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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 November 2021 (25.11.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/237162 A1

(51) International Patent Classification:
A23L 33/00 (2016.01) *A61K 31/702* (2006.01)
A23L 33/135 (2016.01) *A61K 35/74* (2015.01)
A61K 9/00 (2006.01) *A61K 35/741* (2015.01)

(21) International Application Number:
PCT/US2021/033762

(22) International Filing Date:
21 May 2021 (21.05.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/028,495 21 May 2020 (21.05.2020) US

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(54) Title: HIGH-COMPLEXITY SYNTHETIC GUT BACTERIAL COMMUNITIES

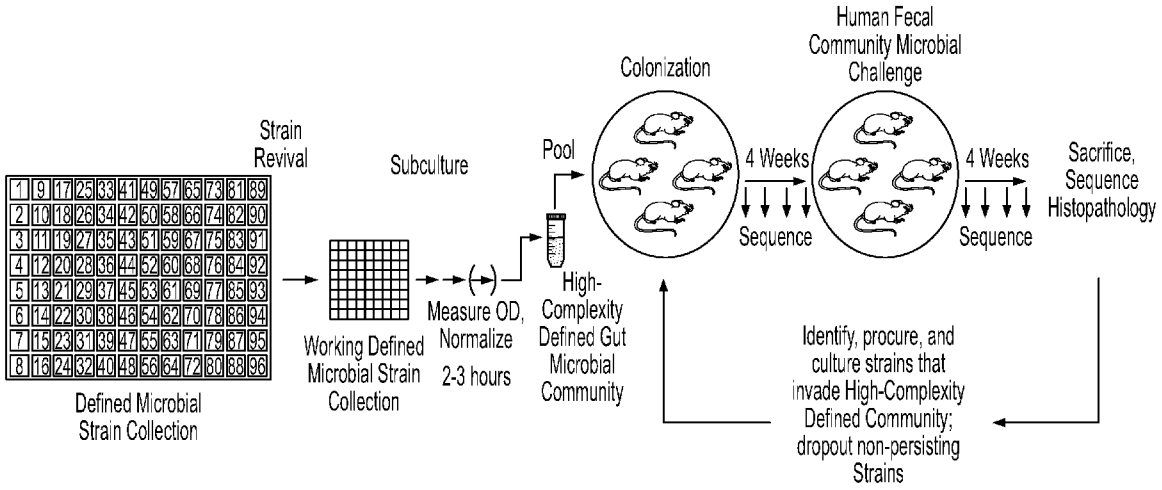

FIG. 1

(57) Abstract: The present invention provides high-complexity defined gut microbial communities capable of achieving substantial engraftment and having stability following human fecal community microbial challenge and methods of producing the same. Also provided are methods of using high-complexity defined gut microbial communities for the treatment of dysbiosis or a pathological condition in an animal.

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

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

Published:

— *with international search report (Art. 21(3))*

HIGH-COMPLEXITY SYNTHETIC GUT BACTERIAL COMMUNITIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 63/028,495, filed May 21, 2020, the disclosure of which is hereby incorporated by reference in its entirety for all purposes.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made with Government support under Grant No: DK113598 awarded by the National Institutes of Health (NIH). The Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Fecal microbiota transplantation (FMT) is a promising therapeutic approach that has proved highly effective for treating conditions such as recurrent *C. difficile* infection (CDI). To avoid the disadvantages of using stool, Allen-Vercoe and Petrof proposed treatment of recurrent CDI using a synthetic bacterial ecosystem of 33 strains developed from a subset of isolates. Allen-Vercoe, E. and Petrof, EO, 2013, "Artificial stool transplantation: progress towards a safer, more effective and acceptable alternative," *Expert Rev. Gastroenterol. Hepatol.* 7(4), 291-293 (2013); WO 2013/037068 Al.

[0004] FMT has been proposed by Fischbach and colleagues as a therapeutic intervention to change the spectrum of metabolites in a patient's bloodstream, urine, bile and/or feces by engineering the molecular output of the gut bacterial community. Dodd *et al.*, 2017, "A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites," *Nature* 551: 648-652; Fischbach MA, 2018, "Microbiome: Focus on Causation and Mechanism," *Cell* 174(4):785-790.

[0005] Although FMT shows great promise as a therapeutic modality, better transplantable compositions are needed, as are better methods for developing therapeutic agents with a desired activity.

SUMMARY OF THE INVENTION

[0006] In one aspect, provided herein is a high-complexity defined gut microbial community, comprising: a plurality of between 40 and 500 defined microbial strains, wherein the defined microbial strains comprise at least 3 of 4 phyla selected from the group consisting of Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria. In some embodiments, the defined gut microbial community is capable of: (a) metabolizing at least 90% of enumerated substrates selected from the group consisting of: a-mannan (yeast), acetate, agarose, alanine, alginate, anthocyanin, arabinan, arabinogalactan, arabinoxylan, arginine, asparagine, Aspartate, b-glucans, butyrate, carrageenan, chitin, chlorogenic acids, chondroitin sulfate, cinnamic acid, Cysteine, dextran (40), Dihydrochalcones, Enterodiol, flavan-3-ols, flavanones, flavones, flavonols, folate, formate, galactomannan (carob), galacturonan (homo), galacturonate, glucomannan (konjac), glutamate, Glutamine, Glycine, Histidine, hyaluronan, hydrogen, hydroxycinnamic acids, hydroxyproline, inulin, isoflavones/isoflavanones, Isoleucine, lactate, laminarin, Leucine, levan, Lysine, Methionine, mucin O-linked glycans, Ornithine, Phenylalanine, porphyran, Proline, propionate, rhamnogalacturonan I, rhamnogalacturonan II, Secoisolariciresinol diglucoside, Serine, starch (potato), starch (structure 1), thiamine, Threonine, tryptophan, Tyrosine, Valine, xyloglucan, and xylooligosaccharides (XOS), and/or (b) producing at least 90% of enumerated metabolites selected from the group consisting of: formate, acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, 2-methylbutyrate, caporate, isocaporate, 3-methylvaleric acid, L-phenylalanine, 3-phenylpropionic acid, phyenypyruvate, DL-3-phenyllactic acid, trans-cinnamic acid, phenyllactic acid, phenethylamine, L-tyrosine, 3-(4-hydroxyphenyl)propionic acid, 3-(4-hydroxyphenyl) pyruvic acid, DL-p-hydroxyphenyl lactic acid, p-coumaric acid, 4-hydroxyphenyl acetic acid, tyramine, phenol, p-cresol, 4-ethylphenol, 4-vinylphenol, 4-hydroxybenzoic acid, L-tryptophan, 3-indolepropionic acid, 3-indolepyruvic acid, DL-indole-3-lactic acid, trans-3-indoleacrylic acid, 3-indoleacetic acid, tryptamine, indole, skatol, indole-3-carboxylic acid, indole-3-carboxyaldehyde, N-acetyl-L-phenylalanine, phenylpropionylglycine, 3-(3-hydroxyphenyl) propionic acid, cinnamoylglycine, phenylacetylglycine, phenylacetylglutamine, hippuric acid, 2-hydroxyhippuric acid, 3-hydroxyhippuric acid, 4-hydroxyhippuric acid, 4-hydroxyphenylacetylglycine, phenyl sulfate, phenyl glucuronide, p-cresol sulfate, p-cresol glucuronide, 4-ethylphenol sulfate, 4-ethylphenol glucuronide, N-acetyl-L-tryptophan, 5-hydroxy-L-typtophan, N-acetylserotonin, 3-indolepriopionylglycine, indolyl-3-acryloylglycine, indoxyl sulfate, indoxyl glucuronide, 5-hydroxyindole-3-acetic acid, indoleacetylglycine, lithocholic acid, murocholic acid, ursodeoxycholic acid, hyodeoxycholic acid, deoxycholic acid, 7-oxocholic acid, ω-muricholic acid, α-muricholic acid, β-muricholic

acid, γ-muricholic acid, 7βcholic acid, taurolithocholic acid, tauroursodeoxycholic acid, taurohyodeoxycholic acid, taurochenodeoxycholic acid, taurodeoxycholic acid, tauro-β-muricholic acid, tauro- ω-muricholic acid, and taurocholic acid. In some embodiments, the high-complexity defined gut microbial community achieves substantial engraftment when administered to a gnotobiotic mouse. In some embodiments, the engrafted high-complexity defined gut microbial community is stable following a human fecal community microbial challenge.

[0007] In some embodiments, metabolization of a substrate and/or production of a metabolite can be determined by culturing the defined gut microbial community *in vitro* and measuring whether the substrate is metabolized and/or the metabolite is produced by liquid chromatography-mass spectrometry analysis. In some embodiments, metabolization of a substrate and/or production of a product can be determined by administering the defined gut microbial community to a gnotobiotic mouse and measuring whether the substrate is metabolized and/or the product is produced after a defined period of time by liquid chromatography-mass spectrometry analysis of a sample obtained from the mouse. In certain embodiments, the defined gut microbial community is administered via a route selected from the group consisting of oral, rectal, fecal (by enema), and naso/oro-gastric gavage. In certain embodiments, the defined period of time is about 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours, 2 days, 7 days, 14 days, 1 month, or 2 months. In certain embodiments, the sample is selected from the group consisting of a fecal sample, urine sample, blood sample, or serum sample.

[0008] In another aspect, provided herein is a high-complexity defined gut microbial community, comprising: a plurality of between 40 and 500 defined microbial strains, wherein the defined microbial strains comprise at least 3 of 4 phyla selected from the group consisting of Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria; wherein the defined gut microbial community encode the enzymes catalyzing all reactions for at least 90% of the enumerated MetaCyc metabolic pathways selected from the group consisting of: 1CMET2-PWY, 2.6.1.32-RXN, AEROBACTINSYN-PWY, ALACAT2-PWY, ALADEG-PWY, ALANINE-DEG3-PWY, ALANINE-SYN2-PWY, ALANINE-VALINESYN-PWY, ANAPHENOXI-PWY, ARGASEDEG-PWY, ARGDEG-III-PWY, ARGDEG-IV-PWY, ARGDEGRAD-PWY, ARGDEG-V-PWY, ARG-GLU-PWY, ARGININE-SYN4-PWY, ARG-PRO-PWY, ARGSYNBSUB-PWY, ARGSYN-PWY, ASPARAGINE-BIOSYNTHESIS, ASPARAGINE-DEG1-PWY, ASPARAGINE-DEG1-PWY-1, ASPARAGINESYN-PWY, ASPARTATE-DEG1-PWY, ASPARTATESYN-PWY, ASPASN-PWY, ASPSYNII-PWY, AST-PWY, BETA-

ALA-DEGRADATION-I-PWY, CAMALEXIN-SYN, CITRULBIO-PWY, CITRULLINE-DEG-PWY, COA-PWY, CODH-PWY, CYSTEINE-DEG-PWY, CYSTSYN-PWY, DAPLYSINESYN-PWY, ENTBACSYN-PWY, ETHYL-PWY, FAO-PWY, FERMENTATION-PWY, GLNSYN-PWY, GLUDEG-I-PWY, GLUGLNSYN-PWY, GLUTAMATE-DEG1-PWY, GLUTAMATE-SYN2-PWY, GLUTAMINDEG-PWY, GLUTAMINEFUM-PWY, GLUTATHIONESYN-PWY, GLUTDEG-PWY, GLUTORN-PWY, GLUTSYNIII-PWY, GLUTSYN-PWY, GLYCGREAT-PWY, GLYSYN-ALA-PWY, GLYSYN-PWY, GLYSYN-THR-PWY, HISDEG-PWY, HISHP-PWY, HISTDEG-PWY, HISTSYN-PWY, HOMOCYSDEGR-PWY, HOMOSER-METSYN-PWY, HOMOSERSYN-PWY, HSERMETANA-PWY, HYDROXYPRODEG-PWY, ILEUDEG-PWY, ILEUSYN-PWY, LARABITOLUTIL-PWY, LCYSDEG-PWY, LEU-DEG2-PWY, LEUSYN-PWY, LYSDEGII-PWY, LYSINE-AMINOAD-PWY, LYSINE-DEG1-PWY, MALATE-ASPARTATE-SHUTTLE-PWY, METH-ACETATE-PWY, METHANOGENESIS-PWY, METHIONINE-DEG1-PWY, MGLDLCTANA-PWY, ORN-AMINOPENTANOATE-CAT-PWY, ORNDEG-PWY, P101-PWY, P162-PWY, P163-PWY, P181-PWY, P261-PWY, P283-PWY, P401-PWY, P541-PWY, PHENYLALANINE-DEG1-PWY, PHESYN, PHOSLIPSYN2-PWY, PHOSPHONOTASE-PWY, PROSYN-PWY, PROUT-PWY, PWY0-1021, PWY0-1221, PWY0-1299, PWY0-1303, PWY0-1305, PWY0-1313, PWY0-1317, PWY0-1321, PWY0-1338, PWY0-1347, PWY0-1355, PWY0-1356, PWY0-1534, PWY0-1544, PWY0-1565, PWY0-1576, PWY0-1577, PWY0-1578, PWY0-1585, PWY0-1601, PWY0-42, PWY0-461, PWY0-823, PWY0-901, PWY-1, PWY-1061, PWY-1121, PWY-1186, PWY1-2, PWY-1263, PWY-1622, PWY-1722, PWY-1781, PWY-181, PWY-1881, PWY-1962, PWY-1981, PWY1F-467, PWY1F-FLAVSYN, PWY1G-0, PWY-2021, PWY-2161, PWY-2181, PWY-2201, PWY-2821, PWY-2941, PWY-2942, PWY-3, PWY-3022, PWY-3081, PWY-3161, PWY-3162, PWY-3181, PWY-3301, PWY-3341, PWY-3385, PWY-3461, PWY-3462, PWY-3581, PWY-361, PWY-3661, PWY-3661-1, PWY-381, PWY-3841, PWY-3941, PWY3DJ-12, PWY3O-4107, PWY3O-4108, PWY-4, PWY-40, PWY-4002, PWY-4041, PWY-4201, PWY-4281, PWY-43, PWY-4321, PWY-4341, PWY-4361, PWY-46, PWY490-3, PWY490-4, PWY-4981, PWY-4983, PWY-4984, PWY4FS-6, PWY-5, PWY-5022, PWY-5024, PWY-5028, PWY-5029, PWY-5030, PWY-5031, PWY-5041, PWY-5048, PWY-5049, PWY-5057, PWY-5075, PWY-5076, PWY-5078, PWY-5079, PWY-5081, PWY-5082, PWY-5087, PWY-5097, PWY-5101, PWY-5103, PWY-5104, PWY-5108, PWY-5109, PWY-5129, PWY-5135, PWY-5136, PWY-5151, PWY-5154, PWY-5155, PWY-5159, PWY-5176, PWY-5188, PWY-5189, PWY-5196, PWY-5199, PWY-5207, PWY-5250, PWY-5254, PWY-5265, PWY-5280, PWY-5283, PWY-5290, PWY-

5297, PWY-5298, PWY-5311, PWY-5314, PWY-5316, PWY-5319, PWY-5324, PWY-5329, PWY-5331, PWY-5332, PWY-5364, PWY-5381, PWY-5382, PWY-5386, PWY-5394, PWY-5399, PWY-5436, PWY-5437, PWY-5441, PWY-5443, PWY-5458, PWY-5467, PWY-5468, PWY-5473, PWY-5474, PWY-5494, PWY-5497, PWY-5499, PWY-5629, PWY-5651, PWY-5653, PWY-5665, PWY-5669, PWY-5675, PWY-5679, PWY-5686, PWY-5710, PWY-5736, PWY-5737, PWY-5739, PWY-5740, PWY-5742, PWY-5747, PWY-5748, PWY-5751, PWY-5754, PWY-5766, PWY-5770, PWY-5784, PWY-5788, PWY-5797, PWY-5800, PWY-581, PWY-5811, PWY-5818, PWY-5826, PWY-5877, PWY-5883, PWY-5886, PWY-5912, PWY-5913, PWY-5921, PWY-5936, PWY-5940, PWY-5958, PWY-5963, PWY-5968, PWY-5978, PWY-5980, PWY-5990, PWY-6003, PWY-6004, PWY-601, PWY-6030, PWY-6039, PWY-6045, PWY-6052, PWY-6053, PWY-6054, PWY-6055, PWY-6068, PWY-6069, PWY-6082, PWY-6120, PWY-6121, PWY-6122, PWY-6123, PWY-6124, PWY-6133, PWY-6134, PWY-6141, PWY-6143, PWY-6148, PWY-6151, PWY-6160, PWY-6173, PWY-6196, PWY-6219, PWY-622, PWY-6220, PWY-6233, PWY-6273, PWY-6277, PWY-6281, PWY-6307, PWY-6309, PWY-6313, PWY-6318, PWY-6320, PWY-6321, PWY-6322, PWY-6324, PWY-6328, PWY-6334, PWY-6339, PWY-6343, PWY-6344, PWY-6345, PWY-6346, PWY-6375, PWY-6376, PWY-6381, PWY-6386, PWY-6387, PWY-6397, PWY-6403, PWY-6407, PWY-6408, PWY-6409, PWY-6431, PWY-6435, PWY-6444, PWY-6455, PWY-6456, PWY-6457, PWY-6466, PWY-6471, PWY-6473, PWY-6481, PWY-6486, PWY-6493, PWY-6495, PWY-6511, PWY-6512, PWY-6519, PWY-6533, PWY-6535, PWY-6536, PWY-6537, PWY-6543, PWY-6549, PWY-6559, PWY-6562, PWY-6572, PWY-6573, PWY-6574, PWY-6578, PWY-6588, PWY-6614, PWY-6627, PWY66-301, PWY66-375, PWY-6638, PWY66-391, PWY-6642, PWY66-420, PWY66-421, PWY66-425, PWY66-426, PWY66-428, PWY-6643, PWY-6661, PWY-6673, PWY-6682, PWY-6690, PWY-6696, PWY-6711, PWY-6717, PWY-6720, PWY-6724, PWY-6728, PWY-6731, PWY-6735, PWY-6749, PWY-6769, PWY-6771, PWY-6772, PWY-6773, PWY-6781, PWY-6784, PWY-6790, PWY-6791, PWY-6802, PWY-6807, PWY-6808, PWY-6813, PWY-6815, PWY-6816, PWY-6817, PWY-6818, PWY-6821, PWY-6822, PWY-6823, PWY-6831, PWY-6832, PWY-6834, PWY-6840, PWY-6845, PWY-6853, PWY-6854, PWY-6855, PWY-6891, PWY-6892, PWY-6896, PWY-6898, PWY-6902, PWY-6907, PWY-6908, PWY-6920, PWY-6922, PWY-6936, PWY-6942, PWY-6949, PWY-6953, PWY-6955, PWY-6963, PWY-6964, PWY-6965, PWY-6968, PWY-6969, PWY-6981, PWY-6982, PWY-6986, PWY-6994, PWY-7000, PWY-701, PWY-7014, PWY-7015, PWY-7016, PWY-7018, PWY-7019, PWY-702, PWY-7022, PWY-7025, PWY-7028, PWY-7040, PWY-7046, PWY-7052, PWY-7054, PWY-7064, PWY-7072, PWY-7088, PWY-7090, PWY-7097, PWY-

7104, PWY-7115, PWY-7117, PWY-7118, PWY-7126, PWY-7147, PWY-7153, PWY-7158, PWY-7176, PWY-7177, PWY-7185, PWY-7186, PWY-7219, PWY-7221, PWY-7234, PWY-7246, PWY-7248, PWY-7250, PWY-7255, PWY-7274, PWY-7275, PWY-7282, PWY-7288, PWY-7297, PWY-7304, PWY-7315, PWY-7316, PWY-7318, PWY-7342, PWY-7351, PWY-7356, PWY-7376, PWY-7377, PWY-7383, PWY-7387, PWY-7397, PWY-7398, PWY-7400, PWY-7414, PWY-7425, PWY-7430, PWY-7432, PWY-7440, PWY-7441, PWY-7456, PWY-7467, PWY-7498, PWY-7501, PWY-7506, PWY-7510, PWY-7514, PWY-7518, PWY-7520, PWY-7525, PWY-7531, PWY-7532, PWY-7533, PWY-7536, PWY-7542, PWY-7543, PWY-7547, PWY-7549, PWY-7550, PWY-7555, PWY-7561, PWY-7565, PWY-7570, PWY-7571, PWY-7600, PWY-7605, PWY-761, PWY-7612, PWY-7626, PWY-7645, PWY-7648, PWY-7649, PWY-7650, PWY-7665, PWY-7667, PWY-7668, PWY-7669, PWY-7671, PWY-7674, PWY-7688, PWY-7690, PWY-7693, PWY-7694, PWY-7701, PWY-7704, PWY-7706, PWY-7708, PWY-7717, PWY-7718, PWY-7719, PWY-7733, PWY-7734, PWY-7735, PWY-7737, PWY-7751, PWY-7761, PWY-7765, PWY-7767, PWY-7769, PWY-7770, PWY-7782, PWY-7790, PWY-7791, PWY-7793, PWY-7797, PWY-7811, PWY-7814, PWY-7822, PWY-7824, PWY-7826, PWY-7842, PWY-7850, PWY-7851, PWY-7855, PWY-7860, PWY-7861, PWY-7863, PWY-7867, PWY-7870, PWY-7880, PWY-7888, PWY-7889, PWY-7891, PWY-7892, PWY-7897, PWY-7901, PWY-7904, PWY-7907, PWY-7909, PWY-7910, PWY-7913, PWY-7917, PWY-7930, PWY-7931, PWY-7936, PWY-7953, PWY-7955, PWY-7956, PWY-7957, PWY-7958, PWY-7959, PWY-7960, PWY-7962, PWY-7977, PWY-7985, PWY-7986, PWY-7987, PWY-7988, PWY-7990, PWY-8002, PWY-8003, PWY-8006, PWY-8007, PWY-8008, PWY-8009, PWY-801, PWY-8010, PWY-8011, PWY-8013, PWY-8014, PWY-8015, PWY-8016, PWY-8017, PWY-8024, PWY-8032, PWY-8040, PWY-8043, PWY-8045, PWY-8071, PWY-8072, PWY-8080, PWY-8081, PWY-8082, PWY-8083, PWY-8086, PWY-8088, PWY-842, PWY-861, PWY-862, PWY8J2-1, PWY8J2-22, PWYDQC-4, PWYG-321, PWY-I9, PWYQT-4450, PWYQT-4476, PYRIDNUCSAL-PWY, PYRIDNUCSYN-PWY, PYRIDOXSYN-PWY, SAM-PWY, SERDEG-PWY, SERSYN-PWY, SPHINGOLIPID-SYN-PWY, TAURINEDEG-PWY, THRDLCTCAT-PWY, THREONINE-DEG2-PWY, TRNA-CHARGING-PWY, TRPCAT-PWY, TRPIAACAT-PWY, TRPKYNCAT-PWY, TRPSYN-PWY, TRYPDEG-PWY, TYRFUMCAT-PWY, TYRSYN, UDPNACETYLGALSYN-PWY, UDPNAGSYN-PWY, VALDEG-PWY, and VALSYN-PWY.

[0009] In some embodiments, encoding the enzymes catalyzing all reactions of a MetaCyc metabolic pathway can be determined by culturing the defined gut microbial community *in vitro* and measuring whether a substrate in the pathway is metabolized, a metabolite in the pathway is

produced, and/or a reaction intermediate in the pathway is produced by liquid chromatography-mass spectrometry analysis. In some embodiments, encoding the enzymes catalyzing all reactions of a MetaCyc metabolic pathway can be determined by administering the defined gut microbial community to a gnotobiotic mouse and measuring whether a substrate in the pathway is metabolized, a metabolite in the pathway is produced, and/or a reaction intermediate in the pathway is produced after a defined period of time by liquid chromatography-mass spectrometry analysis of a sample obtained from the mouse. In certain embodiments, the defined gut microbial community is administered via a route selected from the group consisting of oral, rectal, fecal (by enema), and naso/oro-gastric gavage. In certain embodiments, the defined period of time is about 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours, 2 days, 7 days, 14 days, 1 month, or 2 months. In certain embodiments, the sample is selected from the group consisting of a fecal sample, urine sample, blood sample, or serum sample.

[0010] In some embodiments, the at least 3 of 4 phyla comprise Bacteroidetes, Firmicutes, and Actinobacteria. In certain embodiments, the high complexity defined gut microbial community comprises Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria. In certain embodiments, the defined microbial strains comprise phyla selected from the group consisting of Bateriodales, Clostridiales, Lactobacillales, Negativicutes, Eggerthellales, Bifidobacteriales, and Proteobacteria.

[0011] In some embodiments, the defined microbial strains comprise a genus selected from the group consisting of: Acidaminococcus, Adlercreutzia, Akkermansia, Alistipes, Anaerobutyricum, Anaerofustis, Anaerostipes, Anaerotruncus, Bacteroides, Parabacteroides, Bifidobacterium, Bilophila, Blautia, Catenibacterium, Clostridium, Tyzzerella, Absiella, Collinsella, Coprococcus, Dialister, Eubacterium, Holdemanella, Intestinibacter, Megasphaera, Odoribacter, Parabacteroides, Granulicatella, Holdemania, Hungatella, Intestinimonas, Solobacterium, Mitsuokella, Olsenella, Parabacteroides, Prevotella, Roseburia, Ruminococcus, Slackia, Butyrivibrio, Subdoligranulum, Turicibacter, Butyricimonas, Streptococcus, Dorea, Oscillibacter, Desulfovibrio, Ethanoligenens, Marvinbryantia, Lactobacillus, and Faecalibacterium.

[0012] In some embodiments, the defined microbial strains are selected from the group consisting of: Acidaminococcus fermentans, Acidaminococcus sp., Adlercreutzia equolifaciens, Akkermansia muciniphila, Alistipes finegoldii, Alistipes indistinctus, Alistipes onderdonkii, Anaerobutyricum hallii, Anaerofustis stercorihominis, Anaerostipes caccae, Anaerotruncus colihominis, Bacteroides caccae, Bacteroides cellulosilyticus, Bacteroides coprocola, Bacteroides coprophilus, Bacteroides dorei, Bacteroides eggerthii, Bacteroides finegoldii,

Bacteroides fragilis, Bacteroides intestinalis, Bacteroides ovatus, Bacteroides rodentium, Bacteroides thetaiotaomicron, Bacteroides xylanisolvens, Parabacteroides distasonis, Bacteroides dorea, Bacteroides stercoris, Bacteroides uniformis, Bacteroides vulgatus, Bifidobacterium pseudocatenulatum, Bilophila wadsworthia, Blautia hansenii, Blautia hydrogenotrophica, Blautia obeum, Blautia sp., Blautia wexlerae, Catenibacterium mitsuokai, Clostridium asparagiforme, Clostridium hylemonae, Clostridium leptum, Tyzzerella nexilis, Clostridium saccharolyticum, Absiella dolichum, Collinsella aerofaciens, Collinsella stercoris, Coprococcus comes, Dialister invisus, Eubacterium rectale, Eubacterium siraeum, Eubacterium ventriosum, Coprococcus eutactus, Holdemanella biformis, Intestinibacter bartlettii, Megasphaera sp., Odoribacter splanchnicus, Parabacteroides merdae, Parabacteroides sp., Granulicatella adiacens, Holdemania filiformis, Hungatella hathewayi, Intestinimonas butyriciproducens, Solobacterium moorei, Mitsuokella multacida, Olsenella uli, Parabacteroides johnsonii, Prevotella buccalis, Prevotella copri, Roseburia inulinivorans, Clostridium sp., Ruminococcus gauvreauii, Ruminococcus lactaris, Ruminococcus torques, Alistipes putredinis, Alistipes senegalensis, Clostridium spiroforme, Slackia exigua, Bacteroides pectinophilus, Butyrivibrio crossotus, Subdoligranulum variabile, Turicibacter sanguinis, Bifidobacterium breve, Bifidobacterium catenulatum, Butyricimonas virosa, Streptococcus salivarius subsp. thermophilus, Dorea formicigenerans, Bacteroides plebeius, Ruminococcus gnavus, Oscillibacter sp., Clostridium sp., Slackia heliotrinireducens, Desulfovibrio piger, Clostridium methylpentosum, Ethanoligenens harbinense, Marvinbryantia formatexigens, Lactobacillus ruminis, Clostridium bolteae, Clostridium hiranonis, Clostridium scindens, Clostridium sp., Clostridium orbiscindens, Alistipes shahii, and Faecalibacterium prausnitzii.

[0013] In certain embodiments, the defined microbial strains are selected from the group consisting of: Acidaminococcus fermentans -- VR4, Acidaminococcus sp. -- D21, Adlercreutzia equolifaciens -- FJC-B9, Akkermansia muciniphila -- Muc [CIP 107961], Alistipes finegoldii -- AHN 2437, Alistipes indistinctus -- JCM 16068, YIT 12060, Alistipes onderdonkii -- WAL 8169, Anaerobutyricum hallii -- VPI B4-27, Anaerofustis stercorihominis -- ATCC BAA-858, CCUG 47767, CIP 108481, WAL 14563, Anaerostipes caccae -- L1-92, Anaerotruncus colihominis -- 277, Bacteroides caccae -- VPI 3452A [CIP 104201T, JCM 9498], Bacteroides cellulosilyticus -- CRE21, CCUG 44979, Bacteroides coprocola -- M16, Bacteroides coprophilus -- CB42, JCM 13818, Bacteroides dorei -- 175, Bacteroides dorei -- 5_1_36/D4, Bacteroides eggerthii -- ATCC 27754, NCTC 11155, Bacteroides finegoldii -- 199, Bacteroides fragilis -- 3_1_12, Bacteroides intestinalis -- 341, Bacteroides ovatus -- NCTC 11153, Bacteroides rodentium -- ST28, CCUG 59334, JCM 16469, Bacteroides thetaiotaomicron -- 1_1_6,

Bacteroides fragilis -- 2_1_16, Bacteroides xylanisolvens -- 2_1_22, Parabacteroides distasonis -- 3_1_19, Bacteroides dorea -- 9_1_42FAA, Bacteroides ovatus -- D2, Bacteroides stercoris -- VPI B3-21, ATCC 43183, CIP 104203, JCM 9496, Bacteroides thetaiotaomicron -- VPI 5482 [CIP 104206T, E50, NCTC 10582], Bacteroides uniformis -- ATCC 8492, Bacteroides vulgatus -- NCTC 11154, Bifidobacterium pseudocatenulatum -- B1279, ATCC 27919, Bilophila wadsworthia -- WAL 7959 [Lab 88-130H], Blautia hansenii -- VPI C7-24, Blautia hydrogenotrophica -- S5a33, Blautia obeum -- ATCC 29174, KCTC 15206, VPI B3-21, Blautia sp. -- KLE 1732, Blautia wexlerae -- ATCC BAA-1564, JCM 17041, KCTC 5965, WAL 14507, Catenibacterium mitsuokai -- RCA14-39, CIP 106738, JCM 10609, Clostridium asparagiforme -- N6, CCUG 48471, Clostridium hylemonae -- TN-271, JCM 10539, Clostridium leptum -- VPI T7-24-1, ATCC 29065, Tyzzerella nexilis DSM 1787, Clostridium saccharolyticum -- WM1, ATCC 35040, NRC 2533, Absiella dolichum DSM 3991, Collinsella aerofaciens -- VPI 1003 [DSM 3979, JCM 10188], Collinsella stercoris -- RCA 55-54, JCM 10641, Coprococcus comes -- VPI CI-38, Dialister invisus -- E7.25, CCUG 47026, Eubacterium rectale -- VPI 0990 [CIP 105953], Eubacterium siraeum -- VPI T9-50-2, ATCC 29066, DSM 3996, Eubacterium ventriosum -- VPI 1013B, Coprococcus eutactus -- VPI C33-22, Holdemanella biformis -- VPI C17-5, ATCC 27806, KCTC 5969, Intestinibacter bartlettii -- WAL 16138, ATCC BAA-827, CCUG 48940, Megasphaera sp. -- Sanger 24, Sanger_24, Odoribacter splanchnicus -- 1651/6, ATCC 29572, CCUG 21054, CIP 104287, LMG 8202, NCTC 10825, Parabacteroides distasonis -- NCTC 11152, Parabacteroides merdae -- VPI T4-1, ATCC 43184, CCUG 38734, CIP 104202, JCM 9497, Parabacteroides sp. -- D13, Granulicatella adiacens -- GaD [CIP 103243, DSM 9848], Holdemania filiformis -- VPI J1-31B-1, ATCC 51649, Hungatella hathewayi -- 1313, CCUG 43506, CIP 109440, MTCC 10951, Intestinimonas butyriciproducens -- SRB-521-5-l, CCUG 63529, Solobacterium moorei -- RCA59-74, CIP 106864, JCM 10645, Mitsuokella multacida -- A 405-1, ATCC 27723, NCTC 10934, Olsenella uli -- D76D-27C, ATCC 49627, CIP 109912, Parabacteroides johnsonii -- M-165, CIP 109537, JCM 13406, Prevotella buccalis -- HS4, ATCC 35310, NCDO 2354, Prevotella copri -- CB7, JCM 13464, Roseburia inulinivorans -- A2-194, CIP 109405, JCM 17584, NCIMB 14030, Clostridium sp. -- VPI C48-50 (unassigned Clostridiales), Ruminococcus gauvreauii -- CCRI-16110, CCUG 54292, JCM 14987, NML 060141, Ruminococcus lactaris -- VPI X6-29, Ruminococcus torques -- VPI B2-51, Alistipes putredinis -- CCUG 45780, CIP 104286, ATCC 29800, Carlier 10203, VPI 3293, Alistipes senegalensis -- CSUR P150, JCM 32779, JC50, Clostridium spiroforme -- VPI C28-23-1A, ATCC 29900, NCTC 11211, Slackia exigua -- S-7, ATCC 700122, JCM 11022, KCTC 5966, Bacteroides pectinophilus -- N3, Butyrivibrio crossotus -- T9-40A, ATCC 29175,

Subdoligranulum variabile -- BI-114, CCUG 47106, Turicibacter sanguinis -- MOL361, NCCB 100008, Bifidobacterium breve -- S1, ATCC 15700, NCTC 11815, Bifidobacterium catenulatum -- B669, ATCC 27539, CECT 7362, CIP 104175, DSM 20103, Butyricimonas virosa -- MT12, CCUG 56611, JCM 15149, Streptococcus salivarius subsp. thermophilus -- LMD-9, Dorea formicigenerans -- VPI C8-13 [JCM 9500], Bacteroides plebeius -- M12, Ruminococcus gnavus -- VPI C7-9, Oscillibacter sp. -- KLE 1728, Clostridium sp. -- M62/1, Slackia heliotrinireducens -- RHS 1, ATCC 29202, NCTC 11029, Desulfovibrio piger -- VPI C3-23 [DSM 749], Clostridium methylpentosum -- R2, ATCC 43829, Ethanoligenens harbinense -- YUAN-3, CGMCC 1.5033, JCM 12961, Marvinbryantia formatexigens -- I-52, CCUG 46960, Lactobacillus ruminis -- E 194e, Clostridium bolteae -- WAL 16351, [CCUG 46953], ATCC BAA-613, Song et al. 2003, Clostridium hiranonis -- TO-931, JCM 10541, KCTC 15199, Clostridium scindens -- VPI 13733, ATCC 35704, 19, Bacteroides xylanisolvens -- XB1A, CCUG 53782, Clostridium sp. -- L2-50, Clostridium orbiscindens -- 1_3_50AFAA, Alistipes shahii -- WAL 8301, and Faecalibacterium prausnitzii -- A2-165, JCM 31915.

[0014] In some embodiments, the defined gut microbial community comprises Acidaminococcus, Adlercreutzia, Akkermansia, Anaerostipes, Anaerotruncus, Bacteroides, Bifidobacterium, Bilophila, Blautia, Butyrivibrio, Clostridium, Collinsella, Coprococcus, Desulfovibrio, Eggerthella, Eubacterium, Faecalibacterium, Marvinbryantia, Mitsuokella, Odoribacter, Parabacteroides, Roseburia, Ruminococcus, Slackia, and Solobacterium. In certain embodiments, the defined gut microbial community comprises Acidaminococcus fermentans, Adlercreutzia equolifaciens, Akkermansia muciniphila, Anaerostipes caccae, Anaerotruncus colihominis, Bacteroides caccae, Bacteroides cellulosilyticus, Bacteroides dorei, Bacteroides eggerthii, Bacteroides fragilis, Bacteroides intestinalis, Bacteroides ovatus, Bacteroides pectinophilus, Bacteroides plebeius, Bacteroides stercoris, Bacteroides thetaiotaomicron, Bacteroides uniformis, Bacteroides vulgatus, Bifidobacterium breve, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bilophila wadsworthia, Blautia hansenii, Blautia hydrogenotrophica, Butyrivibrio crossotus, Clostridium asparagiforme, Clostridium hiranonis, Clostridium hylemonae, Clostridium leptum, Clostridium orbiscindens, Clostridium saccharolyticum, Clostridium scindens, Collinsella aerofaciens, Coprococcus comes, Desulfovibrio piger, Eggerthella lenta, Eubacterium rectale, Eubacterium siraeum, Eubacterium ventriosum, Faecalibacterium prausnitzii, Marvinbryantia formatexigens, Mitsuokella multacida, Odoribacter splanchnicus, Parabacteroides distasonis, Parabacteroides johnsonii, Parabacteroides merdae, Roseburia inulinivorans, Ruminococcus gnavus, Ruminococcus lactaris, Ruminococcus torques, Slackia exigua, and Solobacterium moorei.

[0015] In certain embodiments, the defined gut microbial community comprises Acidaminococcus fermentans -- VR4,Acidaminococcus sp. -- D21,Adlercreutzia equolifaciens -- FJC-B9,Akkermansia muciniphila -- Muc [CIP 107961],Alistipes finegoldii -- AHN 2437,Alistipes indistinctus -- JCM 16068, YIT 12060,Alistipes onderdonkii -- WAL 8169,Anaerobutyricum hallii -- VPI B4-27,Anaerofustis stercorihominis -- ATCC BAA-858, CCUG 47767, CIP 108481, WAL 14563,Anaerostipes caccae -- L1-92,Anaerotruncus colihominis -- 277,Bacteroides caccae -- VPI 3452A [CIP 104201T, JCM 9498],Bacteroides cellulosilyticus -- CRE21, CCUG 44979,Bacteroides coprocola -- M16,Bacteroides coprophilus -- CB42, JCM 13818,Bacteroides dorei -- 175,Bacteroides dorei -- 5_1_36/D4,Bacteroides eggerthii -- ATCC 27754, NCTC 11155,Bacteroides finegoldii -- 199,Bacteroides fragilis -- 3_1_12,Bacteroides intestinalis -- 341,Bacteroides ovatus -- NCTC 11153,Bacteroides rodentium -- ST28, CCUG 59334, JCM 16469,Bacteroides thetaiotaomicron -- 1_1_6,Bacteroides fragilis -- 2_1_16,Bacteroides xylanisolvens -- 2_1_22,Parabacteroides distasonis -- 3_1_19,Bacteroides dorea -- 9_1_42FAA,Bacteroides ovatus -- D2,Bacteroides stercoris -- VPI B3-21, ATCC 43183, CIP 104203, JCM 9496,Bacteroides thetaiotaomicron -- VPI 5482 [CIP 104206T, E50, NCTC 10582],Bacteroides uniformis -- ATCC 8492,Bacteroides vulgatus -- NCTC 11154,Bifidobacterium pseudocatenulatum -- B1279, ATCC 27919,Bilophila wadsworthia -- WAL 7959 [Lab 88-130H],Blautia hansenii -- VPI C7-24,Blautia hydrogenotrophica -- S5a33,Blautia obeum -- ATCC 29174, KCTC 15206, VPI B3-21,Blautia sp. -- KLE 1732,Blautia wexlerae -- ATCC BAA-1564, JCM 17041, KCTC 5965, WAL 14507,Catenibacterium mitsuokai -- RCA14-39, CIP 106738, JCM 10609,Clostridium asparagiforme -- N6, CCUG 48471,Clostridium hylemonae -- TN-271, JCM 10539,Clostridium leptum -- VPI T7-24-1, ATCC 29065,Tyzzerella nexilis DSM 1787,Clostridium saccharolyticum -- WM1, ATCC 35040, NRC 2533,Absiella dolichum DSM 3991,Collinsella aerofaciens -- VPI 1003 [DSM 3979, JCM 10188],Collinsella stercoris -- RCA 55-54, JCM 10641,Coprococcus comes -- VPI CI-38,Dialister invisus -- E7.25, CCUG 47026,Eubacterium rectale -- VPI 0990 [CIP 105953],Eubacterium siraeum -- VPI T9-50-2, ATCC 29066, DSM 3996,Eubacterium ventriosum -- VPI 1013B,Coprococcus eutactus -- VPI C33-22,Holdemanella biformis -- VPI C17-5, ATCC 27806, KCTC 5969,Intestinibacter bartlettii -- WAL 16138, ATCC BAA-827, CCUG 48940,Megasphaera sp. -- Sanger 24, Sanger_24,Odoribacter splanchnicus -- 1651/6, ATCC 29572, CCUG 21054, CIP 104287, LMG 8202, NCTC 10825,Parabacteroides distasonis -- NCTC 11152,Parabacteroides merdae -- VPI T4-1, ATCC 43184, CCUG 38734, CIP 104202, JCM 9497,Parabacteroides sp. -- D13,Granulicatella adiacens -- GaD [CIP 103243, DSM 9848],Holdemania filiformis -- VPI J1-31B-1, ATCC 51649,Hungatella hathewayi -- 1313,

CCUG 43506, CIP 109440, MTCC 10951,Intestinimonas butyriciproducens -- SRB-521-5-l, CCUG 63529,Solobacterium moorei -- RCA59-74, CIP 106864, JCM 10645,Mitsuokella multacida -- A 405-1, ATCC 27723, NCTC 10934,Olsenella uli -- D76D-27C, ATCC 49627, CIP 109912,Parabacteroides johnsonii -- M-165, CIP 109537, JCM 13406,Prevotella buccalis -- HS4, ATCC 35310, NCDO 2354,Prevotella copri -- CB7, JCM 13464,Roseburia inulinivorans -- A2-194, CIP 109405, JCM 17584, NCIMB 14030,Clostridium sp. -- VPI C48-50 (unassigned Clostridiales),Ruminococcus gauvreauii -- CCRI-16110, CCUG 54292, JCM 14987, NML 060141,Ruminococcus lactaris -- VPI X6-29,Ruminococcus torques -- VPI B2-51,Alistipes putredinis -- CCUG 45780, CIP 104286, ATCC 29800, Carlier 10203, VPI 3293,Alistipes senegalensis -- CSUR P150, JCM 32779, JC50,Clostridium spiroforme -- VPI C28-23-1A, ATCC 29900, NCTC 11211,Slackia exigua -- S-7, ATCC 700122, JCM 11022, KCTC 5966,Bacteroides pectinophilus -- N3,Butyrivibrio crossotus -- T9-40A, ATCC 29175,Subdoligranulum variabile -- BI-114, CCUG 47106,Turicibacter sanguinis -- MOL361, NCCB 100008,Bifidobacterium breve -- S1, ATCC 15700, NCTC 11815,Bifidobacterium catenulatum -- B669, ATCC 27539, CECT 7362, CIP 104175, DSM 20103,Butyricimonas virosa -- MT12, CCUG 56611, JCM 15149,Streptococcus salivarius subsp. thermophilus -- LMD-9,Dorea formicigenerans -- VPI C8-13 [JCM 9500],Bacteroides plebeius -- M12,Ruminococcus gnavus -- VPI C7-9,Oscillibacter sp. -- KLE 1728,Clostridium sp. -- M62/1,Slackia heliotrinireducens -- RHS 1, ATCC 29202, NCTC 11029,Desulfovibrio piger -- VPI C3-23 [DSM 749],Clostridium methylpentosum -- R2, ATCC 43829,Ethanoligenens harbinense -- YUAN-3, CGMCC 1.5033, JCM 12961,Marvinbryantia formatexigens -- I-52, CCUG 46960,Lactobacillus ruminis -- E 194e,Clostridium bolteae -- WAL 16351, [CCUG 46953], ATCC BAA-613, Song et al. 2003,Clostridium hiranonis -- TO-931, JCM 10541, KCTC 15199,Clostridium scindens -- VPI 13733, ATCC 35704, 19,Bacteroides xylanisolvens -- XB1A, CCUG 53782,Clostridium sp. -- L2-50,Clostridium orbiscindens -- 1_3_50AFAA,Alistipes shahii -- WAL 8301, and Faecalibacterium prausnitzii -- A2-165, JCM 31915.

[0016] In some embodiments, community stability is characterized by up to 10% of the defined microbial strains dropping out following the microbial challenge. In some embodiments, community stability is characterized by the appearance of up to 10% of new strains contributed from the human fecal community appearing following the microbial challenge. In certain embodiments, at least 50% of the defined microbial strains are detectable following the microbial challenge. In certain embodiments, at least 60% of the defined microbial strains are detectable following the microbial challenge. In certain embodiments, at least 70% of the defined microbial strains are detectable following the microbial challenge. In certain

embodiments, at least 80% of the defined microbial strains are detectable following the microbial challenge. In certain embodiments, at least 90% of the defined microbial strains are detectable following the microbial challenge. In certain embodiments, at least 95% of the defined microbial strains are detectable following the microbial challenge. In certain embodiments, at least 99% of the defined microbial strains are detectable following the microbial challenge.

[0017] In some embodiments, community stability is characterized by metagenomic analysis of a fecal sample obtained from the mouse following the microbial challenge. In certain embodiments, the metagenomic analysis is selected from whole genome sequencing, ribosomal gene sequencing, or ribosomal RNA sequencing. In certain embodiments, the whole genome sequencing is whole genome shotgun sequencing.

[0018] In some aspects of the above embodiments, the defined gut microbial community comprises between 100 and 200 defined microbial strains. In some embodiments, the defined gut microbial community comprises between 100 and 150 defined microbial strains.

[0019] In some aspects of the above embodiments, each defined microbial strain is molecularly identified. In some embodiments, the molecular identification comprises identification of a nucleic acid sequence that uniquely identifies each of the defined microbial strains. In certain embodiments, the nucleic acid sequence comprises a 16S rRNA sequence. In certain embodiments, the nucleic acid sequence comprises a whole genomic sequence. In certain embodiments, the molecular identification comprises Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry.

[0020] In another aspect, provided herein is a method of treating an animal having a dysbiosis or pathological condition comprising administering a high-complexity defined gut microbial community according to any of the above embodiments. In certain embodiments, the animal is a mammal. In certain embodiments, the animal is a human. In certain embodiments, the high-complexity defined gut microbial community is administered via a route selected from the group consisting of oral, rectal, fecal (by enema), and naso/oro-gastric gavage.

[0021] In another aspect, provided herein is a method of making a high-complexity defined gut microbial community, wherein each of the plurality of defined microbial strains is individually cultured then combined to form the defined gut microbial community.

[0022] In another aspect, provided herein is a method of making a high-complexity defined gut microbial community, wherein all of the plurality of defined microbial strains are cultured together to form the defined gut microbial community.

[0023] In another aspect, provided herein is a method of making a high-complexity defined gut microbial community, wherein one or more of the plurality of defined microbial strains is individually cultured and two or more of the defined microbial strains are cultured together, and wherein the individually cultured defined microbial strains and the co-cultured defined microbial strains are combined together to form the defined gut microbial community.

[0024] In another aspect, provided herein is a formulation comprising the high-complexity defined gut microbial community and a pharmaceutically acceptable carrier or excipient.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] FIGURE 1 is a schematic illustrating a workflow to preparing a high-complexity defined gut microbial community.

[0026] FIGURE 2 shows the relative abundance of microbial strains in mice colonized with a high-complexity defined microbial community and challenged with fecal samples prepared from 3 different human donors.

[0027] FIGURE 3A shows a schematic of a treatment schedule of gnotobiotic mice colonized with human fecal samples, inoculated with *C. difficile*, and treated with a high-complexity defined gut microbial community. FIGURE 3B shows a dot plot of *C. difficile* concentrations in the stool of mice treated in accordance with the treatment schedule of FIGURE 3A.

[0028] FIGURE 4 shows bar graphs of bile acid concentrations in stool (FIGURE 4A) and cecum (FIGURE 4B) from mice treated with human stool sample or high-complexity defined gut microbial community.

[0029] FIGURE 5 shows bar graphs of metabolite concentrations in urine samples from mice treated with human stool sample or high-complexity defined gut microbial community.

DETAILED DESCRIPTION

1. Definitions

[0030] The term “a” and “an” as used herein mean “one or more” and include the plural unless the context is appropriate.

[0031] As used herein, "abundance" of a specific gut microorganism refers to the number of individual organisms in an individual animal's gut. Abundance can be described as a proportion of the total gut population (*e.g.*, number of organisms relative to the total gut population, the mass of the organism relative to the mass of the total gut population).

[0032] As used herein, "animal" refers to an organism to be treated with a microbial community, *e.g.*, a high-complexity defined gut microbial community. Animals include, but are not limited to, mammals (*e.g.*, murines, simians, equines, bovines, porcines, canines, felines, and the like), and more preferably include humans.

[0033] As used herein, "dysbiosis" refers to a state of a microbiome of the gut of an animal in which normal diversity and/or function is perturbed. In some instances, dysbiosis may be attributed to a decrease in the diversity of the gut microbiota, overabundance of one or more pathogens or pathobionts, or presence of pathogenic symbionts.

[0034] As used herein, the term "effective amount" refers to an amount sufficient to achieve a beneficial or desired result.

[0035] As used herein, a "humanized mouse" refers to a mouse with a human gut microbiome. A humanized mouse can be produced by removing the mouse's gut flora (*e.g.*, by administering PEG-3350 and electrolytes, *e.g.*, GoLYTELY® (Braintree Laboratories, Inc., Braintree, MA)) and/or administering broad spectrum antibiotics, and colonizing the mouse with a preparation of microorganisms from human feces. A humanized mouse can also refer to a gnotobiotic mouse that has been colonized with a human fecal sample. In some embodiments, the gut of the humanized mouse can be flushed (*e.g.*, by administration of PEG-3350) before inoculation with a high-complexity gut microbial community described herein.

[0036] As used herein, an "isogenic gnotobiotic control mouse" refers to a mouse used as an experimental control that shares the same genotype as a mouse receiving administration of a microbial community, *e.g.*, a high-complexity defined gut microbial community, but to which a vehicle control, or other experimental negative control, has been administered.

[0037] As used herein, the term "pharmaceutically acceptable carrier" refers to any of the standard pharmaceutical carriers, such as phosphate buffered saline (PBS) solution, water, emulsions (*e.g.*, such as oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers, and adjuvants, *see e.g.,* Martin, Remington's Pharmaceutical Sciences, 15th Ed. Mack Publ. Co., Easton, PA [1975].

[0038] As used herein, "prevalence" of a gut microorganism refers to the frequency (*e.g.*, number of individuals in a population) at which the organism is found in the human gut.

[0039] As used herein, "significantly" or "significant" refers to a change or alteration in a measurable parameter to a statistically significant degree as determined in accordance with an appropriate statistically relevant test. For example, in some embodiments, a change or alteration

is significant if it is statistically significant in accordance with, *e.g.*, a Student's t-test, chi-square, or Mann Whitney test.

[0040] As used herein, "minimal difference" refers to a change or alteration in a measurable parameter to a degree that is not statistically significant as determined in accordance with an appropriate statistically relevant test. For example, in some embodiments, a change or alteration is minimally different if it is not statistically significant in accordance with, *e.g.*, a Student's t-test, chi-square, or Mann Whitney test.

[0041] As used herein, the term "metabolizing a substrate" means that a measurable reduction in the amount of the substrate can be demonstrated following contacting of the substrate with a microbial community, *in vivo* or *in vitro*, as compared to contacting with a vehicle control. In embodiments, the reduction in amount is determined using mass spectrometry. In embodiments, the contacting *in vivo* is achieved through introduction of the community into a gnotobiotic organism such as, *e.g.*, a gnotobiotic mouse.

[0042] As used herein, the term "producing a metabolite" means that a measurable increase in the amount of the metabolite can be demonstrated following contacting one or more metabolite precursor molecules with a microbial community, *in vivo* or *in vitro*, as compared to contacting with a vehicle control. In embodiments, the increase in amount is determined using mass spectrometry. In embodiments, the contacting *in vivo* is achieved through introduction of the community into a gnotobiotic organism such as, *e.g.*, a gnotobiotic mouse.

2. Fecal Microbiota Transplantation

[0043] Fecal microbiota transplantation (FMT) is remarkable in two ways that suggest its generality: 1) there has been a very low rate of acute adverse events, suggesting that this modality is likely to be generally safe; and 2) even though no concerted effort has been made to optimize the process of engraftment, it already works quite well for treating certain conditions. Taken together, these observations suggested to the inventors that, counterintuitively, one single community could in principle be transplanted stably into the gut of millions of patients and administration of a high-complexity defined gut microbial community may be safer and more predictable than seemingly simpler perturbations to the gut (*e.g.*, addition or removal of one or a few strains). This is exciting, since administration of a high-complexity defined gut microbial community would be the biggest 'lever' one could pull in terms of controlling human biology linked to the microbiota. However, the current state of the art is fecal transplantation, which cannot be scaled. This calls for a new technology that enables the design and assembly of

transplantable communities that are, on the one hand, completely defined, and on the other hand, approach the complexity of a native gut community.

3. Microbial Communities

[0044] As used herein, "community" or "microbial community" refers to a physical combination of a plurality of different microorganisms, usually a plurality of different bacterial strains, sometimes comprising one or more strains or archaea. A naturally occurring gut microbiome is one example of a community. An artificially created mixture of strains of known identity is another example of a community. A defined gut microbial community is yet another example of a community. As used herein, a "defined gut microbial community" means a combined plurality of microbial strains for engraftment in a gut of an animal wherein each microbial strain has been molecularly identified.

[0045] As used herein, a "microbial strain" refers to a type or sub-type of a microbe. As used herein, a "defined microbial strain" is a microbial strain that has been molecularly identified; *e.g.,* a microbial strain whose whole genome has been sequenced. As used herein, a "plurality of defined microbial strains" means two or more microbial strains from two or more distinct microbial species. In some embodiments, multiple microbial strains in a plurality may represent a single microbial species.

[0046] As used herein, "complexity" means the number of strains in a community without regard to abundance. A community comprising 50 strains is more complex than a community comprising 15 strains. As used herein, "high-complexity" means a community having at least 40 defined microbial strains. In some embodiments, a high-complexity community comprises between 40 and 500, between 40 and 400, between 40 and 300, between 40 and 200, between 40 and 150, between 40 and 140, between 40 and 130, between 40 and 120, between 40 and 110, between 40 and 100, between 50 and 500, between 50 and 400, between 50 and 300, between 50 and 200, between 50 and 150, between 50 and 140, between 50 and 130, between 50 and 120, between 50 and 110, between 50 and 100, between 60 and 500, between 60 and 400, between 60 and 300, between 60 and 200, between 60 and 150, between 60 and 140, between 60 and 130, between 60 and 120, between 60 and 110, between 60 and 100, between 70 and 500, between 70 and 400, between 70 and 300, between 70 and 200, between 70 and 150, between 70 and 140, between 70 and 130, between 70 and 120, between 70 and 110, between 70 and 100, between 80 and 500, between 80 and 400, between 80 and 300, between 80 and 200, between 80 and 150, between 80 and 140, between 80 and 130, between 80 and 120, between 80 and 110, between 80 and 100, between 90 and 500, between 90 and 400, between 90 and 300, between 90 and 200,

between 90 and 150, between 90 and 140, between 90 and 130, between 90 and 120, between 90 and 110, between 90 and 100, between 100 and 500, between 100 and 400, between 100 and 300, between 100 and 200, between 100 and 150, between 100 and 140, between 100 and 130, between 100 and 120, or between 100 and 110 defined microbial strains.

3.1. Culturing Microbial Strains and Communities

[0047] As used herein, "culture" (and grammatical variants thereof, *e.g.*, "cultured," and "culturing") refers to the maintenance and/or growth of a microbial strain or microbial community in a liquid medium, or on a solid medium. For example, in some embodiments, culturing of purchased microbial strains is performed in accordance with the manufacturer's instructions.

[0048] As used herein, "aliquot," refers to an *in vitro* bacterial population that is physically separated from other populations for storage, culture, analysis and the like. "Aliquot" may refer to separate populations in vessels, compartments, tubes, wells of multiwell plates, emulsion clonal, such as a stock of a strain isolate, or may be a mixture of strains, such as an artificial community or defined gut microbial community.

[0049] In certain embodiments, microbial strains or microbial communities are maintained or grown in specially formulated media such as the media described in any one of Tables 1-6 below.

TABLE 1 – MEDIUM A

Component	**Amount (in 500 mL)**	**Final Concentration**
Trypticase™ Peptone	5 g	1% (w/v)
Yeast Extract	2.5 g	0.5% (w/v)
D-(+)-Glucose	1 g	0.2% (w/v)
L-Cysteine hydrochloride	0.25 g	0.05% (w/v)
1M Potassium phosphate buffer, pH 7.2**	50 mL	10% (v/v)

TYG Salts solution**	20 mL	4% (w/v)
Vitamin K solution**	500 µL of 1 mg/mL	0.000001% (w/v)
0.8% (w/v) $CaCl_2$**	500 µL	
$FeSO_4 \cdot 7\ H_2O$**	500 µL of 0.4 mg/mL	
Resazurin**	2 mL of 0.25 mg/mL	0.000001% (w/v)
Histidine – Hematin**	500 µl	
D-(+)-Cellobiose	0.5 g	0.1% (w/v)
D-(+)-Maltose monohydrate	0.5 g	0.1% (w/v)
D-(-)-Fructose	0.5 g	0.1% (w/v)
Soluble starch**	12.5 mL of 2% (w/v)	0.05% (w/v)
Tween 80	1 mL of 25% (v/v)	0.05% (v/v)
Meat extract	2.5 g	0.5% (w/v)
Trace Mineral Supplement	5 mL	1% (v/v)
Vitamin Supplement	5 mL	1% (v/v)
SCFA supplement**	1.4 mL	0.28% (v/v)
Milli-Q water (dH_2O)*	150 mL	

TABLE 2 – MEDIUM B

Component	**Amount (in 1000 mL)**	**Final Concentration**
Lean Ground Beef (Fat Free)	500 g	50% (w/v)
NaOH	25 mL	2.5% (v/v)
Casitone	30 g	3% (w/v)
Yeast Extract	5 g	0.5% (w/v)
K_2HPO_4	5 g	0.5% (w/v)
Resazurin	1 mg	0.01% (w/v)
(±) Haemin Solution [1 mL 1N NaOH in 100 mL of dH_2O]	10.0 mL	1% (v/v)
(±) Vitamin K_1 Solution [0.1 mL Vitamin K_1 in 20 mL of 95% Ethanol] or Vitamin K_3 Solution [0.05 mg/mL Vitamin K_3 ub 95% Ethanol]	10.0 mL	1% (v/v)
$NaHCO_3$	1 g	0.1% (w/v)
Milli-Q water (dH_2O)*	To Final Volume of 1000 mL	
pH adjusted to 7.2		

TABLE 3 – MEDIUM C

Component	Amount (in 1000 mL)	Final Concentration
Trypticase Peptone	5 g	0.5% (w/v)
Peptone	5 g	0.5% (w/v)
Yeast Extract	10 g	1% (w/v)
Beef Extract	5 g	0.5% (w/v)
Glucose	5 g	0.5% (w/v)
K_2HPO_4	2 g	0.2% (w/v)
Tween-80	1 mL	0.1% (v/v)
Cysteine-HCl x H_2O	0.5 g	0.05% (w/v)
Resazurin	1 mg	0.01% (w/v)
Salt Solution [0.25 g $CaCl_2$ x 2 H_2O, 0.5 g $MgSO_4$ x 7 H_2O, 1 g K_2HPO_4, 1 g KH_2PO_4, 10 g $NaHCO_3$, 2 g NaCl, to 1000 mL in dH_2O]	40 mL	4 % (v/v)
Haemin Solution [1 mL 1N NaOH in 100 mL of dH_2O]	10.0 mL	1% (v/v)
Vitamin K_1 Solution [0.1 mL Vitamin K_1 in 20 mL of 95% Ethanol]	0.2 mL	0.02% (v/v)

Milli-Q water (dH_2O)*	To Final Volume of 1000 mL	
pH adjusted to 7.2 using 8N NaOH		

TABLE 4 – MEDIUM D

Component	Amount (in 1000 mL)	Final Concentration
Tryptose	10 g	1% (w/v)
Beef Extract	10 g	1% (w/v)
Yeast Extract	3 g	0.3% (w/v)
Dextrose	5 g	0.5% (w/v)
NaCl	5 g	0.5% (w/v)
Soluble Starch	1 g	0.1% (w/v)
L-Cysteine HCl	0.5 g	0.05% (v/v)
Sodium Acetate	3 g	0.3% (w/v)
Resazurin (0.025%)	4 mL	0.0001% (w/v)
Milli-Q water (dH_2O)*	To Final Volume of 1000 mL	
pH adjusted to 6.8		

TABLE 5 – MEDIUM F

Component	Amount (in 1000 mL)	Final Concentration
Casein peptone, tryptic digest	10 g	1% (w/v)
Yeast Extract	5 g	0.5% (w/v)
Meat Extract	5 g	0.5% (w/v)
Bacto Soytone	5 g	0.5% (w/v)
Glucose	10 g	1% (w/v)
K_2HPO_4	2 g	0.2% (w/v)

$MgSO_4$ x 7 H_2O	0.2 g	0.02% (w/v)
$MnSO_4$ x H_2O	0.05 g	0.005% (w/v)
Tween 80	1 ml	0.1% (v/v)
NaCl	5 g	0.5% (w/v)
Cysteine-HCl x H_2O	0.5 g	0.05% (w/v)
Salt Solution [0.25 g $CaCl_2$ x 2 H_2O, 0.5 g $MgSO_4$ x 7 H_2O, 1 g K_2HPO_4, 1 g KH_2PO_4, 10 g $NaHCO_3$, 2 g NaCl, to 1000 mL in dH_2O]	40 mL	4 % (v/v)
Resazurin (25 mg/100 mL)	4 mL	0.01% (w/v)
Milli-Q water (dH_2O)*	To Final Volume of 1000 mL	
pH adjusted to 6.8 using 8N NaOH		

TABLE 6 – YCFAC Broth

Component	**Amount (in 1000 mL)**	**Final Concentration**
Casitone	10 g	1% (w/v)
Yeast Extract	2.5 g	0.25% (w/v)
Sodium Bicarbonate	4 g	0.4% (w/v)
Glucose	2 g	0.2% (w/v)
Cellobiose	2 g	0.2% (w/v)
Maltose	2 g	0.2% (w/v)

Potassium Phosphate Monobasic	0.45 g	0.045% (w/v)
Potassium Phosphate Dibasic	0.45 g	0.045% (w/v)
Sodium Chloride	0.9 g	0.09% (w/v)
Ammonium Sulfate	0.9 g	0.09% (w/v)
Magnesium Sulfate Heptahydrate	0.09 g	0.009% (w/v)
Calcium chloride	0.09 g	0.009% (w/v)
Hemin (0.1% solution)	10 mL	0.001% (w/v)
Vitamin Supplement [Folic acid 2.0 mg/l, Pyridoxine hydrochloride 10.0 mg/L, Riboflavin 5.0 mg/L, Biotin 2.0 mg/L. Thiamine 5.0 mg/L, Nicotinic acid 5.0 mg/L, Calcium Pantothenate 5.0 mg/liter, Vitamin B12 0.1 mg/L, p-	10 mL	1 % (v/v)

Aminobenzoic acid 5.0 mg/L, Thioctic acid 5.0 mg/L, Monopotassium phosphate 900.0 mg/L]		
Resazurin (0.025% solution)	4 mL	0.0001% (w/v)
L-Cysteine (25% solution)	4 mL	0.01% (w/v)
Volatile Fatty Acid Solution	2.9 mL	0.29% (v/v)
Milli-Q water (dH_2O)*	To Final Volume of 1000 mL	
pH adjusted to 6.8		

3.2. Engraftment

[0050] As used herein, “engraftment” (and grammatical variants thereof, *e.g.*, "engraft") refers to the ability of a microbial strain or microbial community to establish in one or more niches of the gut of an animal. Operationally, a microbial strain or microbial community is “engrafted” if evidence of its establishment, post-administration, can be obtained. In some embodiments, that evidence is obtained by molecular identification (*e.g.*, Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS), liquid chromatography-mass spectrometry (LC-MS), 16S rRNA sequencing, or genomic sequencing) of a sample obtained from the animal. In some embodiments, the sample is a stool sample. In some embodiments, the sample is a biopsy sample taken from the gut of the animal (*e.g.*, from a location along the gastrointestinal tract of the animal). Engraftment may be transient or may be persistent. In some embodiments, transient engraftment means that the microbial strain or microbial community can no longer be detected in an animal to which it has been administered

after the lapse of about 1 week, about 2 weeks, about three weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 6 months, about 8 month, about 10 months, about 1 year, about 1.5 years, or about 2 years.

[0051] As used herein, "substantial engraftment" refers to that at a defined timepoint following administration to an animal (*e.g.*, in some embodiments, a gnotobiotic mouse) of the microbial community (*e.g.*, a high-complexity defined gut microbial community), evidence of the engraftment of at least 70% of the administered defined microbial strains can be demonstrated. For example, in some embodiments, substantial engraftment is achieved when at least 72%, at least 74%, at least 76%, at least 78%, at least 80%, at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or 100% of the administered defined microbial strains can be demonstrated. In some embodiments, such evidence is obtained by metagenomic analysis of a stool sample obtained from the animal. In some embodiments, "substantial engraftment" is achieved when an intended metabolic phenotype is demonstrably present in the recipient post-administration. In some embodiments, the defined timepoint is between 1 week and 52 weeks. For example, in some embodiments, the defined timepoint is between 1 week and 48 weeks, 1 week and 42 weeks, 1 week and 36 weeks, 1 week and 30 weeks, 1 week and 24 week, 1 week and 18 weeks, 1 week and 12 weeks, 1 week and 10 weeks, 1 week and 8 weeks, 1 week and 6 weeks, 1 week and 4 weeks, 1 week and 2 weeks, 2 weeks and 52 weeks, 2 weeks and 48 weeks, 2 weeks and 36 weeks, 2 weeks and 30 weeks, 2 ad 24 weeks, 2 weeks and 18 weeks, 2 weeks and 12 weeks, 2 weeks and 10 weeks, 2 weeks and 8 weeks, 2 weeks and 6 weeks, 2 weeks and 4 weeks, 4 weeks and 52 weeks, 4 weeks and 48 weeks, 4 weeks and 42 weeks, 4 weeks and 36 weeks, 4 weeks and 30 weeks, 4 weeks and 24 weeks, 4 weeks and 18 weeks, 4 weeks and 12 weeks, 4 weeks and 10 weeks, 4 weeks and 8 weeks, 4 weeks and 6 weeks, 6 weeks and 52 weeks, 6 weeks and 48 weeks, 6 weeks and 42 weeks, 6 weeks and 36 weeks, 6 weeks and 30 weeks, 6 weeks and 24 weeks, 6 weeks and 18 weeks, 6 weeks and 12 weeks, 6 weeks and 10 weeks, 6 weeks and 8 weeks, 8 weeks and 52 weeks, 8 weeks and 48 weeks, 8 weeks and 42 weeks, 8 weeks and 36 weeks, 8 weeks and 30 weeks, 8 weeks and 24 weeks, 8 weeks and 18 weeks, 8 weeks and 12 weeks, or 8 weeks and 10 weeks.

3.3. Stability

[0052] As used herein, "human fecal community microbial challenge" refers to administration of a human stool sample into the gut of an animal that has previously been

colonized with a microbial community, *e.g.*, a high-complexity defined gut microbial community.

[0053] In some embodiments, stability of a community refers to the ability of defined microbial strains comprising a community to persist (*i.e.* remain engrafted) in a gut of an animal following microbial challenge. In some embodiments, when given sufficient time to permit colonization of microbial challenge strains in the gut of an animal engrafted with a high-complexity defined gut microbial community, a stable community can be defined as one where at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the defined microbial strains are detectable by metagenomic analysis. For example, in some embodiments, metagenomic analysis comprises whole genome shotgun sequencing analysis.

[0054] In some embodiments, stability can be demonstrated at a time range of between at least 1 week and 52 weeks. For example, in some embodiments, stability can be demonstrated at a time rage of between at least 1 week and 48 weeks, 1 week and 42 weeks, 1 week and 36 weeks, 1 week and 30 weeks, 1 week and 24 week, 1 week and 18 weeks, 1 week and 12 weeks, 1 week and 10 weeks, 1 week and 8 weeks, 1 week and 6 weeks, 1 week and 4 weeks, 1 week and 2 weeks, 2 weeks and 52 weeks, 2 weeks and 48 weeks, 2 weeks and 36 weeks, 2 weeks and 30 weeks, 2 ad 24 weeks, 2 weeks and 18 weeks, 2 weeks and 12 weeks, 2 weeks and 10 weeks, 2 weeks and 8 weeks, 2 weeks and 6 weeks, 2 weeks and 4 weeks, 4 weeks and 52 weeks, 4 weeks and 48 weeks, 4 weeks and 42 weeks, 4 weeks and 36 weeks, 4 weeks and 30 weeks, 4 weeks and 24 weeks, 4 weeks and 18 weeks, 4 weeks and 12 weeks, 4 weeks and 10 weeks, 4 weeks and 8 weeks, 4 weeks and 6 weeks, 6 weeks and 52 weeks, 6 weeks and 48 weeks, 6 weeks and 42 weeks, 6 weeks and 36 weeks, 6 weeks and 30 weeks, 6 weeks and 24 weeks, 6 weeks and 18 weeks, 6 weeks and 12 weeks, 6 weeks and 10 weeks, 6 weeks and 8 weeks, 8 weeks and 52 weeks, 8 weeks and 48 weeks, 8 weeks and 42 weeks, 8 weeks and 36 weeks, 8 weeks and 30 weeks, 8 weeks and 24 weeks, 8 weeks and 18 weeks, 8 weeks and 12 weeks, or 8 weeks and 10 weeks.

[0055] In other embodiments, stability of a community refers to the characteristic of defined microbial strains comprising a community to maintain a metabolic phenotype over a period of time or following microbial challenge. For example, in some embodiments, defined microbial strains comprising a community can maintain a metabolic phenotype for at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 6 weeks, at least 8 weeks, at least 10 weeks, at least 12 weeks, at least 4 months, at least 6 months at least 8 months, at least 10 months, at least 1 year, at least 1.5 years, or at least 2 years.

[0056] In some embodiments, a stable community can be defined as one where the defined microbial strains comprising the community maintain the ability to metabolize at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of substrates selected from the group consisting of: a-mannan (yeast), acetate, agarose, alanine, alginate, anthocyanin, arabinan, arabinogalactan, arabinoxylan, arginine, asparagine, Aspartate, b-glucans, butyrate, carrageenan, chitin, chlorogenic acids, chondroitin sulfate, cinnamic acid, Cysteine, dextran (40), Dihydrochalcones, Enterodiol, flavan-3-ols, flavanones, flavones, flavonols, folate, formate, galactomannan (carob), galacturonan (homo), galacturonate, glucomannan (konjac), glutamate, Glutamine, Glycine, Histidine, hyaluronan, hydrogen, hydroxycinnamic acids, hydroxyproline, inulin, isoflavones/isoflavanones, Isoleucine, lactate, laminarin, Leucine, levan, Lysine, Methionine, mucin O-linked glycans, Ornithine, Phenylalanine, porphyran, Proline, propionate, rhamnogalacturonan I, rhamnogalacturonan II, Secoisolariciresinol diglucoside, Serine, starch (potato), starch (structure 1), thiamine, Threonine, tryptophan, Tyrosine, Valine, xyloglucan, and xylooligosaccharides (XOS), over a period of time or following microbial challenge.

[0057] In some embodiments, a stable community can be defined as one where the defined microbial strains comprising the community maintain the ability to produce at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of metabolites selected from the group consisting of: formate, acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, 2-methylbutyrate, caporate, isocaporate, 3-methylvaleric acid, L-phenylalanine, 3-phenylpropionic acid, phyenypyruvate, DL-3-phenyllactic acid, trans-cinnamic acid, phenyllactic acid, phenethylamine, L-tyrosine, 3-(4-hydroxyphenyl)propionic acid, 3-(4-hydroxyphenyl) pyruvic acid, DL-p-hydroxyphenyl lactic acid, p-coumaric acid, 4-hydroxyphenyl acetic acid, tyramine, phenol, p-cresol, 4-ethylphenol, 4-vinylphenol, 4-hydroxybenzoic acid, L-tryptophan, 3-indolepropionic acid, 3-indolepyruvic acid, DL-indole-3-lactic acid, trans-3-indoleacrylic acid, 3-indoleacetic acid, tryptamine, indole, skatol, indole-3-carboxylic acid, indole-3-carboxyaldehyde, N-acetyl-L-phenylalanine, phenylpropionylglycine, 3-(3-hydroxyphenyl) propionic acid, cinnamoylglycine, phenylacetylglycine, phenylacetylglutamine, hippuric acid, 2-hydroxyhippuric acid, 3-hydroxyhippuric acid, 4-hydroxyhippuric acid, 4-hydroxyphenylacetylglycine, phenyl sulfate, phenyl glucuronide, p-cresol sulfate, p-cresol glucuronide, 4-ethylphenol sulfate, 4-ethylphenol glucuronide, N-acetyl-L-tryptophan, 5-hydroxy-L-typtophan, N-acetylserotonin, 3-indolepriopionylglycine, indolyl-3-acryloylglycine, indoxyl sulfate, indoxyl glucuronide, 5-hydroxyindole-3-acetic acid, indoleacetylglycine, lithocholic acid, murocholic acid,

ursodeoxycholic acid, hyodeoxycholic acid, deoxycholic acid, 7-oxocholic acid, ω-muricholic acid, α-muricholic acid, β-muricholic acid, γ-muricholic acid, 7βcholic acid, taurolithocholic acid, tauroursodeoxycholic acid, taurohyodeoxycholic acid, taurochenodeoxycholic acid, taurodeoxycholic acid, tauro-β-muricholic acid, tauro- ω-muricholic acid, and taurocholic acid, over a period of time or following microbial challenge.

[0058] In some embodiments, a stable community can be defined as one where the ability to utilize at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the MetaCyc metabolic pathways selected from the group consisting of: 1CMET2-PWY, 2.6.1.32-RXN, AEROBACTINSYN-PWY, ALACAT2-PWY, ALADEG-PWY, ALANINE-DEG3-PWY, ALANINE-SYN2-PWY, ALANINE-VALINESYN-PWY, ANAPHENOXI-PWY, ARGASEDEG-PWY, ARGDEG-III-PWY, ARGDEG-IV-PWY, ARGDEGRAD-PWY, ARGDEG-V-PWY, ARG-GLU-PWY, ARGININE-SYN4-PWY, ARG-PRO-PWY, ARGSYNBSUB-PWY, ARGSYN-PWY, ASPARAGINE-BIOSYNTHESIS, ASPARAGINE-DEG1-PWY, ASPARAGINE-DEG1-PWY-1, ASPARAGINESYN-PWY, ASPARTATE-DEG1-PWY, ASPARTATESYN-PWY, ASPASN-PWY, ASPSYNII-PWY, AST-PWY, BETA-ALA-DEGRADATION-I-PWY, CAMALEXIN-SYN, CITRULBIO-PWY, CITRULLINE-DEG-PWY, COA-PWY, CODH-PWY, CYSTEINE-DEG-PWY, CYSTSYN-PWY, DAPLYSINESYN-PWY, ENTBACSYN-PWY, ETHYL-PWY, FAO-PWY, FERMENTATION-PWY, GLNSYN-PWY, GLUDEG-I-PWY, GLUGLNSYN-PWY, GLUTAMATE-DEG1-PWY, GLUTAMATE-SYN2-PWY, GLUTAMINDEG-PWY, GLUTAMINEFUM-PWY, GLUTATHIONESYN-PWY, GLUTDEG-PWY, GLUTORN-PWY, GLUTSYNIII-PWY, GLUTSYN-PWY, GLYCGREAT-PWY, GLYSYN-ALA-PWY, GLYSYN-PWY, GLYSYN-THR-PWY, HISDEG-PWY, HISHP-PWY, HISTDEG-PWY, HISTSYN-PWY, HOMOCYSDEGR-PWY, HOMOSER-METSYN-PWY, HOMOSERSYN-PWY, HSERMETANA-PWY, HYDROXYPRODEG-PWY, ILEUDEG-PWY, ILEUSYN-PWY, LARABITOLUTIL-PWY, LCYSDEG-PWY, LEU-DEG2-PWY, LEUSYN-PWY, LYSDEGII-PWY, LYSINE-AMINOAD-PWY, LYSINE-DEG1-PWY, MALATE-ASPARTATE-SHUTTLE-PWY, METH-ACETATE-PWY, METHANOGENESIS-PWY, METHIONINE-DEG1-PWY, MGLDLCTANA-PWY, ORN-AMINOPENTANOATE-CAT-PWY, ORNDEG-PWY, P101-PWY, P162-PWY, P163-PWY, P181-PWY, P261-PWY, P283-PWY, P401-PWY, P541-PWY, PHENYLALANINE-DEG1-PWY, PHESYN, PHOSLIPSYN2-PWY, PHOSPHONOTASE-PWY, PROSYN-PWY, PROUT-PWY, PWY0-1021, PWY0-1221, PWY0-1299, PWY0-1303, PWY0-1305, PWY0-1313, PWY0-1317, PWY0-1321, PWY0-1338, PWY0-1347, PWY0-1355, PWY0-1356, PWY0-1534, PWY0-1544, PWY0-

1565, PWY0-1576, PWY0-1577, PWY0-1578, PWY0-1585, PWY0-1601, PWY0-42, PWY0-461, PWY0-823, PWY0-901, PWY-1, PWY-1061, PWY-1121, PWY-1186, PWY1-2, PWY-1263, PWY-1622, PWY-1722, PWY-1781, PWY-181, PWY-1881, PWY-1962, PWY-1981, PWY1F-467, PWY1F-FLAVSYN, PWY1G-0, PWY-2021, PWY-2161, PWY-2181, PWY-2201, PWY-2821, PWY-2941, PWY-2942, PWY-3, PWY-3022, PWY-3081, PWY-3161, PWY-3162, PWY-3181, PWY-3301, PWY-3341, PWY-3385, PWY-3461, PWY-3462, PWY-3581, PWY-361, PWY-3661, PWY-3661-1, PWY-381, PWY-3841, PWY-3941, PWY3DJ-12, PWY3O-4107, PWY3O-4108, PWY-4, PWY-40, PWY-4002, PWY-4041, PWY-4201, PWY-4281, PWY-43, PWY-4321, PWY-4341, PWY-4361, PWY-46, PWY490-3, PWY490-4, PWY-4981, PWY-4983, PWY-4984, PWY4FS-6, PWY-5, PWY-5022, PWY-5024, PWY-5028, PWY-5029, PWY-5030, PWY-5031, PWY-5041, PWY-5048, PWY-5049, PWY-5057, PWY-5075, PWY-5076, PWY-5078, PWY-5079, PWY-5081, PWY-5082, PWY-5087, PWY-5097, PWY-5101, PWY-5103, PWY-5104, PWY-5108, PWY-5109, PWY-5129, PWY-5135, PWY-5136, PWY-5151, PWY-5154, PWY-5155, PWY-5159, PWY-5176, PWY-5188, PWY-5189, PWY-5196, PWY-5199, PWY-5207, PWY-5250, PWY-5254, PWY-5265, PWY-5280, PWY-5283, PWY-5290, PWY-5297, PWY-5298, PWY-5311, PWY-5314, PWY-5316, PWY-5319, PWY-5324, PWY-5329, PWY-5331, PWY-5332, PWY-5364, PWY-5381, PWY-5382, PWY-5386, PWY-5394, PWY-5399, PWY-5436, PWY-5437, PWY-5441, PWY-5443, PWY-5458, PWY-5467, PWY-5468, PWY-5473, PWY-5474, PWY-5494, PWY-5497, PWY-5499, PWY-5629, PWY-5651, PWY-5653, PWY-5665, PWY-5669, PWY-5675, PWY-5679, PWY-5686, PWY-5710, PWY-5736, PWY-5737, PWY-5739, PWY-5740, PWY-5742, PWY-5747, PWY-5748, PWY-5751, PWY-5754, PWY-5766, PWY-5770, PWY-5784, PWY-5788, PWY-5797, PWY-5800, PWY-581, PWY-5811, PWY-5818, PWY-5826, PWY-5877, PWY-5883, PWY-5886, PWY-5912, PWY-5913, PWY-5921, PWY-5936, PWY-5940, PWY-5958, PWY-5963, PWY-5968, PWY-5978, PWY-5980, PWY-5990, PWY-6003, PWY-6004, PWY-601, PWY-6030, PWY-6039, PWY-6045, PWY-6052, PWY-6053, PWY-6054, PWY-6055, PWY-6068, PWY-6069, PWY-6082, PWY-6120, PWY-6121, PWY-6122, PWY-6123, PWY-6124, PWY-6133, PWY-6134, PWY-6141, PWY-6143, PWY-6148, PWY-6151, PWY-6160, PWY-6173, PWY-6196, PWY-6219, PWY-622, PWY-6220, PWY-6233, PWY-6273, PWY-6277, PWY-6281, PWY-6307, PWY-6309, PWY-6313, PWY-6318, PWY-6320, PWY-6321, PWY-6322, PWY-6324, PWY-6328, PWY-6334, PWY-6339, PWY-6343, PWY-6344, PWY-6345, PWY-6346, PWY-6375, PWY-6376, PWY-6381, PWY-6386, PWY-6387, PWY-6397, PWY-6403, PWY-6407, PWY-6408, PWY-6409, PWY-6431, PWY-6435, PWY-6444, PWY-6455, PWY-6456, PWY-6457, PWY-6466, PWY-6471, PWY-6473, PWY-6481, PWY-6486, PWY-6493,

PWY-6495, PWY-6511, PWY-6512, PWY-6519, PWY-6533, PWY-6535, PWY-6536, PWY-6537, PWY-6543, PWY-6549, PWY-6559, PWY-6562, PWY-6572, PWY-6573, PWY-6574, PWY-6578, PWY-6588, PWY-6614, PWY-6627, PWY66-301, PWY66-375, PWY-6638, PWY66-391, PWY-6642, PWY66-420, PWY66-421, PWY66-425, PWY66-426, PWY66-428, PWY-6643, PWY-6661, PWY-6673, PWY-6682, PWY-6690, PWY-6696, PWY-6711, PWY-6717, PWY-6720, PWY-6724, PWY-6728, PWY-6731, PWY-6735, PWY-6749, PWY-6769, PWY-6771, PWY-6772, PWY-6773, PWY-6781, PWY-6784, PWY-6790, PWY-6791, PWY-6802, PWY-6807, PWY-6808, PWY-6813, PWY-6815, PWY-6816, PWY-6817, PWY-6818, PWY-6821, PWY-6822, PWY-6823, PWY-6831, PWY-6832, PWY-6834, PWY-6840, PWY-6845, PWY-6853, PWY-6854, PWY-6855, PWY-6891, PWY-6892, PWY-6896, PWY-6898, PWY-6902, PWY-6907, PWY-6908, PWY-6920, PWY-6922, PWY-6936, PWY-6942, PWY-6949, PWY-6953, PWY-6955, PWY-6963, PWY-6964, PWY-6965, PWY-6968, PWY-6969, PWY-6981, PWY-6982, PWY-6986, PWY-6994, PWY-7000, PWY-701, PWY-7014, PWY-7015, PWY-7016, PWY-7018, PWY-7019, PWY-702, PWY-7022, PWY-7025, PWY-7028, PWY-7040, PWY-7046, PWY-7052, PWY-7054, PWY-7064, PWY-7072, PWY-7088, PWY-7090, PWY-7097, PWY-7104, PWY-7115, PWY-7117, PWY-7118, PWY-7126, PWY-7147, PWY-7153, PWY-7158, PWY-7176, PWY-7177, PWY-7185, PWY-7186, PWY-7219, PWY-7221, PWY-7234, PWY-7246, PWY-7248, PWY-7250, PWY-7255, PWY-7274, PWY-7275, PWY-7282, PWY-7288, PWY-7297, PWY-7304, PWY-7315, PWY-7316, PWY-7318, PWY-7342, PWY-7351, PWY-7356, PWY-7376, PWY-7377, PWY-7383, PWY-7387, PWY-7397, PWY-7398, PWY-7400, PWY-7414, PWY-7425, PWY-7430, PWY-7432, PWY-7440, PWY-7441, PWY-7456, PWY-7467, PWY-7498, PWY-7501, PWY-7506, PWY-7510, PWY-7514, PWY-7518, PWY-7520, PWY-7525, PWY-7531, PWY-7532, PWY-7533, PWY-7536, PWY-7542, PWY-7543, PWY-7547, PWY-7549, PWY-7550, PWY-7555, PWY-7561, PWY-7565, PWY-7570, PWY-7571, PWY-7600, PWY-7605, PWY-761, PWY-7612, PWY-7626, PWY-7645, PWY-7648, PWY-7649, PWY-7650, PWY-7665, PWY-7667, PWY-7668, PWY-7669, PWY-7671, PWY-7674, PWY-7688, PWY-7690, PWY-7693, PWY-7694, PWY-7701, PWY-7704, PWY-7706, PWY-7708, PWY-7717, PWY-7718, PWY-7719, PWY-7733, PWY-7734, PWY-7735, PWY-7737, PWY-7751, PWY-7761, PWY-7765, PWY-7767, PWY-7769, PWY-7770, PWY-7782, PWY-7790, PWY-7791, PWY-7793, PWY-7797, PWY-7811, PWY-7814, PWY-7822, PWY-7824, PWY-7826, PWY-7842, PWY-7850, PWY-7851, PWY-7855, PWY-7860, PWY-7861, PWY-7863, PWY-7867, PWY-7870, PWY-7880, PWY-7888, PWY-7889, PWY-7891, PWY-7892, PWY-7897, PWY-7901, PWY-7904, PWY-7907, PWY-7909, PWY-7910, PWY-7913, PWY-7917, PWY-7930, PWY-7931, PWY-7936, PWY-7953, PWY-7955,

PWY-7956, PWY-7957, PWY-7958, PWY-7959, PWY-7960, PWY-7962, PWY-7977, PWY-7985, PWY-7986, PWY-7987, PWY-7988, PWY-7990, PWY-8002, PWY-8003, PWY-8006, PWY-8007, PWY-8008, PWY-8009, PWY-801, PWY-8010, PWY-8011, PWY-8013, PWY-8014, PWY-8015, PWY-8016, PWY-8017, PWY-8024, PWY-8032, PWY-8040, PWY-8043, PWY-8045, PWY-8071, PWY-8072, PWY-8080, PWY-8081, PWY-8082, PWY-8083, PWY-8086, PWY-8088, PWY-842, PWY-861, PWY-862, PWY8J2-1, PWY8J2-22, PWYDQC-4, PWYG-321, PWY-I9, PWYQT-4450, PWYQT-4476, PYRIDNUCSAL-PWY, PYRIDNUCSYN-PWY, PYRIDOXSYN-PWY, SAM-PWY, SERDEG-PWY, SERSYN-PWY, SPHINGOLIPID-SYN-PWY, TAURINEDEG-PWY, THRDLCTCAT-PWY, THREONINE-DEG2-PWY, TRNA-CHARGING-PWY, TRPCAT-PWY, TRPIAACAT-PWY, TRPKYNCAT-PWY, TRPSYN-PWY, TRYPDEG-PWY, TYRFUMCAT-PWY, TYRSYN, UDPNACETYLGALSYN-PWY, UDPNAGSYN-PWY, VALDEG-PWY, and VALSYN-PWY, is maintained over a period of time or following microbial challenge.

[0059] In some embodiments, a stable community can be defined as one maintaining the ability to metabolize at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the substrates described above and produce at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the metabolites described above over a period of time or following microbial challenge.

[0060] In some embodiments, a stable community can be defined as one maintaining the ability to metabolize at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the substrates described above and utilize at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the MetaCyc metabolic pathways described above over a period of time or following microbial challenge.

[0061] In some embodiments, a stable community can be defined as one maintaining the ability to produce at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the metabolites described above and utilize at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the MetaCyc metabolic pathways described above over a period of time or following microbial challenge.

[0062] In some embodiments, a stable community can be defined as one maintaining the ability to metabolize at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the substrates

described above, produce at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the metabolites described above, and utilize at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the MetaCyc metabolic pathways described above over a period of time or following microbial challenge.

[0063] As used herein, "dropping out" refers to an event where a microbial strain in a microbial community does not stably engraft following administration into the gut of an animal. For example, in some embodiments, a microbial community is stable if up to 10% of the defined microbial strains drop out following microbial challenge. In some embodiments, a microbial community is stable if up to 9%, up to 8%, up to 7%, up to 6%, up to 5%, up to 4%, up to 3%, up to 2%, or up to 1% of the defined microbial strains drop out following microbial challenge.

[0064] As used herein, "jumping in" refers to an event where a microbial strain that is not present in a microbial community at the time of being administered into an animal, stably engrafts into one or more niche in the gut of the animal and becomes part of the engrafted microbial community. In some embodiments, a microbial strain that jumps in originates from an animal's gut commensal repertoire, a fecal community microbial challenge, or from an administration into the gut of an animal subsequent to an initial administration of the microbial community. For example, in some embodiments, a microbial community is stable if up to 10% of new strains are contributed by a microbial challenge (*e.g.*, a human fecal community microbial challenge). In some embodiments, a microbial community is stable if up to 9%, up to 8%, up to 7%, up to 6%, up to 5%, up to 4%, up to 3%, up to 2%, or up to 1% of new strains are contributed by a microbial challenge.

4. Metagenomic Analysis and Molecular Identification

[0065] As used herein, "metagenomic analysis" refers to use of massively parallel sequencing for analyzing a microbiome, or defined gut microbial community. As used herein, metagenomic analysis includes, without limitation, whole genome sequencing (for example, in some embodiments, whole genome shotgun sequencing), ribosomal gene sequencing, rRNA sequencing or other sequencing based methods. See, *e.g.*, Thomas *et al.*, 2012, "Metagenomics – A guide from sampling to data analysis," *Microbial Informatics and Experimentation* 2(1):3; Qin *et al.*, 2009. "A human gut microbial gene catalogue established by metagenomic sequencing," *Nature* 464 (7285): 59-65. For example, in some embodiments, metagenomic sequence reads (*i.e.* sequence fragments) obtained from a sequencing method are mapped against

a comprehensive database of complete, sequenced genomes of all the defined microbial strains comprising a gut community.

[0066] As used herein, "molecularly identified" (and grammatical variants thereof, *e.g.*, "molecular identification") refers to characterization of a microbial species for unique identification. In some embodiments, molecular identification can be 16S rRNA sequencing, whole genome sequencing, Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS), liquid chromatography-mass spectrometry (LC-MS) or similar analytical assay capable of differentiating one microbial species from another microbial species. In some embodiments, species identification is done on the level of strain identification. In some embodiments, strain identification is achieved through whole genome shotgun metagenomic sequencing. As used herein, whole genome shotgun metagenomic sequencing refers to a method of sequencing polynucleotides in parallel and with high sequence coverage from a plurality of genomic regions from a complex sample comprising a plurality of microbial species.

5. In vitro and Metabolic Phenotype

[0067] As used herein an "*in vitro* phenotype" refers to a characteristic, such as a metabolic phenotype, of a microbial community that can be measured *in vitro*. In one embodiment a microbial community is recovered from the gut of an animal. In one embodiment a microbial community is recovered from a fecal sample. In one embodiment a microbial community is an artificial community or a high-complexity defined gut microbial community.

[0068] "Metabolic phenotype" is a property of a microbial strain or a microbial community. In one aspect, a metabolic phenotype refers to the ability of a microbial strain or microbial community to transform one or more first compound(s) into one or more second compound(s). In one example a first compound is enzymatically converted by the microbe or community into a second compound, and the metabolic phenotype is an increase in the amount of the second compound. In some embodiments, metabolic phenotypes include metabolization of a-mannan (yeast), acetate, agarose, alanine, alginate, anthocyanin, arabinan, arabinogalactan, arabinoxylan, arginine, asparagine, Aspartate, b-glucans, butyrate, carrageenan, chitin, chlorogenic acids, chondroitin sulfate, cinnamic acid, Cysteine, dextran (40), Dihydrochalcones, Enterodiol, flavan-3-ols, flavanones, flavones, flavonols, folate, formate, galactomannan (carob), galacturonan (homo), galacturonate, glucomannan (konjac), glutamate, Glutamine, Glycine, Histidine, hyaluronan, hydrogen, hydroxycinnamic acids, hydroxyproline, inulin,

isoflavones/isoflavanones, Isoleucine, lactate, laminarin, Leucine, levan, Lysine, Methionine, mucin O-linked glycans, Ornithine, Phenylalanine, porphyran, Proline, propionate, rhamnogalacturonan I, rhamnogalacturonan II, Secoisolariciresinol diglucoside, Serine, starch (potato), starch (structure 1), thiamine, Threonine, tryptophan, Tyrosine, Valine, xyloglucan, and xylooligosaccharides (XOS). For example, in some embodiments, one or more of the defined microbial strains of the high-complexity defined gut microbial community metabolizes at least one, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, at least 60, or all of the substrates described above.

[0069] In some embodiments, metabolic phenotypes include the production of formate, acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, 2-methylbutyrate, caporate, isocaporate, 3-methylvaleric acid, L-phenylalanine, 3-phenylpropionic acid, phyenypyruvate, DL-3-phenyllactic acid, trans-cinnamic acid, phenyllactic acid, phenethylamine, L-tyrosine, 3-(4-hydroxyphenyl)propionic acid, 3-(4-hydroxyphenyl) pyruvic acid, DL-p-hydroxyphenyl lactic acid, p-coumaric acid, 4-hydroxyphenyl acetic acid, tyramine, phenol, p-cresol, 4-ethylphenol, 4-vinylphenol, 4-hydroxybenzoic acid, L-tryptophan, 3-indolepropionic acid, 3-indolepyruvic acid, DL-indole-3-lactic acid, trans-3-indoleacrylic acid, 3-indoleacetic acid, tryptamine, indole, skatol, indole-3-carboxylic acid, indole-3-carboxyaldehyde, N-acetyl-L-phenylalanine, phenylpropionylglycine, 3-(3-hydroxyphenyl) propionic acid, cinnamoylglycine, phenylacetylglycine, phenylacetylglutamine, hippuric acid, 2-hydroxyhippuric acid, 3-hydroxyhippuric acid, 4-hydroxyhippuric acid, 4-hydroxyphenylacetylglycine, phenyl sulfate, phenyl glucuronide, p-cresol sulfate, p-cresol glucuronide, 4-ethylphenol sulfate, 4-ethylphenol glucuronide, N-acetyl-L-tryptophan, 5-hydroxy-L-typtophan, N-acetylserotonin, 3-indolepriopionylglycine, indolyl-3-acryloylglycine, indoxyl sulfate, indoxyl glucuronide, 5-hydroxyindole-3-acetic acid, indoleacetylglycine, lithocholic acid, murocholic acid, ursodeoxycholic acid, hyodeoxycholic acid, deoxycholic acid, 7-oxocholic acid, ω-muricholic acid, α-muricholic acid, β-muricholic acid, γ-muricholic acid, 7βcholic acid, taurolithocholic acid, tauroursodeoxycholic acid, taurohyodeoxycholic acid, taurochenodeoxycholic acid, taurodeoxycholic acid, tauro-β-muricholic acid, tauro- ω-muricholic acid, and taurocholic acid. For example, in some embodiments, one or more of the defined microbial strains of the high-complexity defined gut microbial community produces at least one, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, or all of the metabolites described above.

[0070] In some embodiments, metabolic phenotypes include the encoding the enzymes catalyzing all reactions of any one or more of the 1CMET2-PWY, 2.6.1.32-RXN, AEROBACTINSYN-PWY, ALACAT2-PWY, ALADEG-PWY, ALANINE-DEG3-PWY, ALANINE-SYN2-PWY, ALANINE-VALINESYN-PWY, ANAPHENOXI-PWY, ARGASEDEG-PWY, ARGDEG-III-PWY, ARGDEG-IV-PWY, ARGDEGRAD-PWY, ARGDEG-V-PWY, ARG-GLU-PWY, ARGININE-SYN4-PWY, ARG-PRO-PWY, ARGSYNBSUB-PWY, ARGSYN-PWY, ASPARAGINE-BIOSYNTHESIS, ASPARAGINE-DEG1-PWY, ASPARAGINE-DEG1-PWY-1, ASPARAGINESYN-PWY, ASPARTATE-DEG1-PWY, ASPARTATESYN-PWY, ASPASN-PWY, ASPSYNII-PWY, AST-PWY, BETA-ALA-DEGRADATION-I-PWY, CAMALEXIN-SYN, CITRULBIO-PWY, CITRULLINE-DEG-PWY, COA-PWY, CODH-PWY, CYSTEINE-DEG-PWY, CYSTSYN-PWY, DAPLYSINESYN-PWY, ENTBACSYN-PWY, ETHYL-PWY, FAO-PWY, FERMENTATION-PWY, GLNSYN-PWY, GLUDEG-I-PWY, GLUGLNSYN-PWY, GLUTAMATE-DEG1-PWY, GLUTAMATE-SYN2-PWY, GLUTAMINDEG-PWY, GLUTAMINEFUM-PWY, GLUTATHIONESYN-PWY, GLUTDEG-PWY, GLUTORN-PWY, GLUTSYNIII-PWY, GLUTSYN-PWY, GLYCGREAT-PWY, GLYSYN-ALA-PWY, GLYSYN-PWY, GLYSYN-THR-PWY, HISDEG-PWY, HISHP-PWY, HISTDEG-PWY, HISTSYN-PWY, HOMOCYSDEGR-PWY, HOMOSER-METSYN-PWY, HOMOSERSYN-PWY, HSERMETANA-PWY, HYDROXYPRODEG-PWY, ILEUDEG-PWY, ILEUSYN-PWY, LARABITOLUTIL-PWY, LCYSDEG-PWY, LEU-DEG2-PWY, LEUSYN-PWY, LYSDEGII-PWY, LYSINE-AMINOAD-PWY, LYSINE-DEG1-PWY, MALATE-ASPARTATE-SHUTTLE-PWY, METH-ACETATE-PWY, METHANOGENESIS-PWY, METHIONINE-DEG1-PWY, MGLDLCTANA-PWY, ORN-AMINOPENTANOATE-CAT-PWY, ORNDEG-PWY, P101-PWY, P162-PWY, P163-PWY, P181-PWY, P261-PWY, P283-PWY, P401-PWY, P541-PWY, PHENYLALANINE-DEG1-PWY, PHESYN, PHOSLIPSYN2-PWY, PHOSPHONOTASE-PWY, PROSYN-PWY, PROUT-PWY, PWY0-1021, PWY0-1221, PWY0-1299, PWY0-1303, PWY0-1305, PWY0-1313, PWY0-1317, PWY0-1321, PWY0-1338, PWY0-1347, PWY0-1355, PWY0-1356, PWY0-1534, PWY0-1544, PWY0-1565, PWY0-1576, PWY0-1577, PWY0-1578, PWY0-1585, PWY0-1601, PWY0-42, PWY0-461, PWY0-823, PWY0-901, PWY-1, PWY-1061, PWY-1121, PWY-1186, PWY1-2, PWY-1263, PWY-1622, PWY-1722, PWY-1781, PWY-181, PWY-1881, PWY-1962, PWY-1981, PWY1F-467, PWY1F-FLAVSYN, PWY1G-0, PWY-2021, PWY-2161, PWY-2181, PWY-2201, PWY-2821, PWY-2941, PWY-2942, PWY-3, PWY-3022, PWY-3081, PWY-3161, PWY-3162, PWY-3181, PWY-3301, PWY-3341, PWY-3385, PWY-3461, PWY-3462, PWY-3581, PWY-361, PWY-

3661, PWY-3661-1, PWY-381, PWY-3841, PWY-3941, PWY3DJ-12, PWY3O-4107, PWY3O-4108, PWY-4, PWY-40, PWY-4002, PWY-4041, PWY-4201, PWY-4281, PWY-43, PWY-4321, PWY-4341, PWY-4361, PWY-46, PWY490-3, PWY490-4, PWY-4981, PWY-4983, PWY-4984, PWY4FS-6, PWY-5, PWY-5022, PWY-5024, PWY-5028, PWY-5029, PWY-5030, PWY-5031, PWY-5041, PWY-5048, PWY-5049, PWY-5057, PWY-5075, PWY-5076, PWY-5078, PWY-5079, PWY-5081, PWY-5082, PWY-5087, PWY-5097, PWY-5101, PWY-5103, PWY-5104, PWY-5108, PWY-5109, PWY-5129, PWY-5135, PWY-5136, PWY-5151, PWY-5154, PWY-5155, PWY-5159, PWY-5176, PWY-5188, PWY-5189, PWY-5196, PWY-5199, PWY-5207, PWY-5250, PWY-5254, PWY-5265, PWY-5280, PWY-5283, PWY-5290, PWY-5297, PWY-5298, PWY-5311, PWY-5314, PWY-5316, PWY-5319, PWY-5324, PWY-5329, PWY-5331, PWY-5332, PWY-5364, PWY-5381, PWY-5382, PWY-5386, PWY-5394, PWY-5399, PWY-5436, PWY-5437, PWY-5441, PWY-5443, PWY-5458, PWY-5467, PWY-5468, PWY-5473, PWY-5474, PWY-5494, PWY-5497, PWY-5499, PWY-5629, PWY-5651, PWY-5653, PWY-5665, PWY-5669, PWY-5675, PWY-5679, PWY-5686, PWY-5710, PWY-5736, PWY-5737, PWY-5739, PWY-5740, PWY-5742, PWY-5747, PWY-5748, PWY-5751, PWY-5754, PWY-5766, PWY-5770, PWY-5784, PWY-5788, PWY-5797, PWY-5800, PWY-581, PWY-5811, PWY-5818, PWY-5826, PWY-5877, PWY-5883, PWY-5886, PWY-5912, PWY-5913, PWY-5921, PWY-5936, PWY-5940, PWY-5958, PWY-5963, PWY-5968, PWY-5978, PWY-5980, PWY-5990, PWY-6003, PWY-6004, PWY-601, PWY-6030, PWY-6039, PWY-6045, PWY-6052, PWY-6053, PWY-6054, PWY-6055, PWY-6068, PWY-6069, PWY-6082, PWY-6120, PWY-6121, PWY-6122, PWY-6123, PWY-6124, PWY-6133, PWY-6134, PWY-6141, PWY-6143, PWY-6148, PWY-6151, PWY-6160, PWY-6173, PWY-6196, PWY-6219, PWY-622, PWY-6220, PWY-6233, PWY-6273, PWY-6277, PWY-6281, PWY-6307, PWY-6309, PWY-6313, PWY-6318, PWY-6320, PWY-6321, PWY-6322, PWY-6324, PWY-6328, PWY-6334, PWY-6339, PWY-6343, PWY-6344, PWY-6345, PWY-6346, PWY-6375, PWY-6376, PWY-6381, PWY-6386, PWY-6387, PWY-6397, PWY-6403, PWY-6407, PWY-6408, PWY-6409, PWY-6431, PWY-6435, PWY-6444, PWY-6455, PWY-6456, PWY-6457, PWY-6466, PWY-6471, PWY-6473, PWY-6481, PWY-6486, PWY-6493, PWY-6495, PWY-6511, PWY-6512, PWY-6519, PWY-6533, PWY-6535, PWY-6536, PWY-6537, PWY-6543, PWY-6549, PWY-6559, PWY-6562, PWY-6572, PWY-6573, PWY-6574, PWY-6578, PWY-6588, PWY-6614, PWY-6627, PWY66-301, PWY66-375, PWY-6638, PWY66-391, PWY-6642, PWY66-420, PWY66-421, PWY66-425, PWY66-426, PWY66-428, PWY-6643, PWY-6661, PWY-6673, PWY-6682, PWY-6690, PWY-6696, PWY-6711, PWY-6717, PWY-6720, PWY-6724, PWY-6728, PWY-6731, PWY-6735, PWY-6749, PWY-6769, PWY-6771, PWY-6772,

PWY-6773, PWY-6781, PWY-6784, PWY-6790, PWY-6791, PWY-6802, PWY-6807, PWY-6808, PWY-6813, PWY-6815, PWY-6816, PWY-6817, PWY-6818, PWY-6821, PWY-6822, PWY-6823, PWY-6831, PWY-6832, PWY-6834, PWY-6840, PWY-6845, PWY-6853, PWY-6854, PWY-6855, PWY-6891, PWY-6892, PWY-6896, PWY-6898, PWY-6902, PWY-6907, PWY-6908, PWY-6920, PWY-6922, PWY-6936, PWY-6942, PWY-6949, PWY-6953, PWY-6955, PWY-6963, PWY-6964, PWY-6965, PWY-6968, PWY-6969, PWY-6981, PWY-6982, PWY-6986, PWY-6994, PWY-7000, PWY-701, PWY-7014, PWY-7015, PWY-7016, PWY-7018, PWY-7019, PWY-702, PWY-7022, PWY-7025, PWY-7028, PWY-7040, PWY-7046, PWY-7052, PWY-7054, PWY-7064, PWY-7072, PWY-7088, PWY-7090, PWY-7097, PWY-7104, PWY-7115, PWY-7117, PWY-7118, PWY-7126, PWY-7147, PWY-7153, PWY-7158, PWY-7176, PWY-7177, PWY-7185, PWY-7186, PWY-7219, PWY-7221, PWY-7234, PWY-7246, PWY-7248, PWY-7250, PWY-7255, PWY-7274, PWY-7275, PWY-7282, PWY-7288, PWY-7297, PWY-7304, PWY-7315, PWY-7316, PWY-7318, PWY-7342, PWY-7351, PWY-7356, PWY-7376, PWY-7377, PWY-7383, PWY-7387, PWY-7397, PWY-7398, PWY-7400, PWY-7414, PWY-7425, PWY-7430, PWY-7432, PWY-7440, PWY-7441, PWY-7456, PWY-7467, PWY-7498, PWY-7501, PWY-7506, PWY-7510, PWY-7514, PWY-7518, PWY-7520, PWY-7525, PWY-7531, PWY-7532, PWY-7533, PWY-7536, PWY-7542, PWY-7543, PWY-7547, PWY-7549, PWY-7550, PWY-7555, PWY-7561, PWY-7565, PWY-7570, PWY-7571, PWY-7600, PWY-7605, PWY-761, PWY-7612, PWY-7626, PWY-7645, PWY-7648, PWY-7649, PWY-7650, PWY-7665, PWY-7667, PWY-7668, PWY-7669, PWY-7671, PWY-7674, PWY-7688, PWY-7690, PWY-7693, PWY-7694, PWY-7701, PWY-7704, PWY-7706, PWY-7708, PWY-7717, PWY-7718, PWY-7719, PWY-7733, PWY-7734, PWY-7735, PWY-7737, PWY-7751, PWY-7761, PWY-7765, PWY-7767, PWY-7769, PWY-7770, PWY-7782, PWY-7790, PWY-7791, PWY-7793, PWY-7797, PWY-7811, PWY-7814, PWY-7822, PWY-7824, PWY-7826, PWY-7842, PWY-7850, PWY-7851, PWY-7855, PWY-7860, PWY-7861, PWY-7863, PWY-7867, PWY-7870, PWY-7880, PWY-7888, PWY-7889, PWY-7891, PWY-7892, PWY-7897, PWY-7901, PWY-7904, PWY-7907, PWY-7909, PWY-7910, PWY-7913, PWY-7917, PWY-7930, PWY-7931, PWY-7936, PWY-7953, PWY-7955, PWY-7956, PWY-7957, PWY-7958, PWY-7959, PWY-7960, PWY-7962, PWY-7977, PWY-7985, PWY-7986, PWY-7987, PWY-7988, PWY-7990, PWY-8002, PWY-8003, PWY-8006, PWY-8007, PWY-8008, PWY-8009, PWY-801, PWY-8010, PWY-8011, PWY-8013, PWY-8014, PWY-8015, PWY-8016, PWY-8017, PWY-8024, PWY-8032, PWY-8040, PWY-8043, PWY-8045, PWY-8071, PWY-8072, PWY-8080, PWY-8081, PWY-8082, PWY-8083, PWY-8086, PWY-8088, PWY-842, PWY-861, PWY-862, PWY8J2-1, PWY8J2-22, PWYDQC-4, PWYG-321, PWY-I9,

PWYQT-4450, PWYQT-4476, PYRIDNUCSAL-PWY, PYRIDNUCSYN-PWY, PYRIDOXSYN-PWY, SAM-PWY, SERDEG-PWY, SERSYN-PWY, SPHINGOLIPID-SYN-PWY, TAURINEDEG-PWY, THRDLCTCAT-PWY, THREONINE-DEG2-PWY, TRNA-CHARGING-PWY, TRPCAT-PWY, TRPIAACAT-PWY, TRPKYNCAT-PWY, TRPSYN-PWY, TRYPDEG-PWY, TYRFUMCAT-PWY, TYRSYN, UDPNACETYLGALSYN-PWY, UDPNAGSYN-PWY, VALDEG-PWY, and VALSYN-PWY MetaCyc pathways. For example, in some embodiments, one or more of the defined microbial strains of the high-complexity defined gut microbial community utilizes at least one, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 125, at least 150, at least 175, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 550, at least 600, at least 650, or all of the MetaCyc metabolic pathways described above.

6. Microbial Community Backfill

[0071] "Backfill" methods for producing high-complexity defined gut microbial communities are described in International Application Number PCT/US2019/062,689, which is incorporated herein in its entirety. Backfill methods include "*in vitro* backfill" and "*in vivo* backfill." *In vitro* backfill and *in vivo* backfill may be used in combination as described below. In some embodiments, only *in* vitro backfill is used to produce a community. In some embodiments only *in vivo* backfill is performed to produce a community. The specification also describes compositions used in, or produced by, these backfill processes.

[0072] For convenience, the term "backfilling" is used to describe the process of carrying out an *in vitro* or *in vivo* backfill, and the term "backfilled community" refers to a community produced by a backfill process.

6.1 Producing a Complex Community by in vitro Backfilling

[0073] In one aspect, the invention involves producing a complex microbial community by *in vitro* backfilling. A community produced by one or more rounds of *in vitro* backfilling may be used as the starting stock for one or more rounds of *in vivo* backfilling.

6.2 The Microbial Pantry

[0074] As discussed below, a backfill process includes several steps in which an artificial community is prepared by combining several individually selected bacterial strains in the same aliquot. We have designed an initial collection of 109 organisms found in the human gut (including 104 bacterial strains most prevalent in the population and 4 archaea strains). In one aspect, the invention provides, as a useful tool for practicing the backfill method, a "Microbial Pantry," *i.e.* an array, such as a multiwell plate, of aliquots containing clonal isolates in which a substantial portion of the strains in Table 7, *e.g.*, at least 80, at least 90, at least 95, or at least 100 strains, are contained in aliquots of the array. In some embodiments the array is a multiwell plate. Also contemplated is a system in which any combination of individual strains in the "pantry" can be automatically robotically retrieved and combined in an aliquot. Thus, in one aspect the invention includes a system comprising an array and a robot under control of a computer for transferring bacteria. The term "Microbial Pantry" can also refer to a collection of clonal aliquots (*e.g.*, tubes) together containing at least a substantial portion of strains listed in Table 7 even if not physically associated in an array, provided the aliquots are in the same location such that any combination of strains can be retrieved. A Microbial Pantry is typically stored frozen until use. In some cases microorganisms are provided as spores.

TABLE 7: Exemplary Strains of a Microbial Pantry

Alistipes putredinis DSM 17216	*Clostridium scindens* ATCC 35704
Acidaminococcus fermentans DSM 20731	*Clostridium sp.* L2-50
Acidaminococcus sp. D21	*Clostridium sp.* M62/1
Akkermansia muciniphila ATCC BAA-835	*Clostridium spiroforme* DSM 1552
Anaerococcus lactolyticus DSMZ 7456	*Clostridium sporogenes* ATCC 15579
Anaerofustis stercorihominis DSM 17244	*Collinsella aerofaciens* ATCC 25986
Anaerostipes caccae DSM 14662	*Collinsella stercoris* DSM 13279
Anaerotruncus colihominis DSM 17241	*Coprococcus comes* ATCC 27758
Bacteroides capillosus ATCC 29799	*Coprococcus eutactus* ATCC 27759
Bacteroides cellulosilyticus DSM 14838	*Desulfovibrio piger* ATCC 29098
Bacteroides coprocola DSM 17136	*Dialister invisus* DSM 15470
Bacteroides coprophilus DSM 18228	*Dorea formicigenerans* ATCC 27755
Bacteroides dorei 5_1_36/D4 (HM 29)	*Dorea longicatena* DSM 13814
Bacteroides dorei DSM 17855	*Eggerthella lenta* DSM 2243
Bacteroides eggerthii DSM 20697	*Ethanoligenens harbinense* DSMZ 18485
Bacteroides finegoldii DSM 17565	*Eubacterium biforme* DSM 3989

Bacteroides fragilis 3_1_12	*Eubacterium dolichum* DSM 3991
Bacteroides intestinalis DSM 17393	*Eubacterium eligens* ATCC 27750
Bacteroides ovatus ATCC 8483	*Eubacterium hallii* DSM 3353
Bacteroides pectinophilus ATCC 43243	*Eubacterium rectale* ATCC 33656
Bacteroides plebeius DSM 17135	*Eubacterium siraeum* DSM 15702
Bacteroides sp. 1_1_6	*Eubacterium ventriosum* ATCC 27560
Bacteroides sp. 2_1_16	*Faecalibacterium prausnitzii* A2-165
Bacteroides sp. 2_1_22	*Granulicatella adiacens* ATCC 49175
Bacteroides sp. 3_1_19	*Holdemania filiformis* DSM 12042
Bacteroides sp. 4_3_ 47FAA	*Lactobacillus ruminis* ATCC 25644
Bacteroides sp. 9_1_ 42FAA	*Lactococcus lactis* DSMZ 20729
Bacteroides sp. D2	*Mitsuokella multacida* DSM 20544
Bacteroides stercoris ATCC 43183	*Olsenella uli* DSM 7084
Bacteroides stercoris DSMZ 19555	*Parabacteroides distasonis* ATCC 8503
Bacteroides thetaiotaomicron VPl-5482	*Parabacteroides johnsonii* DSM 18315
Bacteroides uniformis ATCC 8492	*Parabacteroides merdae* DSMZ 19495
Bacteroides vulgatus ATCC 8482	*Parabacteroides sp.* D13
Bacteroides xylanisolvens DSMZ 23964	*Prevotella buccae* D17
Bifidobacterium adolescentis L2-32	*Prevotella buccalis* DSMZ 20616
Bifidobacterium longum infantis ATCC 55813	*Prevotella copri* DSM 18205
	Roseburia intestinalis Ll-82
Bifidobacterium pseudocatenulatum DSM 20438	*Roseburia inulinivorans* DSM 16841
	Ruminococcus albus strain 8
Bilophila wadsworthia DSM 11045	*Ruminococcus bromii* ATCC 27255
Blautia hansenii DSM 20583	*Ruminococcus flavefaciens* FD 1
Blautia hydrogenotrophica DSM 10507	*Ruminococcus gnavus* ATCC 29149
Bryantella formatexigens DSM 14469	*Ruminococcus lactaris* ATCC 29176
Butyrivibrio crossotus DSM 2876	*Ruminococcus obeum* ATCC 29174
Catenibacterium mitsuokai DSM 15897	*Ruminococcus torques* ATCC 27756
Clostridium asparagiforme DSM 15981	*Slackia exigua* DSMZ 15923
Clostridium bartlettii DSM 16795	*Slackia heliotrinireducens* DSM 20476
Clostridium bolteae ATCC BAA-613	*Solobacterium moorei* DSM 22971
Clostridium hathewayi DSM 13479	*Streptococcus thermophilus* LMD-9
Clostridium hylemonae DSM 15053	*Subdoligranulum variabile* DSM 15176

Clostridium leptum DSM 753 *Clostridium methylpentosum* DSM 5476 *Clostridium nexile* DSM 1787 *Clostridium saccharolyticum* WMl DSMZ 2544 *Methanobrevibacter smithii* Balch and Wolfe 1981 strain ALI (DSMZ 2375) *Methanobrevibacter smithii* Balch and Wolfe 1981 strain Fl (DSMZ 2374)	*Veillonella dispar* ATCC 17748 *Veillonella sp.* 6_1_27 *Methanobrevibacter smithii* Balch and Wolfe 1981 strain B181 (DSMZ 11975) *Methanobrevibacter smithii* Balch and Wolfe 1981 strain PS (DSMZ 861)

[0075] In addition to the strains listed in Table 7, it is contemplated that other bacterial strains (which will typically be anaerobes or facultative anaerobes) may be used in backfill methods, including non-naturally occurring genetically modified organisms. Exemplary genetic modifications include, without limitation, mutation or knock out of enzyme-encoding genes and expression of heterologous genes.

6.3 First Backfill Community

[0076] Backfilling is an iterative process. A "first backfill community" is prepared by combining strains of a "scaffold community" with "backfill strains." Broadly speaking, and without intending to be bound by a particular mechanism, the scaffold community is a combination of strains selected to produce a desired metabolic phenotype. Backfill strains are a combination of strains selected to include strains that contribute to the stability of the first backfill community *in vitro* and contribute to the stability of a resulting transplantable community in the human gut. Without intending to be bound by a particular mechanism, it is believed that the backfill processes increase the complexity of the community and that communities with higher complexity tend to inhabit more niches in the gut and be more stable.

6.4 Scaffold Community

[0077] A scaffold community comprises a plurality of strains common in the human gut microbiome. A given scaffold community typically contains 5-100 strains, usually 10-30 strains. The scaffold community may comprise one or more strains listed in Table 7 such as, for example, at least 5, at least 10, at least 20, or at least 30 strains listed in Table 7. In some

approaches, at least 50%, 75%, 90% or all of the strains in a scaffold community are selected from Table 7.

[0078] The scaffold community is selected to exhibit a desired phenotype, typically a desired metabolic phenotype. A "metabolic phenotype" of a community, as described above, refers to the production or consumption of metabolites by the community. An exemplary metabolic phenotype is the ability to increase or decrease the concentration of a compound or compounds in the environment as a result of microbial metabolic processes. For example, a scaffold community comprising *Clostridium sporogenes* may consume phenylalanine and produce tyrosine, in which case the metabolic phenotype could be "produce tyrosine." Similarly, a community comprising *Proteus mirabilis* in an environment containing urea may decrease the concentration of urea and increase the concentration of ammonia, and a community comprising *Bacillus subtilis* in an environment containing sucrose may decrease the concentration of sucrose and increase the concentration of glucose. Importantly, however, these simple illustrations vastly oversimplify the metabolic processes that occur in a microbial ecosystem. For example, the metabolic product of a first member of a microbial community may be a metabolic substrate for a second member of the community, or the metabolic product of one member of the microbial community may be a transcriptional activator in another microbe or, alternatively, may be toxic to the other microbe. In a complex microbial ecosystem comprising hundreds of different strains, it is not possible, using current methods, to accurately predict the network of interactions of strains, metabolites, and environmental factors of a particular microbial ecosystem even if the identity of each species present is known. Further, unless or until a microbial ecosystem is at homeostasis, the combination of strains in the population will be unstable and may change in unpredictable ways, which may change the metabolic phenotype of the community.

6.5 Creating First in vitro Backfill Communities by Adding Backfill Strains to Scaffold Communities

[0079] To create a first *in vitro* backfill community, the designed scaffold community is supplemented with additional microbial strains referred to as "backfill strains." For example, each scaffold community may be combined with 35 to 495 additional strains. In some embodiments, each scaffold community may be combined with between 40 and 400, between 40 and 300, between 40 and 200, between 40 and 150, between 40 and 140, between 40 and 130, between 40 and 120, between 40 and 110, between 40 and 100, between 50 and 400, between 50

and 300, between 50 and 200, between 50 and 150, between 50 and 140, between 50 and 130, between 50 and 120, between 50 and 110, between 50 and 100, between 60 and 400, between 60 and 300, between 60 and 200, between 60 and 150, between 60 and 140, between 60 and 130, between 60 and 120, between 60 and 110, between 60 and 100, between 70 and 500, between 70 and 400, between 70 and 300, between 70 and 200, between 70 and 150, between 70 and 140, between 70 and 130, between 70 and 120, between 70 and 110, between 70 and 100, between 80 and 400, between 80 and 300, between 80 and 200, between 80 and 150, between 80 and 140, between 80 and 130, between 80 and 120, between 80 and 110, between 80 and 100, between 90 and 400, between 90 and 300, between 90 and 200, between 90 and 150, between 90 and 140, between 90 and 130, between 90 and 120, between 90 and 110, between 90 and 100, between 100 and 400, between 100 and 300, between 100 and 200, between 100 and 150, between 100 and 140, between 100 and 130, between 100 and 120, or between 100 and 110 defined microbial strains. The backfill strains and the strains of the scaffold community may be combined in any order. For example, backfill strains can be added in a single batch to all of the scaffold community strains. Alternatively, subsets of scaffold community strains may be combined with subsets of the backfill strains, in any desired sequence.

6.6 Parallel Backfill Communities

[0080] *In vitro* backfill methods are carried out according to the methods disclosed herein, by testing many different lineages and combinations in parallel as described in greater detail below. Although in principle a single first *in vitro* backfill community can be produced by combining a single scaffold community with backfill strains, the robustness of the method arises, in part, from parallel processing of multiple communities. Typically a plurality of first *in vitro* backfill communities designed to exhibit a predetermined metabolic phenotype are produced (*e.g.*, typically from 2 to 100 communities, and generally at least 5, at least 10 or at least 15 communities) by combining scaffold communities and backfill communities. In one approach, multiple aliquots of one scaffold community are used. In one approach multiple different scaffold communities are used, where the communities are designed for the same metabolic phenotype but with different (sometimes only slightly different) combinations of strains. In each approach, one combination of backfill strains, or multiple different combinations of backfill strains may be used. Thus, in the *in vitro* backfill process, multiple first backfilled communities may be created, propagated, and assayed in parallel.

[0081] The number of different first backfill communities assayed in parallel can range from 2 to 100 or more. Typically the number is greater than 5, greater than 10, greater than 25, greater then 50, or greater than 100.

6.7 Culturing in vitro Backfill Communities

[0082] The first backfill communities, as well as subsequent *in vitro* backfill communities (described below) are cultured for a period of time and then are assessed as described below. The strains may be cultured for 2 hours to 10 days, although longer or shorter times can be used. For example, the backfill communities can be cultured for 1 to 72 hours, *e.g.*, 12 to 72 hours, 12 to 48 hours, or 24 to 48 hours. Typically the strains are cultured in an environment that mimics the temperature of the human gut (*e.g.*, 36-38 °C) and low pO_2 (*e.g.*, under anaerobic conditions). Preferably a single universal culture medium is used, which may be designed to approach the conditions encountered in the mammalian (*e.g.*, human) gut.

6.8 Assessing and Ranking in vitro Backfill Communities

[0083] At the end of a culture period, or at multiple times during a culture period, one or more properties of the first backfill communities, as well as subsequent *in vitro* backfill communities, can be assessed. For illustration and not limitation, exemplary properties that can be assessed include a metabolic phenotype and antibiotic resistance.

6.9 Assessing Strain Composition

[0084] At the end of a culture period, or at any desired time during culture, the strain composition of a backfill community can be determined. Strain composition can be determined by metagenomic analysis, by quantitative assessments such as qPCR, using microbiological techniques such as colony counting, or combinations of methods. In one aspect, the abundance, or relative proportions, of individual strains can be measured.

6. 10 Assessing Changes in Strain Composition

[0085] By determining the strain composition of a community at different timepoints, changes in composition can be detected. Some strains "drop out" during culture and/or during *in vivo* backfill. Changes in strain composition over different rounds or iterations of *in vitro* or *in*

vivo backfilling, discussed below, can be used as a measure of "Community Composition Stability," *i.e.* stability, as defined above.

6.11 Assessing Metabolic Phenotype

[0086] The metabolic phenotype of a backfill community can be determined at the end of, or during, a culture period. Metabolic phenotype can be assayed in any suitable fashion based on the desired phenotype. For example, in one approach, one or more than one first compound is combined with a community and conversion of the first compound(s) to second compound(s) is measured over time or at an end point. Detection and measurement of compounds or other properties can be made in any of a variety of ways. For example, liquid chromatogray mass spectrometry (LC-MS), immunoassay (ELISA), tracing radiolabeled metabolites, etc., may be used to detect compounds produced or consumed by a community. Assays may be carried out under conditions that mimic those of the mammalian (*e.g.*, human) gut, or over multiple conditions that mimic variation in the guts of individuals in a population.

[0087] Changes in metabolic phenotype over different rounds or iterations of *in vitro* or *in vivo* backfilling, discussed below, can be used as a measure of "Community Phenotype Stability."

6.12 Other Assessments

[0088] The backfilled communities may also be tested for antibiotic susceptibility or resistance, contamination, and the like. In some cases, a backfilled community may be challenged with a pathogen or other microorganism to determine whether addition of the, *e.g.*, pathogen perturbs or overgrows the community. In some cases, a backfill community may be introduced into the gut of a humanized mouse to determine whether the community can displace the enteric microbiome.

6.13 Ranking Communities

[0089] The first, and subsequent, backfill communities may be ranked according to assessed properties such as metabolic phenotype. For example, if the desired community phenotype is production of metabolite X under defined conditions, the ability of the community to produce X, the rate at which X is produced or other kinetic measurements, and the like, can be measured and the Backfill Communities in which the desired phenotype is more robust can be ranked higher

than communities in which the desired phenotype is absent or less robust. Multiple properties or criteria can be considered and may be assigned equal or unequal weights and used for ranking.

6.14 Selection of Backfilled Communities

[0090] As noted above, backfill communities may be ranked according to any combination of properties, weighed in any manner. In one approach, the highest ranked backfill community or communities are selected for further processing. In one approach, the highest ranked community is selected for further processing. In one approach, the highest ranked 1%, 5%, 10% or 25% of communities are processed for further development. In one approach, communities exhibiting properties above a predetermined threshold may be selected for further processing. Communities that are not selected may be discarded.

[0091] A backfill community selected for further processing can be called a "selected backfill community."

6.15 Further Processing: Subsequent Backfill Communities

[0092] The selected (most highly ranked) first backfill community or communities may be further processed in subsequent iterations, or rounds, of the *in vitro* backfill process. In one approach, the selected first backfill communities are processed in a manner analogous to the treatment of the scaffold community. In some embodiments, each selected first backfill community is divided into multiple aliquots for parallel processing, and a small number of backfill strains (*e.g.*, 1-50 strains) are added to each aliquot, thereby producing a "subsequent backfill community." The backfill strains added to each aliquot are not the same for all aliquots of a first backfill community; rather different combinations and different complexities of backfill strains may be added. The process of adding backfill strains to one backfill community (*e.g.*, a first backfill community) to produce a subsequent backfill community can be referred to as "challenging" or "evolving" the community.

[0093] The subsequent backfill communities are cultured for a period of time ("culture period"), and at the end of a culture period, or at multiple times during a culture period, one or more properties of the subsequent community is assessed as described above, and subsequent communities are ranked for additional iterations or rounds of further processing. The properties assessed, and used for ranking, in one round of processing may be the same or different from properties assessed in previous or subsequent rounds.

6.16 Iterations

[0094] When developing a complex community for transplantation, multiple iterations of the backfilling process may be carried out. As used in this context, producing the first backfill community is a first iteration, and subsequent iterations are used to produce subsequent backfill communities are denoted by ordinal numbers (second backfill community, third backfill community, etc.). As used in this context, second or subsequent "iterations" include the process of (1) adding at least one backfill strain to an existing backfill population to produce a next generation population, (2) culturing the next generation population, (3) optionally determining a characteristic of the population.

[0095] The number of iterations of producing subsequent backfill communities (*i.e.* not including the first backfill community) may range from 1 to 20. Typically the number of iterations is in the range 5-10 iterations. In general, there are at least 1, 2, 3, 4, 5, 6, or 7 iterations producing subsequent *in vitro* backfill communities.

[0096] As noted above, a selected backfill community can be divided into multiple aliquots each of which is combined with one or more backfill strains (*e.g.*, where not all aliquots receive the same backfill strains). It is sometimes useful to describe the lineage of a community. In any subsequent backfill iteration, communities produced from the same selected backfill community are referred to as "sibling communities" of each other and as "progeny" of the selected backfill community. The selected backfill community can be referred to as an "ancestor" of the progeny communities.

6.17 Producing a Transplantable Community by in vivo Backfilling

[0097] After a final iteration of *in vitro* backfilling, one or more of the subsequent backfill communities may be identified as having desirable properties (*e.g.*, a desired metabolic phenotype), and may be used as a first *in vivo* backfill community. The *in vivo* backfill process parallels the *in vitro* process described above in several respects. Many of the *in vivo* backfill steps are the same as, or analogous to, corresponding in vitro steps discussed above. The chief differences are:

- the first *in vivo* backfill community is usually a community produced by *in vitro* backfill, rather than a scaffold community;
- backfill communities are engrafted into a non-human animal (typically a gnotobiotic mouse) rather than cultured *in vitro*; and

- backfill communities are challenged, or evolved, by combining an engrafted backfill community with human fecal transplant material comprising a complex mixture of strains. Optionally, backfill strains may also be administered.

[0098] Analogous with the *in vitro* method, multiple first *in vivo* backfill communities may be developed in parallel as described in greater detail below. Thus, for example and not limitation, one approach to *in vivo* backfill includes the following steps:

i. engraft a selected *in vitro* backfill community into the gut(s) of one mouse or a plurality of mice or other non-human animal;

iia. introduce human fecal transplant material into the gut(s) of the one mouse or the plurality of mice (*i.e.* challenge the engrafted community) prior to or after step (i);

iib. optionally, backfill strains (*e.g.*, from the Microbial Pantry) may also be administered into the mouse or the plurality of mice;

iii. maintain the mouse or the plurality of mice for a period of time during which time the engrafted and introduced strains colonize the gut, resulting in a "gut community;"

iv. assess one or more properties of the gut communities including composition (*i.e.* the presence of strains that "jump in" or "drop out" relative to the *in vitro* backfill community engrafted in step (i);

v. optionally, rank gut communities, and select one or more gut communities for further processing;

vi. for each selected gut community, engraft a plurality of mice with the community; and

vii. challenge the mice in (vi) by introducing human fecal transplant material (as in step ii, above) and carry out additional iterations of steps (ii) - (vi) until a desired endpoint.

[0099] Certain aspects of the *in vivo* backfill method are described in more detail below.

[0100] *In vivo* backfill is usually carried out in gnotobiotic mice, humanized mice, or other mammals (*e.g.*, simians, equines, bovines, porcines, canines, felines, and the like). Gnotobiotic mice are known in the art and commercially available. In some embodiments, *in vivo* backfill may be carried out in human subjects.

[0101] A selected *in vitro* community or subsequent *in vivo* communities can be engrafted into mice using standard methods such as gavage.

[0102] An engrafted community can be challenged with human fecal material when developing treatments for human patients. Fecal preparations from other species may be used in model systems or in development of treatments for veterinary purposes (see Hu, J *et al.*, 2018,

"Standardized Preparation for Fecal Microbiota Transplantation in Pigs," *Front. Microbiol.* 9:1328.

[0103] The feces donor may be selected or screened for certain characteristics such as the health of the donor.

[0104] Fecal material is processed for transplantation using art-known methods. In some cases, fecal material from more than one individual will be pooled for engraftment.

[0105] Fecal material may be introduced into the mouse gut by gavage. The engrafted mouse is housed under germ free conditions for 1 day to 4 weeks. This interval may be referred to as the "colonization period."

[0106] At the end of a colonization period, or at multiple times during a colonization period, one or more properties of the first backfill communities, as well as subsequent *in vitro* backfill communities, can be assessed.

[0107] For purposes of assessment, a community may be recovered from the animal (*e.g.*, mouse) gut in any fashion that maintains the integrity of the microbiome including (1) recovery of strains from feces; (2) recovery of gut contents; and (3) recovery of the gut surgically (*e.g.*, by sacrifice of mouse).

[0108] The characteristics of the community that may be assayed and suitable methods include those described for *in vitro* backfill, including changes in strain composition; metabolic phenotype; and/or strain and phenotype stability.

[0109] In addition to analysis of the backfill community, the mouse phenotype can be analyzed. Characteristics include the general health and vigor of the mouse, as well as changes in blood or other tissues, such as a change in plasma levels of a metabolite, especially a metabolite related to the desired metabolic phenotype.

[0110] The *in vivo* backfill communities may be ranked according to assessed properties (such as metabolic phenotype). Multiple properties or criteria can be considered and may be assigned equal or unequal weights and used for ranking.

[0111] The selected (most highly ranked) *in vivo* backfill community or communities may be further processed in subsequent iterations, or rounds, of the *in vivo* backfill process. From 2-10 iterations (usually 2-5, often 2-4, iterations). After a final iteration of *in vivo* backfilling, one or more *in vivo* subsequent backfill community may be identified as suitable for use as a therapeutic agent, referred to as a "therapeutic backfill community."

6.18 In vivo Backfill

[0112] In *in vivo* backfill, one approach is to administer to a non-human animal a defined enteric community that is produced through a series of steps that include the following.

1. Obtaining a first defined microbial community with an *in vitro* phenotype. Usually the first defined microbial community is a product of *in vitro* backfill. The *in vitro* phenotype may be a metabolic phenotype.
2. Engrafting the defined microbial community into the gut of an animal, typically a mouse such as a germ-free mouse. This engrafting step may be carried out in a plurality (*i.e.* two or more) of animals in parallel.
3. Challenging the animal with a human fecal community (*e.g.*, feces from a human). In this context, "challenging" means introducing the human fecal community into the gut of the animal previously engrafted with the defined microbial community so that the two communities mix. Alternatively, the two communities can be combined prior to engraftment and the mixture engrafted into the animal. The challenged engrafted animal is maintained for a time sufficient to establish in the gut a community comprising microorganisms from both the human fecal community and the defined microbial community, which may be referred to as a "gut community." The gut community may contain fewer or more strains than the defined microbial community. The gut community may comprise strains contributed from the human fecal community (strains that have "jumped in"). The gut community may not comprise strains (strains that have "dropped out") that were present in the defined microbial community. If more than one animal is challenged, they may be challenged with the same human fecal preparation or with different human fecal preparations. In one approach, not all of the animals are challenged with the same human fecal community.
4. Carrying out a metagenomic analysis to detect strains in the gut community and determining whether there are or are not differences between the gut community and the defined community. If there are differences (strains have jumped in or dropped out), a new defined microbial community (a "subsequent defined microbial community") is prepared (*e.g.*, using strains from the microbial pantry and/or other sources). The subsequent defined microbial community is engrafted into an animal (*e.g.*, an animal not previously engrafted) and processed as the first defined microbial community as discussed above. These steps can be repeated for a plurality of iterations. For example, they can be repeated 1, 2, 3, 4, 5 or 6 times (*e.g.*, typically 1-4 times).
5. Carrying out one or more assays to confirm that the gut community retains the desired phenotype (*i.e.* the phenotype that will provide therapeutic benefit to a patient). Gut

communities that do not retain the phenotype are abandoned. In some approaches, multiple different gut communities can be ranked based on the results of the assays, *e.g.*, within communities strongly expressing the phenotype being ranked higher. In some approaches, higher ranked communities are processed further and lower ranked communities are abandoned.

6. If a defined microbial community is stable, *e.g.*, when engrafted and challenged a minimal difference of strains jump in or drop out, and retains the desired phenotype, it may be used as a therapeutic agent. In some approaches, a defined microbial community is deemed stable if fewer than a threshold number of strains jump in and/or fewer than a threshold number of strains drop out. In some embodiments the threshold numbers for jump in and drop out are independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 strains. In some embodiments, the threshold numbers for jump in and drop out are independently selected from 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10% of the strains in the engrafted defined microbial community.

6.19 Variations

[0113] In certain embodiments, a mammal can be engrafted with first *in vitro* communities (produced by combining a scaffold community with backfill strains) without undertaking an *in vitro* backfill process.

7. Producing a High-Complexity Defined Gut Microbial Community

[0114] In some embodiments, a high-complexity defined gut microbial community can be produced by an *in vivo* backfill process comprising: i) combining a plurality of defined microbial strains; ii) engrafting the combined plurality of defined microbial strains into the gut of an animal to produce an engrafted animal; iii) challenging the engrafted animal with a human fecal sample; iv) maintaining the challenged engrafted animal for a time sufficient for enteric colonization of the animal by microbial strains of the human fecal sample, thereby producing an enteric community in the gut of the animal; v) identifying microbial strains of the enteric community by metagenomic analysis; vi) identifying whether there are differences between the microbial strains comprising the enteric community and the microbial strains comprising the combined plurality of defined microbial strains; vii) if there is a significant difference between the microbial strains comprising the enteric community and the microbial strains comprising the combined plurality of defined microbial strains, adding one or more than one additional defined

microbial strain that was not present in step i) to the combined plurality of defined microbial strains, or removing a defined microbial strain that was present in the combined plurality of defined microbial strains of step i), to produce a modified, combined plurality of defined microbial strains and repeating steps ii) to vi) in an animal that has never been engrafted, using the modified, combined plurality of defined microbial strains as the combined plurality of defined microbial strains, and if there are minimal differences, the modified, defined, microbial community in the final step vii) is a high-complexity defined gut microbial community. In some embodiments, defined microbial strains are selected for combining to form a plurality for engraftment based on the metabolic phenotype of the microbial strains. By selecting defined microbial strains having known metabolic phenotypes, high-complexity defined metabolic communities can be formed that have improved engraftment and/or stability in one or more gut niches.

[0115] In some embodiments, a high-complexity defined gut microbial community can comprise microbial strains belonging to the phyla consisting of Bacteroidetes, Firmicutes, Actinobacteria. In some embodiments, a high-complexity defined gut microbial community can comprise microbial strains belonging to the phyla consisting of Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria. n some embodiments, a high-complexity defined gut microbial community can comprise microbial strains belonging to Bateriodales, Clostridiales, Lactobacillales, Negativicutes, Eggerthellales, Bifidobacteriales, or Proteobacteria.

[0116] In certain embodiments, a high-complexity defined gut microbial community can comprise microbial strains selected from, or consist of the microbial strains: *Acidaminococcus fermentans* DSM 20731, *Acidaminococcus sp.* D21, *Akkermansia muciniphila* ATCC BAA-835, *Alistipes putredinis* DSM 17216, *Anaerofustis stercorihominis* DSM 17244, *Anaerostipes caccae* DSM 14662, *Anaerotruncus colihominis* DSM 17241, *Bacteroides caccae* ATCC 43185, *Bacteroides cellulosilyticus* DSM 14838, *Bacteroides coprocola* DSM 17136, *Bacteroides coprophilus* DSM 18228, *Bacteroides dorei* 5_1_36/D4 (HM 29), *Bacteroides dorei* DSM 17855, *Bacteroides eggerthii* DSM 20697, *Bacteroides finegoldii* DSM 17565, *Bacteroides fragilis* 3_1_12, *Bacteroides intestinalis* DSM 17393, *Bacteroides ovatus* ATCC 8483, *Bacteroides pectinophilus* ATCC 43243, *Bacteroides plebeius* DSM 17135, *Bacteroides sp.* 1_1_6, *Bacteroides sp.* 2_1_16, *Bacteroides sp.* 2_1_22, *Bacteroides sp.* 3_1_19, *Bacteroides sp.* 9_1_42FAA, *Bacteroides sp.* D2, *Bacteroides stercoris* ATCC 43183 DSMZ 19555, *Bacteroides thetaiotaomicron* VPI-5482, *Bacteroides uniformis* ATCC 8492, *Bacteroides vulgatus* ATCC 8482, *Bacteroides xylanisolvens* SD CC 1b -> subbed w/ DSMZ 18836, *Bifidobacterium adolescentis* L2-32, *Bifidobacterium breve* DSM 20213, *Bifidobacterium catenulatum* DSM

16992, *Bifidobacterium longum infantis* ATCC 55813, *Bifidobacterium pseudocatenulatum* DSM 20438, *Blautia hansenii* DSM 20583, *Blautia hydrogenotrophica* DSM 10507, *Bryantella formatexigens* DSM 14469, *Butyrivibrio crossotus* DSM 2876, *Catenibacterium mitsuokai* DSM 15897, *Clostridium asparagiforme* DSM 15981, *Clostridium bartlettii* DSM 16795, *Clostridium bolteae* ATCC BAA-613, *Clostridium hathewayi* DSM 13479, *Clostridium hylemonae* DSM 15053, *Clostridium leptum* DSM 753, *Clostridium methylpentosum* DSM 5476, *Clostridium nexile* DSM 1787, *Clostridium saccharolyticum* WM1 DSMZ 2544, *Clostridium scindens* ATCC 35704, *Clostridium sp.* L2-50, *Clostridium sp.* M62/1, *Clostridium spiroforme* DSM 1552, *Clostridium sporogenes* ATCC 15579, *Collinsella aerofaciens* ATCC 25986, *Collinsella stercoris* DSM 13279, *Coprococcus comes* ATCC 27758, *Coprococcus eutactus* ATCC 27759, *Desulfovibrio piger* ATCC 29098, *Dialister invisus* DSM 15470, *Dorea formicigenerans* ATCC 27755, *Dorea longicatena* DSM 13814, *Eggerthella lenta* DSM 2243, *Ethanoligenens harbinense* YUAN-3 DSMZ 18485, *Eubacterium biforme* DSM 3989, *Eubacterium dolichum* DSM 3991, *Eubacterium eligens* ATCC 27750 DSMZ 3376, *Eubacterium hallii* DSM 3353, *Eubacterium rectale* ATCC 33656, *Eubacterium siraeum* DSM 15702, *Eubacterium ventriosum* ATCC 27560 DSM 3988, *Faecalibacterium prausnitzii* A2-165, *Granulicatella adiacens* ATCC 49175 DSMZ 9848, *Holdemania filiformis* DSM 12042, *Lactobacillus ruminis* ATCC 25644, *Lactococcus lactis* subsp. *lactis* Il1403 -> sub DSMZ 20729, *Megasphaera* DSMZ 102144, *Mitsuokella multacida* DSM 20544, *Olsenella uli* DSM 7084, *Parabacteroides distasonis* ATCC 8503, *Parabacteroides johnsonii* DSM 18315, *Parabacteroides merdae* ATCC 43184 DSMZ 19495, *Parabacteroides sp.* D13, *Prevotella buccae* D17, *Prevotella buccalis* ATCC 35310 DSMZ 20616, *Prevotella copri* DSM 18205, *Roseburia intestinalis* L1-82, *Roseburia inulinivorans* DSM 16841, *Ruminococcus albus* strain 8, *Ruminococcus bromii* L2-32, *Ruminococcus flavefaciens* FD 1, *Ruminococcus gnavus* ATCC 29149, *Ruminococcus lactaris* ATCC 29176, *Ruminococcus obeum* ATCC 29174, *Ruminococcus torques* ATCC 27756, *Slackia exigua* ATCC 700122 DSMZ 15923, *Slackia heliotrinireducens* DSM 20476, *Solobacterium moorei* DSM 22971, *Streptococcus thermophilus* LMD-9 (ATCC 19258), *Subdoligranulum variabile* DSM 15176, *Veillonella dispar* ATCC 17748, *Veillonella sp.* 3_1_44 HM 64, and *Veillonella sp.* 6_1_27 HM 49.

[0117] In certain embodiments, a high-complexity defined gut microbial community can comprise microbial strains selected from, or consist of the microbial strains: *Acidaminococcus fermentans* DSM 20731, *Acidaminococcus sp.* D21, *Adlercreutzia equolifaciens* DSM 19450, *Akkermansia muciniphila* ATCC BAA-835, *Alistipes finegoldii* DSM 17242, *Alistipes ihumii* AP11, *Alistipes indistinctus* YIT 12060/DSM 22520, *Alistipes onderdonkii* DSM 19147,

Alistipes putredinis DSM 17216, *Alistipes senegalensis* JC50/DSM 25460, *Alistipes shahii* WAL 8301/DSM 19121, *Anaerofustis stercorihominis* DSM 17244, *Anaerostipes caccae* DSM 14662, *Anaerotruncus colihominis* DSM 17241, *Bacteroides caccae* ATCC 43185, *Bacteroides cellulosilyticus* DSM 14838, *Bacteroides coprocola* DSM 17136, *Bacteroides coprophilus* DSM 18228, *Bacteroides dorei* 5_1_36/D4 (HM 29), *Bacteroides dorei* DSM 17855, *Bacteroides eggerthii* DSM 20697, *Bacteroides finegoldii* DSM 17565, *Bacteroides fragilis* 3_1_12, *Bacteroides intestinalis* DSM 17393, *Bacteroides ovatus* ATCC 8483, *Bacteroides pectinophilus* ATCC 43243, *Bacteroides plebeius* DSM 17135, *Bacteroides rodentium* DSM 26882, *Bacteroides sp.* 1_1_6, *Bacteroides sp.* 2_1_16, *Bacteroides sp.* 2_1_22, *Bacteroides sp.* 3_1_19, *Bacteroides sp.* 9_1_42FAA, *Bacteroides sp.* D2, *Bacteroides stercoris* ATCC 43183 DSMZ 19555, *Bacteroides thetaiotaomicron* VPI-5482, *Bacteroides uniformis* , ATCC 8492, *Bacteroides vulgatus* ATCC 8482, *Bacteroides xylanisolvens* SD CC 1b -> subbed w/ DSMZ 18836, *Bifidobacterium breve*, *Bifidobacterium catenulatum* DSM 16992, *Bifidobacterium pseudocatenulatum* DSM 20438, *Bilophila wadsworthia* ATCC 49260, *Blautia hansenii* DSM 20583, *Blautia hydrogenotrophica* DSM 10507, *Blautia sp.* KLE 1732 (HM 1032), *Blautia wexlerae* DSM 19850, *Bryantella formatexigens* DSM 14469, *Burkholderiales bacterium* 1_1_47, *Butyricimonas virosa* DSM 23226, *Butyrivibrio crossotus* DSM 2876, *Catenibacterium mitsuokai* DSM 15897, *Clostridiales bacterium* VE202-03, *Clostridiales bacterium* VE202-14, *Clostridiales bacterium* VE202-27, *Clostridium asparagiforme* DSM 15981, *Clostridium bartlettii* DSM 16795, *Clostridium bolteae* ATCC BAA-613, *Clostridium hathewayi* DSM 13479, *Clostridium hylemonae* DSM 15053, *Clostridium leptum* DSM 753, *Clostridium methylpentosum* DSM 5476, *Clostridium nexile* DSM 1787, *Clostridium saccharolyticum* WM1 DSMZ 2544, *Clostridium scindens* ATCC 35704, *Clostridium sp.* ATCC 29733 VPI C48-50, *Clostridium sp.* L2-50, *Clostridium sp.* M62/1, *Clostridium spiroforme* DSM 1552, *Collinsella aerofaciens* ATCC 25986, *Collinsella stercoris* DSM 13279, *Coprococcus comes* ATCC 27758, *Coprococcus eutactus* ATCC 27759, *Desulfovibrio piger* ATCC 29098, *Dorea formicigenerans* ATCC 27755, *Dorea longicatena* DSM 13814, *Eggerthella lenta* DSM 2243, *Ethanoligenens harbinense* YUAN-3 DSMZ 18485, *Eubacterium biforme* DSM 3989, *Eubacterium dolichum* DSM 3991, *Eubacterium eligens* ATCC 27750 DSMZ 3376, *Eubacterium hallii* DSM 3353, *Eubacterium rectale* ATCC 33656, *Eubacterium siraeum* DSM 15702, *Eubacterium ventriosum* ATCC 27560 DSM 3988, *Faecalibacterium prausnitzii* A2-165, *Granulicatella adiacens* ATCC 49175 DSMZ 9848, *Holdemania filiformis* DSM 12042, *Intestinimonas butyriciproducens* DSM 26588, *Lactobacillus ruminis* ATCC 25644, *Megasphaera* DSMZ 102144, *Mitsuokella multacida* DSM 20544, *Odoribacter splanchnicus* DSM 20712, *Olsenella uli* DSM 7084,

Oscillibacter sp. KLE 1728, *Parabacteroides distasonis* ATCC 8503, *Parabacteroides johnsonii* DSM 18315, *Parabacteroides merdae* ATCC 43184 DSMZ 19495, *Parabacteroides sp.* D13, *Prevotella buccae* D17, *Prevotella buccalis* ATCC 35310 DSMZ 20616, *Prevotella copri* DSM 18205, *Roseburia intestinalis* L1-82, *Roseburia inulinivorans* DSM 16841, *Ruminococcus albus* strain 8, *Ruminococcus bromii* ATCC, *Ruminococcus flavefaciens* FD 1, *Ruminococcus gauvreauii* DSM 19829, *Ruminococcus gnavus* ATCC 29149, *Ruminococcus lactaris* ATCC 29176, *Ruminococcus obeum* ATCC 29174, *Ruminococcus torques* ATCC 27756, *Slackia exigua* ATCC 700122 DSMZ 15923, *Slackia heliotrinireducens* DSM 20476, *Solobacterium moorei* DSM 22971, *Streptococcus thermophilus* LMD-9 (ATCC 19258), *Subdoligranulum sp.* 4_3_54A2FAA, *Subdoligranulum variabile* DSM 15176, and *Veillonella dispar* ATCC 17748.

[0118] In certain embodiments, a high-complexity defined gut microbial community can comprise microbial strains selected from, or consist of the microbial strains described in Table 8.

TABLE 8 – Exemplary High-Complexity Defined Gut Microbial Community Strains

Strain	**Strain Repository ID**	**Source Repository**
Acidaminococcus fermentans -- VR4	DSM 20731	DSMZ
Acidaminococcus sp. -- D21	HM-81	BEI
Adlercreutzia equolifaciens -- FJC-B9	DSM 19450	DSMZ
Akkermansia muciniphila -- Muc [CIP 107961]	ATCC BAA-835	ATCC
Alistipes finegoldii -- AHN 2437	DSM 17242	DSMZ
Alistipes indistinctus -- JCM 16068, YIT 12060	DSM 22520	DSMZ
Alistipes onderdonkii -- WAL 8169	DSM 19147	DSMZ
Anaerobutyricum hallii -- VPI B4-27	DSM 3353	DSMZ
Anaerofustis stercorihominis -- ATCC BAA-858, CCUG 47767, CIP 108481, WAL 14563	DSM 17244	DSMZ
Anaerostipes caccae -- L1-92	DSM 14662	DSMZ
Anaerotruncus colihominis -- 277	DSM 17241	DSMZ
Bacteroides caccae -- VPI 3452A [CIP 104201T, JCM 9498]	ATCC 43185	ATCC
Bacteroides cellulosilyticus -- CRE21, CCUG 44979	DSM 14838	DSMZ
Bacteroides coprocola -- M16	DSM 17136	DSMZ
Bacteroides coprophilus -- CB42, JCM 13818	DSM 18228	DSMZ

Strain	Strain Repository ID	Source Repository
Bacteroides dorei -- 175	DSM 17855	DSMZ
Bacteroides dorei -- 5_1_36/D4	HM-29	BEI
Bacteroides eggerthii -- ATCC 27754, NCTC 11155	DSM 20697	DSMZ
Bacteroides finegoldii -- 199	DSM 17565	DSMZ
Bacteroides fragilis -- 3_1_12	HM-20	BEI
Bacteroides intestinalis -- 341	DSM 17393	DSMZ
Bacteroides ovatus -- NCTC 11153	ATCC 8483	ATCC
Bacteroides rodentium -- ST28, CCUG 59334, JCM 16469	DSM 26882	DSMZ
Bacteroides thetaiotaomicron -- 1_1_6	HM-23	BEI
Bacteroides fragilis -- 2_1_16	HM-58	BEI
Bacteroides xylanisolvens -- 2_1_22	HM-18	BEI
Parabacteroides distasonis -- 3_1_19	HM-19	BEI
Bacteroides dorea -- 9_1_42FAA	HM-27	BEI
Bacteroides ovatus -- D2	HM-28	BEI
Bacteroides stercoris -- VPI B3-21, ATCC 43183, CIP 104203, JCM 9496	DSM 19555	DSMZ
Bacteroides thetaiotaomicron -- VPI 5482 [CIP 104206T, E50, NCTC 10582]	ATCC 29148	ATCC
Bacteroides uniformis -- ATCC 8492	ATCC 8492	ATCC
Bacteroides vulgatus -- NCTC 11154	ATCC 8482	ATCC
Bifidobacterium pseudocatenulatum -- B1279, ATCC 27919	DSM 20438	DSMZ
Bilophila wadsworthia -- WAL 7959 [Lab 88-130H]	ATCC 49260	ATCC
Blautia hansenii -- VPI C7-24	DSM 20583	DSMZ
Blautia hydrogenotrophica -- S5a33	DSM 10507	DSMZ
Blautia obeum -- ATCC 29174, KCTC 15206, VPI B3-21	DSMZ 25238	DSMZ
Blautia sp. -- KLE 1732	HM-1032	BEI
Blautia wexlerae -- ATCC BAA-1564, JCM 17041, KCTC 5965, WAL 14507	DSM 19850	DSMZ
Catenibacterium mitsuokai -- RCA14-39, CIP 106738, JCM 10609	DSM 15897	DSMZ
Clostridium asparagiforme -- N6, CCUG 48471	DSM 15981	DSMZ

Strain	Strain Repository ID	Source Repository
Clostridium hylemonae -- TN-271, JCM 10539	DSM 15053	DSMZ
Clostridium leptum -- VPI T7-24-1, ATCC 29065	DSM 753	DSMZ
Tyzzerella nexilis DSM 1787	DSM 1787	DSMZ
Clostridium saccharolyticum -- WM1, ATCC 35040, NRC 2533	DSM 2544	DSMZ
Absiella dolichum DSM 3991	DSM 3991	DSMZ
Collinsella aerofaciens -- VPI 1003 [DSM 3979, JCM 10188]	ATCC 25986	ATCC
Collinsella stercoris -- RCA 55-54, JCM 10641	DSM 13279	DSMZ
Coprococcus comes -- VPI CI-38	ATCC 27758	ATCC
Dialister invisus -- E7.25, CCUG 47026	DSM 15470	DSMZ
Eubacterium rectale -- VPI 0990 [CIP 105953]	ATCC 33656	ATCC
Eubacterium siraeum -- VPI T9-50-2, ATCC 29066, DSM 3996	DSM 15702	DSMZ
Eubacterium ventriosum -- VPI 1013B	ATCC 27560	ATCC
Coprococcus eutactus -- VPI C33-22	ATCC 27759	ATCC
Holdemanella biformis -- VPI C17-5, ATCC 27806, KCTC 5969	DSM 3989	DSMZ
Intestinibacter bartlettii -- WAL 16138, ATCC BAA-827, CCUG 48940	DSM 16795	DSMZ
Megasphaera sp. -- Sanger 24, Sanger_24	DSM 102144	DSMZ
Odoribacter splanchnicus -- 1651/6, ATCC 29572, CCUG 21054, CIP 104287, LMG 8202, NCTC 10825	DSM 20712	DSMZ
Parabacteroides distasonis -- NCTC 11152	ATCC 8503	ATCC
Parabacteroides merdae -- VPI T4-1, ATCC 43184, CCUG 38734, CIP 104202, JCM 9497	DSM 19495	DSMZ
Parabacteroides sp. -- D13	HM-77	BEI
Granulicatella adiacens -- GaD [CIP 103243, DSM 9848]	ATCC 49175	ATCC
Holdemania filiformis -- VPI J1-31B-1, ATCC 51649	DSM 12042	DSMZ
Hungatella hathewayi -- 1313, CCUG 43506, CIP 109440, MTCC 10951	DSM 13479	DSMZ

Strain	Strain Repository ID	Source Repository
Intestinimonas butyriciproducens -- SRB-521-5-l, CCUG 63529	DSM 26588	DSMZ
Solobacterium moorei -- RCA59-74, CIP 106864, JCM 10645	DSM 22971	DSMZ
Mitsuokella multacida -- A 405-1, ATCC 27723, NCTC 10934	DSM 20544	DSMZ
Olsenella uli -- D76D-27C, ATCC 49627, CIP 109912	DSM 7084	DSMZ
Parabacteroides johnsonii -- M-165, CIP 109537, JCM 13406	DSM 18315	DSMZ
Prevotella buccalis -- HS4, ATCC 35310, NCDO 2354	DSM 20616	DSMZ
Prevotella copri -- CB7, JCM 13464	DSM 18205	DSMZ
Roseburia inulinivorans -- A2-194, CIP 109405, JCM 17584, NCIMB 14030	DSM 16841	DSMZ
Clostridium sp. -- VPI C48-50 (unassigned Clostridiales)	ATCC 29733	ATCC
Ruminococcus gauvreauii -- CCRI-16110, CCUG 54292, JCM 14987, NML 060141	DSM 19829	DSMZ
Ruminococcus lactaris -- VPI X6-29	ATCC 29176	ATCC
Ruminococcus torques -- VPI B2-51	ATCC 27756	ATCC
Alistipes putredinis -- CCUG 45780, CIP 104286, ATCC 29800, Carlier 10203, VPI 3293	DSM 17216	DSMZ
Alistipes senegalensis -- CSUR P150, JCM 32779, JC50	DSM 25460	DSMZ
Clostridium spiroforme -- VPI C28-23-1A, ATCC 29900, NCTC 11211	DSM 1552	DSMZ
Slackia exigua -- S-7, ATCC 700122, JCM 11022, KCTC 5966	DSM 15923	DSMZ
Bacteroides pectinophilus -- N3	ATCC 43243	ATCC
Butyrivibrio crossotus -- T9-40A, ATCC 29175	DSM 2876	DSMZ
Subdoligranulum variabile -- BI-114, CCUG 47106	DSM 15176	DSMZ
Turicibacter sanguinis -- MOL361, NCCB 100008	DSM 14220	DSMZ
Bifidobacterium breve -- S1, ATCC 15700, NCTC 11815	DSM 20213	DSMZ

Strain	Strain Repository ID	Source Repository
Bifidobacterium catenulatum -- B669, ATCC 27539, CECT 7362, CIP 104175, DSM 20103	DSM 16992	DSMZ
Butyricimonas virosa -- MT12, CCUG 56611, JCM 15149	DSM 23226	DSMZ
Streptococcus salivarius subsp. thermophilus -- LMD-9	ATCC BAA-491	ATCC
Dorea formicigenerans -- VPI C8-13 [JCM 9500]	ATCC 27755	ATCC
Bacteroides plebeius -- M12	DSM 17135	DSMZ
Ruminococcus gnavus -- VPI C7-9	ATCC 29149	ATCC
Oscillibacter sp. -- KLE 1728	HM-1030	BEI
Clostridium sp. -- M62/1	HM-635	BEI
Slackia heliotrinireducens -- RHS 1, ATCC 29202, NCTC 11029	DSM 20476	DSMZ
Desulfovibrio piger -- VPI C3-23 [DSM 749]	ATCC 29098	ATCC
Clostridium methylpentosum -- R2, ATCC 43829	DSM 5476	DSMZ
Ethanoligenens harbinense -- YUAN-3, CGMCC 1.5033, JCM 12961	DSM 18485	DSMZ
Marvinbryantia formatexigens -- I-52, CCUG 46960	DSM 14469	DSMZ
Lactobacillus ruminis -- E 194e	ATCC 25644	ATCC
Clostridium bolteae -- WAL 16351, [CCUG 46953], ATCC BAA-613, Song et al. 2003	DSM 15670	DSMZ
Clostridium hiranonis -- TO-931, JCM 10541, KCTC 15199	DSM 13275	DSMZ
Clostridium scindens -- VPI 13733, ATCC 35704, 19	DSM 5676	DSMZ
Bacteroides xylanisolvens -- XB1A, CCUG 53782	DSM 18836	DSMZ
Clostridium sp. -- L2-50	HM-634	BEI
Clostridium orbiscindens -- 1_3_50AFAA	HM-303	BEI
Alistipes shahii -- WAL 8301	DSM 19121	DSMZ
Faecalibacterium prausnitzii -- A2-165, JCM 31915	DSM 17677	DSMZ

[0119] In some embodiments, methods of producing a high-complexity defined gut microbial community comprise individually culturing each of a plurality of defined microbial strains prior to combining the defined microbial strains. In other embodiments, methods of

producing a high-complexity defined gut microbial community comprise culturing all of a plurality of defined microbial strains together. In still other embodiments, methods of producing a high-complexity defined gut microbial community comprise individually culturing one or more defined microbial strains and culturing two or more defined microbial strains, then combining together the individually-cultured defined microbial strains and co-cultured defined microbial strains.

7.1 Pathway-Based Selection of High-Complexity Defined Gut Microbial Communities

[0120] The taxonomic structure of the human gut microbiome is highly variable between individuals, but the functional structure is highly conserved and informs a heuristic for the design of a metabolically comprehensive high-complexity defined gut microbial community. High-complexity defined gut microbial communities disclosed herein contain core functional diversity present in the gut microbiomes of healthy human subjects. In some embodiments high-complexity defined gut microbial communities incorporate metabolic redundancy amongst the constituent defined microbial strains to allow engraftment of the defined gut microbial community independent of the diet or genetics of the subject to which the defined gut microbial community is administered.

[0121] In some embodiments, high-complexity defined gut microbial communities disclosed herein are assembled based on the metabolic pathways utilized by one or more of the defined microbial strains rather than selecting microbial strains based on their specific taxa. In some embodiments, function/pathway-based assembly of high-complexity defined gut microbial communities is achieved by screening genomes of microbes found in donor fecal samples for the presence of: (i) core metabolic pathways of the normal human gut microbiome; and (ii) metabolic pathways involved in the consumption/metabolization of a comprehensive panel of substrates or nutrients, and/or the synthesis/production of a comprehensive panel of metabolites.

[0122] As used herein, “core metabolic pathways” refer to complete MetaCyc pathways (Caspi *et al.* 2018, "The MetaCyc database of metabolic pathways and enzymes", Nucleic Acids Research 46(D1):D633-D639.; MetaCyc: MetaCyc Metabolic Pathway Database [database online] [accessed May 20, 2020]. Retrieved from < https://metacyc.org/>.) that are found in the majority of gut metagenomes annotated in the GutCyc project (Hahn, Altman, Konwar, *et al.* GutCyc: a Multi-Study Collection of Human Gut Microbiome Metabolic Models bioRxiv. (2016); GutCyc: Collection of Pathway/Genome Dabases from the Human Gut [database online] [accessed May 20, 2020]. Retrieved from < http://gutcyc.org/>.). For example, in some

embodiments, "core metabolic pathways" can be pathways where all enzymes encoding all reactions of the pathway are present in the majority of gut metagenomes annotated in the GutCyc project. Metagenomes surveyed in the GutCyc project are derived from 418 healthy human subjects from three large-scale studies (MetaHit, The Human Microbiome Project (Lloyd-Price J, Mahurkar A, Rahnavard G, *et al.* Strains, functions and dynamics in the expanded Human Microbiome Project. Nature. 2017;550(7674):61-66.), and the Beijing Genomics Institute Diabetes Study Junjie Qin, Ruiqiang Li, Jeroen Raes, *et al.* A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010 March 4; 464(7285):59-65.)).

[0123] In some embodiments, core metabolic pathways can include any one or more of the 1CMET2-PWY, 2.6.1.32-RXN, AEROBACTINSYN-PWY, ALACAT2-PWY, ALADEG-PWY, ALANINE-DEG3-PWY, ALANINE-SYN2-PWY, ALANINE-VALINESYN-PWY, ANAPHENOXI-PWY, ARGASEDEG-PWY, ARGDEG-III-PWY, ARGDEG-IV-PWY, ARGDEGRAD-PWY, ARGDEG-V-PWY, ARG-GLU-PWY, ARGININE-SYN4-PWY, ARG-PRO-PWY, ARGSYNBSUB-PWY, ARGSYN-PWY, ASPARAGINE-BIOSYNTHESIS, ASPARAGINE-DEG1-PWY, ASPARAGINE-DEG1-PWY-1, ASPARAGINESYN-PWY, ASPARTATE-DEG1-PWY, ASPARTATESYN-PWY, ASPASN-PWY, ASPSYNII-PWY, AST-PWY, BETA-ALA-DEGRADATION-I-PWY, CAMALEXIN-SYN, CITRULBIO-PWY, CITRULLINE-DEG-PWY, COA-PWY, CODH-PWY, CYSTEINE-DEG-PWY, CYSTSYN-PWY, DAPLYSINESYN-PWY, ENTBACSYN-PWY, ETHYL-PWY, FAO-PWY, FERMENTATION-PWY, GLNSYN-PWY, GLUDEG-I-PWY, GLUGLNSYN-PWY, GLUTAMATE-DEG1-PWY, GLUTAMATE-SYN2-PWY, GLUTAMINDEG-PWY, GLUTAMINEFUM-PWY, GLUTATHIONESYN-PWY, GLUTDEG-PWY, GLUTORN-PWY, GLUTSYNIII-PWY, GLUTSYN-PWY, GLYCGREAT-PWY, GLYSYN-ALA-PWY, GLYSYN-PWY, GLYSYN-THR-PWY, HISDEG-PWY, HISHP-PWY, HISTDEG-PWY, HISTSYN-PWY, HOMOCYSDEGR-PWY, HOMOSER-METSYN-PWY, HOMOSERSYN-PWY, HSERMETANA-PWY, HYDROXYPRODEG-PWY, ILEUDEG-PWY, ILEUSYN-PWY, LARABITOLUTIL-PWY, LCYSDEG-PWY, LEU-DEG2-PWY, LEUSYN-PWY, LYSDEGII-PWY, LYSINE-AMINOAD-PWY, LYSINE-DEG1-PWY, MALATE-ASPARTATE-SHUTTLE-PWY, METH-ACETATE-PWY, METHANOGENESIS-PWY, METHIONINE-DEG1-PWY, MGLDLCTANA-PWY, ORN-AMINOPENTANOATE-CAT-PWY, ORNDEG-PWY, P101-PWY, P162-PWY, P163-PWY, P181-PWY, P261-PWY, P283-PWY, P401-PWY, P541-PWY, PHENYLALANINE-DEG1-PWY, PHESYN, PHOSLIPSYN2-PWY, PHOSPHONOTASE-PWY, PROSYN-PWY, PROUT-PWY, PWY0-1021, PWY0-1221, PWY0-1299, PWY0-1303, PWY0-1305, PWY0-1313, PWY0-1317, PWY0-1321, PWY0-1338,

PWY0-1347, PWY0-1355, PWY0-1356, PWY0-1534, PWY0-1544, PWY0-1565, PWY0-1576, PWY0-1577, PWY0-1578, PWY0-1585, PWY0-1601, PWY0-42, PWY0-461, PWY0-823, PWY0-901, PWY-1, PWY-1061, PWY-1121, PWY-1186, PWY1-2, PWY-1263, PWY-1622, PWY-1722, PWY-1781, PWY-181, PWY-1881, PWY-1962, PWY-1981, PWY1F-467, PWY1F-FLAVSYN, PWY1G-0, PWY-2021, PWY-2161, PWY-2181, PWY-2201, PWY-2821, PWY-2941, PWY-2942, PWY-3, PWY-3022, PWY-3081, PWY-3161, PWY-3162, PWY-3181, PWY-3301, PWY-3341, PWY-3385, PWY-3461, PWY-3462, PWY-3581, PWY-361, PWY-3661, PWY-3661-1, PWY-381, PWY-3841, PWY-3941, PWY3DJ-12, PWY3O-4107, PWY3O-4108, PWY-4, PWY-40, PWY-4002, PWY-4041, PWY-4201, PWY-4281, PWY-43, PWY-4321, PWY-4341, PWY-4361, PWY-46, PWY490-3, PWY490-4, PWY-4981, PWY-4983, PWY-4984, PWY4FS-6, PWY-5, PWY-5022, PWY-5024, PWY-5028, PWY-5029, PWY-5030, PWY-5031, PWY-5041, PWY-5048, PWY-5049, PWY-5057, PWY-5075, PWY-5076, PWY-5078, PWY-5079, PWY-5081, PWY-5082, PWY-5087, PWY-5097, PWY-5101, PWY-5103, PWY-5104, PWY-5108, PWY-5109, PWY-5129, PWY-5135, PWY-5136, PWY-5151, PWY-5154, PWY-5155, PWY-5159, PWY-5176, PWY-5188, PWY-5189, PWY-5196, PWY-5199, PWY-5207, PWY-5250, PWY-5254, PWY-5265, PWY-5280, PWY-5283, PWY-5290, PWY-5297, PWY-5298, PWY-5311, PWY-5314, PWY-5316, PWY-5319, PWY-5324, PWY-5329, PWY-5331, PWY-5332, PWY-5364, PWY-5381, PWY-5382, PWY-5386, PWY-5394, PWY-5399, PWY-5436, PWY-5437, PWY-5441, PWY-5443, PWY-5458, PWY-5467, PWY-5468, PWY-5473, PWY-5474, PWY-5494, PWY-5497, PWY-5499, PWY-5629, PWY-5651, PWY-5653, PWY-5665, PWY-5669, PWY-5675, PWY-5679, PWY-5686, PWY-5710, PWY-5736, PWY-5737, PWY-5739, PWY-5740, PWY-5742, PWY-5747, PWY-5748, PWY-5751, PWY-5754, PWY-5766, PWY-5770, PWY-5784, PWY-5788, PWY-5797, PWY-5800, PWY-581, PWY-5811, PWY-5818, PWY-5826, PWY-5877, PWY-5883, PWY-5886, PWY-5912, PWY-5913, PWY-5921, PWY-5936, PWY-5940, PWY-5958, PWY-5963, PWY-5968, PWY-5978, PWY-5980, PWY-5990, PWY-6003, PWY-6004, PWY-601, PWY-6030, PWY-6039, PWY-6045, PWY-6052, PWY-6053, PWY-6054, PWY-6055, PWY-6068, PWY-6069, PWY-6082, PWY-6120, PWY-6121, PWY-6122, PWY-6123, PWY-6124, PWY-6133, PWY-6134, PWY-6141, PWY-6143, PWY-6148, PWY-6151, PWY-6160, PWY-6173, PWY-6196, PWY-6219, PWY-622, PWY-6220, PWY-6233, PWY-6273, PWY-6277, PWY-6281, PWY-6307, PWY-6309, PWY-6313, PWY-6318, PWY-6320, PWY-6321, PWY-6322, PWY-6324, PWY-6328, PWY-6334, PWY-6339, PWY-6343, PWY-6344, PWY-6345, PWY-6346, PWY-6375, PWY-6376, PWY-6381, PWY-6386, PWY-6387, PWY-6397, PWY-6403, PWY-6407, PWY-6408, PWY-6409, PWY-6431, PWY-6435, PWY-6444, PWY-6455, PWY-6456, PWY-6457, PWY-

6466, PWY-6471, PWY-6473, PWY-6481, PWY-6486, PWY-6493, PWY-6495, PWY-6511, PWY-6512, PWY-6519, PWY-6533, PWY-6535, PWY-6536, PWY-6537, PWY-6543, PWY-6549, PWY-6559, PWY-6562, PWY-6572, PWY-6573, PWY-6574, PWY-6578, PWY-6588, PWY-6614, PWY-6627, PWY66-301, PWY66-375, PWY-6638, PWY66-391, PWY-6642, PWY66-420, PWY66-421, PWY66-425, PWY66-426, PWY66-428, PWY-6643, PWY-6661, PWY-6673, PWY-6682, PWY-6690, PWY-6696, PWY-6711, PWY-6717, PWY-6720, PWY-6724, PWY-6728, PWY-6731, PWY-6735, PWY-6749, PWY-6769, PWY-6771, PWY-6772, PWY-6773, PWY-6781, PWY-6784, PWY-6790, PWY-6791, PWY-6802, PWY-6807, PWY-6808, PWY-6813, PWY-6815, PWY-6816, PWY-6817, PWY-6818, PWY-6821, PWY-6822, PWY-6823, PWY-6831, PWY-6832, PWY-6834, PWY-6840, PWY-6845, PWY-6853, PWY-6854, PWY-6855, PWY-6891, PWY-6892, PWY-6896, PWY-6898, PWY-6902, PWY-6907, PWY-6908, PWY-6920, PWY-6922, PWY-6936, PWY-6942, PWY-6949, PWY-6953, PWY-6955, PWY-6963, PWY-6964, PWY-6965, PWY-6968, PWY-6969, PWY-6981, PWY-6982, PWY-6986, PWY-6994, PWY-7000, PWY-701, PWY-7014, PWY-7015, PWY-7016, PWY-7018, PWY-7019, PWY-702, PWY-7022, PWY-7025, PWY-7028, PWY-7040, PWY-7046, PWY-7052, PWY-7054, PWY-7064, PWY-7072, PWY-7088, PWY-7090, PWY-7097, PWY-7104, PWY-7115, PWY-7117, PWY-7118, PWY-7126, PWY-7147, PWY-7153, PWY-7158, PWY-7176, PWY-7177, PWY-7185, PWY-7186, PWY-7219, PWY-7221, PWY-7234, PWY-7246, PWY-7248, PWY-7250, PWY-7255, PWY-7274, PWY-7275, PWY-7282, PWY-7288, PWY-7297, PWY-7304, PWY-7315, PWY-7316, PWY-7318, PWY-7342, PWY-7351, PWY-7356, PWY-7376, PWY-7377, PWY-7383, PWY-7387, PWY-7397, PWY-7398, PWY-7400, PWY-7414, PWY-7425, PWY-7430, PWY-7432, PWY-7440, PWY-7441, PWY-7456, PWY-7467, PWY-7498, PWY-7501, PWY-7506, PWY-7510, PWY-7514, PWY-7518, PWY-7520, PWY-7525, PWY-7531, PWY-7532, PWY-7533, PWY-7536, PWY-7542, PWY-7543, PWY-7547, PWY-7549, PWY-7550, PWY-7555, PWY-7561, PWY-7565, PWY-7570, PWY-7571, PWY-7600, PWY-7605, PWY-761, PWY-7612, PWY-7626, PWY-7645, PWY-7648, PWY-7649, PWY-7650, PWY-7665, PWY-7667, PWY-7668, PWY-7669, PWY-7671, PWY-7674, PWY-7688, PWY-7690, PWY-7693, PWY-7694, PWY-7701, PWY-7704, PWY-7706, PWY-7708, PWY-7717, PWY-7718, PWY-7719, PWY-7733, PWY-7734, PWY-7735, PWY-7737, PWY-7751, PWY-7761, PWY-7765, PWY-7767, PWY-7769, PWY-7770, PWY-7782, PWY-7790, PWY-7791, PWY-7793, PWY-7797, PWY-7811, PWY-7814, PWY-7822, PWY-7824, PWY-7826, PWY-7842, PWY-7850, PWY-7851, PWY-7855, PWY-7860, PWY-7861, PWY-7863, PWY-7867, PWY-7870, PWY-7880, PWY-7888, PWY-7889, PWY-7891, PWY-7892, PWY-7897, PWY-7901, PWY-7904, PWY-7907, PWY-7909, PWY-7910, PWY-7913, PWY-

7917, PWY-7930, PWY-7931, PWY-7936, PWY-7953, PWY-7955, PWY-7956, PWY-7957, PWY-7958, PWY-7959, PWY-7960, PWY-7962, PWY-7977, PWY-7985, PWY-7986, PWY-7987, PWY-7988, PWY-7990, PWY-8002, PWY-8003, PWY-8006, PWY-8007, PWY-8008, PWY-8009, PWY-801, PWY-8010, PWY-8011, PWY-8013, PWY-8014, PWY-8015, PWY-8016, PWY-8017, PWY-8024, PWY-8032, PWY-8040, PWY-8043, PWY-8045, PWY-8071, PWY-8072, PWY-8080, PWY-8081, PWY-8082, PWY-8083, PWY-8086, PWY-8088, PWY-842, PWY-861, PWY-862, PWY8J2-1, PWY8J2-22, PWYDQC-4, PWYG-321, PWY-I9, PWYQT-4450, PWYQT-4476, PYRIDNUCSAL-PWY, PYRIDNUCSYN-PWY, PYRIDOXSYN-PWY, SAM-PWY, SERDEG-PWY, SERSYN-PWY, SPHINGOLIPID-SYN-PWY, TAURINEDEG-PWY, THRDLCTCAT-PWY, THREONINE-DEG2-PWY, TRNA-CHARGING-PWY, TRPCAT-PWY, TRPIAACAT-PWY, TRPKYNCAT-PWY, TRPSYN-PWY, TRYPDEG-PWY, TYRFUMCAT-PWY, TYRSYN, UDPNACETYLGALSYN-PWY, UDPNAGSYN-PWY, VALDEG-PWY, and VALSYN-PWY MetaCyc pathways. For example, in some embodiments, core metabolic pathways can include at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or all the MetaCyc pathways described above.

[0124] The common names of the core MetaCyc pathways described above are provided in Table 9 below.

TABLE 9 – Core MetaCyc Pathways

MetaCyc Pathway ID	**Pathway Common Name**
12DICHLORETHDEG-PWY	1,2-dichloroethane degradation
1CMET2-PWY	formylTHF biosynthesis I
2OXOBUTYRATECAT-PWY	2-oxobutanoate degradation II
ACETOACETATE-DEG-PWY	acetoacetate degradation (to acetyl CoA)
ALACAT2-PWY	alanine degradation II (to D-lactate)
ALANINE-SYN2-PWY	alanine biosynthesis II
ALANINE-VALINESYN-PWY	alanine biosynthesis I
ANAGLYCOLYSIS-PWY	glycolysis III (from glucose)
ANARESP1-PWY	respiration (anaerobic)
ARABCAT-PWY	L-arabinose degradation I
ARG-PRO-PWY	arginine degradation VI (arginase 2 pathway)
ARGASEDEG-PWY	arginine degradation I (arginase pathway)

MetaCyc Pathway ID	Pathway Common Name
ARGDEG-III-PWY	arginine degradation IV (arginine decarboxylase/agmatine deiminase pathway)
ARGDEG-V-PWY	arginine degradation X (arginine monooxygenase pathway)
ARGSYNBSUB-PWY	arginine biosynthesis II (acetyl cycle)
ASPARAGINE-BIOSYNTHESIS	asparagine biosynthesis I
ASPARAGINE-DEG1-PWY	asparagine degradation I
ASPARAGINESYN-PWY	asparagine biosynthesis II
AST-PWY	arginine degradation II (AST pathway)
BGALACT-PWY	lactose degradation III
BSUBPOLYAMSYN-PWY	spermidine biosynthesis I
CATECHOL-ORTHO-CLEAVAGE-PWY	catechol degradation to beta-ketoadipate
CENTBENZCOA-PWY	benzoyl-CoA degradation II (anaerobic)
CENTFERM-PWY	pyruvate fermentation to butanoate
CHLOROPHYLL-SYN	chlorophyllide a biosynthesis I (aerobic, light-dependent)
CITRULLINE-DEG-PWY	citrulline degradation
COA-PWY	coenzyme A biosynthesis
COBALSYN-PWY	adenosylcobalamin salvage from cobinamide I
CRNFORCAT-PWY	creatinine degradation I
CYSTSYN-PWY	cysteine biosynthesis I
DAPLYSINESYN-PWY	lysine biosynthesis I
DARABCATK12-PWY	D-arabinose degradation I
DENITRIFICATION-PWY	nitrate reduction I (denitrification)
DETOX1-PWY	superoxide radicals degradation
DTDPRHAMSYN-PWY	dTDP-L-rhamnose biosynthesis I
ETOH-ACETYLCOA-ANA-PWY	ethanol degradation I
FASYN-ELONG-PWY	fatty acid elongation -- saturated
FERMENTATION-PWY	mixed acid fermentation
FUCCAT-PWY	fucose degradation
GALACTARDEG-PWY	D-galactarate degradation I

MetaCyc Pathway ID	Pathway Common Name
GALACTCAT-PWY	D-galactonate degradation
GALACTUROCAT-PWY	D-galacturonate degradation I
GLUAMCAT-PWY	N-acetylglucosamine degradation I
GLUCARDEG-PWY	D-glucarate degradation I
GLUCONEO-PWY	gluconeogenesis I
GLUCONSUPER-PWY	D-gluconate degradation
GLUCOSE1PMETAB-PWY	glucose and glucose-1-phosphate degradation
GLUTAMINDEG-PWY	glutamine degradation I
GLUTAMINEFUM-PWY	glutamine degradation II
GLUTATHIONESYN-PWY	glutathione biosynthesis
GLUTDEG-PWY	glutamate degradation II
GLUTORN-PWY	ornithine biosynthesis
GLUTSYN-PWY	glutamate biosynthesis I
GLUTSYNIII-PWY	glutamate biosynthesis III
GLYCEROLMETAB-PWY	glycerol degradation V
GLYCLEAV-PWY	glycine cleavage
GLYCOCAT-PWY	glycogen degradation I
GLYCOGENSYNTH-PWY	glycogen biosynthesis I (from ADP-D-Glucose)
GLYCOLYSIS	glycolysis I (from glucose-6P)
GLYSYN-PWY	glycine biosynthesis I
GLYSYN-THR-PWY	glycine biosynthesis IV
HEME-BIOSYNTHESIS-II	heme biosynthesis from uroporphyrinogen-III I
HEMESYN2-PWY	heme biosynthesis from uroporphyrinogen-III II
HISDEG-PWY	histidine degradation I
HISTSYN-PWY	histidine biosynthesis
HOMOSER-METSYN-PWY	methionine biosynthesis I
HOMOSER-THRESYN-PWY	threonine biosynthesis from homoserine
HOMOSERSYN-PWY	homoserine biosynthesis
ILEUDEG-PWY	isoleucine degradation I
ILEUSYN-PWY	isoleucine biosynthesis I (from threonine)
KDO-LIPASYN-PWY	(KDO)2-lipid A biosynthesis I
LACTOSECAT-PWY	lactose and galactose degradation I

MetaCyc Pathway ID	Pathway Common Name
LACTOSEUTIL-PWY	lactose degradation II
LARABITOLUTIL-PWY	xylitol degradation
LCYSDEG-PWY	L-cysteine degradation II
LEUSYN-PWY	leucine biosynthesis
LIPAS-PWY	triacylglycerol degradation
LIPASYN-PWY	phospholipases
MALTOSECAT-PWY	maltose degradation
MANNCAT-PWY	D-mannose degradation
MANNIDEG-PWY	mannitol degradation I
MENAQUINONESYN-PWY	menaquinol-8 biosynthesis
METH-ACETATE-PWY	methanogenesis from acetate
METHFORM-PWY	methyl-coenzyme M reduction to methane
METHIONINE-DEG1-PWY	methionine degradation I (to homocysteine)
N2FIX-PWY	nitrogen fixation
NAD-BIOSYNTHESIS-III	NAD biosynthesis III
NAGLIPASYN-PWY	lipid IVA biosynthesis
NONMEVIPP-PWY	methylerythritol phosphate pathway
NONOXIPENT-PWY	pentose phosphate pathway (non-oxidative branch)
NPGLUCAT-PWY	Entner-Doudoroff pathway II (non-phosphorylative)
OXIDATIVEPENT-PWY	pentose phosphate pathway (oxidative branch)
P105-PWY	TCA cycle IV (2-oxoglutarate decarboxylase)
P121-PWY	adenine and adenosine salvage I
P122-PWY	heterolactic fermentation
P124-PWY	Bifidobacterium shunt
P162-PWY	glutamate degradation V (via hydroxyglutarate)
P163-PWY	lysine fermentation to acetate and butyrate
P164-PWY	purine nucleobases degradation I (anaerobic)
P2-PWY	2-(5-phosphoribosyl)-3-dephospho-CoA biosynthesis I (citrate lyase)
P21-PWY	pentose phosphate pathway (partial)
P283-PWY	hydrogen oxidation I (aerobic)

MetaCyc Pathway ID	Pathway Common Name
P3-PWY	gallate degradation III (anaerobic)
P302-PWY	L-sorbose degradation
P321-PWY	benzoyl-CoA degradation III (anaerobic)
P42-PWY	incomplete reductive TCA cycle
P562-PWY	myo-inositol degradation I
PANTO-PWY	phosphopantothenate biosynthesis I
PHENYLALANINE-DEG1-PWY	phenylalanine degradation I (aerobic)
PHESYN	phenylalanine biosynthesis I
PHOSPHONOTASE-PWY	2-aminoethylphosphonate degradation I
PLPSAL-PWY	pyridoxal 5-phosphate salvage I
PPGPPMET-PWY	ppGpp biosynthesis
PROPIONMET-PWY	methylmalonyl pathway
PROSYN-PWY	proline biosynthesis I
PROUT-PWY	proline degradation
PWY-1001	cellulose biosynthesis
PWY-1081	homogalacturonan degradation
PWY-1269	CMP-KDO biosynthesis I
PWY-1622	formaldehyde assimilation I (serine pathway)
PWY-1722	formaldehyde oxidation V (tetrahydrofolate pathway)
PWY-1881	formate oxidation to CO2
PWY-2161	folate polyglutamylation
PWY-2221	Entner-Doudoroff pathway III (semi-phosphorylative)
PWY-2301	myo-inositol biosynthesis
PWY-2361	3-oxoadipate degradation
PWY-2622	trehalose biosynthesis IV
PWY-2661	trehalose biosynthesis V
PWY-2941	lysine biosynthesis II
PWY-2942	lysine biosynthesis III
PWY-3221	dTDP-L-rhamnose biosynthesis II
PWY-3561	choline biosynthesis III

MetaCyc Pathway ID	Pathway Common Name
PWY-3781	aerobic respiration (cytochrome c)
PWY-3982	uracil degradation I (reductive)
PWY-4	UDP-D-galacturonate biosynthesis II (from D-galacturonate)
PWY-4081	glutathione redox reactions I
PWY-4101	D-sorbitol degradation I
PWY-4121	glutathionylspermidine biosynthesis
PWY-4261	glycerol degradation I
PWY-43	putrescine biosynthesis II
PWY-4341	glutamate biosynthesis V
PWY-4381	fatty acid biosynthesis initiation I
PWY-4621	arsenate detoxification II (glutaredoxin)
PWY-4722	creatinine degradation II
PWY-4921	protein citrullination
PWY-4984	urea cycle
PWY-5022	4-aminobutyrate degradation V
PWY-5041	S-adenosyl-L-methionine cycle II
PWY-5046	2-oxoisovalerate decarboxylation to isobutanoyl-CoA
PWY-5057	valine degradation II
PWY-5084	2-oxoglutarate decarboxylation to succinyl-CoA
PWY-5097	lysine biosynthesis VI
PWY-5101	isoleucine biosynthesis II
PWY-5104	isoleucine biosynthesis IV
PWY-5122	geranyl diphosphate biosynthesis
PWY-5142	acyl-ACP thioesterase pathway
PWY-5143	fatty acid activation
PWY-5148	acyl-CoA hydrolysis
PWY-5155	beta-alanine biosynthesis III
PWY-5162	2-oxopentenoate degradation
PWY-5188	tetrapyrrole biosynthesis I (from glutamate)
PWY-5194	siroheme biosynthesis

MetaCyc Pathway ID	Pathway Common Name
PWY-5207	coenzyme B/coenzyme M regeneration
PWY-5261	methanogenesis from tetramethylammonium
PWY-5278	sulfite oxidation III
PWY-5340	sulfate activation for sulfonation
PWY-5344	homocysteine biosynthesis
PWY-5350	thiosulfate disproportionation III (rhodanese)
PWY-5382	hydrogen oxidation II (aerobic, NAD)
PWY-5384	sucrose degradation IV (sucrose phosphorylase)
PWY-5386	methylglyoxal degradation I
PWY-5436	threonine degradation IV
PWY-5480	pyruvate fermentation to ethanol I
PWY-5481	pyruvate fermentation to lactate
PWY-5493	reductive monocarboxylic acid cycle
PWY-5497	purine nucleobases degradation II (anaerobic)
PWY-5508	adenosylcobalamin biosynthesis from cobyrinate a,c-diamide II
PWY-5509	adenosylcobalamin biosynthesis from cobyrinate a,c-diamide I
PWY-5659	GDP-mannose biosynthesis
PWY-5667	CDP-diacylglycerol biosynthesis I
PWY-5668	cardiolipin biosynthesis I
PWY-5669	phosphatidylethanolamine biosynthesis I
PWY-5674	nitrate reduction IV (dissimilatory)
PWY-5677	succinate fermentation to butyrate
PWY-5686	UMP biosynthesis
PWY-5695	urate biosynthesis/inosine 5-phosphate degradation
PWY-5698	allantoin degradation to ureidoglycolate II (ammonia producing)
PWY-5703	urea degradation I
PWY-5704	urea degradation II
PWY-5743	3-hydroxypropionate cycle
PWY-5751	phenylethanol biosynthesis

MetaCyc Pathway ID	Pathway Common Name
PWY-5783	octaprenyl diphosphate biosynthesis
PWY-5785	di-trans,poly-cis-undecaprenyl phosphate biosynthesis
PWY-5789	3-hydroxypropionate/4-hydroxybutyrate cycle
PWY-5791	1,4-dihydroxy-2-naphthoate biosynthesis II (plants)
PWY-5794	malonate degradation I (biotin-independent)
PWY-5807	heptaprenyl diphosphate biosynthesis
PWY-5831	CDP-abequose biosynthesis
PWY-5833	CDP-3,6-dideoxyhexose biosynthesis
PWY-5837	1,4-dihydroxy-2-naphthoate biosynthesis I
PWY-5839	menaquinol-7 biosynthesis
PWY-5844	menaquinol-9 biosynthesis
PWY-5849	menaquinol-6 biosynthesis
PWY-5851	demethylmenaquinol-9 biosynthesis
PWY-5852	demethylmenaquinol-8 biosynthesis I
PWY-5853	demethylmenaquinol-6 biosynthesis
PWY-5875	staphyloxanthin biosynthesis
PWY-5886	4-hydroxyphenylpyruvate biosynthesis
PWY-5890	menaquinol-10 biosynthesis
PWY-5891	menaquinol-11 biosynthesis
PWY-5892	menaquinol-12 biosynthesis
PWY-5895	menaquinol-13 biosynthesis
PWY-5901	2,3-dihydroxybenzoate biosynthesis
PWY-5913	TCA cycle VI (obligate autotrophs)
PWY-5921	L-glutamine biosynthesis II (tRNA-dependent)
PWY-5940	streptomycin biosynthesis
PWY-5941	glycogen degradation II
PWY-5964	guanylyl molybdenum cofactor biosynthesis
PWY-5971	palmitate biosynthesis II (bacteria and plants)
PWY-5973	cis-vaccenate biosynthesis
PWY-5988	wound-induced proteolysis I

MetaCyc Pathway ID	Pathway Common Name
PWY-5989	stearate biosynthesis II (bacteria and plants)
PWY-6012	acyl carrier protein metabolism
PWY-6018	seed germination protein turnover
PWY-6019	pseudouridine degradation
PWY-6028	acetoin degradation
PWY-6038	citrate degradation
PWY-6121	5-aminoimidazole ribonucleotide biosynthesis I
PWY-6122	5-aminoimidazole ribonucleotide biosynthesis II
PWY-6131	glycerol degradation II
PWY-6139	CMP-N-acetylneuraminate biosynthesis II (bacteria)
PWY-6143	CMP-pseudaminate biosynthesis
PWY-6147	6-hydroxymethyl-dihydropterin diphosphate biosynthesis I
PWY-6153	autoinducer AI-2 biosynthesis I
PWY-6154	autoinducer AI-2 biosynthesis II (Vibrio)
PWY-6163	chorismate biosynthesis from 3-dehydroquinate
PWY-6164	3-dehydroquinate biosynthesis I
PWY-6173	histamine biosynthesis
PWY-6193	3-chlorocatechol degradation II (ortho)
PWY-6196	serine racemization
PWY-621	sucrose degradation III (sucrose invertase)
PWY-622	starch biosynthesis
PWY-6268	adenosylcobalamin salvage from cobalamin
PWY-6269	adenosylcobalamin salvage from cobinamide II
PWY-6282	palmitoleate biosynthesis I
PWY-6317	galactose degradation I (Leloir pathway)
PWY-6322	phosphinothricin tripeptide biosynthesis
PWY-6344	ornithine degradation II (Stickland reaction)
PWY-6348	phosphate acquisition
PWY-6349	CDP-archaeol biosynthesis
PWY-6357	phosphate utilization in cell wall regeneration

MetaCyc Pathway ID	Pathway Common Name
PWY-6386	UDP-N-acetylmuramoyl-pentapeptide biosynthesis II (lysine-containing)
PWY-6387	UDP-N-acetylmuramoyl-pentapeptide biosynthesis III (meso-DAP-containing)
PWY-6397	mycolyl-arabinogalactan-peptidoglycan complex biosynthesis
PWY-6430	thymine degradation
PWY-6461	peptidoglycan cross-bridge biosynthesis II (E. faecium)
PWY-6465	omega-hydroxylation of caprate and laurate
PWY-6476	cytidylyl molybdenum cofactor biosynthesis
PWY-6507	5-dehydro-4-deoxy-D-glucuronate degradation
PWY-6512	hydrogen oxidation III (anaerobic, NADP)
PWY-6518	glycocholate metabolism (bacteria)
PWY-6519	8-amino-7-oxononanoate biosynthesis I
PWY-6543	4-aminobenzoate biosynthesis
PWY-6545	pyrimidine deoxyribonucleotides de novo biosynthesis III
PWY-6559	spermidine biosynthesis II
PWY-6562	norspermidine biosynthesis
PWY-6572	chondroitin sulfate and dermatan sulfate degradation I (bacterial)
PWY-6578	8-amino-7-oxononanoate biosynthesis III
PWY-6583	pyruvate fermentation to butanol I
PWY-6587	pyruvate fermentation to ethanol III
PWY-6599	guanine and guanosine salvage II
PWY-66	GDP-L-fucose biosynthesis I (from GDP-D-mannose)
PWY-6608	guanosine nucleotides degradation III
PWY-6609	adenine and adenosine salvage III
PWY-6610	adenine and adenosine salvage IV

MetaCyc Pathway ID	Pathway Common Name
PWY-6613	tetrahydrofolate salvage from 5,10-methenyltetrahydrofolate
PWY-6614	tetrahydrofolate biosynthesis
PWY-6617	adenosine nucleotides degradation III
PWY-6620	guanine and guanosine salvage I
PWY-6627	salinosporamide A biosynthesis
PWY-6638	sulfolactate degradation III
PWY-6642	(R)-cysteate degradation
PWY-6643	coenzyme M biosynthesis II
PWY-6649	glycolate and glyoxylate degradation III
PWY-6695	oxalate degradation II
PWY-6700	queuosine biosynthesis
PWY-6703	preQ0 biosynthesis
PWY-6708	ubiquinol-8 biosynthesis (prokaryotic)
PWY-6737	starch degradation V
PWY-6744	hydrogen production I
PWY-6756	S-methyl-5-thioadenosine degradation II
PWY-6758	hydrogen production II
PWY-6769	rhamnogalacturonan type I degradation I (fungi)
PWY-6772	hydrogen production V
PWY-6780	hydrogen production VI
PWY-6785	hydrogen production VIII
PWY-6815	porphyran degradation
PWY-6816	agarose degradation
PWY-6823	molybdenum cofactor biosynthesis
PWY-6827	gellan degradation
PWY-6855	chitin degradation I (archaea)
PWY-6890	4-amino-2-methyl-5-diphosphomethylpyrimidine biosynthesis
PWY-6892	thiazole biosynthesis I (E. coli)
PWY-6893	thiamin diphosphate biosynthesis II (Bacillus)
PWY-6894	thiamin diphosphate biosynthesis I (E. coli)

MetaCyc Pathway ID	Pathway Common Name
PWY-6896	thiamin salvage I
PWY-6898	thiamin salvage III
PWY-6899	base-degraded thiamin salvage
PWY-6902	chitin degradation II
PWY-6906	chitin derivatives degradation
PWY-6907	thiamin diphosphate biosynthesis III (Staphylococcus)
PWY-6910	hydroxymethylpyrimidine salvage
PWY-6932	selenate reduction
PWY-6938	NADH repair
PWY-6943	testosterone and androsterone degradation to androstendione
PWY-6944	androstenedione degradation
PWY-6946	cholesterol degradation to androstenedione II (cholesterol dehydrogenase)
PWY-6948	sitosterol degradation to androstenedione
PWY-6951	docosahexanoate biosynthesis II
PWY-6952	glycerophosphodiester degradation
PWY-6961	L-ascorbate degradation II (bacterial, aerobic)
PWY-6964	ammonia assimilation cycle II
PWY-6969	TCA cycle V (2-oxoglutarate:ferredoxin oxidoreductase)
PWY-6984	lipoate salvage II
PWY-6986	alginate degradation
PWY-6987	lipoate biosynthesis and incorporation III (Bacillus)
PWY-6999	theophylline degradation
PWY-701	methionine degradation II
PWY-7028	UDP-N,N-diacetylbacillosamine biosynthesis
PWY-7054	N-acetylglutaminylglutamine amide biosynthesis
PWY-7096	triclosan resistance

MetaCyc Pathway ID	Pathway Common Name
PWY-7159	chlorophyllide a biosynthesis III (aerobic, light independent)
PWY-7174	S-methyl-5-thio-alpha-D-ribose 1-phosphate degradation II
PWY-7176	UTP and CTP de novo biosynthesis
PWY-7179	purine deoxyribonucleosides degradation I
PWY-7180	2-deoxy-alpha-D-ribose 1-phosphate degradation
PWY-7181	pyrimidine deoxyribonucleosides degradation
PWY-7183	pyrimidine nucleobases salvage I
PWY-7184	pyrimidine deoxyribonucleotides de novo biosynthesis I
PWY-7185	UTP and CTP dephosphorylation I
PWY-7187	pyrimidine deoxyribonucleotides de novo biosynthesis II
PWY-7193	pyrimidine ribonucleosides salvage I
PWY-7197	pyrimidine deoxyribonucleotide phosphorylation
PWY-7199	pyrimidine deoxyribonucleosides salvage
PWY-7205	CMP phosphorylation
PWY-7210	pyrimidine deoxyribonucleotides biosynthesis from CTP
PWY-7219	adenosine ribonucleotides de novo biosynthesis
PWY-7220	adenosine deoxyribonucleotides de novo biosynthesis II
PWY-7221	guanosine ribonucleotides de novo biosynthesis
PWY-7222	guanosine deoxyribonucleotides de novo biosynthesis II
PWY-7224	purine deoxyribonucleosides salvage
PWY-7242	D-fructuronate degradation
PWY-7246	pectin degradation II
PWY-7247	beta-D-glucuronide and D-glucuronate degradation
PWY-7248	pectin degradation III

MetaCyc Pathway ID	Pathway Common Name
PWY-7250	[2Fe-2S] iron-sulfur cluster biosynthesis
PWY-7285	methylwyosine biosynthesis
PWY-7286	7-(3-amino-3-carboxypropyl)-wyosine biosynthesis
PWY-7294	xylose degradation IV
PWY-7295	L-arabinose degradation IV
PWY-7308	acrylonitrile degradation I
PWY-7330	UDP-N-acetyl-beta-L-fucosamine biosynthesis
PWY-7331	UDP-N-acetyl-beta-L-quinovosamine biosynthesis
PWY-7333	UDP-N-acetyl-alpha-D-fucosamine biosynthesis
PWY-7334	UDP-N-acetyl-alpha-D-quinovosamine biosynthesis
PWY-7335	UDP-N-acetyl-alpha-D-mannosaminouronate biosynthesis
PWY-7343	UDP-glucose biosynthesis
PWY-7344	UDP-D-galactose biosynthesis
PWY-7346	UDP-alpha-D-glucuronate biosynthesis (from UDP-glucose)
PWY-7347	sucrose biosynthesis III
PWY-7353	4-methyl-5(beta-hydroxyethyl)thiazole salvage (yeast)
PWY-7356	thiamin salvage IV (yeast)
PWY-7367	phosphatidylcholine resynthesis via glycerophosphocholine
PWY-901	methylglyoxal degradation II
PWY0-1021	alanine biosynthesis III
PWY0-1182	trehalose degradation II (trehalase)
PWY0-1241	ADP-L-glycero-beta-D-manno-heptose biosynthesis
PWY0-1261	1,6-anhydro-N-acetylmuramic acid recycling
PWY0-1264	biotin-carboxyl carrier protein assembly
PWY0-1275	lipoate biosynthesis and incorporation II

MetaCyc Pathway ID	Pathway Common Name
PWY0-1280	ethylene glycol degradation
PWY0-1295	pyrimidine ribonucleosides degradation
PWY0-1296	purine ribonucleosides degradation
PWY0-1299	arginine dependent acid resistance
PWY0-1300	2-O-alpha-mannosyl-D-glycerate degradation
PWY0-1301	melibiose degradation
PWY0-1305	glutamate dependent acid resistance
PWY0-1312	acetate formation from acetyl-CoA I
PWY0-1313	acetate conversion to acetyl-CoA
PWY0-1314	fructose degradation
PWY0-1315	L-lactaldehyde degradation (anaerobic)
PWY0-1317	L-lactaldehyde degradation (aerobic)
PWY0-1319	CDP-diacylglycerol biosynthesis II
PWY0-1324	N-acetylneuraminate and N-acetylmannosamine degradation
PWY0-1334	NADH to cytochrome bd oxidase electron transfer
PWY0-1338	polymyxin resistance
PWY0-1353	succinate to cytochrome bd oxidase electron transfer
PWY0-1477	ethanolamine utilization
PWY0-1479	tRNA processing
PWY0-1507	biotin biosynthesis from 8-amino-7-oxononanoate
PWY0-1535	D-serine degradation
PWY0-1545	cardiolipin biosynthesis III
PWY0-1546	muropeptide degradation
PWY0-321	phenylacetate degradation I (aerobic)
PWY0-42	2-methylcitrate cycle I
PWY0-43	conversion of succinate to propionate
PWY0-461	lysine degradation I
PWY0-501	lipoate biosynthesis and incorporation I
PWY0-522	lipoate salvage I
PWY0-541	cyclopropane fatty acid (CFA) biosynthesis

MetaCyc Pathway ID	Pathway Common Name
PWY0-662	PRPP biosynthesis I
PWY0-823	arginine degradation III (arginine decarboxylase/agmatinase pathway)
PWY0-862	cis-dodecenoyl biosynthesis
PWY0-901	selenocysteine biosynthesis I (bacteria)
PWY1-2	alanine degradation IV
PWY1A0-6325	actinorhodin biosynthesis
PWY3O-4106	NAD salvage pathway III
PWY490-4	asparagine biosynthesis III (tRNA-dependent)
PWY66-21	ethanol degradation II
PWY66-400	glycolysis VI (metazoan)
PWYG-321	mycolate biosynthesis
PWYQT-4429	CO2 fixation into oxaloacetate (anapleurotic)
PYRIDNUCSYN-PWY	NAD biosynthesis I (from aspartate)
PYRIDOXSYN-PWY	pyridoxal 5-phosphate biosynthesis I
PYRUVDEHYD-PWY	pyruvate decarboxylation to acetyl CoA
PYRUVOX-PWY	pyruvate oxidation pathway
REDCITCYC	TCA cycle III (helicobacter)
RHAMCAT-PWY	L-rhamnose degradation I
RIBOSYN2-PWY	flavin biosynthesis I (bacteria and plants)
SALVADEHYPOX-PWY	adenosine nucleotides degradation II
SALVPURINE2-PWY	xanthine and xanthosine salvage
SAM-PWY	S-adenosyl-L-methionine biosynthesis
SERDEG-PWY	L-serine degradation
SERSYN-PWY	serine biosynthesis
SORBDEG-PWY	D-sorbitol degradation II
SUCROSEUTIL2-PWY	sucrose degradation VII (sucrose 3-dehydrogenase)
SUCUTIL-PWY	sucrose degradation I (sucrose phosphotransferase)
TEICHOICACID-PWY	teichoic acid (poly-glycerol) biosynthesis
THIOREDOX-PWY	thioredoxin pathway
THREONINE-DEG2-PWY	threonine degradation II

MetaCyc Pathway ID	Pathway Common Name
TREDEGLOW-PWY	trehalose degradation I (low osmolarity)
TRESYN-PWY	trehalose biosynthesis I
TRNA-CHARGING-PWY	tRNA charging
TRPSYN-PWY	tryptophan biosynthesis
TRYPDEG-PWY	tryptophan degradation II (via pyruvate)
TYRFUMCAT-PWY	tyrosine degradation I
TYRSYN	tyrosine biosynthesis I
UDPNAGSYN-PWY	UDP-N-acetyl-D-glucosamine biosynthesis I
VALDEG-PWY	valine degradation I
VALSYN-PWY	valine biosynthesis
XYLCAT-PWY	xylose degradation I

[0125] In some embodiments, in addition to core metabolic pathways, high-complexity defined gut microbial communities can further comprise one or more microbes utilizing one or more variable metabolic pathways. As used herein, "variable metabolic pathways" refers to metabolic pathways that are found in some gut metagenomes annotated in the GutCyc project. For example, in some embodiments variable metabolic pathways can include any one or more of the 2AMINOBENZDEG-PWY, 2PHENDEG-PWY, 3-HYDROXYPHENYLACETATE-DEGRADATION-PWY, 7ALPHADEHYDROX-PWY, AEROBACTINSYN-PWY, ALADEG-PWY, ALKANEMONOX-PWY, AMMOXID-PWY, ANAPHENOXI-PWY, ARG-GLU-PWY, ARGDEG-IV-PWY, ARGSPECAT-PWY, ASPARTATE-DEG1-PWY, ASPARTATESYN-PWY, BETSYN-PWY, CALVIN-PWY, CARNMET-PWY, CHOLINE-BETAINE-ANA-PWY, CO2FORM-PWY, CODH-PWY, CYANCAT-PWY, DARABCAT-PWY, DARABITOLUTIL-PWY, DHGLUCONATE-PYR-CAT-PWY, DISSULFRED-PWY, ECASYN-PWY, ENTNER-DOUDOROFF-PWY, FAO-PWY, FESULFOX-PWY, FORMASS-PWY, GALDEG-PWY, GDPRHAMSYN-PWY, GLUCUROCAT-PWY, GLUT-REDOX-PWY, GLUTAMATE-SYN2-PWY, GLYCOLATEMET-PWY, GLYOXDEG-PWY, GLYOXYLATE-BYPASS, GLYSYN-ALA-PWY, HCAMHPDEG-PWY, HOMOCYSDEGR-PWY, HSERMETANA-PWY, IDNCAT-PWY, KDOSYN-PWY, LEU-DEG2-PWY, LIPA-CORESYN-PWY, METHANOGENESIS-PWY, NADPHOS-DEPHOS-PWY, OCTOPINEDEG-PWY, ORN-AMINOPENTANOATE-CAT-PWY, P1-PWY, P101-PWY, P108-PWY, P141-PWY, P161-PWY, P181-PWY, P183-PWY, P184-PWY, P201-PWY, P224-PWY, P241-PWY, P261-PWY, P303-PWY, P341-PWY, P344-PWY, P483-PWY, P541-PWY, P561-PWY, P621-PWY, P641-

PWY, PARATHION-DEGRADATION-PWY, PCEDEG-PWY, PROTOCATECHUATE-ORTHO-CLEAVAGE-PWY, PUTDEG-PWY, PWY-101, PWY-1263, PWY-1281, PWY-1341, PWY-1361, PWY-1641, PWY-1723, PWY-1781, PWY-1801, PWY-181, PWY-1861, PWY-2, PWY-2201, PWY-2242, PWY-2503, PWY-2721, PWY-2722, PWY-283, PWY-3161, PWY-3181, PWY-3462, PWY-3602, PWY-3661, PWY-3722, PWY-3941, PWY-40, PWY-4181, PWY-4521, PWY-4601, PWY-481, PWY-4821, PWY-4861, PWY-5025, PWY-5026, PWY-5028, PWY-5033, PWY-5055, PWY-5074, PWY-5080, PWY-5087, PWY-5103, PWY-5111, PWY-5120, PWY-5123, PWY-5154, PWY-5159, PWY-5169, PWY-5177, PWY-5189, PWY-5197, PWY-5198, PWY-5209, PWY-5247, PWY-5250, PWY-5254, PWY-5274, PWY-5276, PWY-5277, PWY-5279, PWY-5280, PWY-5302, PWY-5331, PWY-5332, PWY-5352, PWY-5358, PWY-5364, PWY-5372, PWY-5392, PWY-5437, PWY-5453, PWY-5482, PWY-5484, PWY-5486, PWY-5489, PWY-5490, PWY-5499, PWY-5517, PWY-5519, PWY-5521, PWY-5526, PWY-5530, PWY-5531, PWY-5532, PWY-5533, PWY-5534, PWY-5535, PWY-5651, PWY-5654, PWY-5656, PWY-5662, PWY-5663, PWY-5670, PWY-5675, PWY-5687, PWY-5691, PWY-5697, PWY-5726, PWY-5731, PWY-5739, PWY-5740, PWY-5744, PWY-5747, PWY-5755, PWY-5766, PWY-5782, PWY-5796, PWY-5805, PWY-5810, PWY-5834, PWY-5907, PWY-5915, PWY-5917, PWY-5927, PWY-5929, PWY-5938, PWY-5939, PWY-5951, PWY-5963, PWY-5966, PWY-5979, PWY-5981, PWY-5983, PWY-5985, PWY-5986, PWY-6004, PWY-6021, PWY-6048, PWY-6050, PWY-6060, PWY-6077, PWY-6082, PWY-6107, PWY-6120, PWY-6123, PWY-6125, PWY-6126, PWY-6130, PWY-6137, PWY-6148, PWY-6160, PWY-6166, PWY-6167, PWY-6174, PWY-6183, PWY-6184, PWY-6190, PWY-6213, PWY-6223, PWY-6262, PWY-6281, PWY-6328, PWY-6373, PWY-6383, PWY-6388, PWY-6390, PWY-6406, PWY-6409, PWY-6416, PWY-6419, PWY-6424, PWY-6454, PWY-6455, PWY-6458, PWY-6463, PWY-6464, PWY-6466, PWY-6478, PWY-6481, PWY-6482, PWY-6497, PWY-6499, PWY-6502, PWY-6510, PWY-6523, PWY-6535, PWY-6536, PWY-6537, PWY-6550, PWY-6556, PWY-6580, PWY-6588, PWY-6605, PWY-6611, PWY-6618, PWY-6619, PWY-6622, PWY-6626, PWY-6637, PWY-6644, PWY-6646, PWY-6654, PWY-6655, PWY-6672, PWY-6675, PWY-6679, PWY-6682, PWY-6687, PWY-6690, PWY-6696, PWY-6698, PWY-6711, PWY-6713, PWY-6717, PWY-6728, PWY-6731, PWY-6748, PWY-6753, PWY-6754, PWY-6755, PWY-6759, PWY-6767, PWY-6771, PWY-6789, PWY-6790, PWY-6793, PWY-6795, PWY-6797, PWY-6805, PWY-6813, PWY-6814, PWY-6821, PWY-6825, PWY-6891, PWY-6945, PWY-6966, PWY-6972, PWY-6978, PWY-6993, PWY-6994, PWY-7013, PWY-7014, PWY-7022, PWY-7032, PWY-7046, PWY-7052, PWY-7072, PWY-7097, PWY-7098, PWY-7104, PWY-7106, PWY-7130, PWY-7177, PWY-7198, PWY-7206, PWY-

722, PWY-7241, PWY-7254, PWY-7255, PWY-7301, PWY-7309, PWY-7312, PWY-7315, PWY-7316, PWY-7318, PWY-761, PWY-822, PWY-922, PWY0-1221, PWY0-1297, PWY0-1298, PWY0-1321, PWY0-1329, PWY0-1335, PWY0-1336, PWY0-1337, PWY0-1347, PWY0-1348, PWY0-1352, PWY0-1355, PWY0-1356, PWY0-1391, PWY0-1433, PWY0-1465, PWY0-1466, PWY0-1471, PWY0-1533, PWY0-1544, PWY0-163, PWY0-166, PWY0-181, PWY0-301, PWY0-44, PWY0-521, PWY0-981, PWY1G-0, PWY1G-170, PWY3DJ-11281, PWY3O-246, PWY3O-450, PWY490-3, PWY5F9-12, PWYQT-4427, PYRIDNUCSAL-PWY, QUINATEDEG-PWY, RIBITOLUTIL-PWY, RIBOKIN-PWY, RUMP-PWY, SHIKIMATEDEG-PWY, SUCSYN-PWY, TAURINEDEG-PWY, TCA, THRDLCTCAT-PWY, TOLUENE-DEG-2-OH-PWY, TOLUENE-DEG-3-OH-PWY, TOLUENE-DEG-4-OH-PWY, TRPCAT-PWY, and TRPKYNCAT-PWY MetaCyc pathways.

[0126] The common names of the variable MetaCyc pathways described above are provided in Table 10 below.

TABLE 10 – Variable MetaCyc Pathways

MetaCyc Pathway ID	**Pathway Common Name**
2AMINOBENZDEG-PWY	anthranilate degradation III (anaerobic)
2PHENDEG-PWY	phenylethylamine degradation I
3-HYDROXYPHENYLACETATE-DEGRADATION-PWY	4-hydroxyphenylacetate degradation
7ALPHADEHYDROX-PWY	cholate degradation (bacteria, anaerobic)
AEROBACTINSYN-PWY	aerobactin biosynthesis
ALADEG-PWY	alanine degradation I
ALKANEMONOX-PWY	two-component alkanesulfonate monooxygenase
AMMOXID-PWY	ammonia oxidation I (aerobic)
ANAPHENOXI-PWY	phenylalanine degradation II (anaerobic)
ARG-GLU-PWY	arginine degradation VII (arginase 3 pathway)
ARGDEG-IV-PWY	arginine degradation VIII (arginine oxidase pathway)
ARGSPECAT-PWY	spermine biosynthesis
ASPARTATE-DEG1-PWY	aspartate degradation I
ASPARTATESYN-PWY	aspartate biosynthesis
BETSYN-PWY	glycine betaine biosynthesis I (Gram-negative bacteria)

MetaCyc Pathway ID	Pathway Common Name
CALVIN-PWY	Calvin-Benson-Bassham cycle
CARNMET-PWY	carnitine degradation I
CHOLINE-BETAINE-ANA-PWY	choline degradation I
CO2FORM-PWY	methanogenesis from methanol
CODH-PWY	reductive acetyl coenzyme A pathway
CYANCAT-PWY	cyanate degradation
DARABCAT-PWY	D-arabinose degradation II
DARABITOLUTIL-PWY	D-arabitol degradation
DHGLUCONATE-PYR-CAT-PWY	glucose degradation (oxidative)
DISSULFRED-PWY	sulfate reduction IV (dissimilatory)
ECASYN-PWY	enterobacterial common antigen biosynthesis
ENTNER-DOUDOROFF-PWY	Entner-Doudoroff pathway I
FAO-PWY	fatty acid beta-oxidation I
FESULFOX-PWY	sulfur oxidation II (Fe+3-dependent)
FORMASS-PWY	formaldehyde oxidation IV (thiol-independent)
GALDEG-PWY	galactose degradation II
GDPRHAMSYN-PWY	GDP-D-rhamnose biosynthesis
GLUCUROCAT-PWY	superpathway of _-D-glucuronosides degradation
GLUT-REDOX-PWY	glutathione redox reactions II
GLUTAMATE-SYN2-PWY	glutamate biosynthesis II
GLYCOLATEMET-PWY	glycolate and glyoxylate degradation I
GLYOXDEG-PWY	glycolate and glyoxylate degradation II
GLYOXYLATE-BYPASS	glyoxylate cycle
GLYSYN-ALA-PWY	glycine biosynthesis III
HCAMHPDEG-PWY	3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation to 2-oxopent-4-enoate
HOMOCYSDEGR-PWY	cysteine biosynthesis/homocysteine degradation
HSERMETANA-PWY	methionine biosynthesis III
IDNCAT-PWY	L-idonate degradation
KDOSYN-PWY	KDO transfer to lipid IVA I
LEU-DEG2-PWY	leucine degradation I

MetaCyc Pathway ID	Pathway Common Name
LIPA-CORESYN-PWY	Lipid A-core biosynthesis
METHANOGENESIS-PWY	methanogenesis from CO2
NADPHOS-DEPHOS-PWY	NAD phosphorylation and dephosphorylation
OCTOPINEDEG-PWY	octopine degradation
ORN-AMINOPENTANOATE-CAT-PWY	ornithine degradation I (proline biosynthesis)
P1-PWY	purine and pyrimidine metabolism
P101-PWY	ectoine biosynthesis
P108-PWY	pyruvate fermentation to propionate I
P141-PWY	atrazine degradation I (aerobic)
P161-PWY	acetylene degradation
P181-PWY	nicotine degradation I
P183-PWY	catechol degradation to 2-oxopent-4-enoate I
P184-PWY	protocatechuate degradation I (meta-cleavage pathway)
P201-PWY	nitroglycerin degradation
P224-PWY	sulfate reduction V (dissimilatory)
P241-PWY	coenzyme B biosynthesis
P261-PWY	coenzyme M biosynthesis I
P303-PWY	ammonia oxidation II (anaerobic)
P341-PWY	glycolysis V (Pyrococcus)
P344-PWY	acrylonitrile degradation
P483-PWY	phosphonoacetate degradation
P541-PWY	glycine betaine biosynthesis IV (from glycine)
P561-PWY	stachydrine degradation
P621-PWY	nylon-6 oligomer degradation
P641-PWY	phenylmercury acetate degradation
PARATHION-DEGRADATION-PWY	parathion degradation
PCEDEG-PWY	tetrachloroethene degradation
PROTOCATECHUATE-ORTHO-CLEAVAGE-PWY	protocatechuate degradation II (ortho-cleavage pathway)
PUTDEG-PWY	putrescine degradation I

MetaCyc Pathway ID	Pathway Common Name
PWY-101	photosynthesis light reactions
PWY-1263	taurine degradation I
PWY-1281	sulfoacetaldehyde degradation I
PWY-1341	phenylacetate degradation II (anaerobic)
PWY-1361	benzoyl-CoA degradation I (aerobic)
PWY-1641	methane oxidation to methanol I
PWY-1723	formaldehyde oxidation VI (H4MPT pathway)
PWY-1781	beta-alanine degradation II
PWY-1801	formaldehyde oxidation II (glutathione-dependent)
PWY-181	photorespiration
PWY-1861	formaldehyde assimilation II (RuMP Cycle)
PWY-2	putrescine degradation IV
PWY-2201	folate transformations I
PWY-2242	ammonia oxidation III
PWY-2503	benzoate degradation I (aerobic)
PWY-2721	trehalose degradation III
PWY-2722	trehalose degradation IV
PWY-283	benzoate degradation II (aerobic and anaerobic)
PWY-3161	indole-3-acetate biosynthesis III (bacteria)
PWY-3181	tryptophan degradation VI (via tryptamine)
PWY-3462	phenylalanine biosynthesis II
PWY-3602	carnitine degradation II
PWY-3661	glycine betaine degradation
PWY-3722	glycine betaine biosynthesis II (Gram-positive bacteria)
PWY-3941	beta-alanine biosynthesis II
PWY-40	putrescine biosynthesis I
PWY-4181	glutathione amide metabolism
PWY-4521	arsenite oxidation (respiratory)
PWY-4601	arsenate reduction (respiratory)
PWY-481	ethylbenzene degradation (anaerobic)

MetaCyc Pathway ID	Pathway Common Name
PWY-4821	UDP-D-xylose and UDP-D-glucuronate biosynthesis
PWY-4861	UDP-D-galacturonate biosynthesis I (from UDP-D-glucuronate)
PWY-5025	indole-3-acetate biosynthesis IV (bacteria)
PWY-5026	indole-3-acetate biosynthesis V (bacteria and fungi)
PWY-5028	histidine degradation II
PWY-5033	nicotinate degradation II
PWY-5055	nicotinate degradation III
PWY-5074	mevalonate degradation
PWY-5080	very long chain fatty acid biosynthesis
PWY-5087	glutamate degradation VI (to pyruvate)
PWY-5103	isoleucine biosynthesis III
PWY-5111	CMP-KDO biosynthesis II (from D-arabinose 5-phosphate)
PWY-5120	geranylgeranyl diphosphate biosynthesis
PWY-5123	trans, trans-farnesyl diphosphate biosynthesis
PWY-5154	arginine biosynthesis III
PWY-5159	4-hydroxyproline degradation II
PWY-5169	cyanurate degradation
PWY-5177	glutaryl-CoA degradation
PWY-5189	tetrapyrrole biosynthesis II (from glycine)
PWY-5197	lactate biosynthesis (archaea)
PWY-5198	factor 420 biosynthesis
PWY-5209	methyl-coenzyme M oxidation to CO2
PWY-5247	methanogenesis from methylamine
PWY-5250	methanogenesis from trimethylamine
PWY-5254	methanofuran biosynthesis
PWY-5274	sulfide oxidation II (sulfide dehydrogenase)
PWY-5276	sulfite oxidation I (sulfite oxidoreductase)
PWY-5277	thiosulfate disproportionation I (thiol-dependent)
PWY-5279	sulfite oxidation II

MetaCyc Pathway ID	Pathway Common Name
PWY-5280	lysine degradation IV
PWY-5302	sulfur disproportionation II (aerobic)
PWY-5331	taurine biosynthesis
PWY-5332	sulfur reduction I
PWY-5352	thiosulfate disproportionation II (non thiol-dependent)
PWY-5358	tetrathionate reduction I (to thiosulfate)
PWY-5364	sulfur reduction II (via polysulfide)
PWY-5372	carbon tetrachloride degradation II
PWY-5392	reductive TCA cycle II
PWY-5437	threonine degradation I
PWY-5453	methylglyoxal degradation III
PWY-5482	pyruvate fermentation to acetate II
PWY-5484	glycolysis II (from fructose-6P)
PWY-5486	pyruvate fermentation to ethanol II
PWY-5489	methyl parathion degradation
PWY-5490	paraoxon degradation
PWY-5499	vitamin B6 degradation
PWY-5517	L-arabinose degradation III
PWY-5519	D-arabinose degradation III
PWY-5521	L-ascorbate biosynthesis III
PWY-5526	bacteriochlorophyll a biosynthesis
PWY-5530	sorbitol biosynthesis II
PWY-5531	chlorophyllide a biosynthesis II (anaerobic)
PWY-5532	adenosine nucleotides degradation IV
PWY-5533	acetone degradation II (to acetoacetate)
PWY-5534	propylene degradation
PWY-5535	acetate formation from acetyl-CoA II
PWY-5651	tryptophan degradation to 2-amino-3-carboxymuconate semialdehyde
PWY-5654	2-amino-3-carboxymuconate semialdehyde degradation to 2-oxopentenoate

MetaCyc Pathway ID	Pathway Common Name
PWY-5656	mannosylglycerate biosynthesis I
PWY-5662	glucosylglycerate biosynthesis I
PWY-5663	tetrahydrobiopterin biosynthesis I
PWY-5670	epoxysqualene biosynthesis
PWY-5675	nitrate reduction V (assimilatory)
PWY-5687	pyrimidine ribonucleotides interconversion
PWY-5691	urate degradation to allantoin
PWY-5697	allantoin degradation to ureidoglycolate I (urea producing)
PWY-5726	deethylsimazine degradation
PWY-5731	atrazine degradation III
PWY-5739	GDP-D-perosamine biosynthesis
PWY-5740	GDP-L-colitose biosynthesis
PWY-5744	glyoxylate assimilation
PWY-5747	2-methylcitrate cycle II
PWY-5755	4-hydroxybenzoate biosynthesis II (bacteria and fungi)
PWY-5766	glutamate degradation X
PWY-5782	2-keto-L-gulonate biosynthesis
PWY-5796	2-(5-phosphoribosyl)-3-dephospho-CoA biosynthesis II (malonate decarboxylase)
PWY-5805	nonaprenyl diphosphate biosynthesis I
PWY-5810	usnate biosynthesis
PWY-5834	CDP-tyvelose biosynthesis
PWY-5907	homospermidine biosynthesis
PWY-5915	phycoerythrobilin biosynthesis
PWY-5917	phycocyanobilin biosynthesis
PWY-5927	(4S)-carveol and (4S)-dihydrocarveol degradation
PWY-5929	puromycin biosynthesis
PWY-5938	(R)-acetoin biosynthesis I
PWY-5939	(R)-acetoin biosynthesis II
PWY-5951	(R,R)-butanediol biosynthesis

MetaCyc Pathway ID	Pathway Common Name
PWY-5963	thio-molybdenum cofactor biosynthesis
PWY-5966	fatty acid biosynthesis initiation II
PWY-5979	3-amino-5-hydroxybenzoate biosynthesis
PWY-5981	CDP-diacylglycerol biosynthesis III
PWY-5983	trehalose biosynthesis VI
PWY-5985	trehalose biosynthesis VII
PWY-5986	ammonium transport
PWY-6004	glycine betaine biosynthesis V (from glycine)
PWY-6021	nitrilotriacetate degradation
PWY-6048	methylthiopropionate degradation I (cleavage)
PWY-6050	dimethyl sulfoxide degradation
PWY-6060	malonate degradation II (biotin-dependent)
PWY-6077	anthranilate degradation II (aerobic)
PWY-6082	alginate biosynthesis II
PWY-6107	chlorosalicylate degradation
PWY-6120	tyrosine biosynthesis III
PWY-6123	inosine-5-phosphate biosynthesis I
PWY-6125	guanosine nucleotides de novo biosynthesis
PWY-6126	adenosine nucleotides de novo biosynthesis
PWY-6130	glycerol degradation III
PWY-6137	copper transport II
PWY-6148	tetrahydromethanopterin biosynthesis
PWY-6160	3-dehydroquinate biosynthesis II (archaea)
PWY-6166	calcium transport I
PWY-6167	flavin biosynthesis II (archaea)
PWY-6174	mevalonate pathway II (archaea)
PWY-6183	salicylate degradation I
PWY-6184	methylsalicylate degradation
PWY-6190	2,4-dichlorotoluene degradation
PWY-6213	cadmium transport I
PWY-6223	gentisate degradation
PWY-6262	demethylmenaquinol-8 biosynthesis II

MetaCyc Pathway ID	Pathway Common Name
PWY-6281	selenocysteine biosynthesis II (archaea and eukaryotes)
PWY-6328	lysine degradation X
PWY-6373	acrylate degradation
PWY-6383	mono-trans, poly-cis decaprenyl phosphate biosynthesis
PWY-6388	(S,S)-butanediol degradation
PWY-6390	(S,S)-butanediol biosynthesis
PWY-6406	salicylate biosynthesis I
PWY-6409	pyoverdine I biosynthesis
PWY-6416	quinate degradation II
PWY-6419	shikimate degradation II
PWY-6424	26,27-dehydrozymosterol metabolism
PWY-6454	vancomycin resistance I
PWY-6455	vancomycin resistance II
PWY-6458	benzoyl-CoA biosynthesis
PWY-6463	peptidoglycan cross-bridge biosynthesis IV (Weissella viridescens)
PWY-6464	polyvinyl alcohol degradation
PWY-6466	pyridoxal 5-phosphate biosynthesis II
PWY-6478	GDP-D-glycero-alpha-D-manno-heptose biosynthesis
PWY-6481	L-dopachrome biosynthesis
PWY-6482	diphthamide biosynthesis
PWY-6497	D-galactarate degradation II
PWY-6499	D-glucarate degradation II
PWY-6502	oxidized GTP and dGTP detoxification
PWY-6510	methanol oxidation to formaldehyde II
PWY-6523	intra-aerobic nitrite reduction
PWY-6535	4-aminobutyrate degradation I
PWY-6536	4-aminobutyrate degradation III
PWY-6537	4-aminobutyrate degradation II

MetaCyc Pathway ID	Pathway Common Name
PWY-6550	carbazole degradation
PWY-6556	pyrimidine ribonucleosides degradation II
PWY-6580	L-1-phosphatidyl-inositol biosynthesis (Mycobacteria)
PWY-6588	pyruvate fermentation to acetone
PWY-6605	adenine and adenosine salvage II
PWY-6611	adenine and adenosine salvage V
PWY-6618	guanine and guanosine salvage III
PWY-6619	adenine and adenosine salvage VI
PWY-6622	heptadecane biosynthesis
PWY-6626	CDP-2-glycerol biosynthesis
PWY-6637	sulfolactate degradation II
PWY-6644	fluoroacetate and fluorothreonine biosynthesis
PWY-6646	fluoroacetate degradation
PWY-6654	phosphopantothenate biosynthesis III
PWY-6655	xanthan biosynthesis
PWY-6672	cis-genanyl-CoA degradation
PWY-6675	sulfur oxidation IV (intracellular sulfur)
PWY-6679	jadomycin biosynthesis
PWY-6682	dehydrophos biosynthesis
PWY-6687	mannosylglucosylglycerate biosynthesis II
PWY-6690	cinnamate and 3-hydroxycinnamate degradation to 2-oxopent-4-enoate
PWY-6696	oxalate degradation III
PWY-6698	oxalate degradation V
PWY-6711	archaeosine biosynthesis
PWY-6713	L-rhamnose degradation II
PWY-6717	(1,4)-beta-xylan degradation
PWY-6728	methylaspartate cycle
PWY-6731	starch degradation III
PWY-6748	nitrate reduction VII (denitrification)
PWY-6753	S-methyl-5-thioadenosine degradation III

MetaCyc Pathway ID	Pathway Common Name
PWY-6754	S-methyl-5-thioadenosine degradation I
PWY-6755	S-methyl-5-thio-alpha-D-ribose 1-phosphate degradation I
PWY-6759	hydrogen production III
PWY-6767	4,4-diapolycopenedioate biosynthesis
PWY-6771	rhamnogalacturonan type I degradation II (bacteria)
PWY-6789	(1,3)-beta-D-xylan degradation
PWY-6790	L-arabinan degradation
PWY-6793	demethylmenaquinol-8 biosynthesis III
PWY-6795	diacylglyceryl-N,N,N-trimethylhomoserine biosynthesis
PWY-6797	6-hydroxymethyl-dihydropterin diphosphate biosynthesis II (archaea)
PWY-6805	cellulose degradation I (cellulosome)
PWY-6813	glucuronoarabinoxylan degradation
PWY-6814	acidification and chitin degradation (in carnivorous plants)
PWY-6821	kappa-carrageenan degradation
PWY-6825	phosphatidylcholine biosynthesis V
PWY-6891	thiazole biosynthesis II (Bacillus)
PWY-6945	cholesterol degradation to androstenedione I (cholesterol oxidase)
PWY-6966	methanol oxidation to formaldehyde I
PWY-6972	oleandomycin activation/inactivation
PWY-6978	plastoquinol-9 biosynthesis II
PWY-6993	nicotine degradation II
PWY-6994	pyrrolysine biosynthesis
PWY-7013	L-1,2-propanediol degradation
PWY-7014	paromamine biosynthesis I
PWY-7022	paromamine biosynthesis II
PWY-7032	alkane biosynthesis I
PWY-7046	4-coumarate degradation (anaerobic)

MetaCyc Pathway ID	Pathway Common Name
PWY-7052	cyanophycin metabolism
PWY-7072	hopanoid biosynthesis (bacteria)
PWY-7097	vanillin and vanillate degradation I
PWY-7098	vanillin and vanillate degradation II
PWY-7104	dTDP-L-megosamine biosynthesis
PWY-7106	erythromycin D biosynthesis
PWY-7130	L-glucose degradation
PWY-7177	UTP and CTP dephosphorylation II
PWY-7198	pyrimidine deoxyribonucleotides de novo biosynthesis IV
PWY-7206	pyrimidine deoxyribonucleotides dephosphorylation
PWY-722	nicotinate degradation I
PWY-7241	myo-inositol degradation II
PWY-7254	TCA cycle VII (acetate-producers)
PWY-7255	ergothioneine biosynthesis
PWY-7301	dTDP-beta-L-noviose biosynthesis
PWY-7309	acrylonitrile degradation II
PWY-7312	dTDP-D-beta-fucofuranose biosynthesis
PWY-7315	dTDP-N-acetylthomosamine biosynthesis
PWY-7316	dTDP-N-acetylviosamine biosynthesis
PWY-7318	dTDP-3-acetamido-3,6-dideoxy-alpha-D-glucose biosynthesis
PWY-761	rhizobactin 1021 biosynthesis
PWY-822	fructan biosynthesis
PWY-922	mevalonate pathway I
PWY0-1221	putrescine degradation II
PWY0-1297	superpathway of purine deoxyribonucleosides degradation
PWY0-1298	superpathway of pyrimidine deoxyribonucleosides degradation
PWY0-1321	nitrate reduction III (dissimilatory)

MetaCyc Pathway ID	Pathway Common Name
PWY0-1329	succinate to cytochrome bo oxidase electron transfer
PWY0-1335	NADH to cytochrome bo oxidase electron transfer
PWY0-1336	NADH to fumarate electron transfer
PWY0-1337	oleate beta-oxidation
PWY0-1347	NADH to trimethylamine N-oxide electron transfer
PWY0-1348	NADH to dimethyl sulfoxide electron transfer
PWY0-1352	nitrate reduction VIII (dissimilatory)
PWY0-1355	formate to trimethylamine N-oxide electron transfer
PWY0-1356	formate to dimethyl sulfoxide electron transfer
PWY0-1391	S-methyl-5-thioadenosine degradation IV
PWY0-1433	tetrahydromonapterin biosynthesis
PWY0-1465	D-malate degradation
PWY0-1466	trehalose degradation VI (periplasmic)
PWY0-1471	uracil degradation III
PWY0-1533	methylphosphonate degradation
PWY0-1544	proline to cytochrome bo oxidase electron transfer
PWY0-163	salvage pathways of pyrimidine ribonucleotides
PWY0-166	superpathway of pyrimidine deoxyribonucleotides de novo biosynthesis (E. coli)
PWY0-181	salvage pathways of pyrimidine deoxyribonucleotides
PWY0-301	L-ascorbate degradation I (bacterial, anaerobic)
PWY0-44	D-allose degradation
PWY0-521	fructoselysine and psicoselysine degradation
PWY0-981	taurine degradation IV
PWY1G-0	mycothiol biosynthesis
PWY1G-170	formaldehyde oxidation III (mycothiol-dependent)
PWY3DJ-11281	sphingomyelin metabolism
PWY3O-246	(R,R)-butanediol degradation
PWY3O-450	phosphatidylcholine biosynthesis I
PWY490-3	nitrate reduction VI (assimilatory)

MetaCyc Pathway ID	Pathway Common Name
PWY5F9-12	biphenyl degradation
PWYQT-4427	sulfolipid biosynthesis
PYRIDNUCSAL-PWY	NAD salvage pathway I
QUINATEDEG-PWY	quinate degradation I
RIBITOLUTIL-PWY	ribitol degradation
RIBOKIN-PWY	ribose degradation
RUMP-PWY	formaldehyde oxidation I
SHIKIMATEDEG-PWY	shikimate degradation I
SUCSYN-PWY	sucrose biosynthesis
TAURINEDEG-PWY	taurine degradation III
TCA	TCA cycle I (prokaryotic)
THRDLCTCAT-PWY	threonine degradation III (to methylglyoxal)
TOLUENE-DEG-2-OH-PWY	toluene degradation to 2-oxopent-4-enoate I (via o-cresol)
TOLUENE-DEG-3-OH-PWY	toluene degradation to 2-oxopent-4-enoate (via 4-methylcatechol)
TOLUENE-DEG-4-OH-PWY	toluene degradation to protocatechuate (via p-cresol)
TRPCAT-PWY	tryptophan degradation I (via anthranilate)
TRPKYNCAT-PWY	tryptophan degradation IV (via indole-3-lactate)

[0127] In some embodiments, the comprehensive panel of substrates or nutrients metabolized by a metabolic pathway include a-mannan (yeast), acetate, agarose, alanine, alginate, anthocyanin, arabinan, arabinogalactan, arabinoxylan, arginine, asparagine, Aspartate, b-glucans, butyrate, carrageenan, chitin, chlorogenic acids, chondroitin sulfate, cinnamic acid, Cysteine, dextran (40), Dihydrochalcones, Enterodiol, flavan-3-ols, flavanones, flavones, flavonols, folate, formate, galactomannan (carob), galacturonan (homo), galacturonate, glucomannan (konjac), glutamate, Glutamine, Glycine, Histidine, hyaluronan, hydrogen, hydroxycinnamic acids, hydroxyproline, inulin, isoflavones/isoflavanones, Isoleucine, lactate, laminarin, Leucine, levan, Lysine, Methionine, mucin O-linked glycans, Ornithine, Phenylalanine, porphyran, Proline, propionate, rhamnogalacturonan I, rhamnogalacturonan II, Secoisolariciresinol diglucoside, Serine, starch (potato), starch (structure 1), thiamine, Threonine, tryptophan, Tyrosine, Valine, xyloglucan, and xylooligosaccharides (XOS). For

example, in some embodiments, the comprehensive panel of substrates or nutrients metabolized by a metabolic pathway comprises at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all the substrates described above.

[0128] In some embodiments, the comprehensive panel of metabolites synthesized or produced by a metabolic pathway include formate, acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, 2-methylbutyrate, caporate, isocaporate, 3-methylvaleric acid, L-phenylalanine, 3-phenylpropionic acid, phyenypyruvate, DL-3-phenyllactic acid, trans-cinnamic acid, phenyllactic acid, phenethylamine, L-tyrosine, 3-(4-hydroxyphenyl)propionic acid, 3-(4-hydroxyphenyl) pyruvic acid, DL-p-hydroxyphenyl lactic acid, p-coumaric acid, 4-hydroxyphenyl acetic acid, tyramine, phenol, p-cresol, 4-ethylphenol, 4-vinylphenol, 4-hydroxybenzoic acid, L-tryptophan, 3-indolepropionic acid, 3-indolepyruvic acid, DL-indole-3-lactic acid, trans-3-indoleacrylic acid, 3-indoleacetic acid, tryptamine, indole, skatol, indole-3-carboxylic acid, indole-3-carboxyaldehyde, N-acetyl-L-phenylalanine, phenylpropionylglycine, 3-(3-hydroxyphenyl) propionic acid, cinnamoylglycine, phenylacetylglycine, phenylacetylglutamine, hippuric acid, 2-hydroxyhippuric acid, 3-hydroxyhippuric acid, 4-hydroxyhippuric acid, 4-hydroxyphenylacetylglycine, phenyl sulfate, phenyl glucuronide, p-cresol sulfate, p-cresol glucuronide, 4-ethylphenol sulfate, 4-ethylphenol glucuronide, N-acetyl-L-tryptophan, 5-hydroxy-L-typtophan, N-acetylserotonin, 3-indolepriopionylglycine, indolyl-3-acryloylglycine, indoxyl sulfate, indoxyl glucuronide, 5-hydroxyindole-3-acetic acid, indoleacetylglycine, lithocholic acid, murocholic acid, ursodeoxycholic acid, hyodeoxycholic acid, deoxycholic acid, 7-oxocholic acid, ω-muricholic acid, α-muricholic acid, β-muricholic acid, γ-muricholic acid, 7βcholic acid, taurolithocholic acid, tauroursodeoxycholic acid, taurohyodeoxycholic acid, taurochenodeoxycholic acid, taurodeoxycholic acid, tauro-β-muricholic acid, tauro- ω-muricholic acid, and taurocholic acid. For example, in some embodiments, the comprehensive panel of metabolites synthesized or produced by a metabolic pathway comprises at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all the substrates described above.

[0129] In some embodiments, high-complexity defined gut microbial communities disclosed herein are assembled to have the ability to metabolize at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the substrates described above and produce at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the metabolites described above.

[0130] In some embodiments, high-complexity defined gut microbial communities disclosed herein are assembled to have the ability to metabolize at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the substrates described above and utilize at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the MetaCyc metabolic pathways described above.

[0131] In some embodiments, high-complexity defined gut microbial communities disclosed herein are assembled to have the ability to produce at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the metabolites described above and utilize at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the MetaCyc metabolic pathways described above.

[0132] In some embodiments, high-complexity defined gut microbial communities disclosed herein are assembled to have the ability to metabolize at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the substrates described above, produce at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the metabolites described above, and utilize at least 90% (*e.g.*, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or all) of the MetaCyc metabolic pathways described above.

[0133] In some embodiments, the ability to metabolize a substrate, produce a metabolite, or utilize a MetaCyc pathway is experimentally determined by culturing the defined gut microbial community *in vitro* and measuring whether a substrate is metabolized , a metabolite is produced, and/or a reaction intermediate in a MetaCyc pathway is produced by liquid chromatography-mass spectrometry analysis.

[0134] In some embodiments, the ability to metabolize a substrate, produce a metabolite, or utilize a MetaCyc pathway is experimentally determined by administering the defined gut microbial community to a gnotobiotic mouse and measuring whether a substrate is metabolized , a metabolite is produced, and/or a reaction intermediate in a MetaCyc pathway is produced after a defined period of time by liquid chromatography-mass spectrometry (LC-MS) analysis of a sample obtained from the mouse. In some embodiments, the defined gut microbial community is administered via a route selected from the group consisting of oral, rectal, fecal (by enema), and naso/oro-gastric gavage. In some embodiments, the defined period of time is about 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours, 2 days, 7 days, 14 days, 1 month, or 2 months. In

some embodiments, the same obtained from the mouse is selected from the group consisting of a fecal sample, urine sample, blood sample, or serum sample.

8. Microbial Communities for the Treatment of Dysbiosis or a Pathological Condition

[0135] Backfill communities identified using the methods described herein can be used to treat patients by administration of a high-complexity defined gut microbial community. Exemplary patients are patients with dysbiosis or a pathological condition.

8.1 Clostridium difficile Infection

8.1.1 Murine Model

[0136] In some embodiments, when tested in a murine model of *C. difficile* infection, the high-complexity defined microbial community of the present invention reduces the number of *C. difficile* colony forming units (CFU) per µl of stool by at least 1 to 2 logs, at least 2 to 3 logs, at least 3 to 4 logs, at least 4 to 5 logs, or by at least 5 to 6 logs. In some embodiments, when tested in a murine model of *C. difficile* infection, the high-complexity defined microbial community of the present invention reduces the number of *C. difficile* colony forming units (CFU) per gram of stool by at least 1 to 2 logs, at least 2 to 3 logs, at least 3 to 4 logs, at least 4 to 5 logs, or by at least 5 to 6 logs.

8.1.2 Treatment of Persistent C. difficile Infection

[0137] In some embodiments, a high-complexity defined gut microbial community of the present invention can be used to treat an animal having a persistent *C. difficile* infection. For example in some embodiments, the animal may be a mammal, and more particularly a human.

[0138] In some embodiments, a method for producing a high-complexity defined gut microbial community of the present invention for treatment of persistent *C. difficile* infection, may comprise: i) performing a *C. difficile* plate count on a stool sample obtained from an animal having a persistent *C. difficile* infection; ii) engrafting the high-complexity defined gut microbial community into the gut of the animal having a persistent *C. difficile* infection to produce an engrafted, infected animal; iii) maintaining the engrafted, infected animal for a time sufficient for enteric colonization by microbial strains of the high-complexity defined gut microbial community, thereby producing an engrafted, infected community in the gut of the engrafted, infected animal; iv) performing an additional *C. difficile* plate count on a stool sample obtained

from the engrafted, infected animal; v) if the number of *C. difficile* CFUs obtained from the plate count of step iv) is not significantly less than the number of *C. difficile* CFUs obtained from the plate count of step i), adding one or more than one additional defined microbial strain to the high-complexity defined gut microbial community that was not present in step ii) to produce a modified, high-complexity defined gut microbial community and repeating steps i) to iv) in an animal having a persistent *C. difficile* infection that has never been engrafted, using the modified, high-complexity defined gut microbial community as the high-complexity defined gut microbial community; and if there is a statistically significant reduction in the number of *C. difficile* CFUs obtained from the plate count of step iv) as compared to the number of *C. difficile* CFUs obtained from the plate count of step i), the modified, defined, stable enteric community in the final step iv) is a final, high-complexity defined gut microbial community.

[0139] In some embodiments, administration of an effective amount of final, high-complexity defined gut microbial community to an animal having a persistent *C. difficile* infection effectively reduces the number of *C. difficile* CFU/µl of stool in the treated animal. In some embodiments, administration of an effective amount of final, high-complexity defined gut microbial community to an animal having a persistent *C. difficile* infection effectively reduces the number of *C. difficile* CFU/g of stool in the treated animal.

8.2 Bile Acid Metabolism and Cholestatic Disease

[0140] In some embodiments, a high-complexity defined gut microbial community significantly alters the profile and/or concentration of bile acids present in an animal (*e.g.*, mouse) stool sample as compared to an isogenic gnotobiotic control animal (*e.g.*, isogenic gnotobiotic control mouse).

[0141] For example, in some embodiments, a high-complexity defined gut microbial community of the present invention significantly alters the profile and/or concentration of Tβ-MCA, Tα-MCA, TUDCA, THDCA, TCA, 7β-CA, 7-oxo-CA, TCDCA, Tω-MCA, TDCA, α-MCA, β-MCA, ω-MCA, Muro-CA, d4-CA, CA, TLCA, UDCA, HDCA, CDCA, DCA, and LCA in an animal (*e.g.* mouse).

[0142] In some embodiments, a high-complexity defined gut microbial community of the present invention can be used to treat an animal having a cholestatic disease, such as, for example, primary sclerosing cholangitis, primary biliary cholangitis, progressive familial intrahepatic cholestasis, or nonalcoholic steatohepatitis. For example in some embodiments, the animal may be a mammal, and more particularly a human.

9. Modification of metabolites

[0143] In some embodiments, a high-complexity defined gut microbial community significantly alters the concentration of metabolites present in an animal (*e.g.*, mouse) urine sample as compared to an isogenic gnotobiotic control animal (*e.g.* isogenic gnotobiotic control mouse).

[0144] For example in some embodiments, a high-complexity defined gut microbial community of the present invention significantly alters the concentration of 4-hydroxybenzoic acid, L-tyrosine, 4-hydroxyphenylacetic acid, DL-p-hydroxyphenyllactic acid, p-coumaric acid, 3-(4-Hydroxyphenyl) propionic acid, 3-(4-hydroxyphenyl)pyruvic acid, indole-3-carboxylic acid, tyramine, L-phenylalanine, phenylacetic acid, 3-indoleacetic acid, DL-3-phenyllactic acid, L-tryptophan, DL-indole-3-lactic acid, phenylpyruvate, trans-3-indoleacrylic acid, 3-indolepyruvic acid, 3-indolepyropionic acid, 3-phenylproprionic acid, trans-cinnamic acid, tryptamine, phenol, indole-3-carboxaldehyde, p-cresol, indole, 4-vinylphenol, or 4-ethylphenol.

10. Pharmaceutical Compositions

[0145] A product of the *in vivo* backfill process is a defined microbial community (*e.g.*, a stable defined microbial community) with a known phenotype (*e.g.*, a metabolic phenotype) that, when engrafted into a subject, confers benefit to the subject.

[0146] The therapeutic backfill community may be expanded and combined with excipients for administration orally (*e.g.*, as a capsule), by naso/oro-gastric gavage, fecally (*e.g.* by enema), or rectally (*e.g.*, by colonoscopy). Exemplary excipients include normal saline and others known in the art.

[0147] The present disclosure also provides pharmaceutical compositions that contain an effective amount of a microbial community, *e.g.*, a high-complexity defined gut microbial community. The composition can be formulated for use in a variety of delivery systems. One or more physiologically acceptable excipient(s) or carrier(s) can also be included in the composition for proper formulation. Suitable formulations for use in the present disclosure are found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, Pa., 17th ed., 1985. For a brief review of methods for drug delivery, *see, e.g.*, Langer (*Science* 249:1527-1533, 1990).

[0148] In some embodiments a pharmaceutical composition disclosed herein may comprise a microbial community, *e.g.*, a high-complexity defined gut microbial community, of the present invention and one or more than one agent selected from, but not limited to: carbohydrates (*e.g.*,

glucose, sucrose, galactose, mannose, ribose, arabinose, xylose, fructose, maltose, cellobiose, lactose, deoxyribose, hexose); lipids (*e.g.*, lauric acid (12:0) myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16: 1), margaric acid (17:0), heptadecenoic acid (17: 1), stearic acid (18:0), oleic acid (18: 1), linoleic acid (18:2), linolenic acid (l 8:3), octadecatetraenoic acid (18:4), arachidic acid (20:0), eicosenoic acid (20: 1), eicosadienoic acid (20:2), eicosatetraenoic acid (20:4), eicosapentaenoic acid (20:5) (EPA), docosanoic acid (22:0), docosenoic acid (22: 1), docosapentaenoic acid (22:5), docosahexaenoic acid (22:6) (DHA), and tetracosanoic acid (24:0)); minerals (*e.g.*, chloride, sodium, calcium, iron, chromium, copper, iodine, zinc, magnesium, manganese, molybdenum, phosphorus, potassium, and selenium); vitamins (*e.g.*, vitamin C, vitamin A, vitamin E, vitamin B 12, vitamin K, riboflavin, niacin, vitamin D, vitamin B6, folic acid, pyridoxine, thiamine, pantothenic acid, and biotin); buffering agents (*e.g.*, sodium citrate, magnesium carbonate, magnesium bicarbonate, calcium carbonate, and calcium bicarbonate); preservatives (*e.g.*, alpha-tocopherol, ascorbate, parabens, chlorobutanol, and phenol); binders (*e.g.*, starches, pregelatinized starches, gelatin, polyvinylpyrolidone, cellulose, methylcellulose, sodium carboxymethylcellulose, ethylcellulose, polyacrylamides, polyvinyloxoazolidone, polyvinylalcohols, C_{12}-C_{18} fatty acid alcohol, polyethylene glycol, polyols, saccharides, oligosaccharides); lubricants (*e.g.*, magnesium stearate, calcium stearate, zinc stearate, hydrogenated vegetable oils, sterotex, polyoxyethylene monostearate, talc, polyethyleneglycol, sodium benzoate, sodium lauryl sulfate, magnesium lauryl sulfate, and light mineral oil); dispersants (*e.g.*, starch, alginic acid, polyvinylpyrrolidones, guar gum, kaolin, bentonite, purified wood cellulose, sodium starch glycolate, isoamorphous silicate, and microcrystalline cellulose); disintegrants (*e.g.*, com starch, potato starch, pregelatinized and modified starches thereof, sweeteners, clays, such as bentonite, micro-crystalline cellulose, alginates, sodium starch glycolate, gums such as agar, guar, locust bean, karaya, pecitin, tragacanth, sodium bicarbonate in combination with citric acid, and sodium bicarbonate in combination with tartaric acid); flavoring agents; sweeteners; and coloring agents.

[0149] In certain embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community, of the present invention is administered orally as a lyophilized powder, capsule, tablet, troche, lozenge, granule, gel or liquid. In some embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community, of the present invention is administered as a tablet or pill and can be compressed, multiply compressed, multiply layered, and/or coated.

11. Dosages

[0150] In some embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community of the present invention is administered in a dosage form having a total amount of microbial community, *e.g.*, a high-complexity defined gut microbial community, of 1×10^6 to 1×10^{13} CFUs, 1×10^6 to 1×10^{12} CFUs, 1×10^6 to 1×10^{11} CFUs, 1×10^6 to 1×10^{10} CFUs, 1×10^6 to 1×10^9 CFUs, 1×10^6 to 1×10^8 CFUs, 1×10^6 to 1×10^7 CFUs, 5×10^6 to 1×10^{13} CFUs, 5×10^6 to 1×10^{12} CFUs, 5×10^6 to 1×10^{11} CFUs, 5×10^6 to 1×10^{10} CFUs, 5×10^6 to 1×10^9 CFUs, 5×10^6 to 1×10^8 CFUs, 5×10^6 to 1×10^7 CFUs, 1×10^7 to 1×10^{13} CFUs, 1×10^7 to 1×10^{12} CFUs, 1×10^7 to 1×10^{11} CFUs, 1×10^7 to 1×10^{10} CFUs, 1×10^7 to 1×10^9 CFUs, 1×10^7 to 1×10^8 CFUs, 5×10^7 to 1×10^{13} CFUs, 5×10^7 to 1×10^{12} CFUs, 5×10^7 to 1×10^{11} CFUs, 5×10^7 to 1×10^{10} CFUs, 5×10^7 to 1×10^9 CFUs, 5×10^7 to 1×10^8 CFUs, 1×10^8 to 1×10^{13} CFUs, 1×10^8 to 1×10^{12} CFUs, 1×10^8 to 1×10^{11} CFUs, 1×10^8 to 1×10^{10} CFUs, 1×10^8 to 1×10^9 CFUs, 5×10^8 to 1×10^{13} CFUs, 5×10^8 to 1×10^{12} CFUs, 5×10^8 to 1×10^{11} CFUs, 5×10^8 to 1×10^{10} CFUs, 5×10^8 to 1×10^9 CFUs, 1×10^9 to 1×10^{13} CFUs, 1×10^9 to 1×10^{12} CFUs, 1×10^9 to 1×10^{11} CFUs, 1×10^9 to 1×10^{10} CFUs, 5×10^9 to 1×10^{13} CFUs, 5×10^9 to 1×10^{12} CFUs, 5×10^9 to 1×10^{11} CFUs, 5×10^9 to 1×10^{10} CFUs, 1×10^{10} to 1×10^{13} CFUs, 1×10^{10} to 1×10^{12} CFUs, 1×10^{10} to 1×10^{11} CFUs, 5×10^{10} to 1×10^{13} CFUs, 5×10^{10} to 1×10^{12} CFUs or 5×10^{10} to 1×10^{11} CFUs.

[0151] In some embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community of the present invention is administered in a dosage form having a total amount of microbial community, *e.g.*, a high-complexity defined gut microbial community, of 0.1 ng to 500 mg, 0.5 ng to 500 mg, 1 ng to 500 mg, 5 ng to 500 mg, 10 ng to 500 mg, 50 ng to 500 mg, 100 ng to 500 mg, 500 ng to 500 mg, 1 µg to 500 mg, 5 µg to 500 mg, 10 µg to 500 mg, 50 µg to 500 mg, 100 µg to 500 mg, 500 µg to 500 mg, 1 mg to 500 mg, 5 mg to 500 mg, 10 mg to 500 mg, 50 mg to 500 mg, 100 mg to 500 mg, 0.1 ng to 100 mg, 0.5 ng to 100 mg, 1 ng to 100 mg, 5 ng to 100 mg, 10 ng to 100 mg, 50 ng to 100 mg, 100 ng to 100 mg, 500 ng to 500 mg, 1 µg to 100 mg, 5 µg to 100 mg, 10 µg to 100 mg, 50 µg to 100 mg, 100 µg to 100 mg, 500 µg to 100 mg, 1 mg to 500 mg, 5 mg to 100 mg, 10 mg to 100 mg, 50 mg to 100 mg, 0.1 ng to 50 mg, 0.5 ng to 50 mg, 1 ng to 50 mg, 5 ng to 50 mg, 10 ng to 50 mg, 50 ng to 50 mg, 100 ng to 50 mg, 500 ng to 500 mg, 1 µg to 50 mg, 5 µg to 50 mg, 10 µg to 50 mg, 50 µg to 50 mg, 100 µg to 50 mg, 500 µg to 50 mg, 1 mg to 500 mg, 5 mg to 50 mg, 10 mg to 50 mg, 0.1 ng to 10 mg, 0.5 ng to 10 mg, 1 ng to 10 mg, 5 ng to 10 mg, 10 ng to 10 mg, 50 ng to 10 mg, 100 ng to 10 mg, 500 ng to 500 mg, 1 µg to 10 mg, 5 µg to 10 mg, 10 µg to 10 mg, 50 µg to 10 mg, 100 µg to 10 mg, 500 µg to 10 mg, 1 mg to 500 mg, 5 mg to 10 mg, 0.1 ng to 5 mg, 0.5 ng to 5 mg, 1 ng to 5 mg,

5 ng to 5 mg, 10 ng to 5 mg, 50 ng to 5 mg, 100 ng to 5 mg, 500 ng to 500 mg, 1 μg to 5 mg, 5 μg to 5 mg, 10 μg to 5 mg, 50 μg to 5 mg, 100 μg to 5 mg, 500 μg to 5 mg, 1 mg to 500 mg, 0.1 ng to 1 mg, 0.5 ng to 1 mg, 1 ng to 1 mg, 5 ng to 1 mg, 10 ng to 1 mg, 50 ng to 1 mg, 100 ng to 1 mg, 500 ng to 500 mg, 1 μg to 1 mg, 5 μg to 1 mg, 10 μg to 1 mg, 50 μg to 1 mg, 100 μg to 1 mg, 500 μg to 1 mg, 0.1 ng to 500 μg, 0.5 ng to 500 μg, 1 ng to 500 μg, 5 ng to 500 μg, 10 ng to 500 μg, 50 ng to 500 μg, 100 ng to 500 μg, 500 ng to 500 μg, 1 μg to 500 μg, 5 μg to 500 μg, 10 μg to 500 μg, 50 μg to 500 μg, 100 μg to 500 μg, 0.1 ng to 100 μg, 0.5 ng to 100 μg, 1 ng to 100 μg, 5 ng to 100 μg, 10 ng to 100 μg, 50 ng to 100 μg, 100 ng to 100 μg, 500 ng to 100 μg, 1 μg to 100 μg, 5 μg to 100 μg, 10 μg to 100 μg, 50 μg to 100 μg, 0.1 ng to 50 μg, 0.5 ng to 50 μg, 1 ng to 50 μg, 5 ng to 50 μg, 10 ng to 50 μg, 50 ng to 50 μg, 100 ng to 50 μg, 500 ng to 50 μg, 1 μg to 50 μg, 5 μg to 50 μg, 10 μg to 50 μg, 0.1 ng to 10 μg, 0.5 ng to 10 μg, 1 ng to 10 μg, 5 ng to 10 μg, 10 ng to 10 μg, 50 ng to 10 μg, 100 ng to 10 μg, 500 ng to 10 μg, 1 μg to 10 μg, 5 μg to 10 μg, 0.1 ng to 5 μg, 0.5 ng to 5 μg, 1 ng to 5 μg, 5 ng to 5 μg, 10 ng to 5 μg, 50 ng to 5 μg, 100 ng to 5 μg, 500 ng to 5 μg, 1 μg to 5 μg, 0.1 ng to 1 μg, 0.5 ng to 1 μg, 1 ng to 1 μg, 5 ng to 1 μg, 10 ng to 1 μg, 50 ng to 1 μg, 100 ng to 1 μg, 500 ng to 1 μg, 0.1 ng to 500 ng, 0.5 ng to 500 ng, 1 ng to 500 ng, 5 ng to 500 ng, 10 ng to 500 ng, 50 ng to 500 ng, 100 ng to 500 ng, 0.1 ng to 100 ng, 0.5 ng to 100 ng, 1 ng to 100 ng, 5 ng to 100 ng, 10 ng to 100 ng, 50 ng to 100 ng, 0.1 ng to 50 ng, 0.5 ng to 50 ng, 1 ng to 50 ng, 5 ng to 50 ng, 10 ng to 50 ng, 0.1 ng to 10 ng, 0.5 ng to 10 ng, 1 ng to 10 ng, 5 ng to 10 ng, 0.1 ng to 5 ng, 0.5 ng to 5 ng, 1 ng to 5 ng, 0.1 ng to 1 ng, 0.1 ng to 1 ng, or 0.1 ng to 0.5 ng.

[0152] In other embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community of the present invention is consumed at a rate of 1 X 10^6 to 1 X 10^{13} CFUs a day, 1 X 10^6 to 1 X 10^{12} CFUs a day, 1 X 10^6 to 1 X 10^{11} CFUs a day, 1 X 10^6 to 1 X 10^{10} CFUs a day, 1 X 10^6 to 1 X 10^9 CFUs a day, 1 X 10^6 to 1 X 10^8 CFUs a day, 1 X 10^6 to 1 X 10^7 CFUs a day, 5 X 10^6 to 1 X 10^{13} CFUs a day, 5 X 10^6 to 1 X 10^{12} CFUs a day, 5 X 10^6 to 1 X 10^{11} CFUs a day, 5 X 10^6 to 1 X 10^{10} CFUs a day, 5 X 10^6 to 1 X 10^9 CFUs a day, 5 X 10^6 to 1 X 10^8 CFUs a day, 5 X 10^6 to 1 X 10^7 CFUs a day, 1 X 10^7 to 1 X 10^{13} CFUs a day, 1 X 10^7 to 1 X 10^{12} CFUs a day, 1 X 10^7 to 1 X 10^{11} CFUs a day, 1 X 10^7 to 1 X 10^{10} CFUs a day, 1 X 10^7 to 1 X 10^9 CFUs a day, 1 X 10^7 to 1 X 10^8 CFUs a day, 5 X 10^7 to 1 X 10^{13} CFUs a day, 5 X 10^7 to 1 X 10^{12} CFUs a day, 5 X 10^7 to 1 X 10^{11} CFUs a day, 5 X 10^7 to 1 X 10^{10} CFUs a day, 5 X 10^7 to 1 X 10^9 CFUs a day, 5 X 10^7 to 1 X 10^8 CFUs a day, 1 X 10^8 to 1 X 10^{13} CFUs a day, 1 X 10^8 to 1 X 10^{12} CFUs a day, 1 X 10^8 to 1 X 10^{11} CFUs a day, 1 X 10^8 to 1 X 10^{10} CFUs a day, 1 X 10^8 to 1 X 10^9 CFUs a day, 5 X 10^8 to 1 X 10^{13} CFUs a day, 5 X 10^8 to 1 X 10^{12} CFUs a day, 5 X 10^8 to 1 X 10^{11} CFUs a day, 5 X 10^8 to 1 X 10^{10} CFUs a day, 5 X 10^8 to

1 X 10^9 CFUs a day, 1 X 10^9 to 1 X 10^{13} CFUs a day, 1 X 10^9 to 1 X 10^{12} CFUs a day, 1 X 10^9 to 1 X 10^{11} CFUs a day, 1 X 10^9 to 1 X 10^{10} CFUs a day, 5 X 10^9 to 1 X 10^{13} CFUs a day, 5 X 10^9 to 1 X 10^{12} CFUs a day, 5 X 10^9 to 1 X 10^{11} CFUs a day, 5 X 10^9 to 1 X 10^{10} CFUs a day, 1 X 10^{10} to 1 X 10^{13} CFUs a day, 1 X 10^{10} to 1 X 10^{12} CFUs a day, 1 X 10^{10} to 1 X 10^{11} CFUs a day, 5 X 10^{10} to 1 X 10^{13} CFUs a day, 5 X 10^{10} to 1 X 10^{12} CFUs a day or 5 X 10^{10} to 1 X 10^{11} CFUs a day.

[0153] In other embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community of the present invention is consumed at a rate of 0.1 ng to 500 mg a day, 0.5 ng to 500 mg a day, 1 ng to 500 mg a day, 5 ng to 500 mg a day, 10 ng to 500 mg a day, 50 ng to 500 mg a day, 100 ng to 500 mg a day, 500 ng to 500 mg a day, 1 µg to 500 mg a day, 5 µg to 500 mg a day, 10 µg to 500 mg a day, 50 µg to 500 mg a day, 100 µg to 500 mg a day, 500 µg to 500 mg a day, 1 mg to 500 mg a day, 5 mg to 500 mg a day, 10 mg to 500 mg a day, 50 mg to 500 mg a day, 100 mg to 500 mg a day, 0.1 ng to 100 mg a day, 0.5 ng to 100 mg a day, 1 ng to 100 mg a day, 5 ng to 100 mg a day, 10 ng to 100 mg a day, 50 ng to 100 mg a day, 100 ng to 100 mg a day, 500 ng to 500 mg a day, 1 µg to 100 mg a day, 5 µg to 100 mg a day, 10 µg to 100 mg a day, 50 µg to 100 mg a day, 100 µg to 100 mg a day, 500 µg to 100 mg a day, 1 mg to 500 mg a day, 5 mg to 100 mg a day, 10 mg to 100 mg a day, 50 mg to 100 mg a day, 0.1 ng to 50 mg a day, 0.5 ng to 50 mg a day, 1 ng to 50 mg a day, 5 ng to 50 mg a day, 10 ng to 50 mg a day, 50 ng to 50 mg a day, 100 ng to 50 mg a day, 500 ng to 500 mg a day, 1 µg to 50 mg a day, 5 µg to 50 mg a day, 10 µg to 50 mg a day, 50 µg to 50 mg a day, 100 µg to 50 mg a day, 500 µg to 50 mg a day, 1 mg to 500 mg a day, 5 mg to 50 mg a day, 10 mg to 50 mg a day, 0.1 ng to 10 mg a day, 0.5 ng to 10 mg a day, 1 ng to 10 mg a day, 5 ng to 10 mg a day, 10 ng to 10 mg a day, 50 ng to 10 mg a day, 100 ng to 10 mg a day, 500 ng to 500 mg a day, 1 µg to 10 mg a day, 5 µg to 10 mg a day, 10 µg to 10 mg a day, 50 µg to 10 mg a day, 100 µg to 10 mg a day, 500 µg to 10 mg a day, 1 mg to 500 mg a day, 5 mg to 10 mg a day, 0.1 ng to 5 mg a day, 0.5 ng to 5 mg a day, 1 ng to 5 mg a day, 5 ng to 5 mg a day, 10 ng to 5 mg a day, 50 ng to 5 mg a day, 100 ng to 5 mg a day, 500 ng to 500 mg a day, 1 µg to 5 mg a day, 5 µg to 5 mg a day, 10 µg to 5 mg a day, 50 µg to 5 mg a day, 100 µg to 5 mg a day, 500 µg to 5 mg a day, 1 mg to 500 mg a day, 0.1 ng to 1 mg a day, 0.5 ng to 1 mg a day, 1 ng to 1 mg a day, 5 ng to 1 mg a day, 10 ng to 1 mg a day, 50 ng to 1 mg a day, 100 ng to 1 mg a day, 500 ng to 500 mg a day, 1 µg to 1 mg a day, 5 µg to 1 mg a day, 10 µg to 1 mg a day, 50 µg to 1 mg a day, 100 µg to 1 mg a day, 500 µg to 1 mg a day, 0.1 ng to 500 µg a day, 0.5 ng to 500 µg a day, 1 ng to 500 µg a day, 5 ng to 500 µg a day, 10 ng to 500 µg a day, 50 ng to 500 µg a day, 100 ng to 500 µg a day, 500 ng to 500 µg a day, 1 µg to 500 µg a day, 5 µg to 500 µg a day, 10 µg to 500 µg a day, 50 µg to 500 µg a day,

100 μg to 500 μg a day, 0.1 ng to 100 μg a day, 0.5 ng to 100 μg a day, 1 ng to 100 μg a day, 5 ng to 100 μg a day, 10 ng to 100 μg a day, 50 ng to 100 μg a day, 100 ng to 100 μg a day, 500 ng to 100 μg a day, 1 μg to 100 μg a day, 5 μg to 100 μg a day, 10 μg to 100 μg a day, 50 μg to 100 μg a day, 0.1 ng to 50 μg a day, 0.5 ng to 50 μg a day, 1 ng to 50 μg a day, 5 ng to 50 μg a day, 10 ng to 50 μg a day, 50 ng to 50 μg a day, 100 ng to 50 μg a day, 500 ng to 50 μg a day, 1 μg to 50 μg a day, 5 μg to 50 μg a day, 10 μg to 50 μg a day, 0.1 ng to 10 μg a day, 0.5 ng to 10 μg a day, 1 ng to 10 μg a day, 5 ng to 10 μg a day, 10 ng to 10 μg a day, 50 ng to 10 μg a day, 100 ng to 10 μg a day, 500 ng to 10 μg a day, 1 μg to 10 μg a day, 5 μg to 10 μg a day, 0.1 ng to 5 μg a day, 0.5 ng to 5 μg a day, 1 ng to 5 μg a day, 5 ng to 5 μg a day, 10 ng to 5 μg a day, 50 ng to 5 μg a day, 100 ng to 5 μg a day, 500 ng to 5 μg a day, 1 μg to 5 μg a day, 0.1 ng to 1 μg a day, 0.5 ng to 1 μg a day, 1 ng to 1 μg a day, 5 ng to 1 μg a day, 10 ng to 1 μg a day, 50 ng to 1 μg a day, 100 ng to 1 μg a day, 500 ng to 1 μg a day, 0.1 ng to 500 ng a day, 0.5 ng to 500 ng a day, 1 ng to 500 ng a day, 5 ng to 500 ng a day, 10 ng to 500 ng a day, 50 ng to 500 ng a day, 100 ng to 500 ng a day, 0.1 ng to 100 ng a day, 0.5 ng to 100 ng a day, 1 ng to 100 ng a day, 5 ng to 100 ng a day, 10 ng to 100 ng a day, 50 ng to 100 ng a day, 0.1 ng to 50 ng a day, 0.5 ng to 50 ng a day, 1 ng to 50 ng a day, 5 ng to 50 ng a day, 10 ng to 50 ng a day, 0.1 ng to 10 ng a day, 0.5 ng to 10 ng a day, 1 ng to 10 ng a day, 5 ng to 10 ng a day, 0.1 ng to 5 ng a day, 0.5 ng to 5 ng a day, 1 ng to 5 ng a day, 0.1 ng to 1 ng a day, 0.1 ng to 1 ng a day, or 0.1 ng to 0.5 ng a day.

[0154] In some embodiments, the microbial composition of the present invention is administered for a period of at least 1 day to 1 week, 1 week to 1 month, 1 month to 3 months, 3 months to 6 months, 6 months to 1 year, or more than 1 year. For example, in some embodiments, the microbial composition of the present invention is administered for a period of at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, or 1 year.

[0155] In some embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community of the present invention is administered as a single dose or as multiple doses. For example, in some embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community of the present invention, is administered once a day for 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, or 1 year. In some embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community of the present invention, is administered multiple times daily. For example, in some embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community of the present invention, is administered twice daily, three times daily, 4 times daily, or 5 times daily. In some embodiments, a microbial

community, *e.g.*, a high-complexity defined gut microbial community of the present invention, is administered intermittently. For example, in some embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community of the present invention is administered once weekly, once monthly, or when a subject is in need thereof.

12. Combination Therapy

[0156] In some embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community of the present invention, can be administered in combination with other agents. For example, in some embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community of the present invention, can be administered concurrently with or after an antimicrobial agent, an antifungal agent, an antiviral agent, an antiparasitic agent or a prebiotic. Administration may be sequential over a period of hours or days, or simultaneously.

[0157] For example, in some embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community, can be administered concurrently with or after one or more than one antibacterial agent selected from fluoroquinolone antibiotics (*e.g.*, ciprofloxacin, levaquin, floxin, tequin, avelox, and norflox); cephalosporin antibiotics (*e.g.*, cephalexin, cefuroxime, cefadroxil, cefazolin, cephalothin, cefaclor, cefamandole, cefoxitin, cefprozil, and ceftobiprole); penicillin antibiotics (*e.g.*, amoxicillin, ampicillin, penicillin V, dicloxacillin, carbenicillin, vancomycin, and methicillin); tetracycline antibiotics (*e.g.*, tetracycline, minocycline, oxytetracycline, and doxycycline); and carbapenem antibiotics (*e.g.*, ertapenem, doripenem, imipenem/cilastatin, and meropenem).

[0158] For example, in some embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community, can be administered concurrently with or after one or more than one antiviral agent selected from Abacavir, Acyclovir, Adefovir, Amprenavir, Atazanavir, Cidofovir, Darunavir, Delavirdine, Didanosine, Docosanol, Efavirenz, Elvitegravir, Emtricitabine, Enfuviltide, Etravirine, Famciclovir, Foscamet, Fomivirsen, Ganciclovir, Indinavir, Idoxuridine, Lamivudine, Lopinavir Maraviroc, MK-2048, Nelfinavir, Nevirapine, Penciclovir, Raltegravir, Rilpivirine, Ritonavir, Saquinavir, Stavudine, Tenofovir Trifluridine, Valaciclovir, Valganciclovir, Vidarabine, Ibacitabine, Amantadine, Oseltamivir, Rimantidine, Tipranavir, Zalcitabine, Zanamivir, and Zidovudine.

[0159] In some embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community can be administered concurrently with or after one or more than one antifungal agent selected from miconazole, ketoconazole, clotrimazole, econazole, omoconazole,

bifonazole, butoconazole, fenticonazole, isoconazole, oxiconazole, sertaconazole, sulconazole, and tioconazole; triazole antifungals such as fluconazole, itraconazole, isavuconazole, ravuconazole, posaconazole, voriconazok, terconazole, and albaconazole; thiazole antifungals such as abafungin; allylamine antifungals such as terbinafine, naftifine, and butenafine; and echinocandin antifungals such as anidulafungin, caspofungin, and micafungin; polygodial; benzoic acid; ciclopirox; tolnaftate; undecylenic acid; flucytosine or 5-fluorocytosine; griseofulvin; and haloprogin.

[0160] In some embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community, can be administered concurrently with or after one or more than one anti-inflammatory and/or immunosuppressive agent selected from corticosteroids, mesalazine, mesalamine, sulfasalazine, sulfasalazine derivatives, cyclosporin A, mercaptopurine, azathiopurine, prednisone, methotrexate, antihistamines, glucocorticoids, epinephrine, theophylline, cromolyn sodium, anti-leukotrienes, anticholinergics, monoclonal anti-IgE, antibodies, and vaccines.

[0161] In some embodiments, a microbial community, *e.g.*, a high-complexity defined gut microbial community of the present invention, can be administered concurrently with or after one or more than one prebiotic selected from, but not limited to, amino acids, biotin, fructooligosaccharides, galactooligosaccharides, inulin, lactulose, mannan oligosaccharides, oligofructose-enriched inulin, oligofructose, oligodextrose, tagatose, trans-galactooligosaccharide, and xylooligosaccharides.

EXAMPLES

[0162] The disclosure now being generally described, will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present disclosure, and are not intended to limit the scope of the disclosure in any way.

Example 1: Sourcing and identification of active and supportive microbial strains

[0163] Microbial strains were purchased from a depository (*e.g.*, the American Type Culture Collection (ATCC)) or are derived from human donor fecal samples.

[0164] Microbial strains purchased from a depository were cultured according to depository instructions and using the media as described in Table 11.

TABLE 11 – Microbial Strains and Culture Media

Strain	**Media** (*see Tables 1 - 6 above*)
Acidaminococcus fermentans -- VR4	B
Acidaminococcus sp. -- D21	B
Adlercreutzia equolifaciens -- FJC-B9	C2 + L-Arginine
Alistipes finegoldii -- AHN 2437	C2
Alistipes onderdonkii -- WAL 8169	B
Anaerofustis stercorihominis -- ATCC BAA-858, CCUG 47767, CIP 108481, WAL 14563	F
Bilophila wadsworthia -- WAL 7959 [Lab 88-130H]	B
Blautia hansenii -- VPI C7-24	B
Blautia hydrogenotrophica -- S5a33	C2
Blautia obeum -- ATCC 29174, KCTC 15206, VPI B3-21	B
Catenibacterium mitsuokai -- RCA14-39, CIP 106738, JCM 10609	B
Clostridium asparagiforme -- N6, CCUG 48471	C2
Collinsella stercoris -- RCA 55-54, JCM 10641	C2
Coprococcus eutactus -- VPI C33-22	B
Parabacteroides distasonis -- NCTC 11152	B
Holdemania filiformis -- VPI J1-31B-1, ATCC 51649	B
Hungatella hathewayi -- 1313, CCUG 43506, CIP 109440, MTCC 10951	B
Intestinimonas butyriciproducens -- SRB-521-5-l, CCUG 63529	B
Solobacterium moorei -- RCA59-74, CIP 106864, JCM 10645	B
Olsenella uli -- D76D-27C, ATCC 49627, CIP 109912	B
Roseburia inulinivorans -- A2-194, CIP 109405, JCM 17584, NCIMB 14030	B
Alistipes putredinis -- CCUG 45780, CIP 104286, ATCC 29800, Carlier 10203, VPI 3293	B
Clostridium spiroforme -- VPI C28-23-1A, ATCC 29900, NCTC 11211	B
Slackia exigua -- S-7, ATCC 700122, JCM 11022, KCTC 5966	B

Strain	**Media** (*see Tables 1 - 6 above*)
Bacteroides pectinophilus -- N3	B
Butyrivibrio crossotus -- T9-40A, ATCC 29175	B
Subdoligranulum variabile -- BI-114, CCUG 47106	YCFAC
Turicibacter sanguinis -- MOL361, NCCB 100008	B
Streptococcus salivarius subsp. thermophilus -- LMD-9	B
Oscillibacter sp. -- KLE 1728	D
Desulfovibrio piger -- VPI C3-23 [DSM 749]	B
Lactobacillus ruminis -- E 194e	B
Clostridium hiranonis -- TO-931, JCM 10541, KCTC 15199	B
Clostridium sp. -- L2-50	B
Clostridium orbiscindens -- 1_3_50AFAA	B
Alistipes shahii -- WAL 8301	B
Faecalibacterium prausnitzii -- A2-165, JCM 31915	B
Akkermansia muciniphila -- Muc [CIP 107961]	A
Alistipes indistinctus -- JCM 16068, YIT 12060	A
Anaerobutyricum hallii -- VPI B4-27	A
Anaerostipes caccae -- L1-92	A
Anaerotruncus colihominis -- 277	A
Bacteroides caccae -- VPI 3452A [CIP 104201T, JCM 9498]	A
Bacteroides cellulosilyticus -- CRE21, CCUG 44979	A
Bacteroides coprocola -- M16	A
Bacteroides coprophilus -- CB42, JCM 13818	A
Bacteroides dorei -- 175	A
Bacteroides dorei -- 5_1_36/D4	A
Bacteroides eggerthii -- ATCC 27754, NCTC 11155	A
Bacteroides finegoldii -- 199	A
Bacteroides fragilis -- 3_1_12	A
Bacteroides intestinalis -- 341	A
Bacteroides ovatus -- NCTC 11153	A
Bacteroides rodentium -- ST28, CCUG 59334, JCM 16469	A
Bacteroides thetaiotaomicron -- 1_1_6	A

Strain	**Media** (*see Tables 1 - 6 above*)
Bacteroides fragilis -- 2_1_16	A
Bacteroides xylanisolvens -- 2_1_22	A
Parabacteroides distasonis -- 3_1_19	A
Bacteroides dorea -- 9_1_42FAA	A
Bacteroides ovatus -- D2	A
Bacteroides stercoris -- VPI B3-21, ATCC 43183, CIP 104203, JCM 9496	A
Bacteroides thetaiotaomicron -- VPI 5482 [CIP 104206T, E50, NCTC 10582]	A
Bacteroides uniformis -- ATCC 8492	A
Bacteroides vulgatus -- NCTC 11154	A
Bifidobacterium pseudocatenulatum -- B1279, ATCC 27919	A
Blautia sp. -- KLE 1732	A
Blautia wexlerae -- ATCC BAA-1564, JCM 17041, KCTC 5965, WAL 14507	A
Clostridium hylemonae -- TN-271, JCM 10539	A
Clostridium leptum -- VPI T7-24-1, ATCC 29065	A
Tyzzerella nexilis DSM 1787	A
Clostridium saccharolyticum -- WM1, ATCC 35040, NRC 2533	A
Absiella dolichum DSM 3991	A
Collinsella aerofaciens -- VPI 1003 [DSM 3979, JCM 10188]	A
Coprococcus comes -- VPI CI-38	A
Dialister invisus -- E7.25, CCUG 47026	A
Eubacterium rectale -- VPI 0990 [CIP 105953]	A
Eubacterium siraeum -- VPI T9-50-2, ATCC 29066, DSM 3996	A
Eubacterium ventriosum -- VPI 1013B	A
Holdemanella biformis -- VPI C17-5, ATCC 27806, KCTC 5969	A
Intestinibacter bartlettii -- WAL 16138, ATCC BAA-827, CCUG 48940	A
Megasphaera sp. -- Sanger 24, Sanger_24	A

Strain	**Media** (*see Tables 1 - 6 above*)
Odoribacter splanchnicus -- 1651/6, ATCC 29572, CCUG 21054, CIP 104287, LMG 8202, NCTC 10825	A
Parabacteroides merdae -- VPI T4-1, ATCC 43184, CCUG 38734, CIP 104202, JCM 9497	A
Parabacteroides sp. -- D13	A
Granulicatella adiacens -- GaD [CIP 103243, DSM 9848]	A
Mitsuokella multacida -- A 405-1, ATCC 27723, NCTC 10934	A
Parabacteroides johnsonii -- M-165, CIP 109537, JCM 13406	A
Prevotella buccalis -- HS4, ATCC 35310, NCDO 2354	A
Prevotella copri -- CB7, JCM 13464	A
Clostridium sp. -- VPI C48-50 (unassigned Clostridiales)	A
Ruminococcus gauvreauii -- CCRI-16110, CCUG 54292, JCM 14987, NML 060141	A
Ruminococcus lactaris -- VPI X6-29	A
Ruminococcus torques -- VPI B2-51	A
Alistipes senegalensis -- CSUR P150, JCM 32779, JC50	A
Bifidobacterium breve -- S1, ATCC 15700, NCTC 11815	A
Bifidobacterium catenulatum -- B669, ATCC 27539, CECT 7362, CIP 104175, DSM 20103	A
Butyricimonas virosa -- MT12, CCUG 56611, JCM 15149	A
Dorea formicigenerans -- VPI C8-13 [JCM 9500]	A
Bacteroides plebeius -- M12	A
Ruminococcus gnavus -- VPI C7-9	A
Clostridium sp. -- M62/1	A
Slackia heliotrinireducens -- RHS 1, ATCC 29202, NCTC 11029	A
Clostridium methylpentosum -- R2, ATCC 43829	A
Ethanoligenens harbinense -- YUAN-3, CGMCC 1.5033, JCM 12961	A
Marvinbryantia formatexigens -- I-52, CCUG 46960	A
Clostridium bolteae -- WAL 16351, [CCUG 46953], ATCC BAA-613, Song et al. 2003	A

Strain	**Media** (*see Tables 1 - 6 above*)
Clostridium scindens -- VPI 13733, ATCC 35704, 19	A
Bacteroides xylanisolvens -- XB1A, CCUG 53782	A

Isolation of donor-derived active and supportive microbial strains

[0165] Fecal donors are selected based on multiple criteria, including a health and medical history questionnaire, physical exam, and blood and stool tests for assessing pathogen-free status. Stool samples from donors who do not meet the inclusion criteria based on any of the above-mentioned assessment are discarded from quarantine.

[0166] Donors provide a stool sample sealed in a plastic container.. Upon collection, stool samples are immediately transferred to an anaerobic chamber (5% CO_2, 5% H_2, 90% N_2) within 15 minutes of collection.

[0167] Once transferred to the anaerobic chamber, the fresh stool sample is labeled, weighed, evaluated for anomalies (presence of urine, toilet paper, etc.), and scored according to the Bristol scale. A stool sample weighing less than 45 g, or that fails to conform to a Bristol Stool Scale type 2, 3, 4 or 5, is rejected. Stool samples that meet the acceptance criteria are processed and aliquoted. 45 g of the stool sample is transferred into a sterile container for specific pathogen testing. The remainder of the sample is aliquoted into cryovials containing sterile glycerol solution (about 2 g of sample per vial; 6 vials per stool sample). These vials are transferred from the anaerobic chamber to a -80 °C freezer for storage until shipping on dry ice.

[0168] Microbial strain isolation is performed by making serial dilution aliquots of the stool samples and plating on a variety of microbial cultivation media suitable for growth of anaerobes. Specific enrichment techniques are performed for species having particular metabolic capabilities, such as consumption and degradation of oxalate from culture media. Species-specific PCR assays are developed to identify and follow the presence of specific species in the stool samples, isolated colonies, or enrichment culture. When appropriate, the enrichment cultures are plated on appropriate agar media to generate isolated colonies of microbes. After incubation under anaerobic conditions, microbial colonies are picked and transferred to plates with appropriate culture media to isolate the desired strain away from any microbial contaminating strain, followed by anaerobic incubation.

[0169] To identify isolated microbial colonies to the species level, either Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS), 16S rRNA sequencing, or whole genome sequencing will be used. Identified colonies belonging to species

of interest will be re-plated on appropriate culture media and their identify reconfirmed by 16S sequencing prior to their liquid media propagation and storage at -80 °C.

DNA Extraction

[0170] DNA was extracted from fecal samples using a Qiagen DNeasy Power Soil Kit (Qiagen, Germantown, MD) in accordance with the manufacturer's instructions. Alternative methods for extracting DNA from fecal samples are well-known and routinely practiced in the art (*e.g.*, described by Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, 3d ed., 2001).

Whole Genome Shotgun Sequencing

[0171] Sequencing of DNA samples is carried out using the TruSeq Nano DNA Library Preparation kit (Illumina, San Diego, CA, US) and a NextSeq platform (Illumina, San Diego, CA, US). In brief, sequencing libraries are prepared from DNA extracted from each sample. DNA is mechanically fragmented using an ultrasonicator. The fragmented DNA is subjected to end repair and size selection of fragments, adenylation of 3' ends, linked with adaptors, and DNA fragments enriched according to the TruSeq Nano DNA Library Preparation kit manual (Illumina, San Diego, CA, US). Samples were sequenced to generate 30-40 million paired-end reads of 75 bp length.

16S rRNA Sequencing

[0172] Microbial species identification by 16S rRNA sequencing is performed by a method as known by persons of skill in the art (*see*, for example, Turner *et al.*, 1999, "Investigating Deep Phylogenetic Relationships among Cyanobacteria and Plastids by Small Subunit rRNA Sequence Analysis," *J Eukaryot Microbiol.* 46:327-338; Shin *et al.*, 2016, "Analysis of the mouse gut microbiome using full-length 16S rRNA amplicon sequencing," *Sci Rep.* 6:29681.) For each microbial stain, at least 1300 bp of 16S rRNA sequence is obtained for species level identification.

MALDI-TOF MS

[0173] Species level identification by MALDI-TOF MS of microbial strains is performed by a method as known by persons of skill in the art (*see*, for example, Seuylemezian *et al.*, 2018, "Development of a Custom MALDI-TOF MS Database for Species-Level Identification of Bacterial Isolates Collected From Spacecraft and Associated Surfaces," *Front Micrbiol.* 9:780.) In brief, spots of microbial isolates are transferred to a well of a 48-well or 96-well plate, layered with 1 µl of 70 % formic acid and left to air dry. 1 µl of α-Cyano-4-hydroxycinnamic acid matrix in 50% acetonitrile-25% trifluoroacetic acid is layered on the sample and left to air dry. MALDI-TOF MS is performed using, for example, a microbflex LT bench-top mass

spectrometry instrument (Bruker Daltonics, Billerica, MA). Processing of spectral data is performed, for example, using flexAnalysis software (Bruker Daltonics, Billerica, MA). At least 10 spectra are calculated for each isolate to create a main spectral profile, wherein each spectral line that constitutes the main spectral profile has a log score of greater than 2.7 and a peak frequency greater than 75%.

Example 2 – Preparation and Optimization of a High-Complexity Defined Gut Microbial Community

[0174] FIGURE 1 shows a workflow schematic for the preparation and optimization of a high-complexity defined gut microbial community. Defined microbial strains purchased from American Type Culture Collection (ATCC, Manassas, VA) were assembled as a frozen glycerol stock collection in 96-well plate format. Defined microbial strains were revived by culturing in 96-well plate format aliquots in growth medium and culture conditions in accordance with the supplier's instructions ("Working Defined Microbial Strain Collection). Defined microbial strains were sub-cultured for 24 hours, two times. Optical density of cultures was measured and cultures normalized to an O.D. value of 0.1. Defined microbial strains were pooled to form a high-complexity defined gut microbial community, washed and resuspended with PBS, then gavaged into gnotobiotic, 6-8 week old, female, Swiss Webster mice, once per day for 3 days, and permitted to colonize. Stool samples from inoculated mice were collected weekly for 4 consecutive weeks and frozen for subsequent DNA extraction and metagenomic analysis. 4-weeks after inoculation, mice were challenged with human fecal samples obtained from three donors. Human fecal samples were administered by oral gavage. Stool samples from challenged mice were collected weekly for 4 consecutive weeks and frozen for subsequent DNA extraction and metagenomic analysis. 4 weeks after human fecal microbial challenge, mice were sacrificed, and colon samples were prepared for histologic analysis. Strains identified to have "jumped in" to the community were identified (by metagenomic analysis), procured and cultured and optionally added to the high-complexity defined gut microbial community to produce a new high-complexity defined gut microbial community. Conversely, strains that were identified (by metagenomic analysis) to "drop out" of the community were omitted from the new high-complexity defined gut microbial community.

DNA Extraction

[0175] DNA was extracted from fecal samples using a Qiagen DNesay Power Soil Kit (Qiagen, Germantown, MD) in accordance with the manufacturer's instructions. Alternative methods for extracting DNA from fecal samples are well-known and routinely practiced in the

art (*e.g.*, described by Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, 3d ed., 2001).

Metagenomic Analysis

[0176] Sequencing of the DNA samples was carried out using the TruSeq Nano DNA Library Preparation kit (Illumina, San Diego, CA, US) and a NextSeq platform (Illumina, San Diego, CA, US). In brief, sequencing libraries were prepared from DNA extracted from each sample. DNA was mechanically fragmented using an ultrasonicator. The fragmented DNA was subjected to end repair and size selection of fragments, adenylation of 3' ends, linked with adaptors, and DNA fragments enriched according to the TruSeq Nano DNA Library Preparation kit manual (Illumina, San Diego, CA, US). Samples were sequenced to generate 30-40 million paired-end reads of 75 bp length.

[0177] Each metagenome was run through the Metagenomic Intra-Species Diversity Analysis System (MIDAS) (*see* Nayfach *et al.*, 2016, "An integrated metagenomics pipeline for strain profiling reveals novel patterns of bacterial transmission and biogeography," *Genome Res.* 26 (11): 1612-1625.) which estimates the sequencing depth and relative abundance of each microbial species in a fecal sample by mapping reads to a reference database of 15 gene families of 5,952 bacterial species which each occur in nearly all bacterial genomes at one copy per genome.

Backfill

[0178] Defined microbial strains that did not engraft (*i.e.* dropped out) of the microbial community were identified by the metagenomic analysis above. Similarly, microbial strains from the human fecal microbial challenge that engrafted into the mouse gut (*i.e.* jumped in) were identified by the metagenomic analysis above. After a first human fecal microbial challenge, 97 defined microbial strains out of the inoculated 104 defined microbial strains persisted in fecal samples of the challenged mice and 7 defined microbial strains dropped out. In two mice, 26 microbial strains from the human fecal microbial challenge jumped in and in one mouse, 44 microbial strains from the human fecal microbial challenge jumped in. 22 of the 26 microbial strains that jumped into the microbial communities in two of the challenged mice were obtained from ATCC and added to the 97 defined microbial strains that persisted after human fecal microbial challenge to produce a high-complexity defined gut microbial community consisting of 119 defined microbial strains (*See* Table 12 and FIGURE 2; "Invaders" = microbial strains that "dropped in" to community, "Input" = defined microbial strains inoculated into mouse).

TABLE 12

Mouse	Defined Microbial Strains Persisting Post-Microbial Challenge	Defined Microbial Strains Dropping Out	Human Microbial Strains Jumping In
1 (Receiving Human Stool Sample 1)	97	7	26
2 (Receiving Human Stool Sample 2)	97	7	26
3 (Receiving Human Stool Sample 3)	97	7	44

Example 3 – Treatment of Mice with Persistent *C. Difficile* Infection

[0179] Gnotobiotic, 6-8 week old, female, Swiss Webster mice were colonized with human stool samples (200 µl of human stool diluted with an equal volume of PBS) by oral gavage. Stool samples from colonized mice were collected weekly for 4 consecutive weeks and frozen for subsequent DNA extraction and metagenomic analysis (as described in Example 2). 4 weeks following human fecal colonization, mice were treated with 200 µl of 1 mg/ml clindamycin by oral gavage. 24 hours after clindamycin treatment, mice were orally gavaged with 200 µl of turbid, overnight cultures of *C. difficile*, and maintained on a high-sugar diet. Stool samples from the inoculated mice were collected daily for 3 days post-inoculation for CFU plating and frozen for subsequent DNA extraction and metagenomic analysis. 3 days post-inoculation with *C. difficile*, mice were treated with human stool sample, the 119 strain high-complexity defined gut microbial community, or phosphate buffered saline (PBS) vehicle control. Stool samples from treated mice were collected daily for 4 days for CFU plating and frozen for subsequent DNA extraction and metagenomic analysis. 4 days post-treatment, mice were sacrificed, and colon samples (*e.g.*, ceca) were prepared for mass spectrometry and histologic analysis. *See* FIGURE 3A for schematic workflow of *C. difficile* infection and treatment schedule.

CFU plating

[0180] Stool samples were diluted in PBS, homogenized using a vortex mixer, and left to sediment. The supernatant was used to make serial 10-fold dilutions in PBS from 1 X 10^{-1} to 10^{-5}. A 100 µl aliquot of each dilution was plated onto CDDC selective agar (*see*, Table 13)

TABLE 13

Component	Amount (in 500 mL)
C. difficile agar base	34.5 g
Cysteine	250 mg
Cefoxitin	8 mg
D-cycloserine	125 mg
Defibrinated horse blood	35 ml
Milli-Q water (dH_2O)*	to total volume of 500 mL

[0181] After 48 h of anaerobic incubation at 37 °C, plates were inspected for growth of colonies with morphology characteristic of *C. difficile*. Plates with 30 to 300 colonies were counted with a detection limit of 3.0 $\log_{10}$ CFU/g. For each dilution, the average of the two duplicate plates was calculated. When two successive dilutions yielded 30 to 300 colonies, the average count of both dilutions was calculated.

[0182] As shown in FIGURE 3B, mice receiving treatment with human stool sample or the 119 defined microbial strain high-complexity defined gut microbial community, significantly reduced the number of *C. difficile* CFUs/µl in stool samples collected at 6 days post *C. difficile* infection (*i.e.* 3 days post treatment) as compared to mice treated with PBS alone.

Example 4 – Bile Acid Analysis by Mass Spectrometry

[0183] Frozen stool samples or homogenized cecum sections were pelleted in a centrifuge tube and extracted with ethyl acetate. Ethyl acetate was evaporated under vacuum and pellets were re-dissolved in 200 µl of 20% DMSO/MeOH.

[0184] LC-MS/MS was performed on an Agilent 6120 quadrupole mass spectrometer in negative mode using a Kinetex C18 stationary phase (1.7µm) column.

[0185] As shown in FIGURE 4, bile acid concentrations in stool samples (FIGURE 4A) and ceca homogenates (FIGURE 4B) collected from mice treated with human stool sample and

mice treated with the 119 defined microbial strain high-complexity defined gut microbial community had similar bile acid profiles and concentrations as quantified by MS.

Example 5 – Metabolite Analysis by Mass Spectrometry

[0186] Urine samples were thawed at room temperature and centrifuged at 13,000 × *g* for 15 min at 4 °C to remove particulate matter. 2 volumes of ethyl acetate was added per volume of urine sample, and the solution was vortex mixed to precipitate proteins. Ethyl acetate was removed by rotary evaporation. Dried material was dissolved in 80% MeOH/DMSO and separated by reverse phase HPLC (Agilent 1200 series) for small molecule purification. NMR spectra were collected on either a Bruker Avance DRX500 or a Bruker AvanceIII 600-I spectrometer. Purification of the ethyl acetate fraction was carried on by gradient HPLC on a C18 reverse phase column.

[0187] As shown in FIGURE 5, urine samples collected from mice treated with human stool sample and mice treated with the 119 defined microbial strain high-complexity defined gut microbial community had similar bile acid profiles and concentrations as quantified by MS

Example 6 – Molecular Identification of Microbial Species

Whole Genome Shotgun Sequencing

[0188] DNA extraction from isolated microbial cultures or fecal samples and whole genome shotgun sequencing is performed by methods as previously described in Example 2. Sequence reads are mapped against a comprehensive database of complete, sequenced genomes of all the defined microbial strains comprising a gut community.

16S rRNA Sequencing

[0189] Molecular identification by 16S rRNA sequencing of microbial colonies in liquid culture or resuspended in PBS is performed by a method as known by persons of skill in the art (*see*, for example, Turner *et al.*, 1999, "Investigating Deep Phylogenetic Relationships among Cyanobacteria and Plastids by Small Subunit rRNA Sequence Analysis," *J Eukaryot Microbiol.* 46:327-338; Shin *et al.*, 2016, "Analysis of the mouse gut microbiome using full-length 16S rRNA amplicon sequencing," *Sci Rep.* 6:29681.) For each defined microbial stain, at least 1300 bp of 16S rRNA sequence is obtained for species level identification.

MALDI-TOF MS

[0190] Molecular identification by MALDI-TOF MS of microbial colonies in liquid culture or resuspended in PBS is performed by a method as known by persons of skill in the art (*see*, for example, Seuylemezian *et al.*, 2018, "Development of a Custom MALDI-TOF MS Database for Species-Level Identification of Bacterial Isolates Collected From Spacecraft and Associated Surfaces," *Front Micrbiol.* 9:780.) In brief, spots of microbial isolates are transferred to a well of a 48-well or 96-well plate, layered with 1 µl of 70 % formic acid and left to air dry. 1 µl of α-Cyano-4-hydroxycinnamic acid matrix in 50% acetonitrile-25% trifluoroacetic acid is layered on the sample and left to air dry. MALDI-TOF MS is performed using, for example, a microbflex LT bench-top mass spectrometry instrument (Bruker Daltonics, Billerica, MA). Processing of spectral data is performed, for example, using flexAnalysis software (Bruker Daltonics, Billerica, MA). At least 10 spectra are calculated for each isolate to create a main spectral profile, wherein each spectral line that constitutes the main spectral profile has a log score of greater than 2.7 and a peak frequency greater than 75%.

Example 7 – Method of Treatment for Persistent *C. difficile* Infection

[0191] A high-complexity defined gut microbial community of the present invention is administered in an effective amount for the treatment of a persistent *C. difficile* infection in a mammalian subject in need thereof. The high-complexity defined gut microbial community is administered as a composition formulated for oral administration or other non-parenteral route of administration as described herein. The mammalian subject may or may not have been treated with antibiotics in advance of treatment with the high-complexity defined gut microbial community. The mammalian subject is treated once prior to improvement of symptoms associated with persistent *C. difficile* infection or a significant reduction in the number of *C. difficile* CFUs in the gut of the mammalian subject. Alternatively, the mammalian subject is treated two or more times prior to improvement of symptoms associated with persistent *C. difficile* infection or a significant reduction in the number of *C. difficile* CFUs in the gut of the mammalian subject.

Example 8 – Method of Treatment for Cholestatic Disease

[0192] A high-complexity defined gut microbial community of the present invention is administered in an effective amount for the treatment of a cholestatic disease in a mammalian subject in need thereof. The high-complexity defined gut microbial community is administered

as a composition formulated for oral administration or other non-parenteral route of administration as described herein. The mammalian subject may or may not have been treated with antibiotics in advance of treatment with the high-complexity defined gut microbial community. The mammalian subject is treated once prior to improvement of symptoms associated with cholestatic disease or a significant modification in bile acid composition profile and/or concentrations in the gut of the mammalian subject. Alternatively, the mammalian subject is treated two or more times prior to improvement of symptoms associated with cholestatic disease or a significant modification in bile acid composition profile and/or concentrations in the gut of the mammalian subject.

Example 9 – Pathway-based Assembly of a High-complexity Defined Gut Microbial Community from Human Donor Fecal Samples

[0193] A high-complexity defined gut microbial community of the present invention is assembled by assignment of specific MetaCyc pathways to defined microbial stains.

[0194] Species-level compositional profiles of a donor fecal sample is generated using shotgun metagenomic sequencing.

[0195] A complete reference genome from the type-strain of every microbial species in the donor sample is retrieved and annotated using a custom computational pipeline that detects and accurately annotates MetaCyc pathways and the specific genes comprising those pathways. This annotation associates all metabolic pathways of interest with all the microbial strains in the fecal sample that utilize those pathways, thus defining a set of candidate microbes that can be isolated to cover/perform a desired metabolic function or fill a desired functional niche.

[0196] Having identified a set of microbial strains present in a fecal sample that utilizes a desired pathway, and given a set of metabolic pathways to be included in the high complexity-defined gut microbial community (*i.e.* core and substrate/metabolite panel pathways described above), a custom optimization algorithm is used to computationally design communities comprising microbes from donor samples that carry all, or substantially all, of the given set of metabolic pathways in addition to meeting the following criteria: (i) all metabolic pathways are utilized or encoded by at least three different species to incorporate functional redundancy; and (ii) at least three of the four major phyla in the normal human gut microbiome (Bacteroidetes, Actinobacteria, Firmicutes, Proteobacteria) are represented, and no one phylum accounts for more than 60% of the strains in the high-complexity defined gut microbial community (*i.e.* to capture the taxonomic diversity of the normal gut microbiome).

INCORPORATION BY REFERENCE

[0197] The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[0198] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

WHAT IS CLAIMED IS:

1. A high-complexity defined gut microbial community, comprising:

a plurality of between 40 and 500 defined microbial strains, wherein the defined microbial strains comprise at least 3 of 4 phyla selected from the group consisting of Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria;

wherein the defined gut microbial community is capable of:

a. metabolizing at least 90% of enumerated substrates selected from the group consisting of: a-mannan (yeast), acetate, agarose, alanine, alginate, anthocyanin, arabinan, arabinogalactan, arabinoxylan, arginine, asparagine, Aspartate, b-glucans, butyrate, carrageenan, chitin, chlorogenic acids, chondroitin sulfate, cinnamic acid, Cysteine, dextran (40), Dihydrochalcones, Enterodiol, flavan-3-ols, flavanones, flavones, flavonols, folate, formate, galactomannan (carob), galacturonan (homo), galacturonate, glucomannan (konjac), glutamate, Glutamine, Glycine, Histidine, hyaluronan, hydrogen, hydroxycinnamic acids, hydroxyproline, inulin, isoflavones/isoflavanones, Isoleucine, lactate, laminarin, Leucine, levan, Lysine, Methionine, mucin O-linked glycans, Ornithine, Phenylalanine, porphyran, Proline, propionate, rhamnogalacturonan I, rhamnogalacturonan II, Secoisolariciresinol diglucoside, Serine, starch (potato), starch (structure 1), thiamine, Threonine, tryptophan, Tyrosine, Valine, xyloglucan, and xylooligosaccharides (XOS), and/or

b. producing at least 90% of enumerated metabolites selected from the group consisting of: formate, acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, 2-methylbutyrate, caporate, isocaporate, 3-methylvaleric acid, L-phenylalanine, 3-phenylpropionic acid, phyenypyruvate, DL-3-phenyllactic acid, trans-cinnamic acid, phenyllactic acid, phenethylamine, L-tyrosine, 3-(4-hydroxyphenyl)propionic acid, 3-(4-hydroxyphenyl) pyruvic acid, DL-p-hydroxyphenyl lactic acid, p-coumaric acid, 4-hydroxyphenyl acetic acid, tyramine, phenol, p-cresol, 4-ethylphenol, 4-vinylphenol, 4-hydroxybenzoic acid, L-tryptophan, 3-indolepropionic acid, 3-indolepyruvic acid, DL-indole-3-lactic acid, trans-3-indoleacrylic acid, 3-indoleacetic acid, tryptamine, indole, skatol, indole-3-carboxylic acid, indole-3-carboxyaldehyde, N-acetyl-L-phenylalanine,

phenylpropionylglycine, 3-(3-hydroxyphenyl) propionic acid, cinnamoylglycine, phenylacetylglycine, phenylacetylglutamine, hippuric acid, 2-hydroxyhippuric acid, 3-hydroxyhippuric acid, 4-hydroxyhippuric acid, 4-hydroxyphenylacetylglycine, phenyl sulfate, phenyl glucuronide, p-cresol sulfate, p-cresol glucuronide, 4-ethylphenol sulfate, 4-ethylphenol glucuronide, N-acetyl-L-tryptophan, 5-hydroxy-L-typtophan, N-acetylserotonin, 3-indolepriopionylglycine, indolyl-3-acryloylglycine, indoxyl sulfate, indoxyl glucuronide, 5-hydroxyindole-3-acetic acid, indoleacetylglycine, lithocholic acid, murocholic acid, ursodeoxycholic acid, hyodeoxycholic acid, deoxycholic acid, 7-oxocholic acid, ω-muricholic acid, α-muricholic acid, β-muricholic acid, γ-muricholic acid, 7βcholic acid, taurolithocholic acid, tauroursodeoxycholic acid, taurohyodeoxycholic acid, taurochenodeoxycholic acid, taurodeoxycholic acid, tauro-β-muricholic acid, tauro- ω-muricholic acid, and taurocholic acid,

wherein the defined gut microbial community achieves substantial engraftment when administered to a gnotobiotic mouse; and

wherein the engrafted defined gut microbial community is stable following a human fecal community microbial challenge.

2. The high complexity defined gut microbial community of claim 1, wherein metabolization of a substrate and/or production of a metabolite can be determined by culturing the defined gut microbial community *in vitro* and measuring whether the substrate is metabolized and/or the metabolite is produced by liquid chromatography-mass spectrometry analysis.

3. The high complexity defined gut microbial community of claim 1 or 2, wherein metabolization of a substrate and/or production of a product can be determined by administering the defined gut microbial community to a gnotobiotic mouse and measuring whether the substrate is metabolized and/or the product is produced after a defined period of time by liquid chromatography-mass spectrometry analysis of a sample obtained from the mouse.

4. The high complexity defined gut microbial community of claim 3, wherein the defined gut microbial community is administered via a route selected from the group consisting of oral, rectal, fecal (by enema), and naso/oro-gastric gavage.

5. The high complexity defined gut microbial community of claim 3 or 4, wherein the defined period of time is about 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours, 2 days, 7 days, 14 days, 1 month, or 2 months.

6. The high complexity defined gut microbial community of any one of claims 3-5, wherein the sample is selected from the group consisting of a fecal sample, urine sample, blood sample, or serum sample.

7. A high-complexity defined gut microbial community, comprising:

a plurality of between 40 and 500 defined microbial strains, wherein the defined microbial strains comprise at least 3 of 4 phyla selected from the group consisting of Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria;

wherein the defined gut microbial community encodes the enzymes catalyzing all reactions at least 90% of the enumerated MetaCyc metabolic pathways selected from the group consisting of: 1CMET2-PWY, 2.6.1.32-RXN, AEROBACTINSYN-PWY, ALACAT2-PWY, ALADEG-PWY, ALANINE-DEG3-PWY, ALANINE-SYN2-PWY, ALANINE-VALINESYN-PWY, ANAPHENOXI-PWY, ARGASEDEG-PWY, ARGDEG-III-PWY, ARGDEG-IV-PWY, ARGDEGRAD-PWY, ARGDEG-V-PWY, ARG-GLU-PWY, ARGININE-SYN4-PWY, ARG-PRO-PWY, ARGSYNBSUB-PWY, ARGSYN-PWY, ASPARAGINE-BIOSYNTHESIS, ASPARAGINE-DEG1-PWY, ASPARAGINE-DEG1-PWY-1, ASPARAGINESYN-PWY, ASPARTATE-DEG1-PWY, ASPARTATESYN-PWY, ASPASN-PWY, ASPSYNII-PWY, AST-PWY, BETA-ALA-DEGRADATION-I-PWY, CAMALEXIN-SYN, CITRULBIO-PWY, CITRULLINE-DEG-PWY, COA-PWY, CODH-PWY, CYSTEINE-DEG-PWY, CYSTSYN-PWY, DAPLYSINESYN-PWY, ENTBACSYN-PWY, ETHYL-PWY, FAO-PWY, FERMENTATION-PWY, GLNSYN-PWY, GLUDEG-I-PWY, GLUGLNSYN-PWY, GLUTAMATE-DEG1-PWY, GLUTAMATE-SYN2-PWY, GLUTAMINDEG-PWY, GLUTAMINEFUM-PWY, GLUTATHIONESYN-PWY, GLUTDEG-PWY, GLUTORN-PWY, GLUTSYNIII-PWY, GLUTSYN-PWY, GLYCGREAT-PWY, GLYSYN-ALA-PWY, GLYSYN-PWY, GLYSYN-THR-PWY, HISDEG-PWY, HISHP-PWY, HISTDEG-PWY, HISTSYN-PWY, HOMOCYSDEGR-PWY, HOMOSER-METSYN-PWY, HOMOSERSYN-PWY, HSERMETANA-PWY, HYDROXYPRODEG-PWY, ILEUDEG-PWY, ILEUSYN-PWY, LARABITOLUTIL-PWY, LCYSDEG-PWY, LEU-DEG2-PWY, LEUSYN-PWY, LYSDEGII-PWY,

LYSINE-AMINOAD-PWY, LYSINE-DEG1-PWY, MALATE-ASPARTATE-SHUTTLE-PWY, METH-ACETATE-PWY, METHANOGENESIS-PWY, METHIONINE-DEG1-PWY, MGLDLCTANA-PWY, ORN-AMINOPENTANOATE-CAT-PWY, ORNDEG-PWY, P101-PWY, P162-PWY, P163-PWY, P181-PWY, P261-PWY, P283-PWY, P401-PWY, P541-PWY, PHENYLALANINE-DEG1-PWY, PHESYN, PHOSLIPSYN2-PWY, PHOSPHONOTASE-PWY, PROSYN-PWY, PROUT-PWY, PWY0-1021, PWY0-1221, PWY0-1299, PWY0-1303, PWY0-1305, PWY0-1313, PWY0-1317, PWY0-1321, PWY0-1338, PWY0-1347, PWY0-1355, PWY0-1356, PWY0-1534, PWY0-1544, PWY0-1565, PWY0-1576, PWY0-1577, PWY0-1578, PWY0-1585, PWY0-1601, PWY0-42, PWY0-461, PWY0-823, PWY0-901, PWY-1, PWY-1061, PWY-1121, PWY-1186, PWY1-2, PWY-1263, PWY-1622, PWY-1722, PWY-1781, PWY-181, PWY-1881, PWY-1962, PWY-1981, PWY1F-467, PWY1F-FLAVSYN, PWY1G-0, PWY-2021, PWY-2161, PWY-2181, PWY-2201, PWY-2821, PWY-2941, PWY-2942, PWY-3, PWY-3022, PWY-3081, PWY-3161, PWY-3162, PWY-3181, PWY-3301, PWY-3341, PWY-3385, PWY-3461, PWY-3462, PWY-3581, PWY-361, PWY-3661, PWY-3661-1, PWY-381, PWY-3841, PWY-3941, PWY3DJ-12, PWY3O-4107, PWY3O-4108, PWY-4, PWY-40, PWY-4002, PWY-4041, PWY-4201, PWY-4281, PWY-43, PWY-4321, PWY-4341, PWY-4361, PWY-46, PWY490-3, PWY490-4, PWY-4981, PWY-4983, PWY-4984, PWY4FS-6, PWY-5, PWY-5022, PWY-5024, PWY-5028, PWY-5029, PWY-5030, PWY-5031, PWY-5041, PWY-5048, PWY-5049, PWY-5057, PWY-5075, PWY-5076, PWY-5078, PWY-5079, PWY-5081, PWY-5082, PWY-5087, PWY-5097, PWY-5101, PWY-5103, PWY-5104, PWY-5108, PWY-5109, PWY-5129, PWY-5135, PWY-5136, PWY-5151, PWY-5154, PWY-5155, PWY-5159, PWY-5176, PWY-5188, PWY-5189, PWY-5196, PWY-5199, PWY-5207, PWY-5250, PWY-5254, PWY-5265, PWY-5280, PWY-5283, PWY-5290, PWY-5297, PWY-5298, PWY-5311, PWY-5314, PWY-5316, PWY-5319, PWY-5324, PWY-5329, PWY-5331, PWY-5332, PWY-5364, PWY-5381, PWY-5382, PWY-5386, PWY-5394, PWY-5399, PWY-5436, PWY-5437, PWY-5441, PWY-5443, PWY-5458, PWY-5467, PWY-5468, PWY-5473, PWY-5474, PWY-5494, PWY-5497, PWY-5499, PWY-5629, PWY-5651, PWY-5653, PWY-5665, PWY-5669, PWY-5675, PWY-5679, PWY-5686, PWY-5710, PWY-5736, PWY-5737, PWY-5739, PWY-5740, PWY-5742, PWY-5747, PWY-5748, PWY-5751, PWY-5754, PWY-5766, PWY-5770, PWY-5784, PWY-5788, PWY-5797, PWY-5800, PWY-581, PWY-5811, PWY-5818, PWY-5826, PWY-5877, PWY-5883, PWY-5886, PWY-5912, PWY-5913, PWY-5921, PWY-5936,

PWY-5940, PWY-5958, PWY-5963, PWY-5968, PWY-5978, PWY-5980, PWY-5990, PWY-6003, PWY-6004, PWY-601, PWY-6030, PWY-6039, PWY-6045, PWY-6052, PWY-6053, PWY-6054, PWY-6055, PWY-6068, PWY-6069, PWY-6082, PWY-6120, PWY-6121, PWY-6122, PWY-6123, PWY-6124, PWY-6133, PWY-6134, PWY-6141, PWY-6143, PWY-6148, PWY-6151, PWY-6160, PWY-6173, PWY-6196, PWY-6219, PWY-622, PWY-6220, PWY-6233, PWY-6273, PWY-6277, PWY-6281, PWY-6307, PWY-6309, PWY-6313, PWY-6318, PWY-6320, PWY-6321, PWY-6322, PWY-6324, PWY-6328, PWY-6334, PWY-6339, PWY-6343, PWY-6344, PWY-6345, PWY-6346, PWY-6375, PWY-6376, PWY-6381, PWY-6386, PWY-6387, PWY-6397, PWY-6403, PWY-6407, PWY-6408, PWY-6409, PWY-6431, PWY-6435, PWY-6444, PWY-6455, PWY-6456, PWY-6457, PWY-6466, PWY-6471, PWY-6473, PWY-6481, PWY-6486, PWY-6493, PWY-6495, PWY-6511, PWY-6512, PWY-6519, PWY-6533, PWY-6535, PWY-6536, PWY-6537, PWY-6543, PWY-6549, PWY-6559, PWY-6562, PWY-6572, PWY-6573, PWY-6574, PWY-6578, PWY-6588, PWY-6614, PWY-6627, PWY66-301, PWY66-375, PWY-6638, PWY66-391, PWY-6642, PWY66-420, PWY66-421, PWY66-425, PWY66-426, PWY66-428, PWY-6643, PWY-6661, PWY-6673, PWY-6682, PWY-6690, PWY-6696, PWY-6711, PWY-6717, PWY-6720, PWY-6724, PWY-6728, PWY-6731, PWY-6735, PWY-6749, PWY-6769, PWY-6771, PWY-6772, PWY-6773, PWY-6781, PWY-6784, PWY-6790, PWY-6791, PWY-6802, PWY-6807, PWY-6808, PWY-6813, PWY-6815, PWY-6816, PWY-6817, PWY-6818, PWY-6821, PWY-6822, PWY-6823, PWY-6831, PWY-6832, PWY-6834, PWY-6840, PWY-6845, PWY-6853, PWY-6854, PWY-6855, PWY-6891, PWY-6892, PWY-6896, PWY-6898, PWY-6902, PWY-6907, PWY-6908, PWY-6920, PWY-6922, PWY-6936, PWY-6942, PWY-6949, PWY-6953, PWY-6955, PWY-6963, PWY-6964, PWY-6965, PWY-6968, PWY-6969, PWY-6981, PWY-6982, PWY-6986, PWY-6994, PWY-7000, PWY-701, PWY-7014, PWY-7015, PWY-7016, PWY-7018, PWY-7019, PWY-702, PWY-7022, PWY-7025, PWY-7028, PWY-7040, PWY-7046, PWY-7052, PWY-7054, PWY-7064, PWY-7072, PWY-7088, PWY-7090, PWY-7097, PWY-7104, PWY-7115, PWY-7117, PWY-7118, PWY-7126, PWY-7147, PWY-7153, PWY-7158, PWY-7176, PWY-7177, PWY-7185, PWY-7186, PWY-7219, PWY-7221, PWY-7234, PWY-7246, PWY-7248, PWY-7250, PWY-7255, PWY-7274, PWY-7275, PWY-7282, PWY-7288, PWY-7297, PWY-7304, PWY-7315, PWY-7316, PWY-7318, PWY-7342, PWY-7351, PWY-7356, PWY-7376, PWY-7377, PWY-7383, PWY-7387, PWY-7397, PWY-7398, PWY-7400, PWY-7414, PWY-7425, PWY-7430, PWY-7432, PWY-7440, PWY-7441, PWY-7456, PWY-7467, PWY-

7498, PWY-7501, PWY-7506, PWY-7510, PWY-7514, PWY-7518, PWY-7520, PWY-7525, PWY-7531, PWY-7532, PWY-7533, PWY-7536, PWY-7542, PWY-7543, PWY-7547, PWY-7549, PWY-7550, PWY-7555, PWY-7561, PWY-7565, PWY-7570, PWY-7571, PWY-7600, PWY-7605, PWY-761, PWY-7612, PWY-7626, PWY-7645, PWY-7648, PWY-7649, PWY-7650, PWY-7665, PWY-7667, PWY-7668, PWY-7669, PWY-7671, PWY-7674, PWY-7688, PWY-7690, PWY-7693, PWY-7694, PWY-7701, PWY-7704, PWY-7706, PWY-7708, PWY-7717, PWY-7718, PWY-7719, PWY-7733, PWY-7734, PWY-7735, PWY-7737, PWY-7751, PWY-7761, PWY-7765, PWY-7767, PWY-7769, PWY-7770, PWY-7782, PWY-7790, PWY-7791, PWY-7793, PWY-7797, PWY-7811, PWY-7814, PWY-7822, PWY-7824, PWY-7826, PWY-7842, PWY-7850, PWY-7851, PWY-7855, PWY-7860, PWY-7861, PWY-7863, PWY-7867, PWY-7870, PWY-7880, PWY-7888, PWY-7889, PWY-7891, PWY-7892, PWY-7897, PWY-7901, PWY-7904, PWY-7907, PWY-7909, PWY-7910, PWY-7913, PWY-7917, PWY-7930, PWY-7931, PWY-7936, PWY-7953, PWY-7955, PWY-7956, PWY-7957, PWY-7958, PWY-7959, PWY-7960, PWY-7962, PWY-7977, PWY-7985, PWY-7986, PWY-7987, PWY-7988, PWY-7990, PWY-8002, PWY-8003, PWY-8006, PWY-8007, PWY-8008, PWY-8009, PWY-801, PWY-8010, PWY-8011, PWY-8013, PWY-8014, PWY-8015, PWY-8016, PWY-8017, PWY-8024, PWY-8032, PWY-8040, PWY-8043, PWY-8045, PWY-8071, PWY-8072, PWY-8080, PWY-8081, PWY-8082, PWY-8083, PWY-8086, PWY-8088, PWY-842, PWY-861, PWY-862, PWY8J2-1, PWY8J2-22, PWYDQC-4, PWYG-321, PWY-I9, PWYQT-4450, PWYQT-4476, PYRIDNUCSAL-PWY, PYRIDNUCSYN-PWY, PYRIDOXSYN-PWY, SAM-PWY, SERDEG-PWY, SERSYN-PWY, SPHINGOLIPID-SYN-PWY, TAURINEDEG-PWY, THRDLCTCAT-PWY, THREONINE-DEG2-PWY, TRNA-CHARGING-PWY, TRPCAT-PWY, TRPIAACAT-PWY, TRPKYNCAT-PWY, TRPSYN-PWY, TRYPDEG-PWY, TYRFUMCAT-PWY, TYRSYN, UDPNACETYLGALSYN-PWY, UDPNAGSYN-PWY, VALDEG-PWY, and VALSYN-PWY,

wherein the defined gut microbial community achieves substantial engraftment when administered to a gnotobiotic mouse; and

wherein the engrafted defined gut microbial community is stable following a human fecal community microbial challenge.

8. The high complexity defined gut microbial community of claim 7, wherein encoding the enzymes catalyzing all reactions of a MetaCyc metabolic pathway can be determined by culturing the defined gut microbial community *in vitro* and measuring whether a substrate in the pathway is metabolized, a metabolite in the pathway is produced, and/or a reaction intermediate in the pathway is produced by liquid chromatography-mass spectrometry analysis

9. The high complexity defined gut microbial community of claim 1 or 2, wherein encoding the enzymes catalyzing all reactions of a MetaCyc metabolic pathway can be determined by administering the defined gut microbial community to a gnotobiotic mouse and measuring whether a substrate in the pathway is metabolized , a metabolite in the pathway is produced, and/or a reaction intermediate in the pathway is produced after a defined period of time by liquid chromatography-mass spectrometry analysis of a sample obtained from the mouse.

10. The high complexity defined gut microbial community of claim 9, wherein the defined gut microbial community is administered via a route selected from the group consisting of oral, rectal, fecal (by enema), and naso/oro-gastric gavage.

11. The high complexity defined gut microbial community of claim 9 or 10, wherein the defined period of time is about 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours, 2 days, 7 days, 14 days, 1 month, or 2 months.

12. The high complexity defined gut microbial community of any one of claims 9-11, wherein the sample is selected from the group consisting of a fecal sample, urine sample, blood sample, or serum sample.

13. The high-complexity defined gut microbial community of any one of claims 1-12, wherein the at least 3 of 4 phyla comprise Bacteroidetes, Firmicutes, and Actinobacteria.

14. The high complexity defined gut microbial community of any one of claims 1-13, comprising Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria.

15. The high-complexity defined gut microbial community of any one of claims 1-14, wherein the defined microbial strains comprise phyla selected from the group consisting of Bateriodales, Clostridiales, Lactobacillales, Negativicutes, Eggerthellales, Bifidobacteriales, and Proteobacteria.

16. The high complexity defined gut microbial community of any one of claims 1-15, wherein the defined microbial strains comprise a genus selected from the group consisting of: Acidaminococcus, Adlercreutzia, Akkermansia, Alistipes, Anaerobutyricum, Anaerofustis, Anaerostipes, Anaerotruncus, Bacteroides, Parabacteroides, Bifidobacterium, Bilophila, Blautia, Catenibacterium, Clostridium, Tyzzerella, Absiella, Collinsella, Coprococcus, Dialister, Eubacterium, Holdemanella, Intestinibacter, Megasphaera, Odoribacter, Parabacteroides, Granulicatella, Holdemania, Hungatella, Intestinimonas, Solobacterium, Mitsuokella, Olsenella, Parabacteroides, Prevotella, Roseburia, Ruminococcus, Slackia, Butyrivibrio, Subdoligranulum, Turicibacter, Butyricimonas, Streptococcus, Dorea, Oscillibacter, Desulfovibrio, Ethanoligenens, Marvinbryantia, Lactobacillus, and Faecalibacterium.

17. The high complexity defined gut microbial community of any one of claims 1-16, wherein the defined microbial strains are selected from the group consisting of: Acidaminococcus fermentans, Acidaminococcus sp., Adlercreutzia equolifaciens, Akkermansia muciniphila, Alistipes finegoldii, Alistipes indistinctus, Alistipes onderdonkii, Anaerobutyricum hallii, Anaerofustis stercorihominis, Anaerostipes caccae, Anaerotruncus colihominis, Bacteroides caccae, Bacteroides cellulosilyticus, Bacteroides coprocola, Bacteroides coprophilus, Bacteroides dorei, Bacteroides eggerthii, Bacteroides finegoldii, Bacteroides fragilis, Bacteroides intestinalis, Bacteroides ovatus, Bacteroides rodentium, Bacteroides thetaiotaomicron, Bacteroides xylanisolvens, Parabacteroides distasonis, Bacteroides dorea, Bacteroides stercoris, Bacteroides uniformis, Bacteroides vulgatus, Bifidobacterium pseudocatenulatum, Bilophila wadsworthia, Blautia hansenii, Blautia hydrogenotrophica, Blautia obeum, Blautia sp., Blautia wexlerae, Catenibacterium mitsuokai, Clostridium asparagiforme, Clostridium hylemonae, Clostridium leptum, Tyzzerella nexilis, Clostridium saccharolyticum, Absiella dolichum, Collinsella aerofaciens, Collinsella stercoris, Coprococcus comes, Dialister invisus, Eubacterium rectale, Eubacterium siraeum, Eubacterium ventriosum, Coprococcus eutactus, Holdemanella biformis, Intestinibacter bartlettii, Megasphaera sp., Odoribacter splanchnicus, Parabacteroides merdae, Parabacteroides sp., Granulicatella adiacens, Holdemania filiformis, Hungatella hathewayi, Intestinimonas butyriciproducens, Solobacterium moorei, Mitsuokella multacida, Olsenella uli, Parabacteroides johnsonii, Prevotella buccalis, Prevotella copri, Roseburia inulinivorans, Clostridium sp., Ruminococcus gauvreauii, Ruminococcus lactaris, Ruminococcus torques, Alistipes

putredinis, Alistipes senegalensis, Clostridium spiroforme, Slackia exigua, Bacteroides pectinophilus, Butyrivibrio crossotus, Subdoligranulum variabile, Turicibacter sanguinis, Bifidobacterium breve, Bifidobacterium catenulatum, Butyricimonas virosa, Streptococcus salivarius subsp. thermophilus, Dorea formicigenerans, Bacteroides plebeius, Ruminococcus gnavus, Oscillibacter sp., Clostridium sp., Slackia heliotrinireducens, Desulfovibrio piger, Clostridium methylpentosum, Ethanoligenens harbinense, Marvinbryantia formatexigens, Lactobacillus ruminis, Clostridium bolteae, Clostridium hiranonis, Clostridium scindens, Clostridium sp., Clostridium orbiscindens, Alistipes shahii, and Faecalibacterium prausnitzii.

18. The high complexity defined gut microbial community of any one of claims 1-17, wherein the defined microbial strains are selected from the group consisting of: Acidaminococcus fermentans -- VR4, Acidaminococcus sp. -- D21, Adlercreutzia equolifaciens -- FJC-B9, Akkermansia muciniphila -- Muc [CIP 107961], Alistipes finegoldii -- AHN 2437, Alistipes indistinctus -- JCM 16068, YIT 12060, Alistipes onderdonkii -- WAL 8169, Anaerobutyricum hallii -- VPI B4-27, Anaerofustis stercorihominis -- ATCC BAA-858, CCUG 47767, CIP 108481, WAL 14563, Anaerostipes caccae -- L1-92, Anaerotruncus colihominis -- 277, Bacteroides caccae -- VPI 3452A [CIP 104201T, JCM 9498], Bacteroides cellulosilyticus -- CRE21, CCUG 44979, Bacteroides coprocola -- M16, Bacteroides coprophilus -- CB42, JCM 13818, Bacteroides dorei -- 175, Bacteroides dorei -- 5_1_36/D4, Bacteroides eggerthii -- ATCC 27754, NCTC 11155, Bacteroides finegoldii -- 199, Bacteroides fragilis -- 3_1_12, Bacteroides intestinalis -- 341, Bacteroides ovatus -- NCTC 11153, Bacteroides rodentium -- ST28, CCUG 59334, JCM 16469, Bacteroides thetaiotaomicron -- 1_1_6, Bacteroides fragilis -- 2_1_16, Bacteroides xylanisolvens -- 2_1_22, Parabacteroides distasonis -- 3_1_19, Bacteroides dorea -- 9_1_42FAA, Bacteroides ovatus -- D2, Bacteroides stercoris -- VPI B3-21, ATCC 43183, CIP 104203, JCM 9496, Bacteroides thetaiotaomicron -- VPI 5482 [CIP 104206T, E50, NCTC 10582], Bacteroides uniformis -- ATCC 8492, Bacteroides vulgatus -- NCTC 11154, Bifidobacterium pseudocatenulatum -- B1279, ATCC 27919, Bilophila wadsworthia -- WAL 7959 [Lab 88-130H], Blautia hansenii -- VPI C7-24, Blautia hydrogenotrophica -- S5a33, Blautia obeum -- ATCC 29174, KCTC 15206, VPI B3-21, Blautia sp. -- KLE 1732, Blautia wexlerae -- ATCC BAA-1564, JCM 17041, KCTC 5965, WAL 14507, Catenibacterium mitsuokai -- RCA14-39, CIP 106738, JCM 10609, Clostridium asparagiforme -- N6,

CCUG 48471, Clostridium hylemonae -- TN-271, JCM 10539, Clostridium leptum -- VPI T7-24-1, ATCC 29065, Tyzzerella nexilis DSM 1787, Clostridium saccharolyticum -- WM1, ATCC 35040, NRC 2533, Absiella dolichum DSM 3991, Collinsella aerofaciens -- VPI 1003 [DSM 3979, JCM 10188], Collinsella stercoris -- RCA 55-54, JCM 10641, Coprococcus comes -- VPI CI-38, Dialister invisus -- E7.25, CCUG 47026, Eubacterium rectale -- VPI 0990 [CIP 105953], Eubacterium siraeum -- VPI T9-50-2, ATCC 29066, DSM 3996, Eubacterium ventriosum -- VPI 1013B, Coprococcus eutactus -- VPI C33-22, Holdemanella biformis -- VPI C17-5, ATCC 27806, KCTC 5969, Intestinibacter bartlettii -- WAL 16138, ATCC BAA-827, CCUG 48940, Megasphaera sp. -- Sanger 24, Sanger_24, Odoribacter splanchnicus -- 1651/6, ATCC 29572, CCUG 21054, CIP 104287, LMG 8202, NCTC 10825, Parabacteroides distasonis -- NCTC 11152, Parabacteroides merdae -- VPI T4-1, ATCC 43184, CCUG 38734, CIP 104202, JCM 9497, Parabacteroides sp. -- D13, Granulicatella adiacens -- GaD [CIP 103243, DSM 9848], Holdemania filiformis -- VPI J1-31B-1, ATCC 51649, Hungatella hathewayi -- 1313, CCUG 43506, CIP 109440, MTCC 10951, Intestinimonas butyriciproducens -- SRB-521-5-l, CCUG 63529, Solobacterium moorei -- RCA59-74, CIP 106864, JCM 10645, Mitsuokella multacida -- A 405-1, ATCC 27723, NCTC 10934, Olsenella uli -- D76D-27C, ATCC 49627, CIP 109912, Parabacteroides johnsonii -- M-165, CIP 109537, JCM 13406, Prevotella buccalis -- HS4, ATCC 35310, NCDO 2354, Prevotella copri -- CB7, JCM 13464, Roseburia inulinivorans -- A2-194, CIP 109405, JCM 17584, NCIMB 14030, Clostridium sp. -- VPI C48-50 (unassigned Clostridiales), Ruminococcus gauvreauii -- CCRI-16110, CCUG 54292, JCM 14987, NML 060141, Ruminococcus lactaris -- VPI X6-29, Ruminococcus torques -- VPI B2-51, Alistipes putredinis -- CCUG 45780, CIP 104286, ATCC 29800, Carlier 10203, VPI 3293, Alistipes senegalensis -- CSUR P150, JCM 32779, JC50, Clostridium spiroforme -- VPI C28-23-1A, ATCC 29900, NCTC 11211, Slackia exigua -- S-7, ATCC 700122, JCM 11022, KCTC 5966, Bacteroides pectinophilus -- N3, Butyrivibrio crossotus -- T9-40A, ATCC 29175, Subdoligranulum variabile -- BI-114, CCUG 47106, Turicibacter sanguinis -- MOL361, NCCB 100008, Bifidobacterium breve -- S1, ATCC 15700, NCTC 11815, Bifidobacterium catenulatum -- B669, ATCC 27539, CECT 7362, CIP 104175, DSM 20103, Butyricimonas virosa -- MT12, CCUG 56611, JCM 15149, Streptococcus salivarius subsp. thermophilus -- LMD-9, Dorea formicigenerans -- VPI C8-13 [JCM 9500], Bacteroides plebeius -- M12, Ruminococcus gnavus -- VPI C7-9, Oscillibacter sp. -- KLE 1728, Clostridium sp. -- M62/1, Slackia heliotrinireducens --

RHS 1, ATCC 29202, NCTC 11029, Desulfovibrio piger -- VPI C3-23 [DSM 749], Clostridium methylpentosum -- R2, ATCC 43829, Ethanoligenens harbinense -- YUAN-3, CGMCC 1.5033, JCM 12961, Marvinbryantia formatexigens -- I-52, CCUG 46960, Lactobacillus ruminis -- E 194e, Clostridium bolteae -- WAL 16351, [CCUG 46953], ATCC BAA-613, Song et al. 2003, Clostridium hiranonis -- TO-931, JCM 10541, KCTC 15199, Clostridium scindens -- VPI 13733, ATCC 35704, 19, Bacteroides xylanisolvens -- XB1A, CCUG 53782, Clostridium sp. -- L2-50, Clostridium orbiscindens -- 1_3_50AFAA, Alistipes shahii -- WAL 8301, and Faecalibacterium prausnitzii -- A2-165, JCM 31915.

19. The high complexity defined gut microbial community of any one of claims 1-18, wherein the defined gut microbial community comprises Acidaminococcus, Adlercreutzia, Akkermansia, Anaerostipes, Anaerotruncus, Bacteroides, Bifidobacterium, Bilophila, Blautia, Butyrivibrio, Clostridium, Collinsella, Coprococcus, Desulfovibrio, Eggerthella, Eubacterium, Faecalibacterium, Marvinbryantia, Mitsuokella, Odoribacter, Parabacteroides, Roseburia, Ruminococcus, Slackia, and Solobacterium.

20. The high complexity defined gut microbial community of any one of claims 1-19, wherein the defined gut microbial community comprises Acidaminococcus fermentans, Adlercreutzia equolifaciens, Akkermansia muciniphila, Anaerostipes caccae, Anaerotruncus colihominis, Bacteroides caccae, Bacteroides cellulosilyticus, Bacteroides dorei, Bacteroides eggerthii, Bacteroides fragilis, Bacteroides intestinalis, Bacteroides ovatus, Bacteroides pectinophilus, Bacteroides plebeius, Bacteroides stercoris, Bacteroides thetaiotaomicron, Bacteroides uniformis, Bacteroides vulgatus, Bifidobacterium breve, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bilophila wadsworthia, Blautia hansenii, Blautia hydrogenotrophica, Butyrivibrio crossotus, Clostridium asparagiforme, Clostridium hiranonis, Clostridium hylemonae, Clostridium leptum, Clostridium orbiscindens, Clostridium saccharolyticum, Clostridium scindens, Collinsella aerofaciens, Coprococcus comes, Desulfovibrio piger, Eggerthella lenta, Eubacterium rectale, Eubacterium siraeum, Eubacterium ventriosum, Faecalibacterium prausnitzii, Marvinbryantia formatexigens, Mitsuokella multacida, Odoribacter splanchnicus, Parabacteroides distasonis, Parabacteroides johnsonii, Parabacteroides merdae, Roseburia inulinivorans, Ruminococcus gnavus, Ruminococcus lactaris, Ruminococcus torques, Slackia exigua, and Solobacterium moorei.

21. The high complexity defined gut microbial community of any one of claims 1-20, wherein the defined gut microbial community comprises Acidaminococcus fermentans -- VR4,Acidaminococcus sp. -- D21,Adlercreutzia equolifaciens -- FJC-B9,Akkermansia muciniphila -- Muc [CIP 107961],Alistipes finegoldii -- AHN 2437,Alistipes indistinctus -- JCM 16068, YIT 12060,Alistipes onderdonkii -- WAL 8169,Anaerobutyricum hallii -- VPI B4-27,Anaerofustis stercorihominis -- ATCC BAA-858, CCUG 47767, CIP 108481, WAL 14563,Anaerostipes caccae -- L1-92,Anaerotruncus colihominis -- 277,Bacteroides caccae -- VPI 3452A [CIP 104201T, JCM 9498],Bacteroides cellulosilyticus -- CRE21, CCUG 44979,Bacteroides coprocola -- M16,Bacteroides coprophilus -- CB42, JCM 13818,Bacteroides dorei -- 175,Bacteroides dorei -- 5_1_36/D4,Bacteroides eggerthii -- ATCC 27754, NCTC 11155,Bacteroides finegoldii -- 199,Bacteroides fragilis -- 3_1_12,Bacteroides intestinalis -- 341,Bacteroides ovatus -- NCTC 11153,Bacteroides rodentium -- ST28, CCUG 59334, JCM 16469,Bacteroides thetaiotaomicron -- 1_1_6,Bacteroides fragilis -- 2_1_16,Bacteroides xylanisolvens -- 2_1_22,Parabacteroides distasonis -- 3_1_19,Bacteroides dorea -- 9_1_42FAA,Bacteroides ovatus -- D2,Bacteroides stercoris -- VPI B3-21, ATCC 43183, CIP 104203, JCM 9496,Bacteroides thetaiotaomicron -- VPI 5482 [CIP 104206T, E50, NCTC 10582],Bacteroides uniformis -- ATCC 8492,Bacteroides vulgatus -- NCTC 11154,Bifidobacterium pseudocatenulatum -- B1279, ATCC 27919,Bilophila wadsworthia -- WAL 7959 [Lab 88-130H],Blautia hansenii -- VPI C7-24,Blautia hydrogenotrophica -- S5a33,Blautia obeum -- ATCC 29174, KCTC 15206, VPI B3-21,Blautia sp. -- KLE 1732,Blautia wexlerae -- ATCC BAA-1564, JCM 17041, KCTC 5965, WAL 14507,Catenibacterium mitsuokai -- RCA14-39, CIP 106738, JCM 10609,Clostridium asparagiforme -- N6, CCUG 48471,Clostridium hylemonae -- TN-271, JCM 10539,Clostridium leptum -- VPI T7-24-1, ATCC 29065,Tyzzerella nexilis DSM 1787,Clostridium saccharolyticum -- WM1, ATCC 35040, NRC 2533,Absiella dolichum DSM 3991,Collinsella aerofaciens -- VPI 1003 [DSM 3979, JCM 10188],Collinsella stercoris -- RCA 55-54, JCM 10641,Coprococcus comes -- VPI CI-38,Dialister invisus -- E7.25, CCUG 47026,Eubacterium rectale -- VPI 0990 [CIP 105953],Eubacterium siraeum -- VPI T9-50-2, ATCC 29066, DSM 3996,Eubacterium ventriosum -- VPI 1013B,Coprococcus eutactus -- VPI C33-22,Holdemanella biformis -- VPI C17-5, ATCC 27806, KCTC 5969,Intestinibacter bartlettii -- WAL 16138, ATCC BAA-827, CCUG 48940,Megasphaera sp. -- Sanger 24, Sanger_24,Odoribacter splanchnicus -- 1651/6, ATCC 29572, CCUG 21054, CIP 104287, LMG 8202, NCTC

10825,Parabacteroides distasonis -- NCTC 11152,Parabacteroides merdae -- VPI T4-1, ATCC 43184, CCUG 38734, CIP 104202, JCM 9497,Parabacteroides sp. -- D13,Granulicatella adiacens -- GaD [CIP 103243, DSM 9848],Holdemania filiformis -- VPI J1-31B-1, ATCC 51649,Hungatella hathewayi -- 1313, CCUG 43506, CIP 109440, MTCC 10951,Intestinimonas butyriciproducens -- SRB-521-5-l, CCUG 63529,Solobacterium moorei -- RCA59-74, CIP 106864, JCM 10645,Mitsuokella multacida -- A 405-1, ATCC 27723, NCTC 10934,Olsenella uli -- D76D-27C, ATCC 49627, CIP 109912,Parabacteroides johnsonii -- M-165, CIP 109537, JCM 13406,Prevotella buccalis -- HS4, ATCC 35310, NCDO 2354,Prevotella copri -- CB7, JCM 13464,Roseburia inulinivorans -- A2-194, CIP 109405, JCM 17584, NCIMB 14030,Clostridium sp. -- VPI C48-50 (unassigned Clostridiales),Ruminococcus gauvreauii -- CCRI-16110, CCUG 54292, JCM 14987, NML 060141,Ruminococcus lactaris -- VPI X6-29,Ruminococcus torques -- VPI B2-51,Alistipes putredinis -- CCUG 45780, CIP 104286, ATCC 29800, Carlier 10203, VPI 3293,Alistipes senegalensis -- CSUR P150, JCM 32779, JC50,Clostridium spiroforme -- VPI C28-23-1A, ATCC 29900, NCTC 11211,Slackia exigua -- S-7, ATCC 700122, JCM 11022, KCTC 5966,Bacteroides pectinophilus -- N3,Butyrivibrio crossotus -- T9-40A, ATCC 29175,Subdoligranulum variabile -- BI-114, CCUG 47106,Turicibacter sanguinis -- MOL361, NCCB 100008,Bifidobacterium breve -- S1, ATCC 15700, NCTC 11815,Bifidobacterium catenulatum -- B669, ATCC 27539, CECT 7362, CIP 104175, DSM 20103,Butyricimonas virosa -- MT12, CCUG 56611, JCM 15149,Streptococcus salivarius subsp. thermophilus -- LMD-9,Dorea formicigenerans -- VPI C8-13 [JCM 9500],Bacteroides plebeius -- M12,Ruminococcus gnavus -- VPI C7-9,Oscillibacter sp. -- KLE 1728,Clostridium sp. -- M62/1,Slackia heliotrinireducens -- RHS 1, ATCC 29202, NCTC 11029,Desulfovibrio piger -- VPI C3-23 [DSM 749],Clostridium methylpentosum -- R2, ATCC 43829,Ethanoligenens harbinense -- YUAN-3, CGMCC 1.5033, JCM 12961,Marvinbryantia formatexigens -- I-52, CCUG 46960,Lactobacillus ruminis -- E 194e,Clostridium bolteae -- WAL 16351, [CCUG 46953], ATCC BAA-613, Song et al. 2003,Clostridium hiranonis -- TO-931, JCM 10541, KCTC 15199,Clostridium scindens -- VPI 13733, ATCC 35704, 19,Bacteroides xylanisolvens -- XB1A, CCUG 53782,Clostridium sp. -- L2-50,Clostridium orbiscindens -- 1_3_50AFAA,Alistipes shahii -- WAL 8301, and Faecalibacterium prausnitzii -- A2-165, JCM 31915.

22. The high-complexity defined gut microbial community according to any one of claims 1-21 wherein community stability is characterized by up to 10% of the defined microbial strains dropping out following the microbial challenge.

23. The high-complexity defined gut microbial community according to any one of claims 1-22, wherein community stability is characterized by the appearance of up to 10% of new strains contributed from the human fecal community appearing following the microbial challenge.

24. The high-complexity defined gut microbial community according to claim 1, wherein at least 50% of the defined microbial strains are detectable following the microbial challenge.

25. The high-complexity defined gut microbial community according to claim 24, wherein at least 60% of the defined microbial strains are detectable following the microbial challenge.

26. The high-complexity defined gut microbial community according to claim 25, wherein at least 70% of the defined microbial strains are detectable following the microbial challenge.

27. The high-complexity defined gut microbial community according to claim 26, wherein at least 80% of the defined microbial strains are detectable following the microbial challenge.

28. The high-complexity defined gut microbial community according to claim 27, wherein at least 90% of the defined microbial strains are detectable following the microbial challenge.

29. The high-complexity defined gut microbial community according to claim 28, wherein at least 95% of the defined microbial strains are detectable following the microbial challenge.

30. The high-complexity defined gut microbial community according to claim 29, wherein at least 99% of the defined microbial strains are detectable following the microbial challenge.

31. The high-complexity defined gut microbial community according to any one of claims 1-30, wherein community stability is characterized by metagenomic analysis of a fecal sample obtained from the mouse following the microbial challenge.

32. The high-complexity defined gut microbial community of claim 31, wherein metagenomic analysis is selected from whole genome sequencing, ribosomal gene sequencing, or ribosomal RNA sequencing.

33. The high-complexity defined gut microbial community of claim 32, wherein whole genome sequencing is whole genome shotgun sequencing.

34. The high-complexity defined gut microbial community according to any one of claims 1-33, wherein the defined gut microbial community comprises between 100 and 200 defined microbial strains.

35. The high-complexity defined gut microbial community according to claim 34, wherein the defined gut microbial community comprises between 100 and 150 defined microbial strains.

36. The high-complexity defined gut microbial community according to any one of claims 1-35, wherein each defined microbial strain is molecularly identified.

37. The high-complexity defined gut microbial community according to claim 36, wherein the molecular identification comprises identification of a nucleic acid sequence that uniquely identifies each of the defined microbial strains.

38. The high-complexity defined gut microbial community according to claim 37 wherein the nucleic acid sequence comprises a 16S rRNA sequence.

39. The high-complexity defined gut microbial community according to claim 37, wherein the nucleic acid sequence comprises a whole genomic sequence.

40. The high-complexity defined gut microbial community according to claim 36, wherein the molecular identification comprises Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry.

41. A method of treating an animal having a dysbiosis or pathological condition comprising administering a high-complexity defined gut microbial community according to any one of claims 1-40.

42. The method of claim 41, wherein the animal is a mammal.

43. The method of claim 42, wherein the animal is a human.

44. The method according to any one of claims 41-44, wherein the high-complexity defined gut microbial community is administered via a route selected from the group consisting of oral, rectal, fecal (by enema), and naso/oro-gastric gavage.

45. A method of making a high-complexity defined gut microbial community according to any one of claims 1-40, wherein each of the plurality of defined microbial strains is individually cultured then combined to form the defined gut microbial community.

46. A method of making a high-complexity defined gut microbial community according to any one of claims 1-40 wherein all of the plurality of defined microbial strains are cultured together to form the defined gut microbial community.

47. A method of making a high-complexity defined gut microbial community according to any one of claims 1-40, wherein one or more of the plurality of defined microbial strains is individually cultured and two or more of the defined microbial strains are cultured together, and wherein the individually cultured defined microbial strains and the co-cultured defined microbial strains are combined together to form the defined gut microbial community.

48. A formulation comprising the high-complexity defined gut microbial community according to any one of claims 1-40 and a pharmaceutically acceptable carrier or excipient.

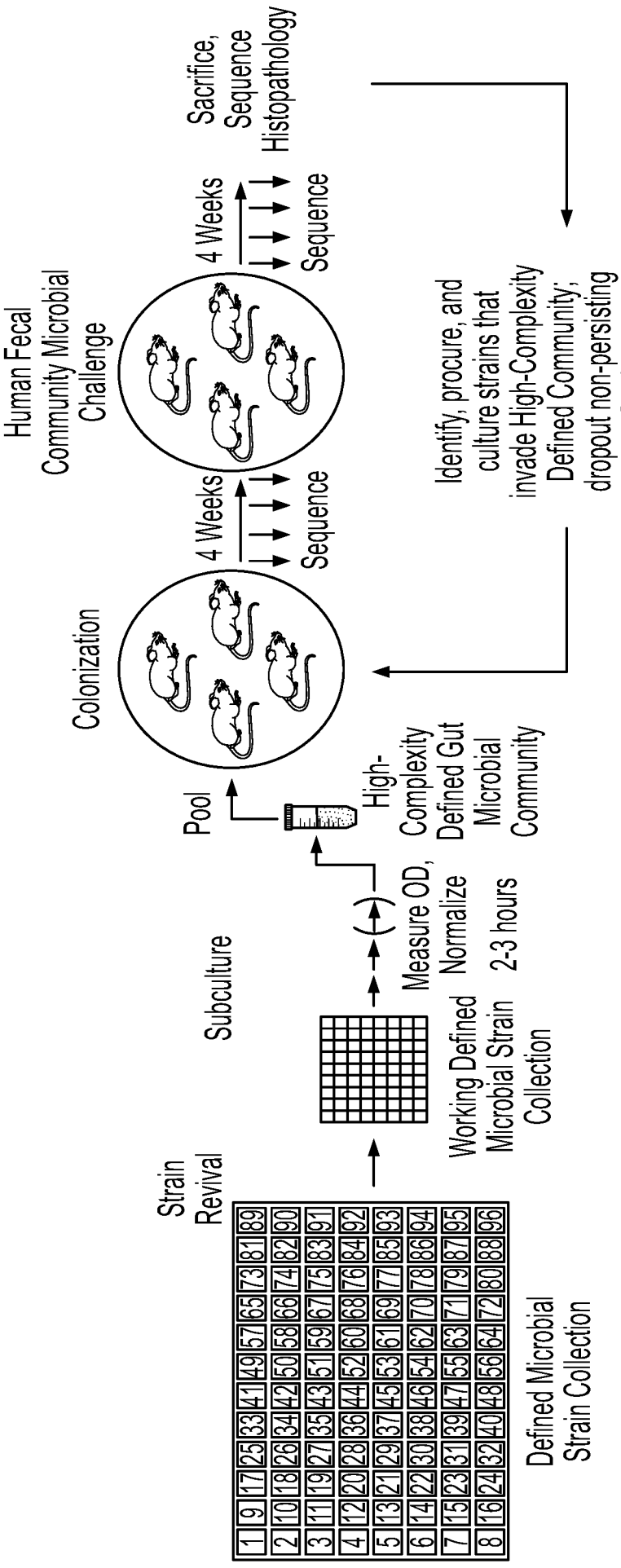
Strain Revival
Defined Microbial Strain Collection
Subculture
Working Defined Microbial Strain Collection
Measure OD, Normalize
2-3 hours
Pool
High-Complexity Defined Gut Microbial Community
Colonization
4 Weeks
Sequence
Human Fecal Community Microbial Challenge
4 Weeks
Sequence
Sacrifice, Sequence Histopathology
Identify, procure, and culture strains that invade High-Complexity Defined Community; dropout non-persisting Strains

FIG. 1

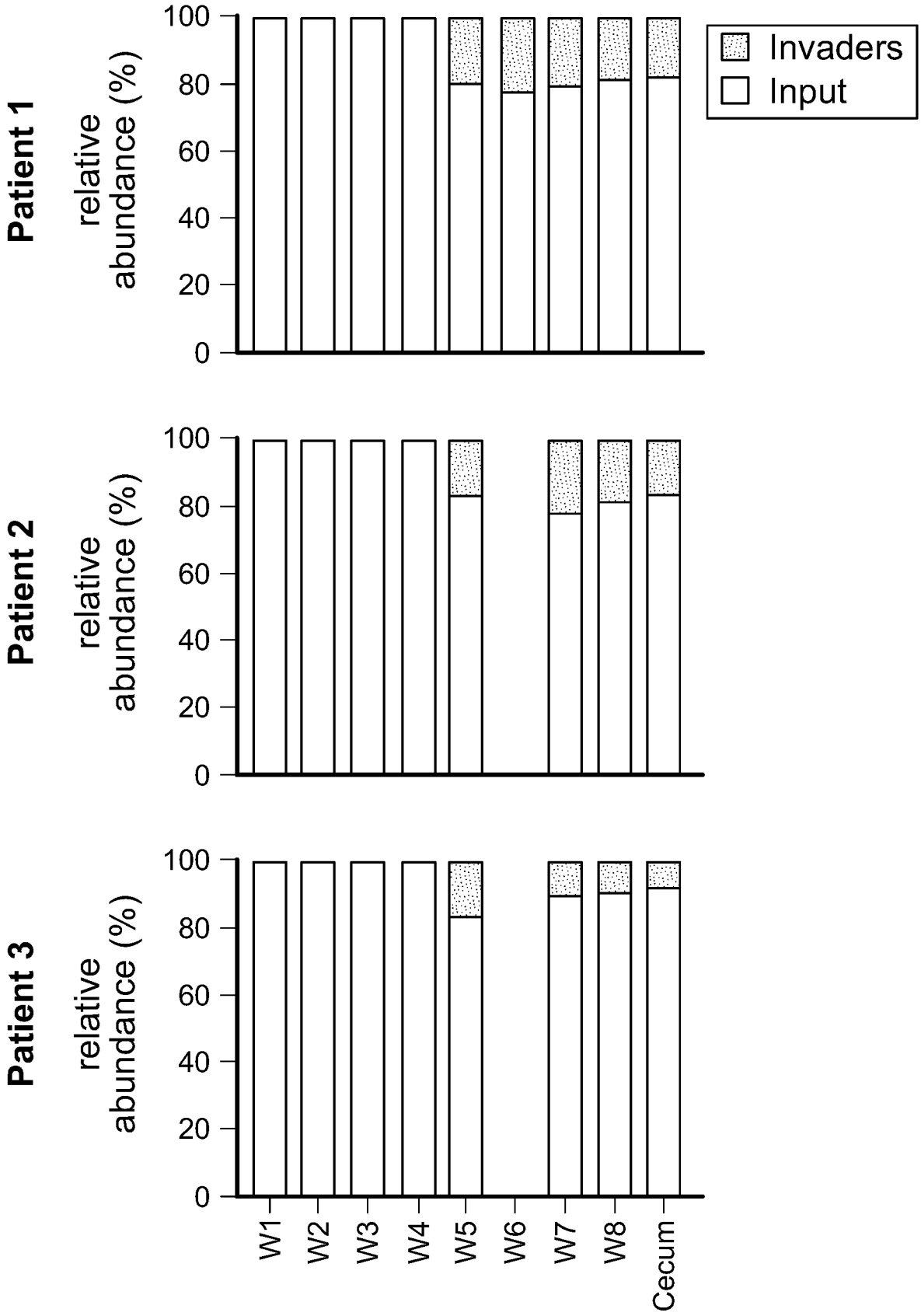
Patient 1
Patient 2
Patient 3
relative abundance (%)
100
80
60
40
20
0
Invaders
Input
W1
W2
W3
W4
W5
W6
W7
W8
Cecum

FIG. 2

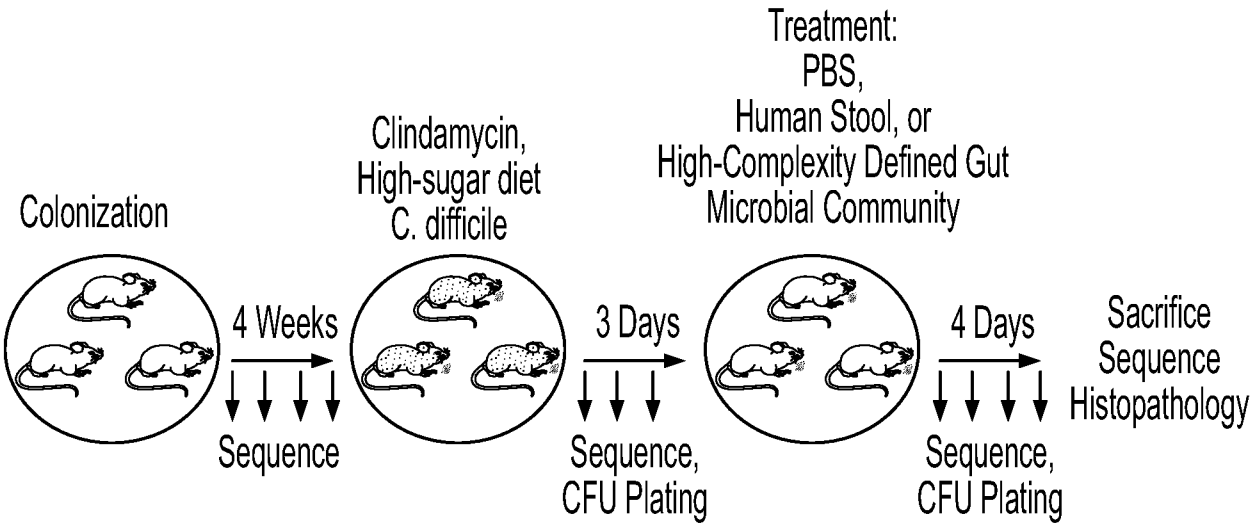
Colonization
4 Weeks
Sequence
Clindamycin,
High-sugar diet
C. difficile
3 Days
Sequence,
CFU Plating
Treatment:
PBS,
Human Stool, or
High-Complexity Defined Gut
Microbial Community
4 Days
Sequence,
CFU Plating
Sacrifice
Sequence
Histopathology

FIG. 3A

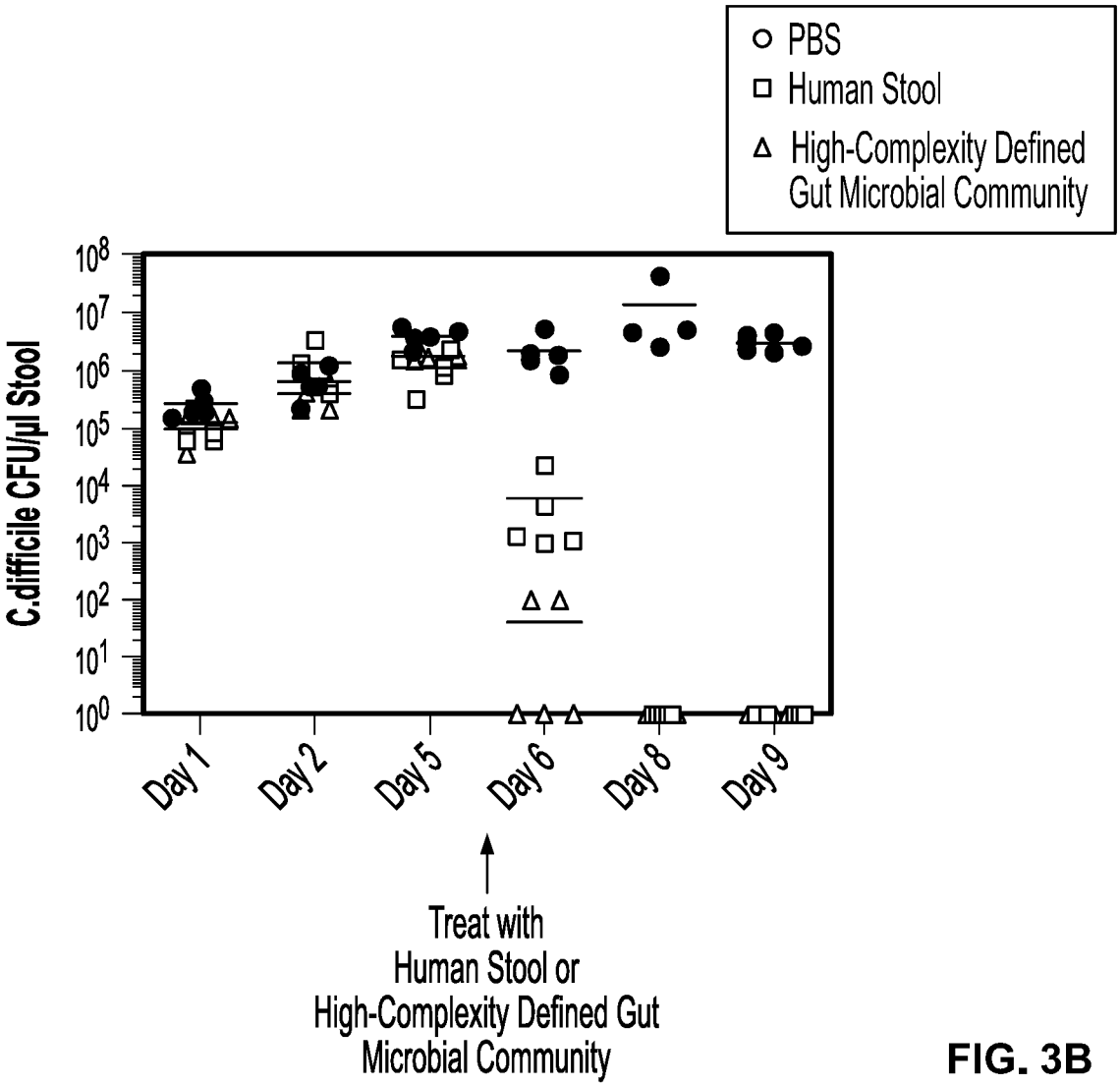
PBS
Human Stool
High-Complexity Defined
Gut Microbial Community
C.difficile CFU/µl Stool
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Day 1
Day 2
Day 5
Day 6
Day 8
Day 9
Treat with
Human Stool or
High-Complexity Defined Gut
Microbial Community

FIG. 3B

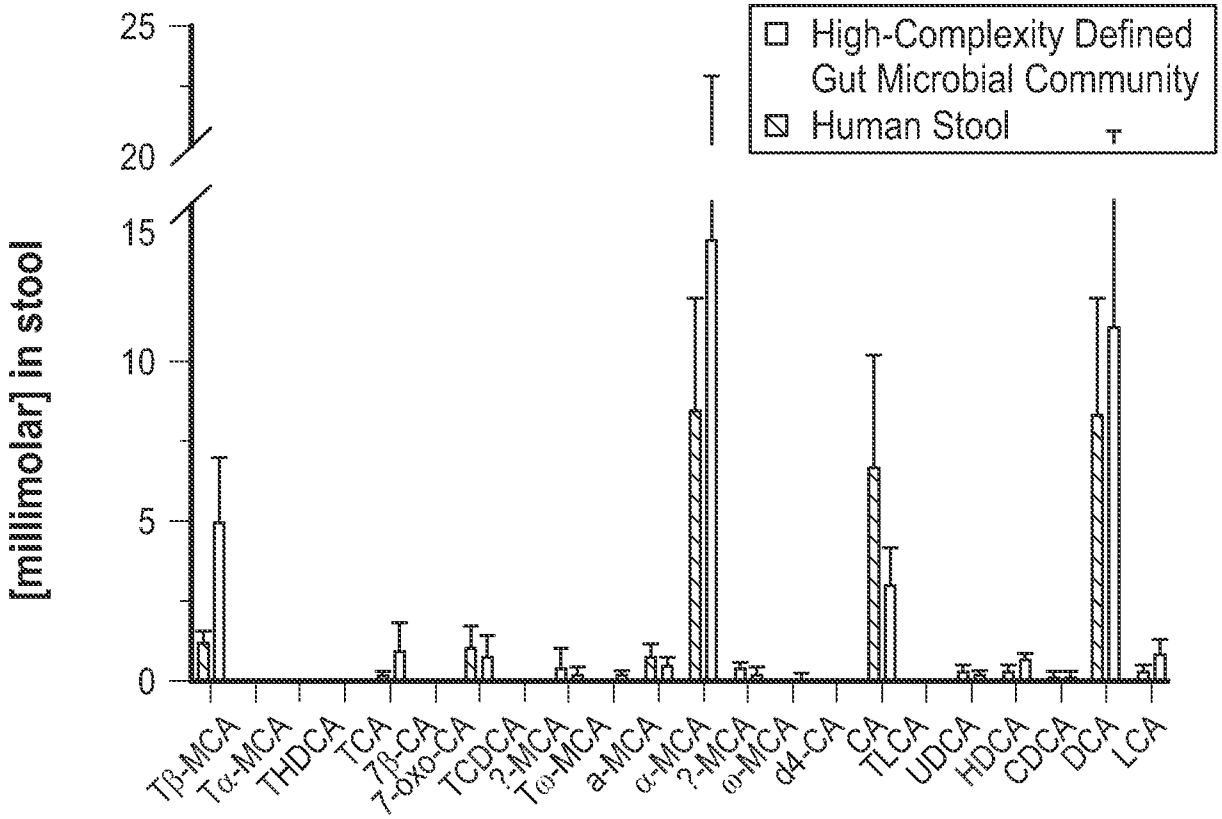
High-Complexity Defined Gut Microbial Community
Human Stool
[millimolar] in stool
25
20
15
10
5
0
Tβ-MCA
Tα-MCA
THDCA
TCA
7β-CA
7-oxo-CA
TCDCA
?-MCA
Tω-MCA
a-MCA
α-MCA
?-MCA
ω-MCA
d4-CA
CA
TLCA
UDCA
HDCA
CDCA
DCA
LCA

FIG. 4A

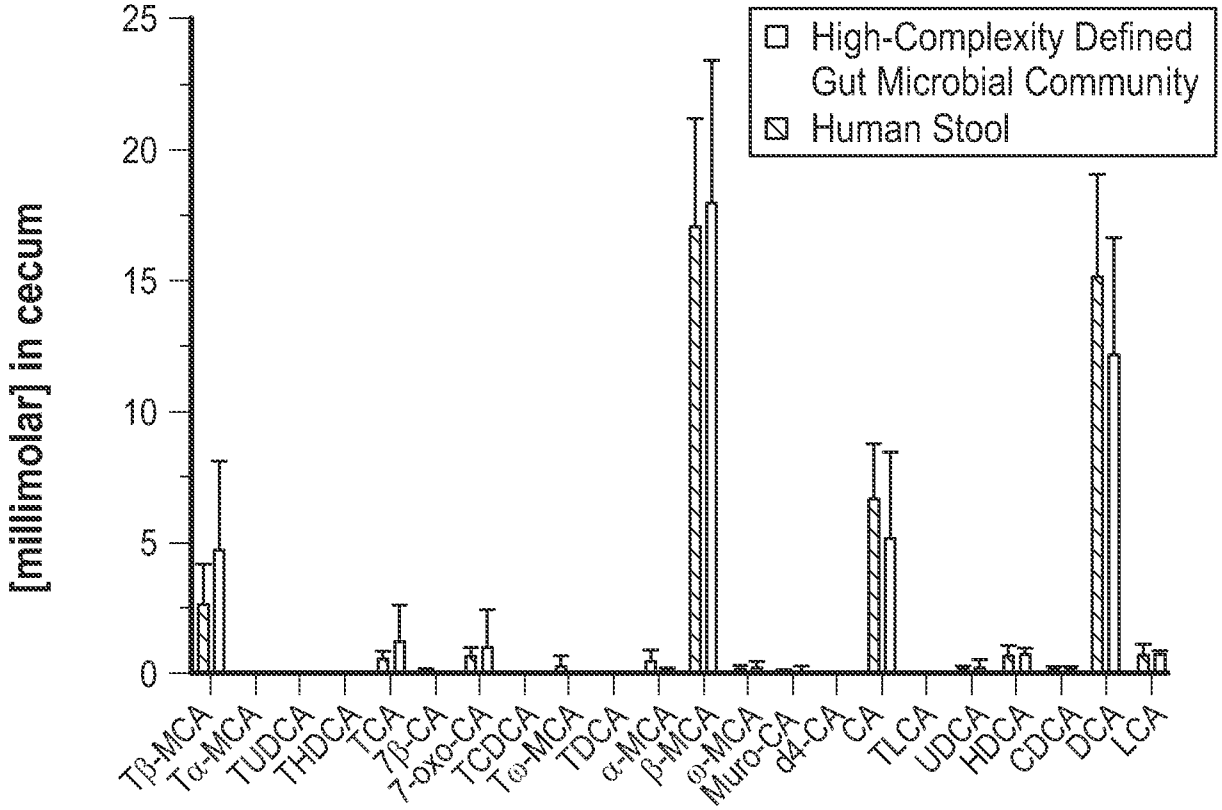
High-Complexity Defined Gut Microbial Community
Human Stool
[millimolar] in cecum
25
20
15
10
5
0
Tβ-MCA
Tα-MCA
TUDCA
THDCA
TCA
7β-CA
7-oxo-CA
TCDCA
Tω-MCA
TDCA
α-MCA
β-MCA
ω-MCA
Muro-CA
d4-CA
CA
TLCA
UDCA
HDCA
CDCA
DCA
LCA

FIG. 4B

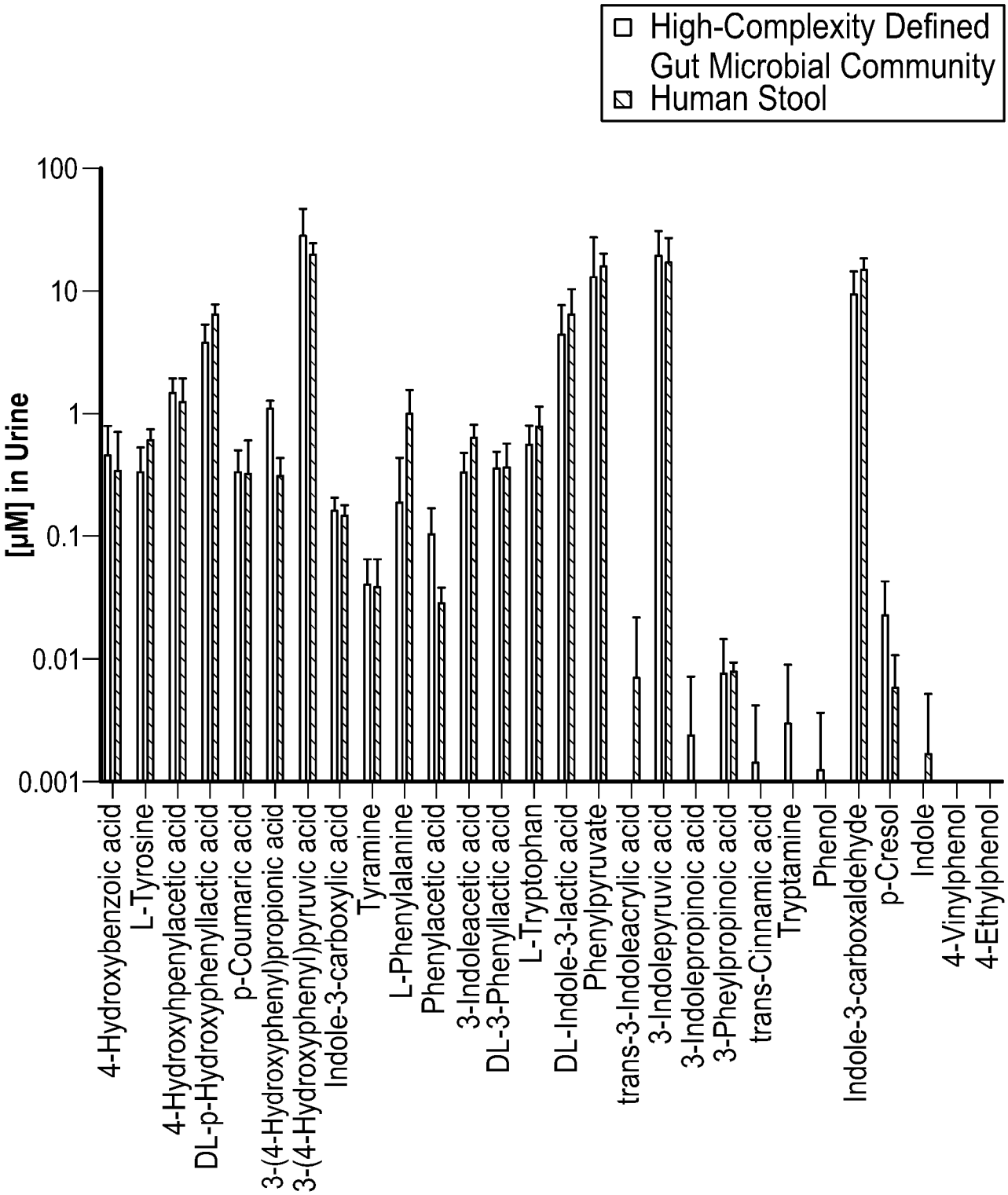
High-Complexity Defined Gut Microbial Community
Human Stool
[µM] in Urine
100
10
1
0.1
0.01
0.001
4-Hydroxybenzoic acid
L-Tyrosine
4-Hydroxyhpenylacetic acid
DL-p-Hydroxyphenyllactic acid
p-Coumaric acid
3-(4-Hydroxyphenyl)propionic acid
3-(4-Hydroxyphenyl)pyruvic acid
Indole-3-carboxylic acid
Tyramine
L-Phenylalanine
Phenylacetic acid
3-Indoleacetic acid
DL-3-Phenyllactic acid
L-Tryptophan
DL-Indole-3-lactic acid
Phenylpyruvate
trans-3-Indoleacrylic acid
3-Indolepyruvic acid
3-Indolepropinoic acid
3-Pheylpropinoic acid
trans-Cinnamic acid
Tryptamine
Phenol
Indole-3-carboxaldehyde
p-Cresol
Indole
4-Vinylphenol
4-Ethylphenol

FIG. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/033762

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A23L 33/00; A23L 33/135; A61K 9/00; A61K 31/702; A61K 35/74; A61K 35/741 (2021.01)
CPC - A23L 33/135; A23L 33/30; A23V 2002/00; A61K 35/74; A61K 35/741; A61K 35/747; A61K 45/06; A61P 1/00; A61P 1/04; A61P 1/12; A61P 1/16; A61P 3/00; A61P 3/04; A61P 3/06; A61P 3/10; C12N 1/20 (2021.05)

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)

see Search History document

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

see Search History document

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

see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2016/0030494 A1 (SERES THERAPEUTICS, INC) 04 February 2016 (04.02.2016) entire document	1-4, 9, 10, 24-30
Y	WO 2015/003001 A1 (THE WASHINGTON UNIVERSITY) 08 January 2015 (08.01.2015) entire document	1-4, 9, 10, 24-30
A	WO 2018/156916 A2 (INTERCEPT PHARMACEUTICALS, INC) 30 August 2018 (30.08.2018) entire document	1-4, 7-10, 24-30
P, A	WO 2020/106999 A1 (CHAN ZUCKERBERG BIOHUB, INC) 28 May 2020 (28.05.2020) entire document	1-4, 7-10, 24-30

☐ Further documents are listed in the continuation of Box C. ☐ 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
"D" document cited by the applicant in the international application
"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
23 July 2021	SEP 09 2021
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Harry Kim Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/033762

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.: 5, 6, 11-23, 31-48
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:

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 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.:

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.