the method further comprises decreasing at least one symptom of a mental disorder or disease of the central nervous system in the subject selected from the group consisting of: fatigue, insomnia, motor dysfunction, stress, persistent anxiety, persistent sadness, social withdrawal, substance withdrawal, irritability, thoughts of suicide, thoughts of self-harm, restlessness, low sex drive, lack of focus, loss of appetite, seizures, memory loss, anger, bouts of emotional reactivity, confusion, pain, and muscle spasms.

Methods of Treatment

[00123] The therapeutic compositions described herein can be administered to a patient in need thereof, for instance for the treatment of a mental illness or disease of the central nervous system. In some embodiments, the method of treatment can comprise first diagnosing a patient who can benefit from treatment by a therapeutic composition described herein. In some embodiments, the method further comprises administering to the patient a therapeutic composition described herein.

Patient Diagnosis

[00124] In some embodiments, the process of identifying a subject with a mental illness or disease of the central nervous system can be carried out by a trained psychologist, psychiatrist, or neurologist. For instance, a psychiatrist, psychologist, or neurologist can diagnose a subject with a mental illness or disease of the central nervous system evaluating the subject's behavior for symptoms of the mental illness or disease of the central nervous system. One of skill in the art will understand that mental illness can also be identified in a subject with the aid of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5), (American Psychiatric Association).

[00125] In one or more embodiments, the process of identifying a subject with a mental illness or disease of the central nervous system can comprise diagnosing the subject with a mental illness or disease of the central nervous system. In some embodiments, the mental illness or disease of the central nervous system is identified or diagnosed using fMRI. In some embodiments, mental illness or disease of the central nervous system can be identified with standard psychological and neurological surveys, or in other methods known to experts in the field.

[00126] In some embodiments, a subject in need of treatment with a therapeutic composition described herein can be identified by identifying low levels of GABA in the subject's blood, serum, stool, or other bodily fluid. In some embodiments, the amount of GABA in the subject's stool (e.g., the initial amount of GABA in the subject's stool) is below about 8 μg GABA per gram of stool. The amount of GABA can be measured using the wet or dry weight of stool by LC/MS or another technique known in the art. In some embodiments, the amount of GABA in the subject's blood or serum (e.g., the initial amount of GABA in the subject's blood or serum) is below about 10 μg/L +/- 5 μg/L

GABA per gram of blood or serum (e.g., as measured by LC/MS). In some embodiments, the amount of GABA in the prefrontal cortex, or other areas of the brain, is below about 1.0 mM/kg, as measured by proton magnetic resonance (PMR), or another similar technique.

[00127] In some embodiments, the percentage of GABA-producing bacteria in the subject's gut (e.g., the initial amount) represents about 10% of total 16S sequences as measured by sequencing using such methods as 16S rDNA gene Illumina sequencing or quantitative PCR. In some embodiments, the percentage of GABA-producing bacteria in the subject's gut represents about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, about 1%, or less than about 1% of the total 16S sequences measured in the subject's gut.

[00128] Determination of the initial amount of GABA in a subject's blood, serum, regions of the brain, or stool can help identify subjects that can benefit from treatment by administration of GABA-producing bacteria. In some embodiments, a subject with an initial amount of GABA in the serum or blood below 10 μg/L GABA can benefit from administration of GABA-producing bacteria. In some embodiments, a subject with an initial amount of GABA in the serum or blood below 100 μg, below 50 μg, below 25 μg, below 20 μg, below 15 μg, below 10 μg, below 9 μg, below 8 μg, below 7 μg, below 6 μg, below 5 μg, below 4 μg, below 3 μg, below 2 μg, below 1 μg, below 0.5 μg, below 0.1 μg, below 0.01 μg, below 10 ng, or below 1 ng, or below 0.1 ng per L of blood or serum can benefit from administration of a GABA-producing bacteria.

[00129] In some embodiments, a subject with an initial amount of GABA in the brain, in regions such as the prefrontal cortex (or other areas of the brain), of about 1.0 mM/kg can benefit from treatment by administration of GABA-producing bacteria. In some embodiments, a subject with an initial amount of GABA in the brain, in such regions as the prefrontal cortex (or other areas of the brain), of below 100 mM, below 50 mM, below 25 mM, below 20 mM, below 15 mM, below 10 mM, below 9 mM, below 8 mM, below 7 mM, below 6 mM, below 5 mM, below 4 mM, below 3 mM, below 2 mM, below 1 mM, below 0.5 mM, below 0.1 mM, or below 0.01 mM, or below 0.001 mM GABA can benefit from treatment by administration of GABA-producing bacteria.

[00130] In some embodiments, a subject with an initial amount of GABA in stool below 8 μg GABA per gram of stool (wet or dry weight) can benefit from administration of GABA-producing bacteria. In some embodiments, a subject with an initial amount of GABA in the stool below 100 μg, below 50 μg, below 25 μg, below 20 μg, below 15 μg, below 10 μg, below 9 μg, below 8 μg, below 7 μg, below 6 μg, below 5 μg, below 4 μg, below 3 μg, below 2 μg, below 1 μg, below 0.5 μg, below 0.1 μg, below 0.01 μg, below 10 ng, or below 1 ng, or below 0.1 ng per gram of stool can benefit from administration of a GABA-producing bacteria.

[00131] In some embodiments of any of the above aspects, the amount of GABA is increased 0.1, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 500, 1000, 2000, 3000, 4000, 5000, or more percent in the subject's stool relative to the initial amount of GABA in the subject's stool, e.g., as measured in step (b) of any of the above-aspects. In some embodiments, the amount of GABA is increased 0.1, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 500, 1000, 2000, 3000, 4000, 5000, or more percent in the subject's blood or serum relative to the initial amount of GABA in the subject's blood or serum, e.g., as measured in step (b) of any of the above-aspects. In some embodiments, the amount of GABA is increased 0.1, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 500, 1000, 2000, 3000, 4000, 5000, or more percent in regions of the subject's brain, such as, but not limited to the prefrontal cortex, relative to the initial amount of GABA in the subject's brain, e.g., as measured in step (b) of any of the above-aspects. In some embodiments, at least one GABA-producing bacteria is increased 0.1, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 500, 1000, 2000, 3000, 4000, 5000, or more percent in the subject's stool relative to the initial amount of GABA-producing bacteria in the subject's stool, e.g., as measured in step (b) in any of the above-aspects. In some embodiments, the level of expression of at least one GABA producing enzyme is increased 0.1, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 500, 1000, 2000, 3000, 4000, 5000, or more percent in the subject's stool relative to the initial level of expression of GABA-producing enzymes in the subject's stool, as measured by qPCR or some other appropriate method, known to those familiar in the field.

[00132] In some embodiments of the disclosure, the amount of GABA-consuming bacteria can be reduced, e.g., reduced in the subject's stool, blood serum, and the like.

The GABA-consuming bacteria can be, for instance, *Evtepia gabavorous* or *Firmicutes bacterium* MGS:114. In some embodiments, GABA-consuming bacteria can be reduced by 0.1, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 500, 1000, 2000, 3000, 4000, 5000, or more percent.

[00133] Accordingly, the present disclosure provides for the treatment of mental illness or disease of the central nervous system comprising administering to the subject GABA-producing bacteria, or prebiotics to stimulate growth or GABA production capabilities of GABA producing bacteria.

Methods of culturing GABA-consuming Bacteria

[00134] In some embodiments, the present disclosure provides a method of culturing bacteria that require GABA for survival and replication. In some cases, these bacteria were previously uncultured or unculturable. In some cases, the bacteria are cultured by supplying endogenous GABA to the growth medium. In some embodiments, the bacteria are cultured by co-culturing the bacteria with a different bacterium capable of producing GABA (e.g., a bacterium as described above).

[00135] In some embodiments, the previously uncultured bacterium is *E. gabavorous*. *E. gabavorous* can be cultured on a suitable substrate such as agar. In some embodiments, the agar can contain added GABA. In some embodiments, the present disclosure provides a method of culturing *E. gabavorous* comprising co-culturing *E. gabavorous* with another bacterial strain, said strain is capable of producing GABA, for instance at conditions that are physiologically relevant and found in to the human gastrointestinal tract (e.g., pH less between about 4.5 and about 7.5).

[00136] Without wishing to be bound by theory, some previously unculturable bacteria (e.g., *E. gabavorous*) may be able to grow in proximity to cultivable organisms producing growth factors necessary for bacteria to survive or grow. Accordingly, the present disclosure teaches the discovery and culture of *E. gabavorous* in the presence of GABA as a necessary growth factor.

[00137] *E. gabavorous* was identified as a late-growing colony in spatial proximity to *Bacteroides fragilis* KLE1758. It was found that growth of *E. gabavorous* KLE1738 was induced in the presence of supernatant derived from *Bacteroides fragilis* KLE1758.

Chemical analysis via HPLC and NMR of *Bacteroides fragilis* KLE1738 supernatant revealed GABA as the necessary growth factor for *E. gabavorous*.

[00138] As set forth in Figure 1, and Example 2, *E. gabavorous* was initially found because it grew in the presence of *Bacteroides fragilis* KLE1758. It was proposed that *Bacteroides fragilis* KLE1758 produces a growth factor that is necessary for the growth and survival of *E. gabavorous*. Figure 1A shows a photograph of an agar plate containing colonies of bacteria after treatment with human stool. The inset at the top right shows a close-up of a colony of KLE1758 along with a colony of 1738 that is growing in the immediate proximity. Figure 1B shows a colony of KLE1758 that is capable of supporting multiple colonies of KLE1738 on an agar plate with no other bacteria. Without wishing to be bound by theory, *Bacteroides fragilis* KLE1758 can support the growth of *E. gabavorous* KLE1738. Without wishing to be bound by theory, *Bacteroides fragilis* KLE1758 and *E. gabavorous* KLE1738 can co-exist in a symbiotic relationship in which *E. gabavorous* KLE1738 can consume the GABA that is produced by *Bacteroides fragilis* KLE1758.

[00139] As shown in Figure 2 and Examples 3-4, the supernatant from a 48-hour culture of *Bacteroides fragilis* KLE1758 was found to support the growth of *E. gabavorous* KLE1738, whereas standard agar was not. After a series of purification and isolation steps of the KLE1758 supernatant, it was discovered that GABA was responsible for the growth of *E. gabavorous* KLE1738. Figure 2A shows that *E. gabavorous* KLE1738 grew in the presence of supernatant of *Bacteroides fragilis*. However, Figure 2B shows that *E. gabavorous* KLE1738 did not grow in the presence of sterile vehicle on standard agar. After a first fractionation of the *Bacteroides fragilis* KLE1758 spent medium, it was found that the most polar fragment was capable of inducing the growth of *E. gabavorous* KLE1738 (Figure 2C), but that less polar fragments could not induce growth (Figure 2D). Figures 2E and 2F show close-up views demonstrating that the most polar fraction of the *Bacteroides fragilis* KLE1758 supernatant could induce growth of *E. gabavorous* KLE1738 (Figure 2E), whereas less polar fragments could not (Figure 2F). As shown in Figure 2G, only GABA was identified as being capable of inducing growth of *E. gabavorous* KLE1738.

[00140] The 16S nucleotide sequence of *E. gabavorous* KLE1738 is given in Seq. ID No. 2286.

[00141] The genetic sequence of *E. gabavorous* KLE1738 was identified as set forth in Example 5. The annotated genome (2,500,009 bp) of *E. gabavorous* is given in the attached Sequence Listing comprising SEQ. ID Nos. 1-2288 and is given in SEQ ID Nos. 2218-2285. Without wishing to be bound by theory, the genome revealed no obvious entry points for metabolism of common sugars or other carbon sources.

[00142] Without wishing to be bound by theory, it was discovered that transport systems for common sugars or other carbon sources were also incomplete. Without wishing to be bound by theory, their absence suggests a recent loss of function. *E. gabavorous* is predicted to have a limited set of transporters, including those for methionine, branched-chain amino acids, dipeptides, oligopeptides, and choline/betaine, as predicted in Table 10.

[00143] Table 10: Predicted Transport Systems in *E. gabavorous*.

Category	Subsystem	Role
Amino Acids and Derivatives	Polyamine Metabolism	ABC transporter, periplasmic spermidine putrescine-binding protein PotD (TC 3.A.1.11.1)
Amino Acids and Derivatives	Polyamine Metabolism	Spermidine Putrescine ABC transporter permease component PotB (TC 3.A.1.11.1)
Amino Acids and Derivatives	Polyamine Metabolism	Spermidine Putrescine ABC transporter permease component PotC (TC 3.A.1.11.1)
Amino Acids and Derivatives	Methionine Biosynthesis	Methionine ABC transporter ATP-binding protein
Amino Acids and Derivatives	Methionine Biosynthesis	Methionine ABC transporter permease protein
Amino Acids and Derivatives	Methionine Biosynthesis	Methionine ABC transporter substrate-binding protein
Amino Acids and Derivatives	Methionine Degradation	Methionine ABC transporter ATP-binding protein
Amino Acids and Derivatives	Methionine Degradation	Methionine ABC transporter permease protein
Amino Acids and Derivatives	Methionine Degradation	Methionine ABC transporter substrate-binding protein
Clustering-based subsystems	PhoR-PhoB two-component regulatory system	Phosphate ABC transporter, periplasmic phosphate-binding protein PstS (TC 3.A.1.7.1)
Membrane Transport	ABC transporter dipeptide (TC 3.A.1.5.2)	Dipeptide-binding ABC transporter, periplasmic substrate-binding component (TC 3.A.1.5.2)
Membrane Transport	ABC transporter oligopeptide (TC 3.A.1.5.1)	Oligopeptide ABC transporter, periplasmic oligopeptide-binding protein OppA (TC 3.A.1.5.1)
Membrane Transport	ABC transporter branched-chain amino acid (TC 3.A.1.4.1)	Branched-chain amino acid ABC transporter, amino acid-binding protein (TC 3.A.1.4.1)
Phosphorus Metabolism	High affinity phosphate transporter and control of PHO regulon	Phosphate ABC transporter, periplasmic phosphate-binding protein PstS (TC 3.A.1.7.1)
Phosphorus Metabolism	Phosphate metabolism	Phosphate ABC transporter, periplasmic phosphate-binding protein PstS (TC 3.A.1.7.1)
Stress Response	Choline and Betaine Uptake and Betaine Biosynthesis	L-proline glycine betaine ABC transport system permease protein ProV (TC 3.A.1.12.1)
Stress Response	Choline and Betaine Uptake and Betaine Biosynthesis	Glycine betaine ABC transport system, glycine-betaine-binding protein OpuAC
Stress Response	Choline and Betaine Uptake and Betaine Biosynthesis	Glycine betaine ABC transport system, permease protein OpuAB
Stress Response	Choline and Betaine Uptake and Betaine Biosynthesis	L-proline glycine betaine binding ABC transporter protein ProX (TC 3.A.1.12.1)

Category	Subsystem	Role
Sulfur Metabolism	Alkanesulfonate assimilation	ABC-type nitrate/sulfonate/bicarbonate transport system, ATPase component
Sulfur Metabolism	Alkanesulfonate assimilation	Alkanesulfonates ABC transporter ATP-binding protein

[00144] Without wishing to be bound by theory, these amino acids are not usually capable of supporting bacterial growth as single carbon sources, unlike serine, threonine, glutamate, and others. This is supported by the inability of *E. gabavorous* to grow on the tested amino acids.

[00145] Without wishing to be bound by theory, the metabolic pathway of *E. gabavorous* is proposed to be similar to that of *Clostridium aminobutyricum*, as set forth in Figure 3, as all enzymes in this pathway were identified in the *E. gabavorous* genome (Table 11).

Table 11. Enzymes in *E. gabavorous* GABA fermentation pathway, predicted by RAST

Enzyme #	Contig	Start	Stop	Length (bp)	Function
1	KLE1738_5	26729	28084	1356	Gamma-aminobutyrate:alpha-ketoglutarate aminotransferase (EC 2.6.1.19)
2	KLE1738_5	25576	26688	1113	NAD-dependent 4-hydroxybutyrate dehydrogenase (EC 1.1.1.61)
3	KLE1738_2	195690	197033	1344	4-hydroxybutyrate:acetyl-CoA CoA transferase (EC 2.8.1.-)
3	KLE1738_28	9343	10665	1323	4-hydroxybutyrate:acetyl-CoA CoA transferase (EC 2.8.1.-)
3	KLE1738_5	24249	25547	1299	4-hydroxybutyrate:acetyl-CoA CoA transferase (EC 2.8.1.-)
3	KLE1738_7	87690	86401	1290	4-hydroxybutyrate:acetyl-CoA CoA transferase (EC 2.8.1.-)
4	KLE1738_5	22352	23902	1551	4-hydroxybutanoyl-CoA dehydratase (EC 4.2.1.-) / Vinylacetyl-CoA Delta-isomerase (EC 5.3.3.3)
5	KLE1738_6	2958	2092	867	Enoyl-CoA hydratase (EC 4.2.1.17)
5	KLE1738_11	1132	356	777	Enoyl-CoA hydratase (EC 4.2.1.17)
6	KLE1738_6	4710	5762	1053	3-hydroxybutyryl-CoA dehydrogenase (EC 1.1.1.35)
6	KLE1738_8	87921	88769	849	3-hydroxybutyryl-CoA dehydrogenase (EC 1.1.1.35)
7	KLE1738_14	4910	6139	1230	Acetyl-CoA acetyltransferase (EC 2.3.1.9)
7	KLE1738_19	28638	27382	1257	Acetyl-CoA acetyltransferase (EC 2.3.1.9)
8	KLE1738_13	26709	25738	972	Phosphate acetyltransferase (EC 2.3.1.8)
9	KLE1738_1	119924	121141	1218	Acetate kinase (EC 2.7.2.1)
1b	KLE1738_30	8650	9999	1350	NADP-specific glutamate dehydrogenase (EC 1.4.1.4)
5b	KLE1738_6	4303	3083	1221	Butyryl-CoA dehydrogenase (EC 1.3.8.1)
6b	KLE1738_1	158124	159767	1644	Acetyl-CoA:acetoacetyl-CoA transferase (EC 2.8.3.8)

[00146] The pH dependency of *B. fragilis* KLE1758's ability to produce GABA was investigated. As set forth in Example 6, *B. fragilis* KLE1758 was grown at various pH values, and the supernatant from that growth was analyzed using LCMS. As shown in Figure 4A, GABA is produced primarily at relatively lower pH (e.g., about 5.5 and below), compared with glutamate.

[00147] Accordingly, it was found that *B. fragilis* KLE1758 can produce GABA at low pH, whereas it was found to produce primarily glutamate at relatively high pH. As set forth in Example 6 and Figure 4A, at a pH of about 5 and about 5.5, *B. fragilis* KLE1758 produced considerably more GABA than glutamate. However, at a pH of about 6 and about 6.5, *B. fragilis* KLE1758 was found to produce primarily glutamate and relatively low quantities of GABA.

Biological Screens for GABA-Producers

[00148] The present disclosure also teaches methods of identifying bacteria that can produce GABA. Given the strict requirement of GABA for the growth of *E. gabavorous*, the present disclosure provides methods of screening for bacteria capable of producing GABA using, for instance, *E. gabavorous* and/or other GABA-dependent bacterial growth as a bioassay. Importantly, by using buffered media (e.g., buffered agar), the assay technique set forth herein can be used to identify bacteria that are capable of producing bacteri at various pH values (e.g., between about 5.5 to about 7.5).

[00149] As set forth in Example 7, a sample that is thought to contain GABA-producing bacteria, such as a human stool sample, can be mixed with molten agar. The agar, containing the bacterial sample, can then be streaked with a dilute solution of *E. gabavorous*. As shown herein, *E. gabavorous* cannot grow in the absence of GABA, and therefore any colonies of *E. gabavorous* that do form will necessarily grow in close spatial proximity to GABA-producers.

[00150] Because GABA production by some bacteria, including *E. coli,* only occurs at a very low pH (e.g., at a pH not relevant to the human gut), the assay method set forth herein was adapted to control the pH of the media, enabling identification of organisms capable of producing GABA at a pH between about 4.5 and about 7.5. Without wishing to be bound by theory, the pH of between about 4.5 and about 7.5 is the relevant pH within the human gut. Accordingly, bacteria that can produce GABA at these pH values can in some embodiments be capable of producing GABA in the human gut.

[00151] In other words, by controlling the pH of the growth medium (e.g., by buffering the molten agar), the present disclosure can allow one to distinguish between GABA-producers that are capable of producing GABA at a physiologically relevant pH (e.g.,

between about 4.5 to about 7.5) from bacteria that are not able to produce GABA at a physiological relevant pH (e.g., bacteria that can only produce substantial quantities of GABA below pH of about 4.5).

[00152] Using this method, a number of representatives from multiple genera, including, but not limited to, *Bacteroides*, *Bifidobacterium*, *Blautia*, *Coprococcus*, *Gordonibacter*, *Dorea*, and *Clostridium* were identified. Figure 4B shows a representative agar plate showing growth of *E. gabavorous* in the presence of a GABA-producing bacteria. Figure 4C shows a phylogenetic tree of GABA-producing bacteria that were identified using this method.

[00153] Figure 5 shows the GABA production capabilities of certain strains of GABA producers identified using the techniques described herein. As set forth in Figure 5, eight strains of GABA produers were grown in buffered media (e.g., between about pH 4.5 and about pH 5.0; and between about pH 6.5 and about pH 7.0). Using the method described in Example 6, the GABA-producing capabilities of the GABA-producing bacteria at various pH values were investigated. As set forth in Figure 5, certain bacteria (e.g., *B. dorei* KLE1912) produced relatively similar quanties of GABA at lower pH (e.g., between about 4.5 and about 5.0). In contrast, certain bacteria produced different amounts of GABA depending on the pH (e.g., *B. vulgatus* KLE1910 and *B. ovatus* KLE1770). Notably, as shown for *B. vulgatus* KLE1910 and *B. ovatus* KLE1770, some bacteria were found to produce relatively more GABA at lower pH than at higher pH, whereas some bacteria were found to produce relatively more GABA at higher pH than at lower pH.

[00154] In some embodiments of the method of identifying bacteria capable of producing GABA, the substrate is agar. In some embodiments, the step of contacting the substrate with *E. gabavorous* comprises streaking the agar with a dilute solution of *E. gabavorous*. The GABA-producing colonies are then identified by growth induction of *E. gabavorous*. As set forth above, growth *E. gabavorous* is used to determine if a bacterial strain produces GABA. However, one of skill in the art will understand that any bacteria that has a strict requirement of GABA for growth and survival can likewise be used as described above to identify bacteria that can produce GABA.

Examples

[00155] The disclosure is further illustrated by the following examples and synthesis examples, which are not to be construed as limiting this disclosure in scope or spirit to the specific procedures herein described. It is to be understood that the examples are provided to illustrate certain embodiments and that no limitation to the scope of the disclosure is intended thereby. It is to be further understood that resort may be had to various other embodiments, modifications, and equivalents thereof which may suggest themselves to those skilled in the art without departing from the spirit of the present disclosure and/or scope of the appended claims.

[00156] Unless otherwise noted, all materials were obtained from commercial suppliers and were used without further purification. Anhydrous solvents were obtained from Sigma-Aldrich (Milwaukee, WI) and used directly.

[00157] Unless otherwise specified, PCR was performed using the general bacterial primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') (set forth in Seq ID No. 2287) and 1492R (5'- TACGGYTACCTTGTTACGACTT-3') (set forth in Seq ID No. 2288) to amplify the 16S rRNA gene. The PCR reaction mixture was 12.5 µL GoTaq Master Mix (Promega), 1 µL 10 µM 27F and 1492R primers, 9.5 µL Nuclease Free Water (Promega), and 1 µL of a colony resuspended in 100 µL sterilized distilled water. The amplification conditions were one cycle of 95°C for 5 min; 30 cycles of 95°C for 30 s, 55°C for 30 s; 72°C for 90 s; and finally one cycle of 72°C for 7 min. Amplification of PCR reactions were then confirmed using gel electrophoresis on a 0.8% agarose gel loaded saturated with ethidium bromide. Successful PCRs were sequenced by Macrogen Corporation using the 27F primer using the Applied Biosystems 3730xl DNA analyzer. Quality control for sequences was performed using DNA Baser (www.DnaBaser.com), in which ends were trimmed until there were more than 75% good bases (defined by having a QV score of higher than 25) in an 18 base window. Identification of phylogenetic neighbors and calculation of pairwise sequence similarity were carried out using the EzTaxon server.

Example 1: Human Stool Collection

[00158] Stool samples from a healthy human donor were collected using a commercially available stool collection vessel. Within 5 minutes of collection, 1 gram of stool was

resuspended in 9 mL of sterile 20% glycerol in PBS and homogenized for 30 seconds using a vortex. 1 mL aliquots of this mixture were loaded in cryotubes and stored at -80°C for cultivation.

Example 2: Cultivation of helper-uncultured pairs from human stool samples

[00159] All cultivation work was performed in a Coy Anaerobic Vinyl chamber with an atmosphere of 5% hydrogen, 10% CO_2, 85% nitrogen. Anaerobically, serial dilutions of thawed stool samples were prepared in PBS and bead spread (7-10 beads/plate) on 1X Fastidious Anaerobic Agar (Accumedia) plates with 2.5% yeast extract (FAAy). Plates were incubated at 37°C anaerobically for one week, and each day appearance of colonies were tracked by spotting the outside of the plates with different colored markers. At the end of the week, serial dilutions of late forming colonies (appearance after 4-7 days) were prepared in PBS and bead spread on FAAy plates. Nearby (< 2 cm), early forming colonies (appearance after 1-3 days) were then resuspended in PBS at a high density. Five µL of this suspension were spotted on the plates with their respective spread-candidate dependent and incubated for up to one week in the chamber, and observed daily. Growth induction of the dependent organism around the spotted helper indicated a positive hit.

[00160] A fecal sample from a healthy human donor was diluted and spread-plated on rich medium, and newly formed colonies were noted daily for a week. Late forming colonies (3-7 days) were diluted and spread on a nutrient agar plate, and a heavy inoculum of a neighboring, early forming colony (1-2 days) was spotted, as shown in Figure 1A. Using this method, a number of helper-uncultured pairs were identified, in which the spread-plated uncultured isolate formed a gradient of growth around the spotted culturable helper.

[00161] One isolate, *E. gabavorous* KLE1738 (93.22% similar to *Flavonifractor plautii* ATCC 29863 by 16S rRNA gene sequence), was dependent on *Bacteroides fragilis* KLE1758 (100% similar to *Bacteroides fragilis* ATCC 25852 by 16S rRNA gene sequence) for growth (Fig. 1B). *E. gabavorous*, is a gram positive organism of the *Clostridia* class.

Example 3: Determination of GABA as Growth Factor for *E. gabavorous*

[00162] The supernatant of a 48-hour culture of *B. fragilis* KLE1758 grown in rich medium induced growth of *E. gabavorous* as shown in Figures 2A and 2B, enabling

bioassay-driven purification of the growth factor. The supernatant was solvent-partitioned with ethyl acetate, and the water residue fraction induced the growth of *E. gabavorous*. The water fraction was then purified using HP-20 column chromatography, and the most polar fraction induced the growth of *E. gabavorous* as shown in Figure 2C. This active fraction was then further fractionated by preparative HPLC. HPLC yielded one active fraction, and NMR showed that it contained 10 compounds, primarily GABA, threonine, lactic acid, valine, glutamine, malonic acid, succinic acid, and alanine. All compounds were spotted on plates where *E. gabavorous* was spread, and only GABA caused growth induction, as shown in Figs. 2E-F. The compounds were identified by NMR analysis including ^{1}H, ^{13}C, ^{1}H-^{1}H COSY, TOCY, HSQC, and HMBC NMR experiments to identify the constituents in the fraction. All NMR experiments were carried out on a Varian INOVA 600 MHz NMR spectrometer equipped with an indirect detection probe.

Example 4: Testing other compounds for induction of *E. gabavorous*

[00163] Multiple compounds were tested for the ability to induce the growth of *E. gabavorous* as shown in Figure 2G. Stocks of each compound (purchased from Sigma, excluding the ATCC Mineral and Vitamin mixes) were prepared dependent on solubility in water at the concentrations shown in Figure 2G. Five µL of the stocks were then spotted on FAAy plates spread with *E. gabavorous*, and incubated anaerobically for one week, and any growth was observed. No compounds induced growth but GABA.

Example 5: Whole genome sequencing and annotation

[00164] DNA from cells of *E. gabavorous* grown 48 hours anaerobically on FAAy plates with 1.0 mg/mL GABA was isolated for genome sequencing using the PowerSoil® DNA Isolation Kit (Mo Bio, San Diego, CA) to manufacturer specifications, yielding ~5.0 µg of high quality DNA. Genomic sequencing and de novo assembly was performed by the Genomic Core at Tufts University in Boston, MA. The genome of *E. gabavorous* was sequenced on an Illumina MiSeq using MiSeq V2 500 cycles chemistry with a paired-end 250 bases format. Briefly, 100 ng of genomic DNA was sheared on a Covaris M220 to an average fragment size of around 600 bases. Using the fragmented DNA as input, a sequencing library was prepared with Illumina TruSeq Nano DNA Sample Preparation Kit per the manufacturer instruction. Base calling and demultiplexing was performed on the raw

data from the MiSeq using CASAVA and fastq files were generated. De novo assembly of the genome was performed using Edena V3.131028 with a customized parameter optimization pipeline. The best assembled genome, as assessed by the contig statistic, was reported. Assembly yielded 68 contigs (n), with all contigs having a sequence length longer than 200 bases (n:200). 7 contigs with a larger value than the N50 (119748), and the minimal contig length is 355 (min). The N20, N50 and N20 are 33403, 119748 and 204670, respectively. The largest contig length (max) was 344080, and the estimated genome size is 2500009. The draft genome was annotated using the RAST server and the KAAS (KEGG Automatic Annotation Server) analysis tool of the KEGG (Kyoto Encyclopedia of Genes and Genomes) database. The genome of *E. gabavorous* was annotated using RAST, and the genomes of CAG:113 and *E. gabavorous* were compared using RAST.

Example 6: Quantification of Glutamate and GABA Production in *B. fragilis*

[00165] The absolute amount of glutamate and GABA contained in the *B. fragilis* KLE1758 supernatant was determined by HPLC, using a fluorophore to aid in detection. Specifically, free amines were labeled for analysis by reacting with the AccQ reagent (Waters) according to manufacturer's protocols. A calibration curve was generated from stock solutions (10 mg/mL) that were prepared by dissolving GABA (2.0 mg) in water (200 µL), glutamate (Glu) (10.2 mg) in water (1020 µL) and cysteic acid (CSA) (16.9 mg) in water (1690 µL). These were serially diluted to generate a concentration gradient. Specifically, stocks were made to final concentrations of 0.1mg/mL, 0.05 mg/mL, 0.01 mg/mL and 0.001 mg/mL. An aliquot of a given stock solution, was added to the AccQ reaction buffer (25 µL final), followed by the addition of the acetonitrile-dissolved AccQ reagent (25 µL). This was reacted for ten minutes at 55-60°C, and then transferred directly to an LCMS vial, fitted with a glass insert. Reaction concentrations of the amino acids were: 20 ng/µL, 10 ng/µL, 5 ng/µL, 2.5 ng/µL, 1 ng/µL, 0.5 ng/µL, 1 ng/µL and 0 ng/µL (control). These samples were injected (10 µL) on an Agilent LCMS, using a gradient of solvent A (water/0.1% formic acid) and B (acetonitrile/0.1% formic acid) over the following time course: 1) 0-40 minutes linear gradient of 2% B to 98% B; 2) 40-45 min isocratic at 98% B; 3) 45-45.5 min linear gradient of 98% B to 2 % B; 4) 45.5-55 min isocratic at 2% B. The CSA-AccA derivative eluted at 9.5 min, the Glu-AccQ derivative eluted at 12.1 min and the GABA-AccQ derivative at 12.5 min. The area under the curve, in

extracted ion (EIC) mode (m/z = 274 for GABA-AccQ, 318 for Glu-AccQ and 340 for CSA-AccQ), was used to develop a calibration curve by plotting area against amount of original Glu, CSA or GABA (in ng) injected. An average of two runs for each tested concentration was used to generate the calibration curve. In the case of GABA and Glu, CSA was added to all reactions, to a final concentration of 2.5 μg/mL, and used as an internal standard.

[00166] Triplicate cultures of *B. fragilis* KLE1758 were grown in BHIych anaerobically for 48 hours, the cells centrifuged, and the supernatant was filtered through a 0.2 μm filter. Samples were stored at 4 °C until analysis. To analyze the samples, an aliquot (2 μL) of each sample was added to AccQ reaction buffer (16 μL), CSA internal standard (2 μL of a 50 μg/mL solution in buffer), followed by the addition of the AccQ reagent (20 μL). These samples were heated to 55°C for ten minutes, and then transferred directly into an LCMS vial fitted with a glass insert. An aliquot of each sample (10 μL) was injected onto the LCMS, and separated following the same injection program as used for the calibration curve. The total EIC area under curves representing GABA, Glu and CSA was determined using ChemStation software (Agilent). Each injection represented 25% of the original media concentration, therefore the total amount of sample determined (in ng) was multiplied by a factor of four to determine the original concentration (in ng/μL = μg/mL). All areas were normalized to the area under the curve of the internal standard (CSA), which was held at constant concentration throughout the experiment. The results are given in Figure 4A.

Example 7: Co-culture Screen for GABA Producers Using *E. gabavorous*

[00167] GABA secretion can allow bacteria to survive acid stress. Decarboxylation of glutamate produces GABA, which is exported from the cell in a protonated form, alkalinizing the cytoplasm. *E. coli*, as well as some *Lactobacillus* and *Bifidobacterium* strains were shown to produce GABA, but these organisms are typically found at a low abundance in the human intestinal tract, and in the case of *E. coli*, is dependent on low pH (e.g., about 4.2 and below). *Bacteroides fragilis*, the helper of *E. gabavorous*, is a common gut bacterium, but it was found that similarly to *E. coli*, GABA production by *Bacteroides fragilis* KLE1758 is only observed at a pH less than about 5.5 as shown in Figure 4A. GABA is shown in left-hand columns and glutamate is shown in right-hand columns at each

pH value. Without wishing to be bound by theory, it was therefore considered useful to identify microorganisms capable of producing GABA at a physiologically relevant pH for the human large intestine (e.g., pH of about 5.5 to about 7.5, or about pH 5.7 to about 7.4).

[00168] To accomplish this, the strict GABA requirement of *E. gabavorous* was utilized to screen for bacteria capable of secreting GABA on heavily buffered medium. Metabolic byproducts of bacterial growth may lower the pH of the medium in the absence of buffer. Stool sample was mixed with molten agar and poured in Petri plates in an anaerobic chamber, and *E. gabavorous* was spread on top of the agar once solidified. By looking for zones of growth induction of *E. gabavorous*, and measuring the pH of the agar, bacteria that produce GABA at a pH of between about 6.0 and about 7.0 were identified, as well as those producing GABA at a pH of about 4.5 to about 5.0, as shown in Figure 4B. The full 16S rRNA gene was amplified and sequenced using the 27F and 1492R universal primers, and annotation with EZTaxon revealed a number of representatives from multiple genera, including *Bacteroides, Bifidobacterium, Blautia, Coprococcus, Gordonibacter, Dorea, and Clostridium* (Fig. 4C). Of these, only *Bifidobacterium adolescentis* was previously reported to produce GABA.

Example 8: Using an Engineered *Escherichia coli* strain to produce GABA and induce the growth of *E. gabavorous*

[00169] GABA can be produced by intestinal epithelial cells and by some bacteria, such as *Escherichia coli* and *Listeria monocytogenes*, by decarboxylation of glutamate. In *E. coli*, the decarboxylation of glutamate serves as a mechanism to decrease intracellular pH, and GABA production generally occurs at a low pH. To survey whether *E. coli* could be engineered to produce GABA, *E. coli* colones harboring native glutamate decarboxylases (gadA, gadB), or the GABA antiporter, (gadC) in the pCA24N IPTG inducible high-copy number vector, were tested for GABA production via co-cultivation assay with *E. gabavorous*. Overexpression of glutamate decarboxylase in *E. coli* (gadA or gadB), resulted in induction of KLE1738 growth to levels seen with *B. fragilis*, while expression of the GABA antiporter, gadC, did not (Figure 6). Altering the pH of growth media for KLE1738 did not change the GABA-dependency phenotype. Without wishing to be bound by theory, this suggests that engineering bacteria to overexpress glutamate decarboxylase or

other GABA producing enzymes, constitutively or inducibly, is an effective way to produce GABA, as well as induce the growth of *E. gabavorous*.

Equivalents

[00170] While the present disclosure has been described in conjunction with the specific embodiments set forth above, many alternatives, modifications and other variations thereof will be apparent to those of ordinary skill in the art. All such alternatives, modifications and variations are intended to fall within the spirit and scope of the present disclosure.

Claims:

1. A therapeutic composition comprising at least one purified bacterial population consisting of bacteria capable of producing GABA in a subject in need thereof.

2. The therapeutic composition of claim 1, wherein the at least one bacterial population consists of a bacteria comprising a 16S rDNA sequence at least about 95% identical to a 16S rDNA sequence selected from one of Seq. ID. Nos. 1-31 set forth in Table 1.

3. The therapeutic composition of claim 1, wherein the at least one purified bacterial population consists of bacteria selected from the group consisting of: *Bacteroides caccae* KLE1911; *Bacteroides clarus* KLE1930; *Bacteroides dorei* KLE1912; *Bacteroides finegoldii* KLE1931; *Bacteroides fragilis* KLE1958; *Bacteroides massiliensis* KLE1932; *Bacteroides ovatus* KLE1770; *Bacteroides stercoris* KLE1933; *Bacteroides thetaiotaomicron* KLE1934; *Bacteroides uniformis* KLE1913; *Bacteroides vulgatus* KLE1910; *Bacteroides xylanisolvens* KLE1935; *Bifidobacterium adolescentis* KLE 1879; *Blautia obeum* KLE1914; *Blautia wexlerae* KLE1916; *Butyricimonas virosa* KLE1938; *Clostridium perfringens* KLE1937; *Clostridium sordellii* KLE1939; *Clostridium sp.* KLE1862; *Clostridium sp.* KLE1918; *Coprobacillus sp.* KLE1779; *Coprococcus sp.* KLE1880; *Dorea longicatena* KLE1917; *Eggerthella lenta* KLE1926; *Eubacterium rectale* KLE1922; *Gordonibacter pamelaeae* KLE1915; *Oscillibacter sp.* KLE1928; *Parabacteroides distasonis* KLE2020; *Parabacteroides merdae* KLE1863; *Ruminococcus gnavus* KLE1940; *Turicibacter sanguinis* KLE1941, and combinations thereof.

4. The therapeutic composition of claim 1, wherein the at least one purified bacterial population consists of a bacteria comprising a 16S rDNA sequence at least about 95% identical to a 16S rDNA sequence selected from one of Seq. ID. Nos. 32-274 set forth in Table 2.

5. The therapeutic composition of claim 1, wherein the at least one purified bacterial population consists of bacteria comprising a 16S rDNA sequence having at least 95% similarity to the 16S rDNA sequence selected from one of Seq. ID. Nos. 305-2217 set forth in Table 10.

6. The therapeutic composition of claim 1, wherein the at least one purified bacterial population consists of bacteria comprising a DNA sequence which encodes an enzyme selected from: glutamate decarboxylase; putrescine aminotransferase; gamma-aminobutyraldehyde dehydrogenase; arginine decarboxylase; agmatinase; ornithine decarboxylase; or a combination thereof.

7. The therapeutic composition of claim 6, wherein the glutamate decarboxylase; putrescine aminotransferase; gamma-aminobutyraldehyde dehydrogenase; arginine decarboxylase; agmatinase; ornithine decarboxylase; or a combination thereof, is encoded by a DNA sequence at least 70% similar in DNA sequence to any one of Seq. ID. Nos. 275-304 set forth in Table 3.

8. The therapeutic composition of claim 6, wherein the glutamate decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 4.

9. The therapeutic composition of claim 6, wherein the putrescine aminotransferase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 5.

10. The therapeutic composition of claim 6, wherein the gamma-aminobutyraldehyde dehydrogenase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 6.

11. The therapeutic composition of claim 6, wherein the arginine decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 7.

12. The therapeutic composition of claim 6, wherein the agmatinase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 8.

13. The therapeutic composition of claim 6, wherein the ornithine decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 9.

14. The therapeutic composition of claim 1, wherein the at least one purified bacterial population consists of bacteria comprising a 16S rDNA sequence having at least 95% similarity to a reference bacterium selected from the group consisting of:

Escherichia coli MG1655;

Escherichia coli Nissle 1917; or a combination thereof.

15. The therapeutic composition of claim 1, wherein the bacterial population consists of bacteria capable of producing GABA at a physiologically relevant pH.

16. The therapeutic composition of claim 1, wherein the bacterial population consists of bacteria capable of producing GABA at a pH range between about 4.5 and about 7.5.

17. The therapeutic composition of claim 1, wherein the bacterial population consists of bacteria capable of producing GABA inside the human gut.

18. The therapeutic composition of claim 1, wherein the composition is in the form of a probiotic, prebiotic, a capsule, a tablet, a caplet, a pill, a troche, a lozenge, a powders, a granule, a medical food, or a combination thereof.

19. The therapeutic composition of claim 1, wherein the composition is administered as a fecal transplant.

20. The therapeutic composition of claim 1, wherein the bacteria are capable of producing GABA via expression of any combination of glutamate decarboxylase, putrescine aminotransferase, gamma-aminobutyraldehyde dehydrogenase, arginine decarboxylase, agmatinase, and/or ornithine decarboxylase.

21. The therapeutic composition of claim 1, further comprising a purified bacterial strain that is cytotoxic or cytostatic to a GABA-consuming bacteria.

22. The therapeutic composition of claim 21, wherein the GABA-consuming bacteria is *Evtepia gabavorous* or *Firmicutes bacterium* MGS:114.

23. The therapeutic composition of claim 1, further comprising a prebiotic capable of stimulating the growth or GABA-production levels of a GABA-producing bacteria.

24. A method of treating a disease or disorder in a subject in need thereof, the method comprising administering to the subject a therapeutic composition comprising at least one purified bacterial population consisting of bacteria capable of producing GABA in a subject in need thereof.

25. The method of claim 24, wherein the disease or disorder is a mental disease or disorder.

26. The method of claim 24, wherein the mental disease or disorder is selected from the group consisting of depression, bipolar disorder, schizophrenia, anxiety, anxiety disorders, addiction, social phobia, treatment-resistant major depressive disorder (TR-MDD), major depressive disorder and its subtypes (melancholic depression, atypical depression, catatonic depression, postpartum depression, and seasonal affective disorder), Neurodegenerative amyloid disorders (Parkinson's, Alzheimer's, and Huntington's diseases) orthostatic tremor, Lafora disease, restless leg syndrome, neuropathic pain, pain disorders, dementia, epilepsy, stiff-person syndrome, premenstrual dysphoric disorder, autism spectrum disorder, sleep disorders, and attention deficit hyperactivity disorder (ADHD), and combinations thereof.

27. The method of claim 24, wherein treating a disease or disorder comprises decreasing at least one symptom of the disease or disorder, such as fatigue, insomnia, motor dysfunction, stress, persistent anxiety, persistent sadness, social withdrawal, substance withdrawal, irritability, thoughts of suicide, thoughts of self-harm, restlessness, low sex drive, lack of focus, seizures, memory loss, anger, bouts of emotional reactivity, confusion, pain, and muscle spasms, loss of appetite, altered intestine motility, and combinations thereof.

28. The method of claim 24, wherein the at least one bacterial population consists of a bacteria comprising a 16S rDNA sequence at least about 95% identical to a 16S rDNA sequence selected from one of Seq. ID. Nos. 1-31 set forth in Table 1.

29. The method of claim 24, wherein the at least one purified bacterial population consists of bacteria selected from the group consisting of: *Bacteroides caccae* KLE1911; *Bacteroides clarus* KLE1930; *Bacteroides dorei* KLE1912; *Bacteroides finegoldii* KLE1931; *Bacteroides fragilis* KLE1958; *Bacteroides massiliensis* KLE1932; *Bacteroides ovatus* KLE1770; *Bacteroides stercoris* KLE1933; *Bacteroides thetaiotaomicron* KLE1934; *Bacteroides uniformis* KLE1913; *Bacteroides vulgatus* KLE1910; *Bacteroides xylanisolvens* KLE1935; *Bifidobacterium adolescentis* KLE 1879; *Blautia obeum* KLE1914; *Blautia wexlerae* KLE1916; *Butyricimonas virosa* KLE1938; *Clostridium perfringens* KLE1937; *Clostridium sordellii* KLE1939; *Clostridium sp.* KLE1862; *Clostridium sp.* KLE1918; *Coprobacillus sp.* KLE1779; *Coprococcus sp.* KLE1880; *Dorea longicatena* KLE1917; *Eggerthella lenta* KLE1926;

Eubacterium rectale KLE1922; *Gordonibacter pamelaeae* KLE1915; *Oscillibacter sp.* KLE1928; *Parabacteroides distasonis* KLE2020; *Parabacteroides merdae* KLE1863; *Ruminococcus gnavus* KLE1940; *Turicibacter sanguinis* KLE1941, and combinations thereof.

30. The method of claim 24, wherein the at least one purified bacterial population consists of a bacteria comprising a 16S rDNA sequence at least about 95% identical to a 16S rDNA sequence selected from one of Seq. ID. Nos. of 32-274 as set forth in Table 2.

31. The method of claim 24, wherein the at least one bacterial population consists of a bacteria comprising a 16S rDNA sequence at least about 95% identical to any one of Seq ID Nos. 305-2217 set forth in Table 10.

32. The method of claim 24, wherein the at least one purified bacterial population consists of bacteria comprising a DNA sequence which encodes an enzyme selected from: glutamate decarboxylase; putrescine aminotransferase; gamma-aminobutyraldehyde dehydrogenase; arginine decarboxylase; agmatinase; ornithine decarboxylase; or a combination thereof.

33. The method of claim 32, wherein the glutamate decarboxylase; putrescine aminotransferase; gamma-aminobutyraldehyde dehydrogenase; arginine decarboxylase; agmatinase; ornithine decarboxylase; or a combination thereof, is encoded by a DNA sequence at least 70% similar in DNA sequence selected from one of Seq. ID. Nos. 275-304 set forth in Table 3.

34. The method of claim 32, wherein the glutamate decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 4.

35. The method of claim 32, wherein the putrescine aminotransferase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 5.

36. The method of claim 32, wherein the gamma-aminobutyraldehyde dehydrogenase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 6.

37. The method of claim 32, wherein the arginine decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 7.

38. The method of claim 32, wherein the agmatinase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 8.

39. The method of claim 32, wherein the ornithine decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 9.

40. The method of claim 24, wherein the bacteria is genetically engineered to produce GABA.

41. The method of claim 40, wherein the bacteria is engineered to produce GABA via expression of glutamate decarboxylase; putrescine aminotransferase; gamma-aminobutyraldehyde dehydrogenase; arginine decarboxylase; agmatinase; ornithine decarboxylase; or a combination thereof.

42. The method of claim 41, wherein the glutamate decarboxylase; putrescine aminotransferase; gamma-aminobutyraldehyde dehydrogenase; arginine decarboxylase; agmatinase; ornithine decarboxylase; or a combination thereof, is encoded by a DNA sequence at least 70% similar in DNA sequence selected from one of Seq. ID. Nos. 275-304 set forth in Table 3.

43. The method of claim 41, wherein the glutamate decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 4.

44. The method of claim 41, wherein the putrescine aminotransferase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 5.

45. The method of claim 41, wherein the gamma-aminobutyraldehyde dehydrogenase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 6.

46. The method of claim 41, wherein the arginine decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 7.

47. The method of claim 41, wherein the agmatinase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 8.

48. The method of claim 41, wherein the ornithine decarboxylase is encoded by a DNA sequence at least 95% similar in DNA sequence to a gene with a EMBL/GENBANK/DDBJ ID found in Table 9.

49. The method of claim 24, wherein the at least one purified bacterial population consists of bacteria comprising a 16S rDNA sequence having at least 95% similarity to a reference bacterium selected from the group consisting of:

Escherichia coli MG1655;

Escherichia coli Nissle 1917; or a combination thereof.

50. The method of claim 24, wherein the bacterial population consists of bacteria capable of producing GABA at a physiologically relevant pH.

51. The method of claim 24, wherein the bacterial population consists of bacteria capable of producing GABA at a pH range between about 4.5 and about 7.5.

52. The method of claim 24, wherein the bacterial population consists of bacteria capable of producing GABA inside the human gut.

53. The method of claim 24, wherein the composition is administered as a fecal transplant.

54. The method of claim 24, wherein the composition is administered as a probiotic.

55. The method of claim 24 wherein the bacteria are capable of producing GABA via expression any combination of glutamate decarboxylase, putrescine aminotransferase, gamma-aminobutyraldehyde dehydrogenase, arginine decarboxylase, agmatinase, ornithine decarboxylase, and combinations thereof.

56. The method of claim 24, wherein the at least one bacterial strain is cytotoxic or cytostatic to a GABA-consuming bacteria.

57. The method of claim 56, wherein the GABA-consuming bacteria is *Evtepia gabavorous* or *Firmicutes bacterium* MGS:114.

58. The method of claim 24, further comprising identifying a subject in need of treatment by determining whether the subject would benefit from an increase in endogenous GABA by measuring an initial amount of GABA in the subject's stool.

59. The method of claim 58, wherein the initial amount of GABA in the subject's stool is below about 8 μg per gram of wet or dry stool.

60. The method of claim 58, wherein the amount of GABA in the subject's stool is increased relative to the initial amount after administering the therapeutic composition.

61. The method of claim 24, further comprising identifying a subject in need of treatment by determining whether the subject would benefit from an increase in endogenous GABA by measuring an initial amount of GABA-producing bacteria in the subject's stool.

62. The method of claim 61, wherein initial amount of GABA-producing bacteria in the subject's stool is less than about 10% of total bacteria as measured by 16S sequence mapping.

63. The method of claim 61, wherein at least one GABA-producing bacteria is increased in the subject's stool relative to the initial amount of GABA-producing bacteria in the subject's stool after administering the therapeutic composition.

64. The method of claim 24, further comprising identifying a subject in need of treatment by determining whether the subject would benefit from an increase in endogenous GABA by measuring an initial amount of GABA in the subject's blood or serum.

65. The method of claim 64, wherein the amount of GABA in the subject's blood or serum is below about 10 μg per liter of blood.

66. The method of claim 64, wherein the amount of GABA in the subject's blood or serum is increased relative to the initial amount after administering the therapeutic composition.

67. The method of claim 24, further comprising identifying a subject in need of treatment by determining whether the subject would benefit from an increase in endogenous GABA by measuring an amount of GABA in the subject's brain.

68. The method of claim 67, wherein the amount of GABA in the subject's brain is below about 1.0 mM/kg.

69. The method of claim 67, wherein the amount of GABA in the subject's brain is increased relative to the initial amount after administering the therapeutic composition.

70. The method of claim 24, further comprising identifying a subject in need of treatment by determining whether the subject would benefit from an increase in endogenous GABA by measuring an initial amount of expression of GABA-producing enzymes in the subject's stool.

71. The method of claim 70, wherein the GABA-producing enzymes are selected from glutamate decarboxylase, putrescine aminotransferase, gamma-aminobutyraldehyde dehydrogenase, arginine decarboxylase, agmatinase, ornithine decarboxylase, and combinations thereof.

72. The method of claim 70, wherein the initial amount of enzyme expression is measured by qPCR.

73. The method of claim 70, wherein the expression of enzymes is increased relative to the initial amount of enzyme expression after administering the therapeutic composition.

74. The method of claim 24, further comprising identifying a subject in need of treatment by determining whether the subject would benefit from an increase in endogenous GABA by measuring an initial amount of GABAergic response in the subject's brain.

75. The method of claim 74, wherein the amount of the GABAergic response in the subject's brain is increased relative to the initial amount after administering the therapeutic composition.

76. The method of claim 24, wherein the therapeutic composition comprises a prebiotic capable of stimulating the growth or GABA production of GABA-producing bacteria.

77. A method of culturing a GABA-dependent bacteria, comprising disposing at least one live GABA-dependent bacterial cell on a suitable substrate, and providing a source of GABA.

78. The method of claim 77, wherein the suitable substrate is agar.

79. The method of claim 77, wherein providing a source of GABA comprises co-culturing with another bacterial strain, said strain is capable of producing GABA.

80. The method of claim 77, wherein GABA is added to the substrate.

81. The method of claim 77, wherein the GABA-dependent bacteria is *E. gabavorous*.

82. A method of identifying a bacterial strain or strains capable of producing GABA at a physiologically relevant pH of the human intestinal tract, comprising:

(a) dispersing a sample believed to contain GABA-producing bacteria within a substrate, the substrate being at least partially permeable to GABA,

(b) contacting the substrate loaded with potential GABA-producing bacteria with a GABA-dependent bacterium; and

(c) identifying a GABA-producing bacteria by observing the formation of a colonies of the GABA-dependent bacteria around potential GABA-producing bacteria in the substrate.

83. The method of claim 82, wherein the substrate is being buffered to maintain the pH at a physiologically range found in the human gastrointestinal tract.

84. The method of claim 82, wherein the GABA-dependent bacteria is *E.gabavorous*.

85. The method of claim 82, wherein the pH range is between about 4.5 and about 7.5.

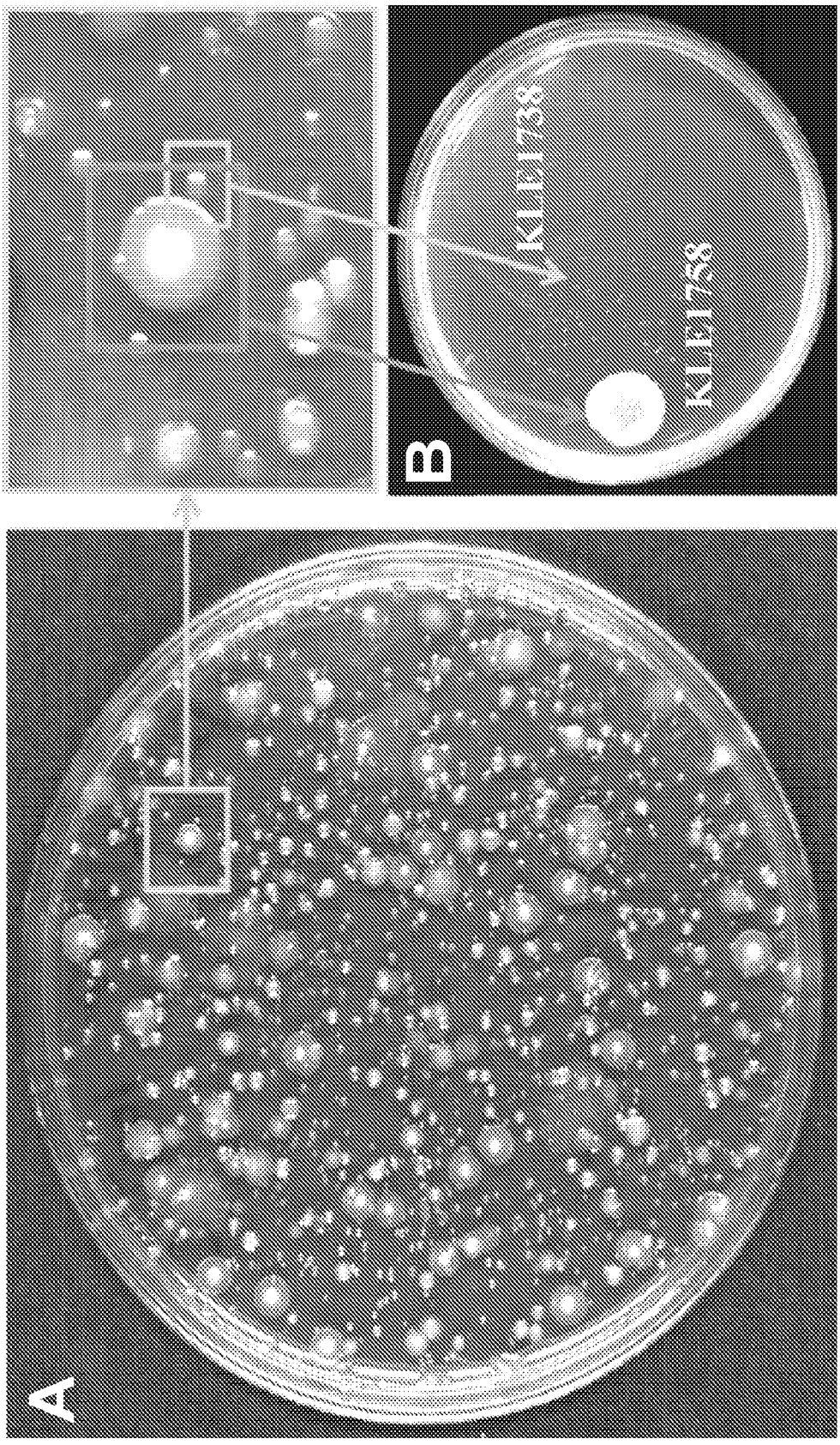


Figure 1

Figure 2A

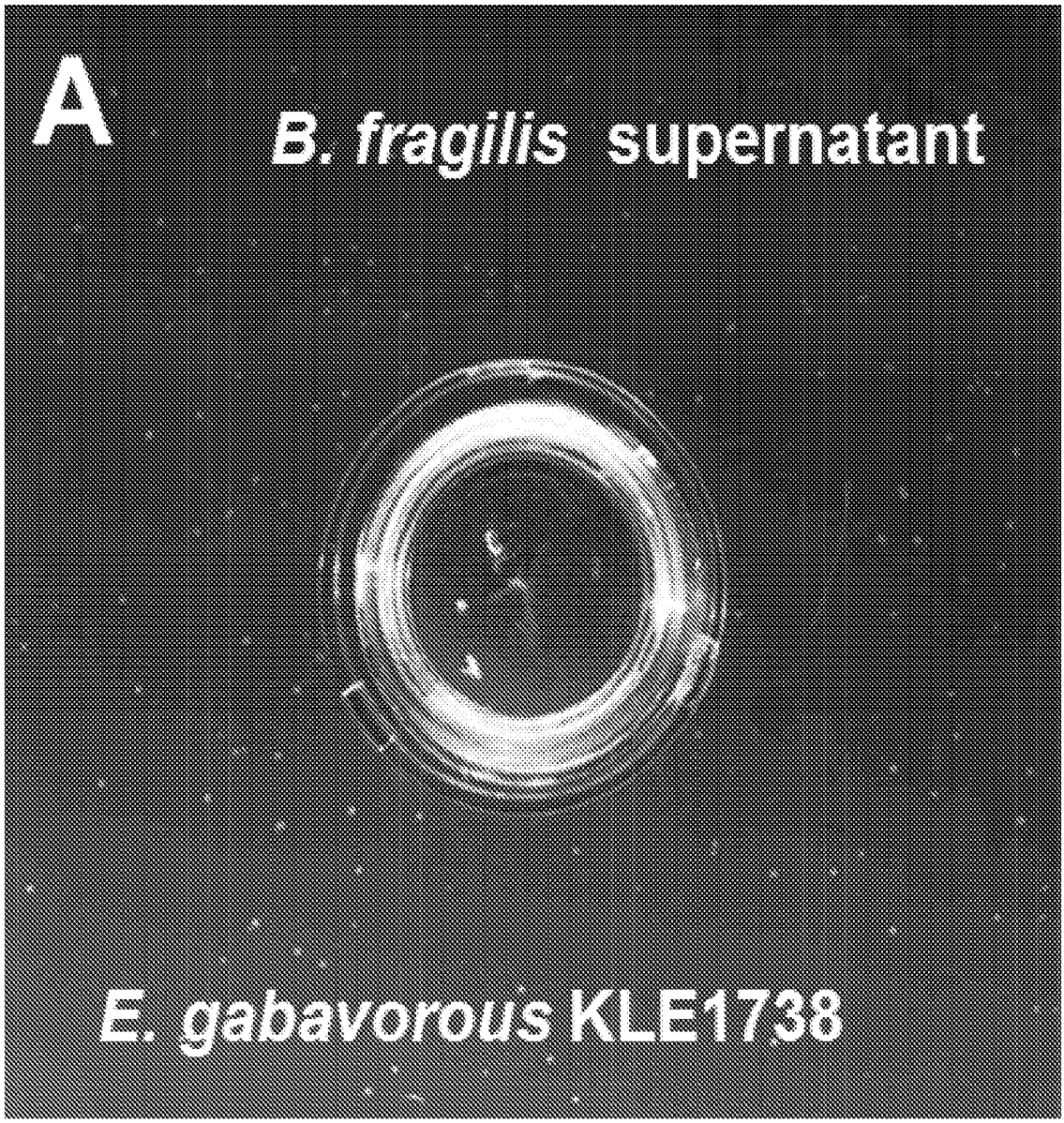


Figure 2B

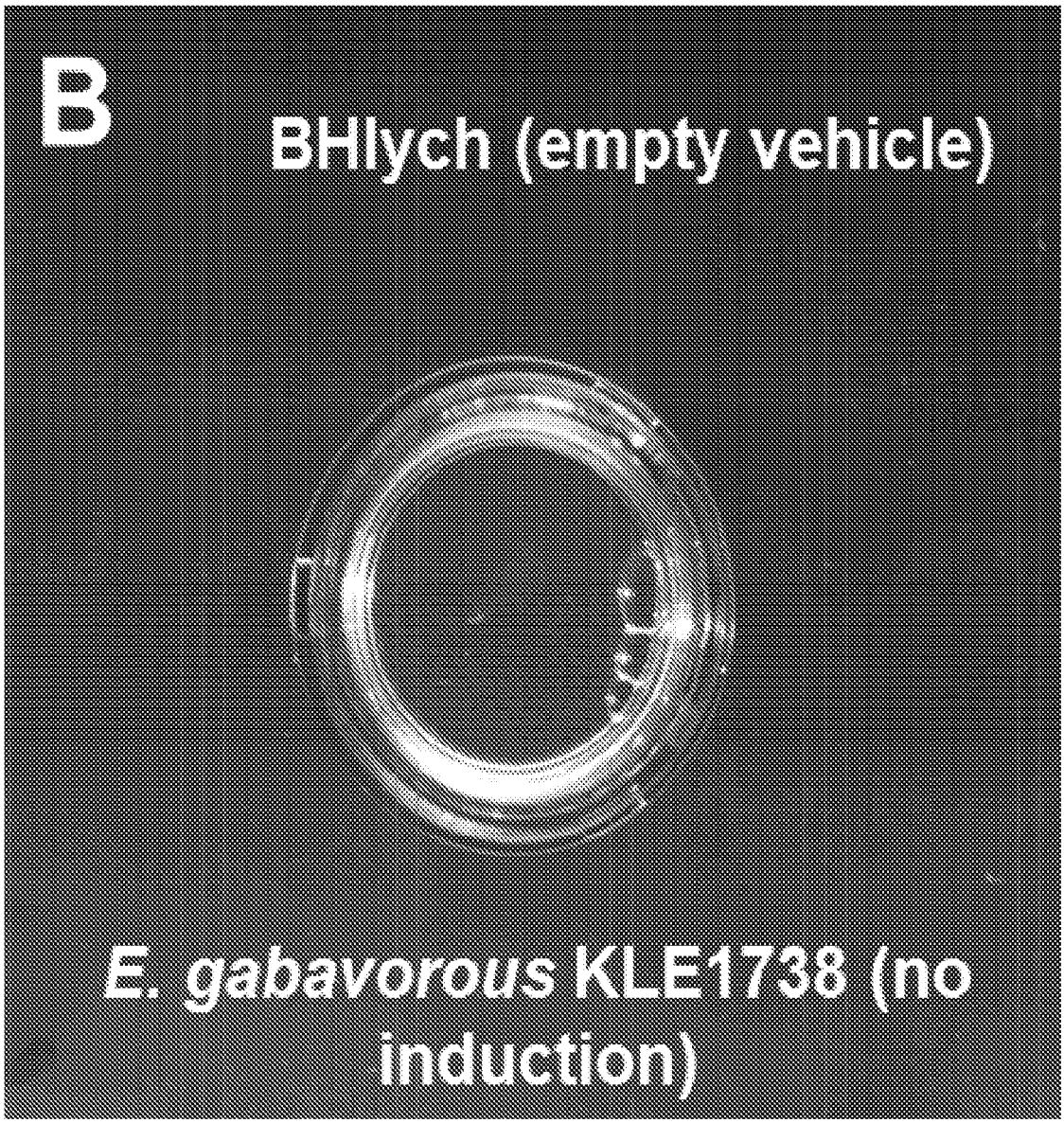


Figure 2C

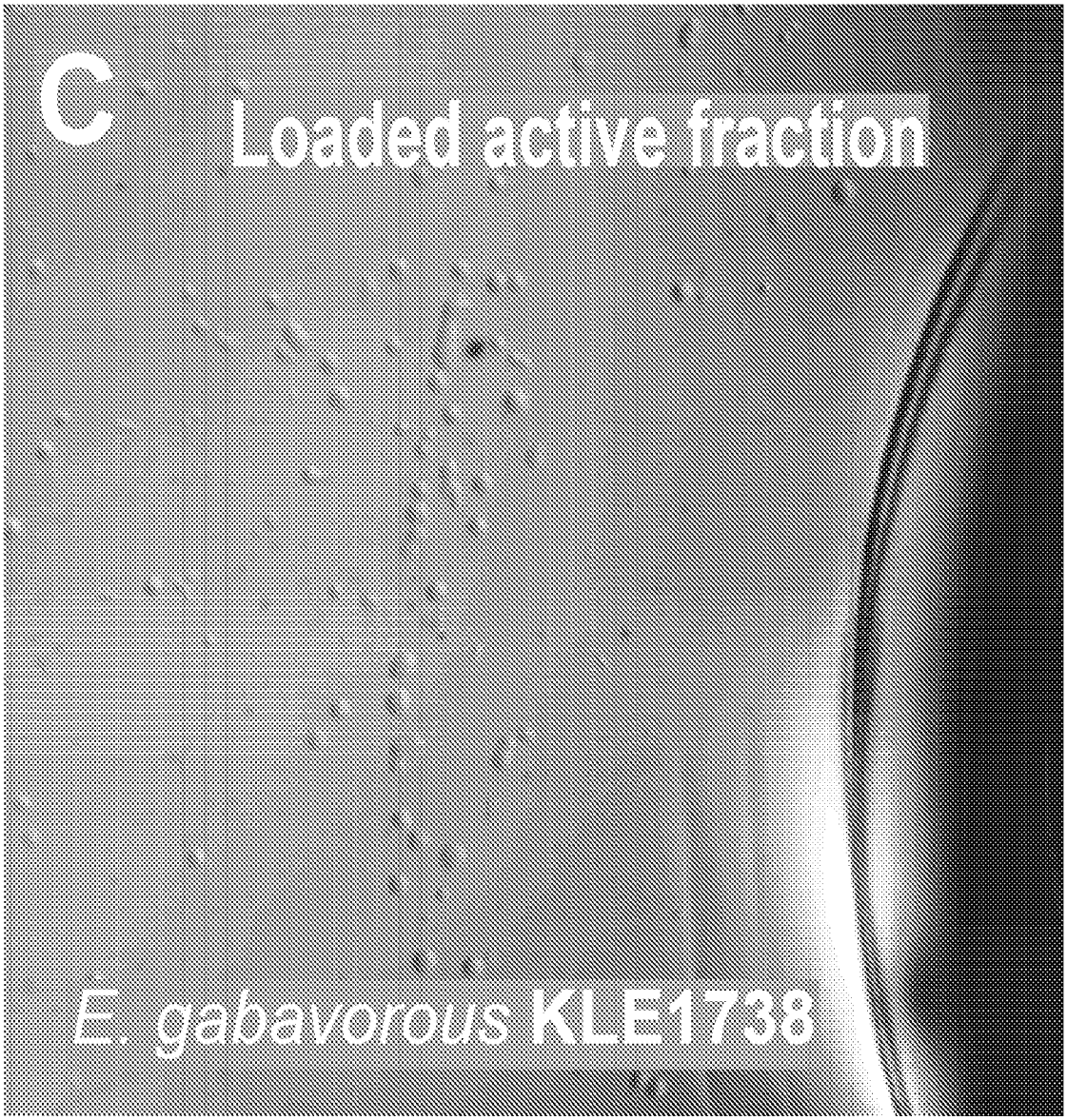


Figure 2D

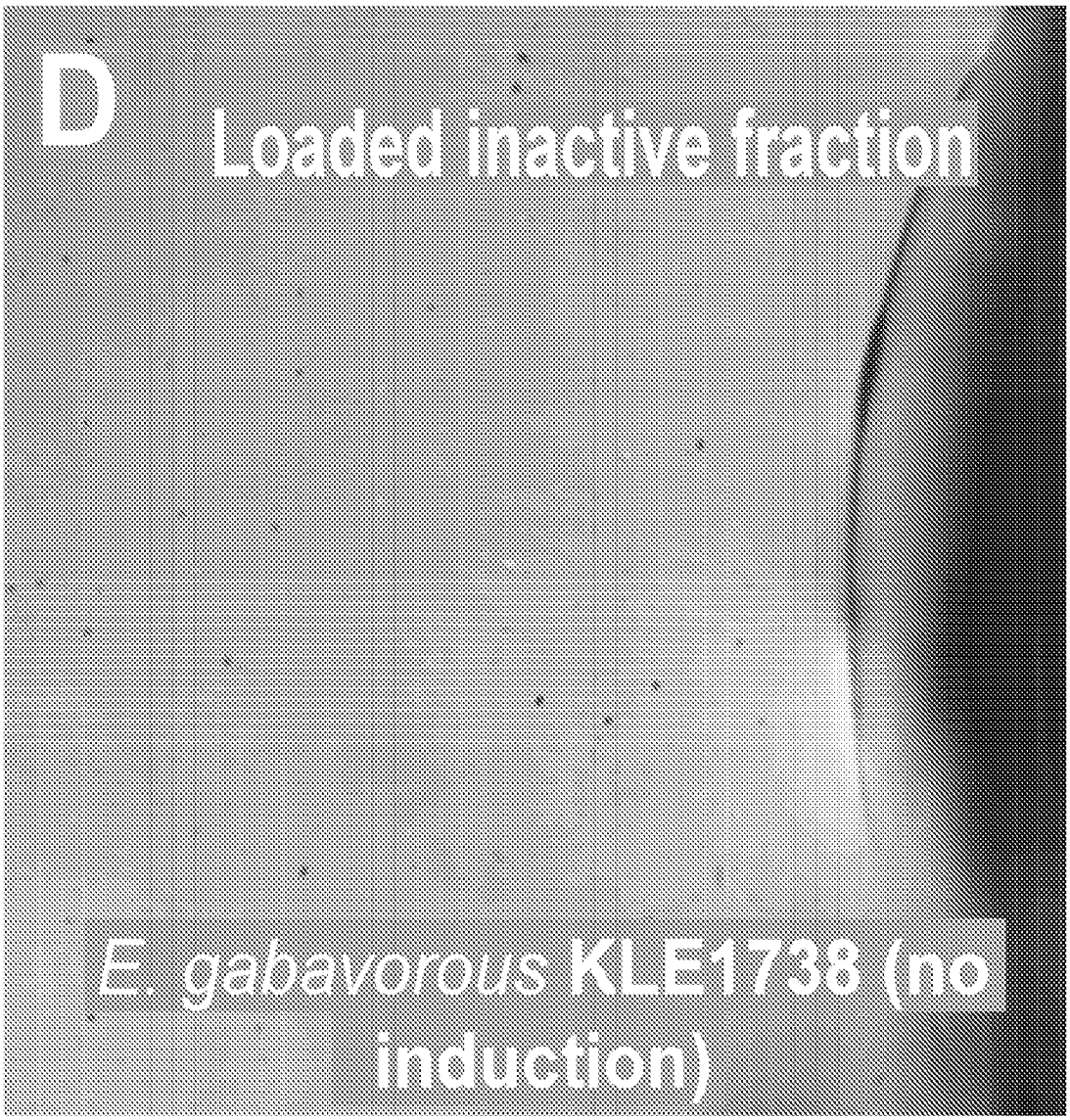

D
Loaded inactive fraction
E. gabavorous KLE1738 (no induction)

Figure 2E

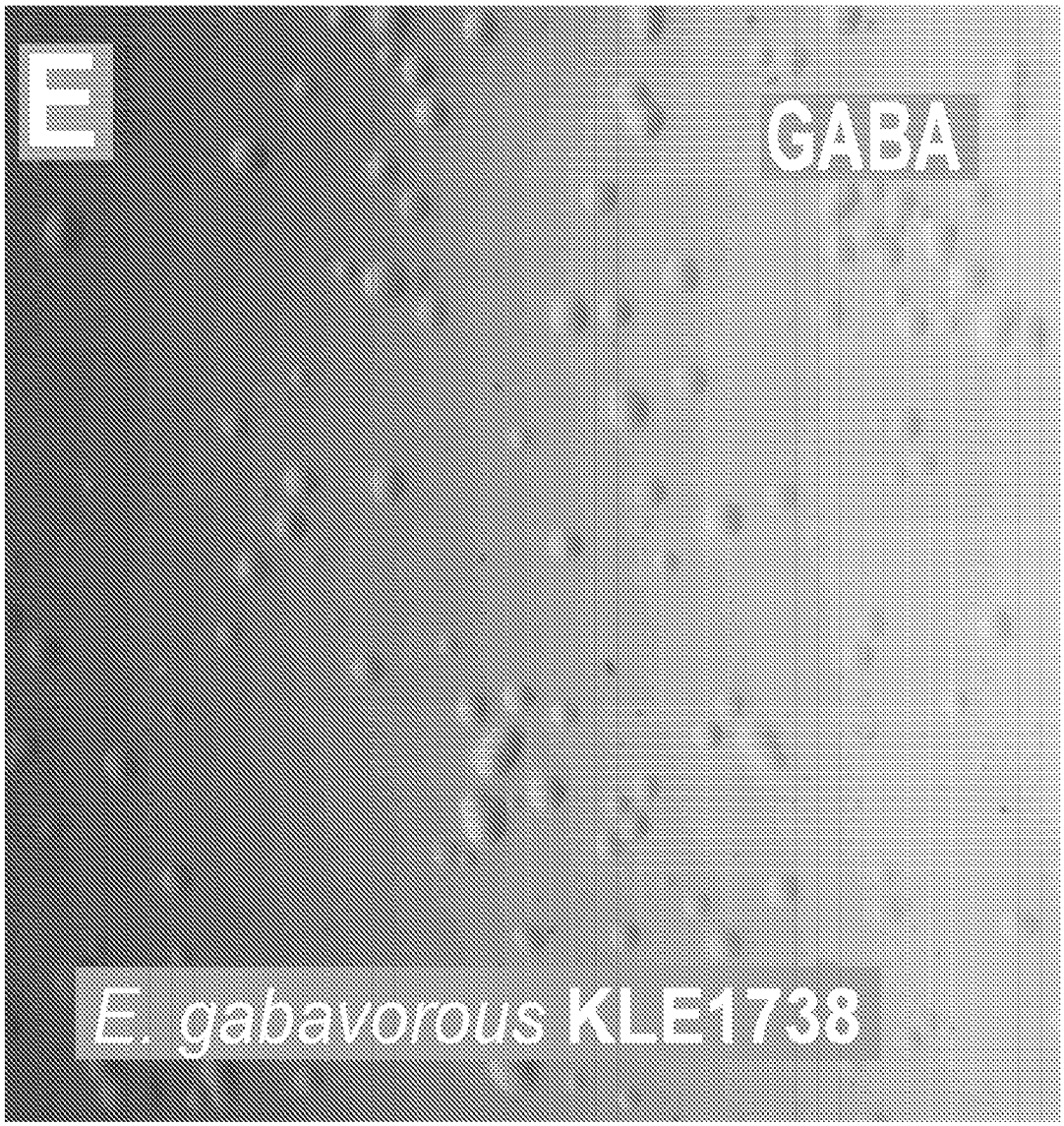


Figure 2F

Figure 2G

Compound	Spotted Conc.	Compound	Spotted Conc.	Compound	Spotted Conc.
γ-amino Butyric Acid (GABA)	100 mg/mL	Fructose	100 mg/mL	L-Alanine	50 mg/mL
γ-Butyrolactone	200 mg/mL	Malate	100 mg/mL	L-Asparagine monohydrate	20 mg/mL
γ-Hydroxybutyric acid	100 mg/mL	Sodium nitrate	100 mg/mL	L-Aspartic Acid	5 mg/mL
Putrescine Dihydrochloride	100 mg/mL	Sodium pyruvate	100 mg/mL	L-Cysteine HCl	50 mg/mL
Spermidine Trihydrocholride	100 mg/mL	Sodium Succinate	100 mg/mL	L-Glutamic Acid	5 mg/mL
Spermine Tetrahydrochloride	100 mg/mL	Sodium Acetate	100 mg/mL	L-Glutamine	20 mg/mL
Succinate Semialdehyde	15% v/v	Sodium Butyrate	100 mg/mL	L-Phenylalanine	20 mg/mL
Butyric Acid	100% soln	Sodium Propionate	100 mg/mL	L-Proline	50 mg/mL
Lactic Acid	100 mg/mL	L-Histidine	40 mg/mL	L-Threonine	50 mg/mL
Malonic Acid	100 mg/mL	L-Isoleucine	40 mg/mL	L-Glycine	20 mg/mL
Succinic Acid	100 mg/mL	L-Leucine	20 mg/mL	Malic Acid	100 mg/mL
Monosodium Glutamate	100 mg/mL	L-Lysine	100 mg/mL	ATCC Mineral Mix	100% solution
Glycerol	99.9% solution	L-Methionine	50 mg/mL	ATCC Vitamin Mix	100% solution
Glucose	100 mg/mL	L-Tryptophan	10 mg/mL	Induction	
Fructose	100 mg/mL	L-Valine	50 mg/mL	No induction	

Figure 3

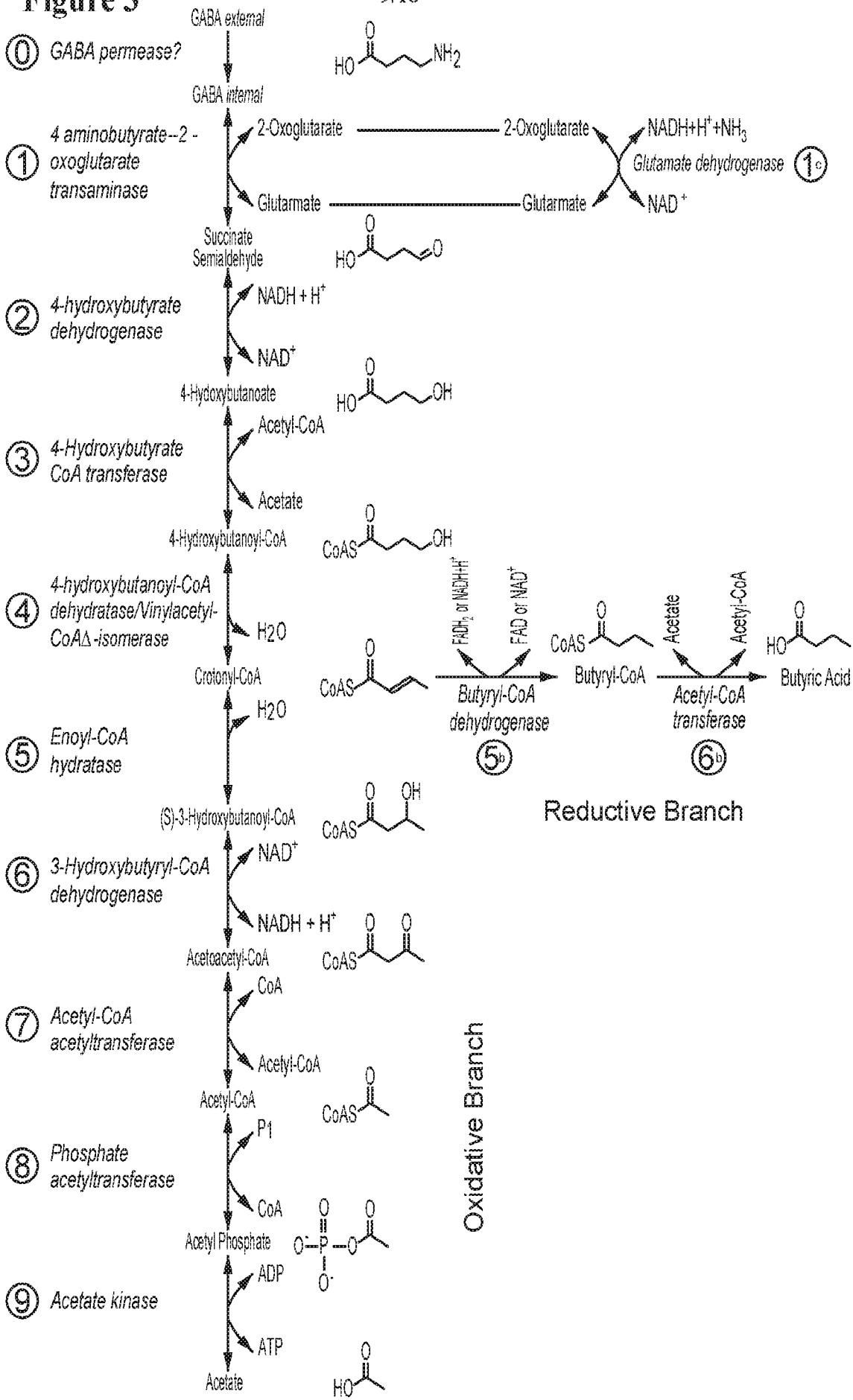

GABA external
⓪ GABA permease?
GABA internal
① 4 aminobutyrate--2 - oxoglutarate transaminase
2-Oxoglutarate
Glutarmate
2-Oxoglutarate
Glutarmate
NADH+H+ +NH3
Glutamate dehydrogenase
NAD +
1c
Succinate Semialdehyde
② 4-hydroxybutyrate dehydrogenase
NADH + H+
NAD+
4-Hydoxybutanoate
③ 4-Hydroxybutyrate CoA transferase
Acetyl-CoA
Acetate
4-Hydroxybutanoyl-CoA
④ 4-hydroxybutanoyl-CoA dehydratase/Vinylacetyl-CoAΔ-isomerase
H2O
Crotonyl-CoA
FADH2 or NADH+H+
FAD or NAD+
Butyryl-CoA dehydrogenase
5b
Butyryl-CoA
Acetate
Acetyl-CoA
Acetyl-CoA transferase
6b
Butyric Acid
Reductive Branch
⑤ Enoyl-CoA hydratase
H2O
(S)-3-Hydroxybutanoyl-CoA
⑥ 3-Hydroxybutyryl-CoA dehydrogenase
NAD+
NADH + H+
Acetoacetyl-CoA
⑦ Acetyl-CoA acetyltransferase
CoA
Acetyl-CoA
Acetyl-CoA
⑧ Phosphate acetyltransferase
P1
CoA
Acetyl Phosphate
⑨ Acetate kinase
ADP
ATP
Acetate
Oxidative Branch

Figure 4A

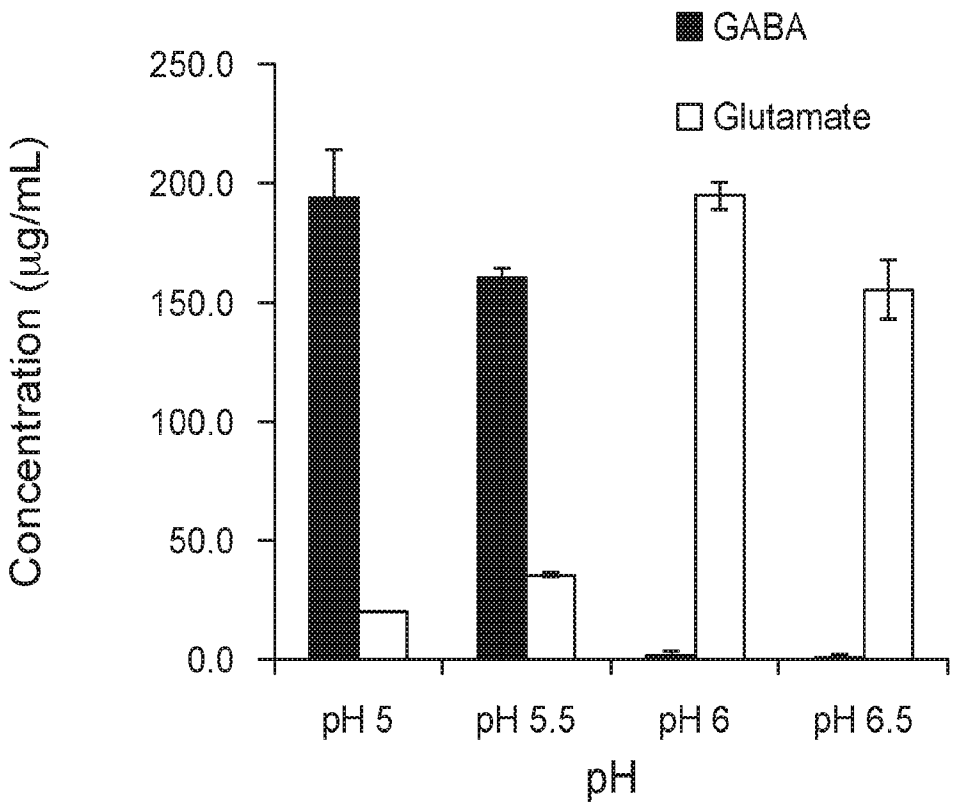

GABA
Glutamate
250.0
200.0
150.0
100.0
50.0
0.0
Concentration (µg/mL)
pH 5
pH 5.5
pH 6
pH 6.5
pH

Figure 4B

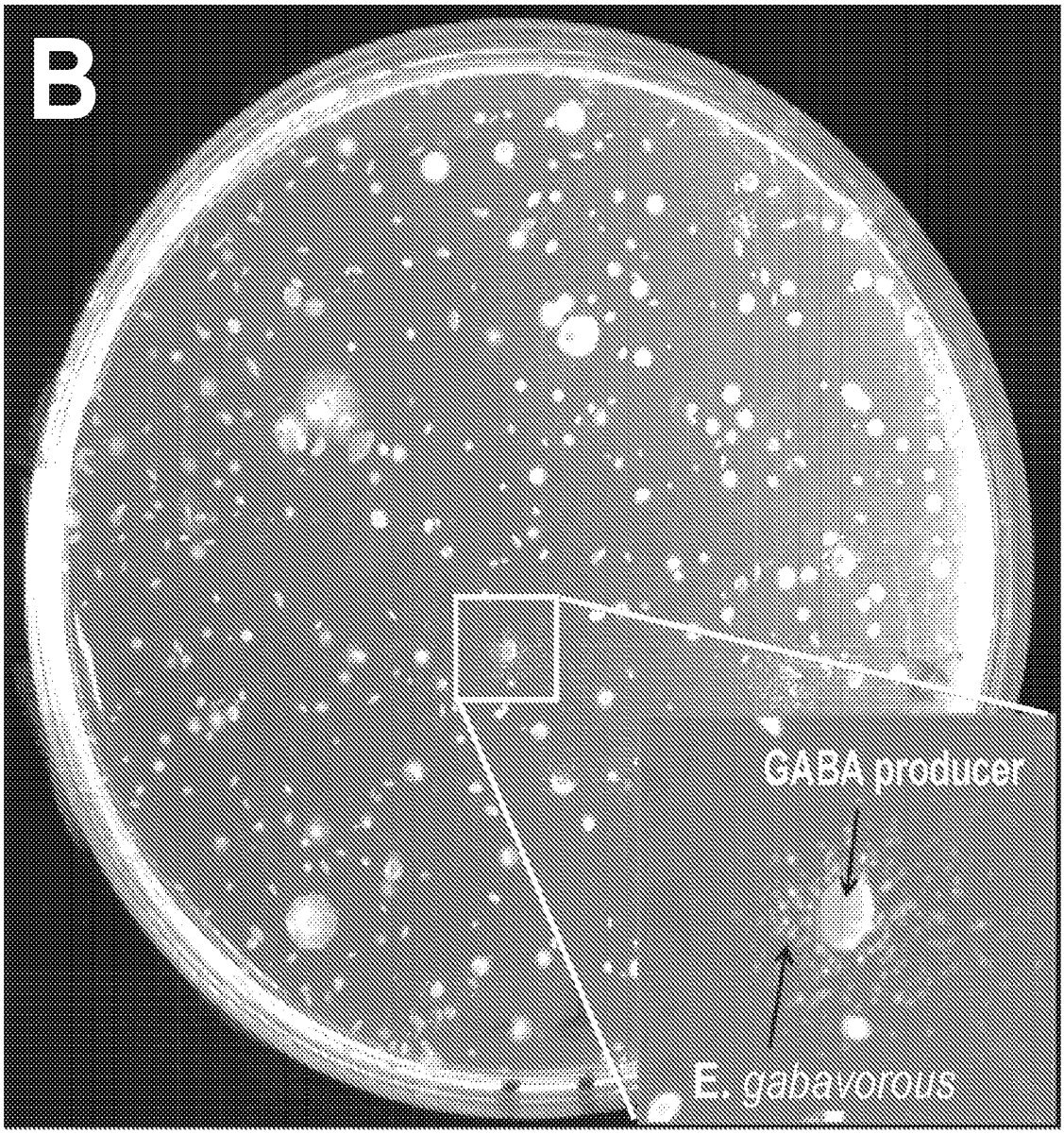
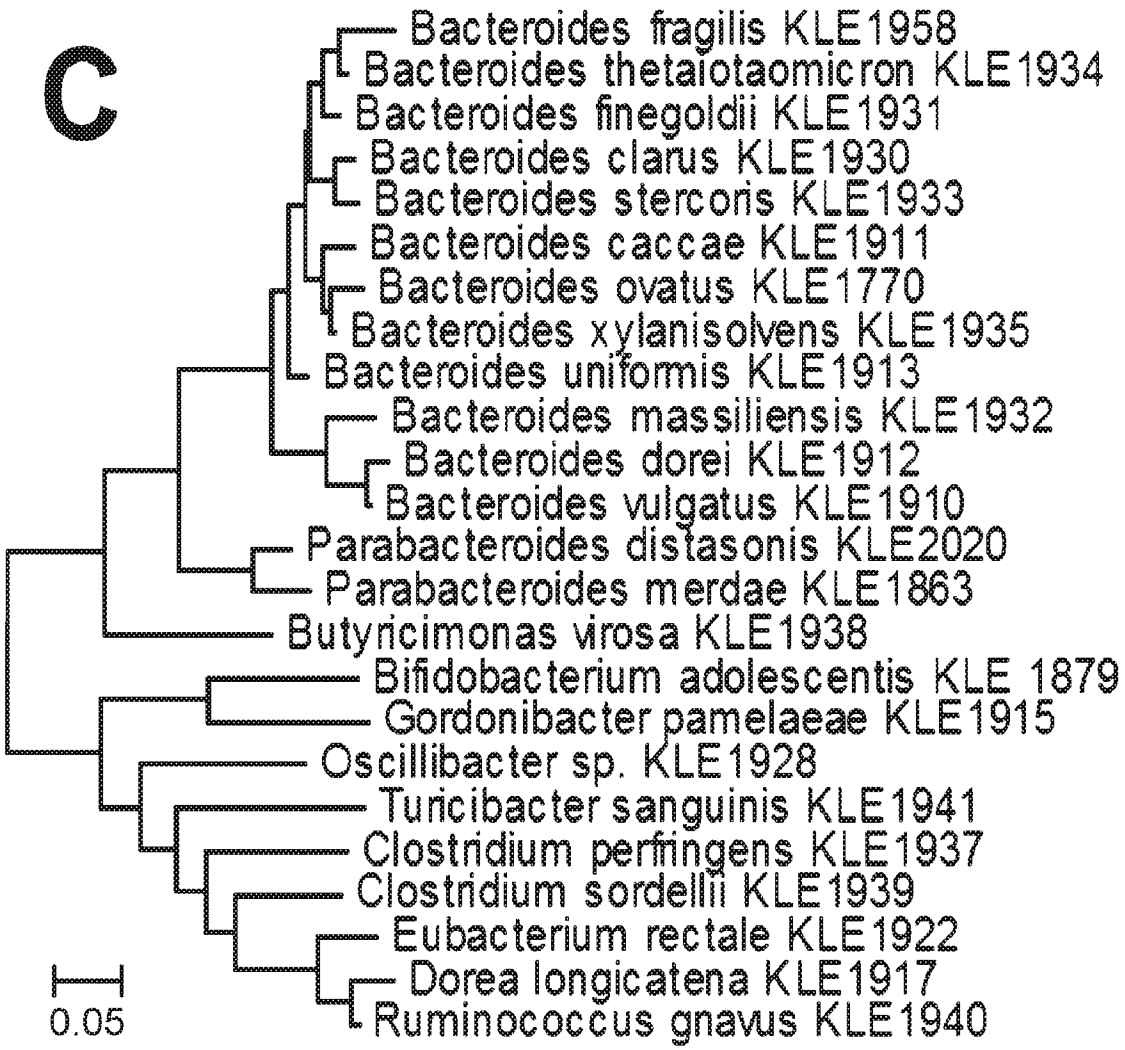


Figure 4C


C
Bacteroides fragilis KLE1958
Bacteroides thetaiotaomicron KLE1934
Bacteroides finegoldii KLE1931
Bacteroides clarus KLE1930
Bacteroides stercoris KLE1933
Bacteroides caccae KLE1911
Bacteroides ovatus KLE1770
Bacteroides xylanisolvens KLE1935
Bacteroides uniformis KLE1913
Bacteroides massiliensis KLE1932
Bacteroides dorei KLE1912
Bacteroides vulgatus KLE1910
Parabacteroides distasonis KLE2020
Parabacteroides merdae KLE1863
Butyricimonas virosa KLE1938
Bifidobacterium adolescentis KLE 1879
Gordonibacter pamelaeae KLE1915
Oscillibacter sp. KLE1928
Turicibacter sanguinis KLE1941
Clostridium perfringens KLE1937
Clostridium sordellii KLE1939
Eubacterium rectale KLE1922
Dorea longicatena KLE1917
Ruminococcus gnavus KLE1940
0.05

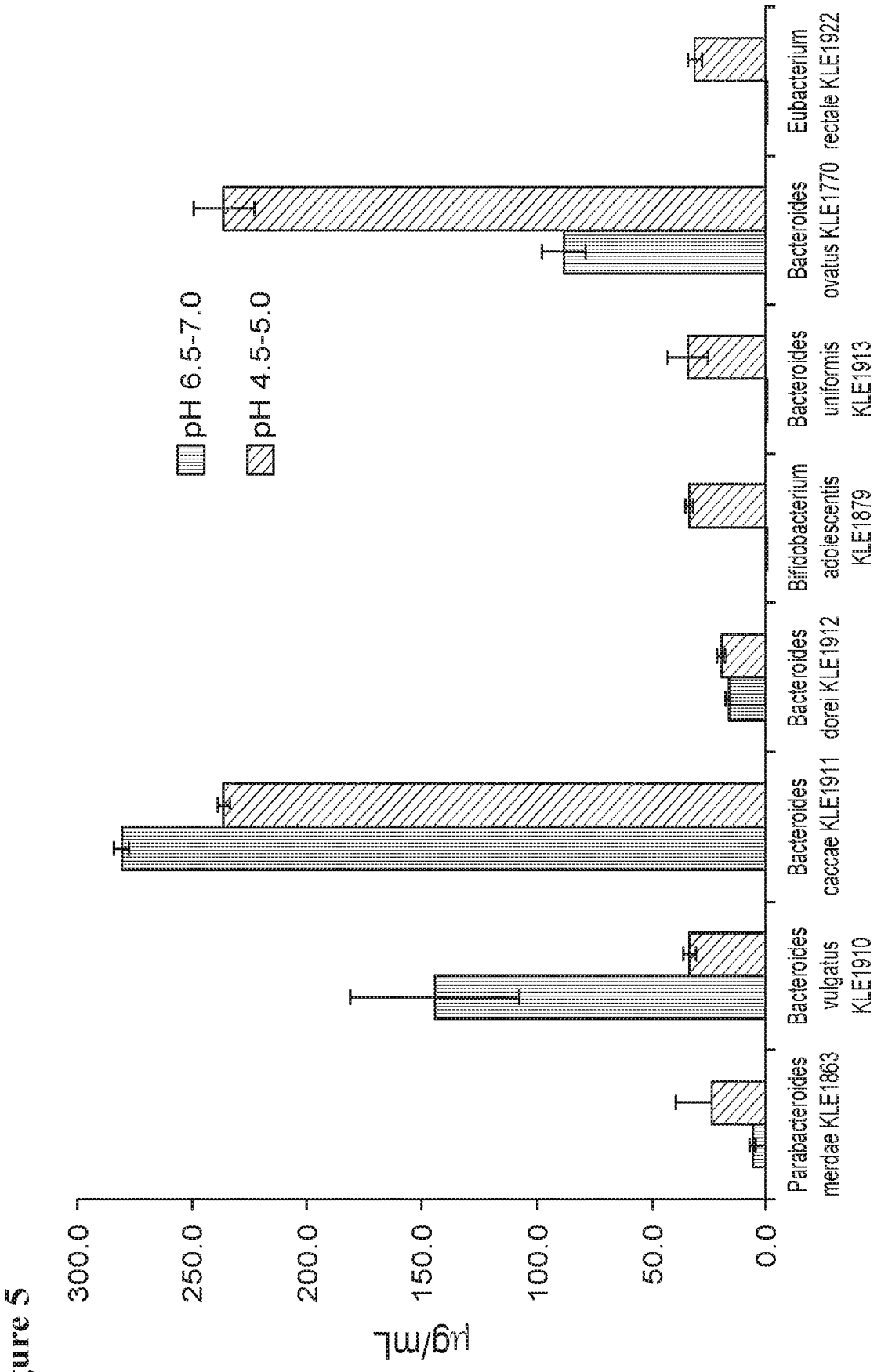

μg/mL
300.0
250.0
200.0
150.0
100.0
50.0
0.0
pH 6.5-7.0
pH 4.5-5.0
Parabacteroides merdae KLE1863
Bacteroides vulgatus KLE1910
Bacteroides caccae KLE1911
Bacteroides dorei KLE1912
Bifidobacterium adolescentis KLE1879
Bacteroides uniformis KLE1913
Bacteroides ovatus KLE1770
Eubacterium rectale KLE1922

Figure 5

Figure 6A

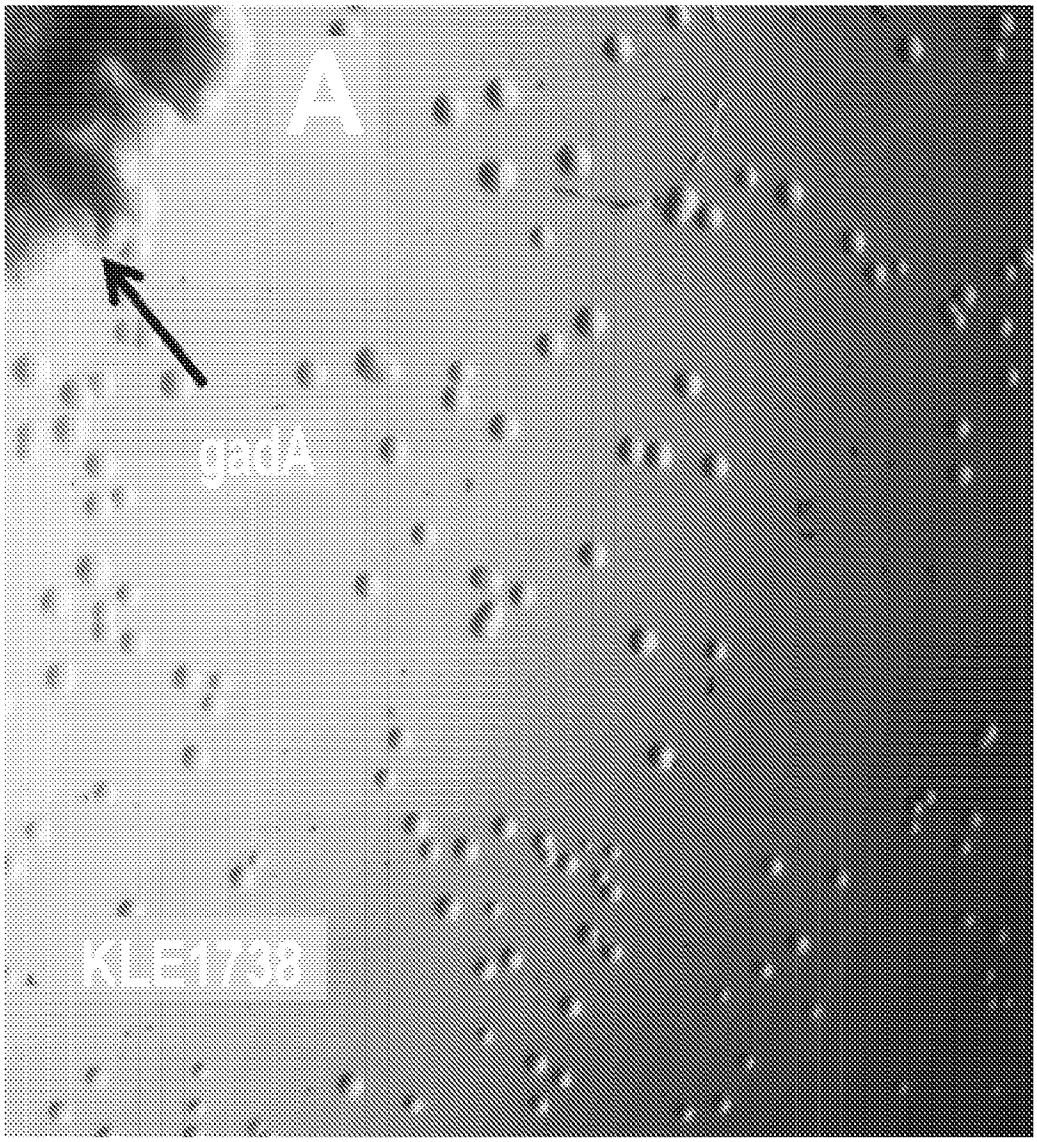


Figure 6B

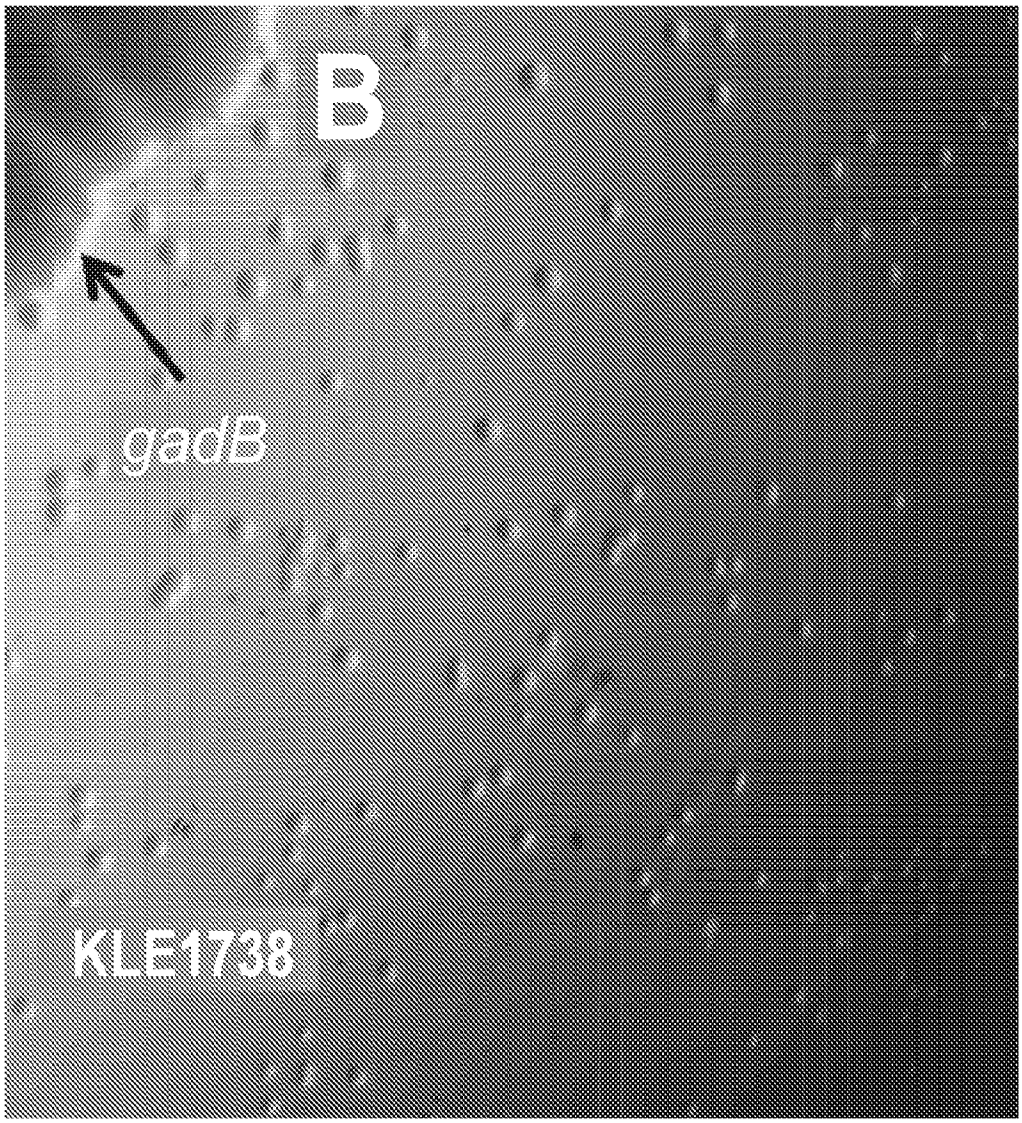


Figure 6C

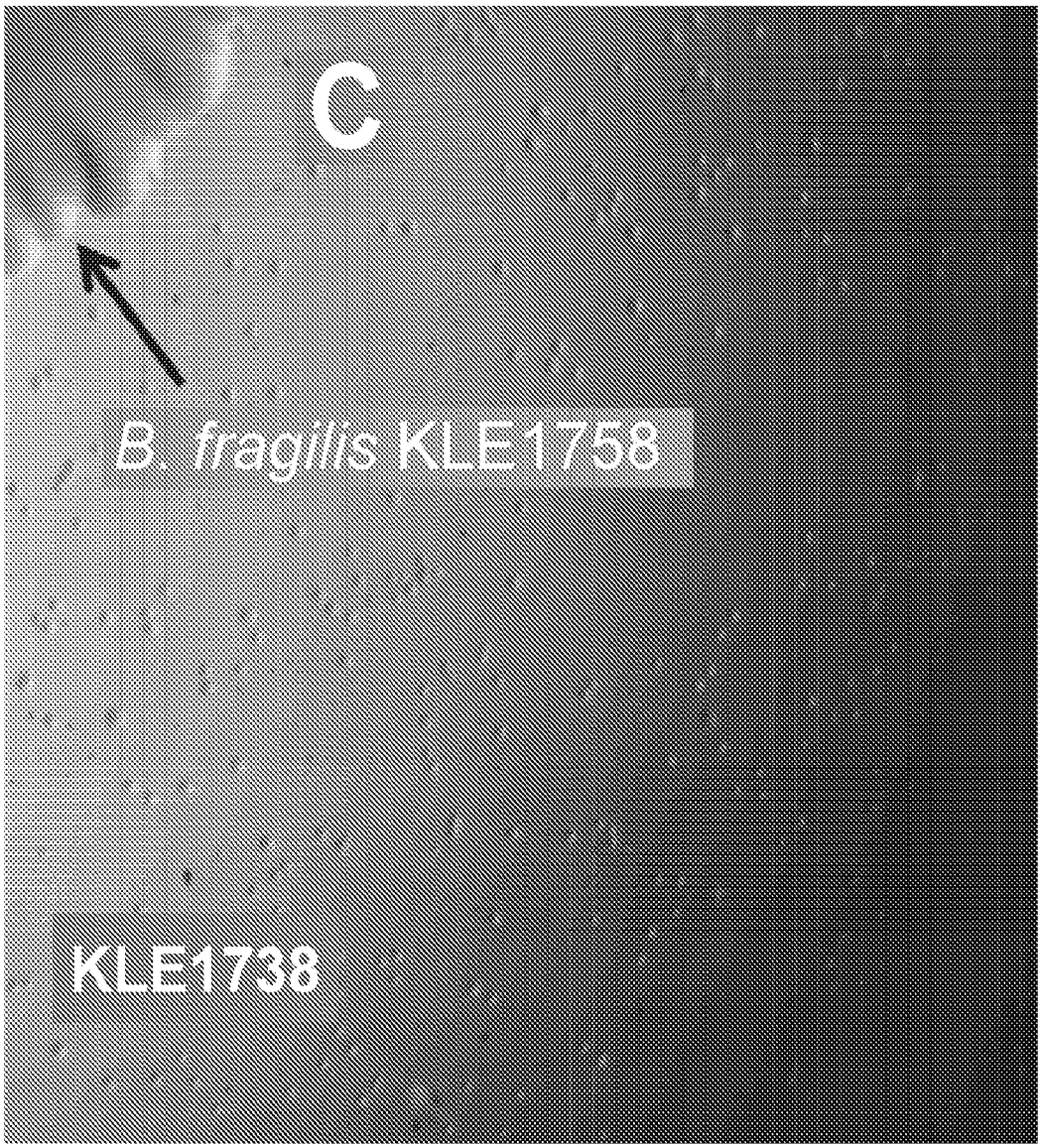


Figure 6D

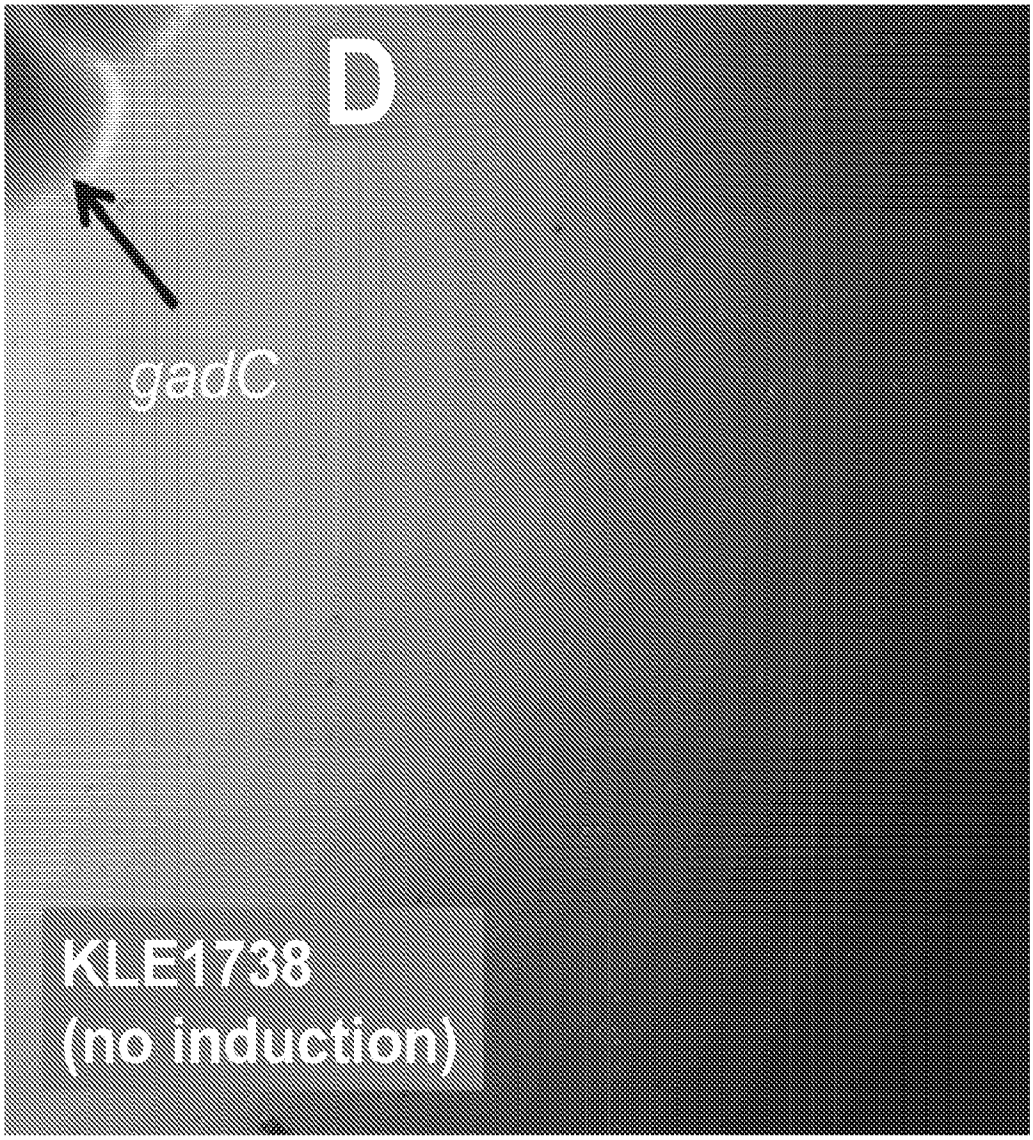
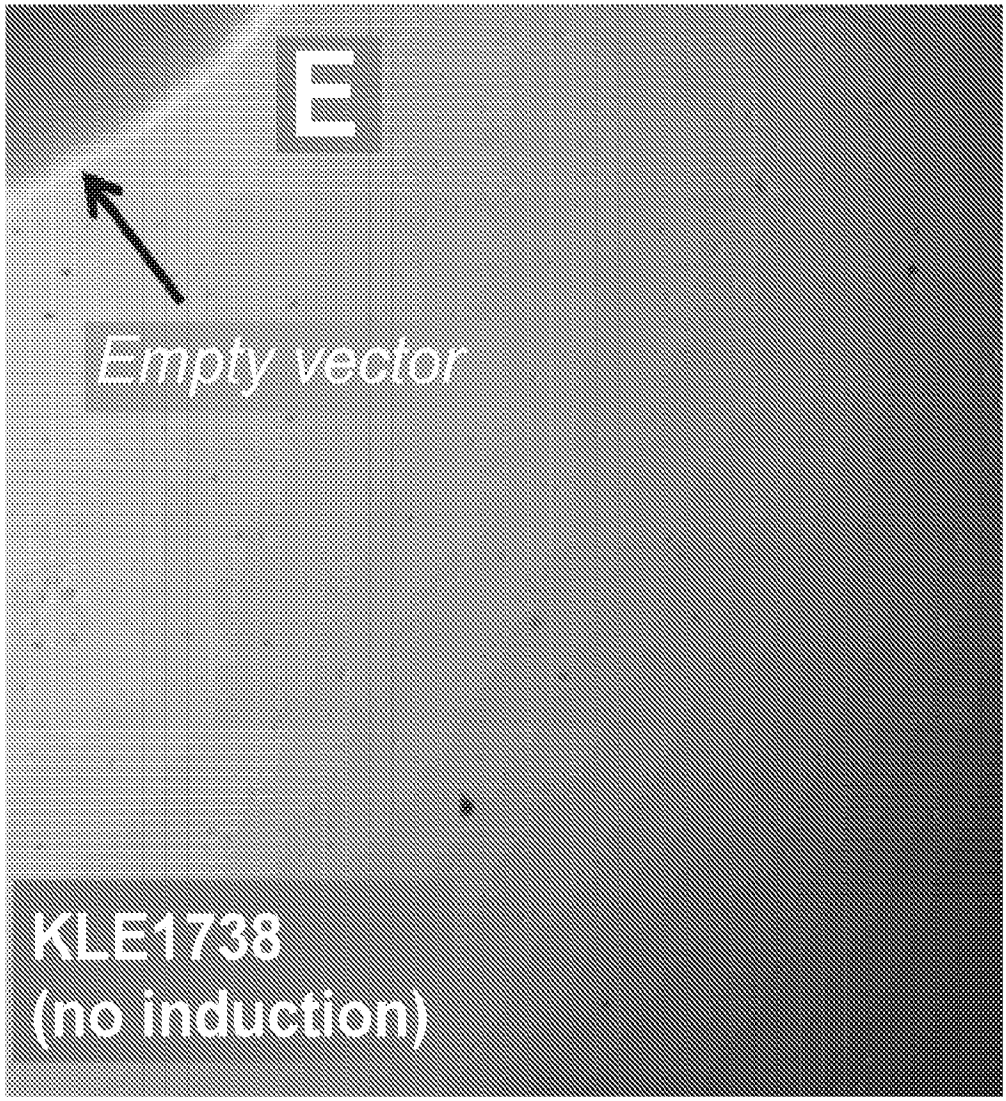


Figure 6E



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/022091

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K35/74 A61K35/745 A61K35/742 A61P25/00 A61P25/18
A61P25/22 A61P25/24 A61P43/00
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, EMBASE, CHEM ABS Data, WPI Data, BIOSIS, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/107913 A1 (UNIV COLLEGE CORK NAT UNIV IE [IE]; AGRICULTURE AND FOOD DEV AUTHORITY) 25 July 2013 (2013-07-25) the whole document	1-76
X	EP 1 988 155 A1 (KIKKOMAN CORP [JP]) 5 November 2008 (2008-11-05) the whole document	1-20

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 June 2017	04/09/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Greif, Gabriela

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/022091

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-76

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KOMATSUZAKI N ET AL: "Production of @c-aminobutyric acid (GABA) by Lactobacillus paracasei isolated from traditional fermented foods", FOOD MICROBIOL, ACADEMIC PRESS LTD, LONDON, GB, vol. 22, no. 6, 1 December 2005 (2005-12-01), pages 497-504, XP004866292, ISSN: 0740-0020, DOI: 10.1016/J.FM.2005.01.002 the whole document	1,2, 4-17,20
X	MARK LYTE: "Microbial endocrinology : Host-microbiota neuroendocrine interactions influencing brain and behavior", GUT MICROBES, vol. 5, no. 3, 29 May 2014 (2014-05-29), pages 381-389, XP055379390, United States ISSN: 1949-0976, DOI: 10.4161/gmic.28682 see the whole document, especially Tables 1 and 2	1-76
X	PARK ET AL: "Cloning, sequencing and expression of a novel glutamate decarboxylase gene from a newly isolated lactic acid bacterium, Lactobacillus brevis OPK-3", BIORESOURCE TECHNOLOGY, ELSEVIER BV, GB, vol. 98, no. 2, 1 January 2007 (2007-01-01), pages 312-319, XP005645219, ISSN: 0960-8524, DOI: 10.1016/J.BIORTECH.2006.01.004 the whole document	1,2,4-8, 15-17,20
X	FENG SHI ET AL: "Synthesis of Î -aminobutyric acid by expressing-derived glutamate decarboxylase in thestrain ATCC 13032", BIOTECHNOLOGY LETTERS, SPRINGER NETHERLANDS, DORDRECHT, vol. 33, no. 12, 9 August 2011 (2011-08-09), pages 2469-2474, XP019978078, ISSN: 1573-6776, DOI: 10.1007/S10529-011-0723-4 the whole document	1,2,4-8, 15-17,20

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HAIXING LI ET AL: "Lactic acid bacterial cell factories for gamma-aminobutyric acid", AMINO ACIDS ; THE FORUM FOR AMINO ACID AND PROTEIN RESEARCH, SPRINGER-VERLAG, VI, vol. 39, no. 5, 3 April 2010 (2010-04-03), pages 1107-1116, XP019856927, ISSN: 1438-2199, DOI: 10.1007/S00726-010-0582-7 the whole document	1,2,4-8, 15-17,20
X	P. B. ECKBURG: "Diversity of the Human Intestinal Microbial Flora", SCIENCE, vol. 308, no. 5728, 10 June 2005 (2005-06-10), pages 1635-1638, XP055050950, ISSN: 0036-8075, DOI: 10.1126/science.1110591 the whole document	1,2
X	E. BARRETT ET AL: "[gamma]-Aminobutyric acid production by culturable bacteria from the human intestine", JOURNAL OF APPLIED MICROBIOLOGY, vol. 113, no. 2, 15 August 2012 (2012-08-15), pages 411-417, XP055066783, ISSN: 1364-5072, DOI: 10.1111/j.1365-2672.2012.05344.x the whole document	1,2,4-8, 15,17,20
X	SIRAGUSA S ET AL: "Synthesis of gamma-aminobutyric acid by lactic acid bacteria isolated from a variety of Italian cheeses", APPLIED AND ENVIRONMENTAL MICROBIOLOGY, , vol. 73, no. 22 1 November 2007 (2007-11-01), pages 7283-7290, XP002677194, ISSN: 0099-2240, DOI: 10.1128/AEM.01064-07 Retrieved from the Internet: URL:http://aem.asm.org/content/73/22/7283 [retrieved on 2007-09-21] the whole document	1,2,4-8, 15-17,20
X	MACIAN M C: "Lactobacillus rennini 16S rRNA gene, strain CECT 5922", EMBL,, 16 July 2003 (2003-07-16), XP002515240, the whole document	1,2

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SATO I ET AL: "Lactobacillus halophilus gene for 16S r RNA, partial sequence", GENBANK, 7 January 2006 (2006-01-07), XP003017201, the whole document -----	1,2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/022091

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013107913 A1	25-07-2013	EP 2828375 A1	28-01-2015
		WO 2013107913 A1	25-07-2013
EP 1988155 A1	05-11-2008	CA 2638783 A1	30-08-2007
		CN 101389751 A	18-03-2009
		EP 1988155 A1	05-11-2008
		ES 2347601 T3	02-11-2010
		JP WO2007097374 A1	16-07-2009
		TW 200811288 A	01-03-2008
		US 2009186388 A1	23-07-2009
		WO 2007097374 A1	30-08-2007

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-76

   A therapeutic composition comprising at least one purified bacterial population consisting of bacteria capable of producing GABA in a subject in need thereof, and a method of treating a disease or disorder in a subject in need thereof, the method comprising administering to the subject a therapeutic composition comprising at least one purified bacterial population consisting of bacteria capable of producing GABA in a subject in need thereof, where the disease is a mental disease or disorder.

   ---

2. claims: 77-81

   A method of culturing a GABA-dependent bacteria, comprising disposing at least one live GABA-dependent bacterial cell on a suitable substrate, and providing a source of GABA

   ---

3. claims: 82-85

   A method of identifying a bacterial strain or strains capable of producing GABA at a physiologically relevant pH of the human intestinal tract, comprising:(a) dispersing a sample believed to contain GABA-producing bacteria within a substrate, the substrate being at least partially permeable to GABA,(b) contacting the substrate loaded with potential GABA-producing bacteria with a GABA-dependent bacterium; and(c) identifying a GABA-producing bacteria by observing the formation of a colonies of the GABA-dependent bacteria around potential GABA-producing bacteria in the substrate.
