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(54) Title: COMPOSITIONS AND METHODS FOR MODULATING GROWTH OF A GENETICALLY MODIFIED GUT BAC-
TERIAL CELL

(57) Abstract: Compositions and methods are provided for modulating growth of a genetically modified bacterial cell present in a
human organ, for modulating growth of a genetically modified bacterial cell in an organ (e.g., gut), for displacing at least a portion of a
population of bacterial cells in an organ, and for facilitating gut colonization by a genetically modified bacterial cell. Also provided are
genetically modified bacterial cells, e.g., cells that include a heterologous carbohydrate-utilization gene or gene set that provides for
the ability to utilize as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% of bacterial
cells present in a human microbiome.

COMPOSITIONS AND METHODS FOR MODULATING GROWTH OF A GENETICALLY MODIFIED GUT BACTERIAL CELL

CROSS-REFERENCE

5 This application claims the benefit of U.S. Provisional Patent Application No. 62/435,048, filed December 15, 2016, which application is incorporated herein by reference in its entirety.

GOVERNMENT RIGHTS

10 This invention was made with Government support under contracts DK085025 and OD006515 awarded by the National Institutes of Health. The Government has certain rights in the invention.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING PROVIDED AS A TEXT FILE

15 A Sequence Listing is provided herewith as a text file, "STAN-1357WO_SeqList_ST25.txt" created on November 27, 2017 and having a size of 732 KB. The contents of the text file are incorporated by reference herein in their entirety.

INTRODUCTION

20 The gut microbiota is integral to many facets of human biology. Gut bacteria produce hundreds of metabolites that accumulate in human tissue, and the composition of the gut microbiota has been linked to pathogen susceptibility, immune disorders, heart disease, central nervous system diseases, diabetes and obesity. The rules governing gut ecology are
25 largely unknown, and colonization level of an introduced bacterial strain could theoretically be influenced by a wide range of factors including competition for a variety of macro- and micro-nutrients and the avoidance of inhibitory interactions from other bacteria, bacteriophage and the immune system.

 There is a need in the art for compositions and methods that facilitate the ability to
30 colonize the gut of a patient with a desired bacterial strain, e.g., a strain that is predicted to improve the patient's health. Prior to this disclosure, it has not been possible to colonize the gut of a patient with an introduced bacterial strain such that the population of the introduced strain can reliably be maintained at a predictable colonization level, despite variations in diet, host genetics, and composition of the bacteria already present.

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SUMMARY

Compositions and methods are provided for modulating growth of a genetically modified bacterial cell present in a human organ, for modulating growth of a genetically modified bacterial cell in an organ, for displacing at least a portion of a population of bacterial cells in an organ, and for facilitating gut colonization by a genetically modified bacterial cell.

For example, in some cases a subject method includes introducing to a human organ (e.g., gut) a genetically modified bacterial cell capable of controlled entrenchment and/or controlled colonization. In some cases a method of the disclosure includes administering to a human a rare carbohydrate of interest that is utilized as a carbon source by a subject genetically modified bacterial cell present in a human organ (e.g., gut), where less than 50% (e.g., less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.2%, less than 0.1%, or none; e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of other bacterial cells present in the organ utilize the rare carbohydrate of interest as a carbon source. In some cases a method of the disclosure includes introducing a subject genetically modified bacterial cell to an organ (e.g., gut) *in vivo*, wherein the genetically modified bacterial cell utilizes as a carbon source a rare carbohydrate of interest, where less than 50% (e.g., less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.2%, less than 0.1%, or none; e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of other bacterial cells present in the organ utilize the rare carbohydrate of interest as a carbon source. In some cases a method of the disclosure includes introducing to an organ (e.g., gut) a genetically modified bacterial cell capable of controlled entrenchment and/or controlled colonization, and displacing at least a portion of the population of bacterial cells in the organ with the genetically modified bacterial cell.

In some cases a method of the disclosure includes (a) introducing a genetically modified bacterial cell into a gut of an individual, where the genetically modified bacterial cell includes a heterologous carbohydrate-utilization gene or gene set that provides the genetically modified bacterial cell with the ability to use a carbohydrate of interest as a carbon source; and (b) administering the carbohydrate of interest to the individual, thereby providing the genetically modified bacterial cell with the carbon source.

Provided are genetically modified bacterial cells. In some embodiments such a cell utilizes as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% (e.g., less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.2%, less than 0.1%,

or none; e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of bacterial cells present in a human microbiome (e.g., gut microbiome). In some cases, such a cell includes a heterologous carbohydrate-utilization gene or gene set that provides the genetically modified bacterial cell with an ability to utilize as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% (e.g., less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.2%, less than 0.1%, or none; e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of bacterial cells present in a human microbiome (e.g., gut microbiome). In some cases, a genetically modified bacterial cell includes (i) a heterologous therapeutic transgene; and (ii) a carbohydrate-utilization gene or gene set that provides the genetically modified bacterial cell with an ability to utilize as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% (e.g., less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.2%, less than 0.1%, or none; e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of bacterial cells present in a human microbiome (e.g., gut microbiome). In some cases, a genetically modified bacterial cell includes a heterologous carbohydrate-utilization gene or gene set that provides the genetically modified bacterial cell with the ability to use a rare carbohydrate of interest as a carbon source.

Provided are methods of genetically modifying a bacterial cell. In some such cases, a subject method includes (i) providing a bacterial cell; and (ii) genetically modifying the bacterial cell to utilize as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% (e.g., less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.2%, less than 0.1%, or none; e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of bacterial cells present in a human microbiome (e.g., gut microbiome). In some cases, a subject method includes (i) providing a bacterial cell that utilizes as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% (e.g., less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.2%, less than 0.1%, or none; e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of bacterial cells present in a human microbiome (e.g., gut microbiome);

and (ii) genetically modifying the bacterial cell to express one or more therapeutic transgenes.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures.

Figure 1. Glycoside hydrolase representation in the healthy American microbiota. The incidence and mean abundance of glycoside hydrolase families in 87 Human Microbiome Project metagenomes are shown. The glycoside hydrolase family GH86 includes enzymes that can break down either porphyrin or agarose, and is underrepresented in these samples, with an incidence and mean abundance of 66% and 0.00015, respectively. GH32 is associated with utilization of inulin and is more highly represented with an incidence and mean abundance of 100% and 0.0049, respectively.

Figure 2 (panels A-E). The abundance of porphyrin-utilizing *Bacteroides* can be modulated by the addition of porphyrin. **(panel A)** A strain of *Bacteroides ovatus* capable of utilizing porphyrin (NB001) was isolated from the environment. **(panel B)** The porphyrin utilizing strain reaches a higher ($P = 0.02$) and more predictable ($P < 0.01$) abundance in eight different complex communities in culture when porphyrin is present. The average of two replicates for each community is shown. **(panel C)** Porphyrin increased abundance of porphyrin utilizer *in vivo*. Two groups of conventional mice, one fed a standard (std) diet and one fed a polysaccharide deficient (pd) diet, were colonized with porphyrin utilizing *Bacteroides*, strain NB006. Porphyrin was administered in the water, and abundance of both total anaerobic culturable bacteria and NB006 in the feces were monitored. Averages and standard deviation for replicates of four mice are shown. **(panel D)** Conventional mice being fed a standard diet (i.e. lacking porphyrin) were administered a *Bacteroides* strain that cannot utilize porphyrin, NB008. After seven days, these mice were challenged with NB006, a *Bacteroides* strain which can utilize porphyrin. NB006 was cleared from the mice after five days. Abundance of NB008 and NB006 in the feces was monitored by selective plating, averages and standard deviation for replicates of three mice are shown. **(panel E)** Porphyrin allowed for stable colonization of a porphyrin utilizer into a resistant microbiota. Conventional mice were administered NB008, and one week later given porphyrin in the water simultaneous to receiving NB006. Porphyrin was removed from the water and then

re-introduced, while abundances of both strains in the feces were monitored via selective plating. NB006 was able to displace NB008 with the addition of porphyrin, and inhibit it from re-invasion on day 48. Averages and standard deviation for replicates of three mice are shown.

5 **Figure 3.** Knockout of an eight-gene operon in the putative NB001 porphyrin PUL prevents growth on porphyrin. Genes corresponding to homologs of BACPLE_1692-1699 from *B. plebeius* were deleted from NB001. End-point cell density measurements were taken after growth in minimal media supplemented with either .2% glucose (blue) or .8% nori extract (orange).

10 **Figure 4.** Nucleotide alignment between the porphyrin mobile elements in *B. plebeius* (Hehemann et al., Nature 464, 908–912 (2010); Hehemann et al., Proc. Natl. Acad. Sci. U. S. A. 109, 19786–19791 (2012)) and NB001. Three truncations of the putative porphyrin polysaccharide utilization locus (PUL), shown in green, were cloned into a conjugation vector and transferred to a naive strain to assay growth on porphyrin.

15 **Figure 5.** Map of the medium-length porphyrin PUL conjugative plasmid pWD036. The medium-length porphyrin PUL genes were cloned from the NB001 genome and are homologs of BACPLE_1683-1706 from the *B. plebeius* genome (see e.g., Tables 5 and 6).

20 **Figure 6.** The medium-length porphyrin PUL is sufficient to confer growth on porphyrin in *B. vulgatus*. A *B. vulgatus* strain (NB004) containing either the small (pWD037) or medium-length (pWD036) porphyrin PUL were grown in minimal media supplemented with either .2% glucose (blue) or .8% nori extract (orange). End-point cell density measurements are shown. pWD037 contains homologs of BACPLE_1688-1699. pWD036 contains homologs of BACPLE_1683-1706 (see, e.g., Tables 5 and 6).

25 **Figure 7 (panels A-D).** Niche availability varied by microbiota and could be modulated by addition of a privileged nutrient. **(panel A)**, Schematic of experimental design. Groups of mice with three different gut communities, mouse (RF), or human (Hum-1, Hum-2), were colonized with NB001. NB001 was tracked in feces for seven days, and mice were switched to specialized polysaccharide chow containing either inulin (in.) or porphyrin (por.). **(panel B)**, Density of NB001 in feces in the three gut communities over the course of seven days (n=10) Kruskal-Wallis test, p<0.0001. **(panel C)**, Density of NB001 in feces prior to and upon addition of inulin in the diet (brackets/dotted lines, RF n=4, Hum-1 n=5) Kruskal-Wallis test, p=0.03. **(panel D)**, Density in NB001 in feces prior to and upon addition of nori extract in the diet (brackets/dotted lines, RF n=5, Hum-1 n=7, Hum-2 n=6). Kruskal-Wallis test, p=0.19. Error bars indicate s.e.m.

35 **Figure 8 (panels A-F).** Access to a privileged nutrient mediated population size and overcame isogenic self-exclusion. Density of NB001 (PUL+), NB001 lacking the ability to utilize porphyrin (PUL-), and total culturable anaerobes (Total) in feces of conventional mice.

Periods of administration of porphyrin extract in drinking water indicated by brackets/dotted lines (panels a-c: 1% w/v, panels d-f: 0.1% w/v). **(panel A)**, Mice colonized with PUL+ and fed a MAC-deficient diet or **(panel B)**, MAC-rich diet demonstrated toggling of strain abundance upon administration of porphyrin extract. **(panel C)**, Mice colonized with PUL- and fed a MAC-rich diet showed no change in strain abundance with administration of porphyrin extract. **(panels D-F)** Mice were colonized with PUL- for 6 days, and challenged with PUL+ on day 6. **(panel D)**, PUL+ is excluded by PUL- in the absence of porphyrin extract. **(panel E)**, PUL+ displaces PUL- with access to porphyrin for five days. **(panel F)**, PUL+ and PUL- stably co-exist after a three day pulse of porphyrin extract. Error bars indicate standard deviation, n=4 for all experiments.

Figure 9 (panels A-E). Control over population size was engineered, and was highly tunable. **(panel A)**, Schematic of porphyrin PUL from NB001 aligned to the previously published *B. plebeius* PUL. Shown below are the different minimal PULs (Long, Medium, and Short) designed and tested for ability to confer growth on porphyrin extract. The eight gene region deleted from NB001 PUL- is shown in gray ("Knockout"). **(panel B)**, Growth curves for natural and engineered strains on porphyrin extract as sole carbon source. **(panel C)**, *B. thetaiotaomicron* harboring the medium length PUL colonized in conventional mice demonstrates toggling upon administration of 1% w/v porphyrin extract in the drinking water (brackets/dotted lines, n=4). Error bars indicate standard deviation. **(panel D)**, NB001 PUL+ colonized in conventional mice demonstrates tunable response to porphyrin extract in the drinking water (brackets/dotted lines, n=4 per group). Error bars indicate standard deviation. **(panel E)**, NB001 expressing GFP colonized in conventional mice given 0.01% porphyrin extract (left) or 1% porphyrin extract (right). Host epithelium visualized by DAPI (nuclei, blue), epithelial border visualized by phalloidin (F-actin, white), background microbiota by DAPI segmented from host epithelium (bacteria, magenta), NB001 by endogenous GFP (bacteria, green). Scale bar represents 20 μ m.

Figure 10 (panels A-B). Three model background communities of gut microbes were distinct from each other. **(panel A)**, Principal Coordinates Analysis of weighted UniFrac distance for 16S rDNA amplicons from feces from the three background community groups from Figure 7 before diet switch, conventional (RF), or humanized (Hum-1 and Hum-2), n=10. **(panel B)**, Comparison of weighted UniFrac distances within each group (Intra) or across groups (AxB, AxC, BxC). One- way ANOVA, p<0.0001.

Figure 11 (panels A-C). NB001 can utilize both inulin and porphyrin extract as sole carbon sources for growth. NB001 demonstrates growth in minimal media with only one specified carbon source, either **(panel A)**, glucose (doubling time = 157 minutes), **(panel B)**,

inulin (doubling time = 127 minutes), or **(panel C)**, porphyran extract (doubling time = 98 minutes).

Figure 12 (panels A-B). Porphyran extract did not significantly impact the gut microbiota in the absence of a known utilizer. Weighted UniFrac analysis was performed on fecal 16S data for conventional mice colonized with a porphyran utilization knockout (Figure 8c) before addition of porphyran extract (Pre, n=8) or after (Post, n=9). **(panel A)**, Principal Coordinates Analysis **(panel B)**, Unpaired two-tailed t-test, p=0.25

Figure 13 (panels A-B). Primary colonizer displacement was robust and contingent upon access to porphyran. **(panel A)**, Conventional mice (n=7) colonized with NB001 (PUL- 1) containing an eight-gene deletion abrogating its ability to utilize porphyran (Figure 9b) demonstrate resistance to subsequent challenge with an isogenic knockout strain (PUL- 2) in the presence of 1% porphyran extract in the drinking water. Notably, conventionally raised mice were permissive to colonization by this strain and other species of *Bacteroides* tested (*B. thetaiotaomicron*, *B. fragilis*, *B. uniformis*, *B. vulgatus*, stable colonization range of 8×10^5 – 3×10^8 c.f.u. per ml feces), which differs from reports of tests on other conventionally raised mice, potentially reflecting inter-colony microbiota differences. **(panel B)**, Mice from Figure 8e were challenged with the originally colonizing porphyran utilization knockout (PUL-) that was displaced by the utilizer (PUL+) and demonstrated colonization resistance to the previously displaced knockout strain.

Figure 14 (panels A-C). Data showing that access to porphyran allowed for crypt invasion and stable maintenance in the presence of an exclusionary primary colonizing strain.

DETAILED DESCRIPTION

As summarized above, compositions and methods are provided for modulating growth of a genetically modified bacterial cell present in a human organ, for modulating growth of a genetically modified bacterial cell in an organ (e.g., gut), for displacing at least a portion of a population of bacterial cells in an organ, and for facilitating gut colonization by a genetically modified bacterial cell. Also provided are genetically modified bacterial cells, e.g., cells that include a heterologous carbohydrate-utilization gene or gene set that provides for the ability to utilize as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% of bacterial cells present in a human microbiome.

Before the present methods and compositions are described, it is to be understood that this invention is not limited to a particular method or composition described, and as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose

of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, some potential and preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. It is understood that the present disclosure supercedes any disclosure of an incorporated publication to the extent there is a contradiction.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present invention. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells (e.g., a population of such cells) and reference to "the protein" includes reference to one or more proteins and equivalents thereof, e.g. polypeptides, known to those skilled in the art, and so forth.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

METHODS AND COMPOSITIONS

Provided are methods and compositions for modulating growth of a genetically modified bacterial cell present in an organ (e.g., human organ) of an individual. Modulation of growth can be achieved by administering an energy and/or carbon source (e.g., a nutrient, a carbohydrate, and the like) of interest to the individual, thereby providing the genetically modified bacterial cell with the energy and/or carbon source. The genetically modified bacterial cell can include a gene or gene set (e.g., a carbohydrate-utilization gene or gene set, e.g., in some cases a heterologous gene or gene set) that provides the genetically modified bacterial cell with the ability to use the energy and/or carbon source. As an example, in some cases a subject genetically modified bacterial cell includes a heterologous gene or gene set that provides the genetically modified bacterial cell with the ability to use a carbohydrate of interest as a carbon source.

In some cases a subject method includes a step of introducing the genetically modified bacterial into the organ (e.g., into a gut of an individual) prior to the modulating growth step. Thus, in some embodiments a genetically modified bacterial cell is introduced into an individual (e.g., into the individual's gut). The individual can be any mammalian species, e.g. rodent (e.g., mouse, rat), ungulate, cow, pig, sheep, camel, rabbit, horse, dog, cat, primate, non-human primate, human, etc. Thus, in some cases the individual is human. The individual may be a neonate, a juvenile, or an adult. In some cases, the introduction is by oral administration. Any convenient type of oral administration can be used. For example, oral administration can include delivery via eating (e.g., incorporated into food), drinking (e.g., incorporated into a solution such as drinking water), oral gavage (e.g., using a stomach tube), aerosol spray, tablets, capsules, pills, powders, and the like. In some embodiments, a genetically modified bacterial cell is introduced into an individual (e.g., into the individual's gut) by delivery into the individual's colon. Any convenient number of genetically modified cells can be introduced. For example, in some cases 10^3 or more cells (e.g., 10^4 or more, 10^5 or more, 10^6 or more, 10^7 or more, 10^8 or more cells, 10^9 or more, or 10^{10} or more) cells are introduced. In some cases 10^{11} or more cells are introduced. In some cases, between 10^7 and 10^{13} cells are introduced (e.g., between 10^8 - 10^{12} , 10^9 - 10^{12} , or 10^{10} - 10^{12} cells).

i. Growth control

Provided are methods for modulating growth of a genetically modified bacterial cell present in an organ (e.g., a gut) of an individual (e.g., a human). Growth can be controlled by providing (e.g., administering to the individual) a carbohydrate that can be utilized by the genetically modified bacterial cell as a carbon source. For example, growth can be controlled by adjusting the amount of carbohydrate provided and/or frequency with which the

carbohydrate is provided.

In some cases, a privileged niche is created by using a carbon source (e.g., carbohydrate) that is uncommon in the diet of the individual and is either rarely or not consumed by the gut bacteria in the population of interest (e.g., human population of interest).

5 The term 'privileged niche' is used herein to refer to a situation in which the genetically modified bacteria have 'privileged' access to a resource (e.g., a carbon source), which thereby provides them with a growth advantage (at least with respect to that resource). The use of a carbon source (e.g., a carbohydrate such as porphyran) to create a privileged niche for an introduced bacteria allows the bacteria to establish a population (e.g., colonize, entrench, etc.)

10 in the target organ (e.g., gut). Moreover, because the introduced bacteria has privileged access, the amount of the carbon source (e.g., carbohydrate) that is made available to the introduced bacteria can be manipulated (e.g., increased, decreased, or maintained) to control the growth of the population of introduced bacteria (e.g., in some cases without perturbing resident bacterial populations). In some embodiments, this results in a situation in which the

15 abundance of introduced bacteria at any given time is predictable, e.g., based on the amount of privileged resource (e.g., carbohydrate such as porphyran) that is being provided (e.g., introduced into the diet of the individual) (see, e.g., Figure 2, panel B). For example, in some cases the deviation in abundance of the introduced bacteria between different microbial communities by the 7th day after introduction (or 7th day after introduction, where the

20 introduced bacteria had access to the privileged resource) spans a range of 4-fold or less (e.g., 3-fold or less).

In some embodiments, e.g., to provide a privileged niche to the introduced genetically modified bacteria, the carbohydrate of interest is a rare carbohydrate of interest. By 'rare' carbohydrate of interest, it is meant a carbohydrate of interest that is utilized by less than 50%

25 (e.g., less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.2%, less than 0.1%, or none; e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of other bacterial cells present in a target organ such as the gut (i.e., cells 'other' than

30 the introduced/genetically modified bacteria, e.g., cells of the resident population prior to introduction). Thus, in some cases (e.g., in any of the methods of the disclosure), a rare carbohydrate of interest is one that can be utilized by less than 50% (e.g., less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.2%, less than 0.1%, or none; e.g., less than 30%, less than

35 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of other bacterial cells present in the organ. In some cases (e.g., in any of the methods of the disclosure), the

rare carbohydrate of interest is one that can be utilized by less than 20% (e.g., less than 10%, less than 5%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.2%, less than 0.1%, or none) of other bacterial cells present in the organ. In some cases (e.g., in any of the methods of the disclosure), the rare carbohydrate of interest is one that can be utilized by less than 5% (e.g., less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.2%, less than 0.1%, or none) of other bacterial cells present in the organ. In some cases (e.g., in any of the methods of the disclosure), the rare carbohydrate of interest is one that can be utilized by less than 2% (e.g., less than 1%, less than 0.5%, less than 0.2%, less than 0.1%, or none) of other bacterial cells present in the organ. In some cases (e.g., in any of the methods of the disclosure), the rare carbohydrate of interest is one that can be utilized by less than 0.5% (e.g., less than 0.2%, less than 0.1%, or none) of other bacterial cells present in the organ. In some cases (e.g., in any of the methods of the disclosure), the rare carbohydrate of interest is one that can be utilized by none of the other bacterial cells present in the organ.

A method of the disclosure can include a step of administering to an individual (e.g., a human) a carbohydrate of interest (e.g., a rare carbohydrate of interest) that is utilized as a carbon source by a subject genetically modified bacterial cell present in an organ of the individual (e.g., the gut), wherein less than 50% (e.g., less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.2%, less than 0.1%, or none; e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of other bacterial cells present in the organ utilize the rare carbohydrate of interest as a carbon source. In some cases, such a method includes a step of introducing the genetically modified bacterial cell into the organ of the individual, e.g., prior to administering the carbohydrate of interest. In some embodiments, the organ (e.g., gut) of the individual includes at least 5 other bacterial species (other than the genetically modified bacteria), e.g., at least 8, at least 10, at least 15, at least 20, at least 50, at least 100, at least 200, at least 500, or at least 1,000 other bacterial species. In some cases, the organ (e.g., gut) of the individual includes at least 50 other bacterial species (other than the genetically modified bacteria). In some cases, the organ (e.g., gut) of the individual includes at least 100 other bacterial species (other than the genetically modified bacteria).

In some cases, two or more privileged niches (e.g., in some cases using dietary substrates from distinct geographic regions) can be used, e.g., to increase the effectiveness of this strategy even further. For example, this may allow robust colonization in the event that one of the two rare substrates is utilized by an individual's endogenous microbiota.

ii. Colonization and Entrenchment

In some cases, growth modulation is used to allow a bacterial cell (e.g., population of

bacterial cells) to colonize a target organ (e.g., gut). Thus, provided are methods that facilitate colonization of a bacterial cell (e.g., a population of bacterial cells) in an organ (e.g., gut) of an individual. By "colonize" it is meant that an introduced bacterial cell (e.g., population of bacterial cells) can establish a population of a desired abundance or level or can establish a large enough population in the target organ that the population is detectable, despite the presence of already established bacterial populations. In some cases, the introduced bacteria reaches a 'high level' of colonization (i.e., a 'high abundance'). In such cases, the introduced bacteria reaches an abundance of 10^5 CFU/ μ l or more (e.g., 10^6 CFU/ μ l or more, 10^7 CFU/ μ l or more) (e.g., by day 6 after introduction) (e.g., in the target organ). Thus, in some cases, introduced bacteria reaches an abundance of 10^7 CFU/ μ l or more. In some cases, the introduced bacteria reaches a 'low level' of colonization (i.e., a 'low abundance'), but the population is still detectable (e.g., by day 6 after introduction). For example, in some cases, the introduced bacteria reaches an abundance in a range of from 10^2 CFU/ μ l to 10^4 CFU/ μ l (e.g., 10^2 CFU/ μ l, 10^3 CFU/ μ l, 10^4 CFU/ μ l) (e.g., by day 6 after introduction) (e.g., in the target organ).

In some embodiments, the introduced/genetically modified bacteria reaches an abundance such that it attains a population level of 10% or more (e.g., 20% or more, 30% or more, 40% or more, 50% or more, 60% or more, 70% or more, 80% or more, or 90% or more) of total anaerobic CFUs (see e.g., Figure 2) (e.g., by day 6 after introduction) (e.g., in the target organ). In some embodiments, the introduced bacteria reaches an abundance such the population attains a population level in a range of from 10%-90% (e.g., from 10%-80%, 10%-70%, 10%-60%, 10%-50%, 20%-90%, 20%-80%, 20%-70%, 20%-60%, 20%-50%, 30%-90%, 30%-80%, 30%-70%, 30%-60%, or 30%-50%) of total anaerobic CFUs (e.g., by day 6 after introduction) (e.g., in the target organ).

In some embodiments, the introduced/genetically modified bacteria reaches an abundance such that it attains a population level of 1% or more (e.g., 2% or more, 5% or more, or 10% or more) of total bacterial cells in the organ (e.g., gut). In some embodiments, the introduced/genetically modified bacteria reaches an abundance such that it attains a population level in a range of from 1%-50% of total bacterial cells in the organ (e.g., from 1%-40%, 1%-30%, 1%-20%, 1%-15%, 2%-50%, 2%-40%, 2%-30%, 2%-20%, 2%-15%, 5%-50%, 5%-40%, 5%-30%, 5%-20%, or 5%-15% of total bacterial cells in the organ).

In some cases at least a portion of the population of bacterial cells in an organ (e.g., gut) is displaced as a result of modulating the growth (e.g., by administration of a carbohydrate of interest) of the genetically modified bacterial cell. For example, in some cases 5% or more (e.g., 10% or more, 15% or more, or 20% or more) of the population of bacterial cells in an organ (e.g., gut) is displaced as a result of modulating the growth (e.g., by administration of a carbohydrate of interest) of the genetically modified bacterial cell. In some cases, the

genetically modified bacterial cell and the bacterial cells in the population of bacterial cells are the same species. In some cases, the genetically modified bacterial cell and the bacterial cells in the population of bacterial cells are different species. In some cases, the genetically modified bacterial cell is generated by providing a bacterial cell from the population of bacterial cells and removing or inactivating one or more genes in the bacterial cell.

In some embodiments, colonization is stable for a long period of time. Thus, in some cases a genetically modified bacterial cell of the disclosure becomes entrenched in the gut. The term "entrench" (e.g., "entrenchment") is used herein to refer to a situation in which an introduced species becomes a stable/persistent member of the community into which it was introduced. For example, in some cases when entrenchment is not accomplished, an introduced bacterial species (e.g., introduced into the gut of an individual) might be cleared (reduced to undetectable levels) by approximately one week after introduction. If there is not a niche available to the introduced bacteria, it must compete with species that are already established (entrenched), and that share the same niche with the introduced bacteria (i.e., the introduced bacteria would have to compete for resources with already established species).

In some cases, entrenchment is accomplished when an introduced bacteria (e.g., a wild type species not normally present in the target organ, e.g., gut; a genetically modified bacterial cell; and the like) remains detectable or at a desired abundance or population level in the organ into which it was introduced (e.g., on day 6 after introduction). In other words, in some cases an introduced bacteria is entrenched if it remains detectable or at a desired abundance or population level for 6 or more days (e.g., 7 or more, 8 or more, 9 or more, or 10 or more) days after introduction. By detectable it is meant that the population of introduced bacteria includes a large enough number of individuals that their presence is detectable. In some cases, entrenchment is maintained by providing a sufficient amount of a carbohydrate (e.g., rare carbohydrate of interest) that can be utilized by the genetically modified bacterial cell at a sufficient frequency. In some cases, entrenchment can be maintained in the absence of a carbohydrate (e.g., rare carbohydrate of interest) that can be utilized by the genetically modified bacterial cell.

For any of the above metrics (e.g., abundance level, abundance range, population level, and the like), a time frame can be placed on the metric. For example, in some cases the metric is reached within 5 days of introduction of the bacteria. In some case, the metric is reached within 6 days of introduction of the bacteria. In some case, the metric is reached within 7 days of introduction of the bacteria. However, the time frame need not be relative to introduction of the bacteria. For example, in some cases the metric is relative to the introduction of the resource (e.g., carbohydrate of interest, rare carbohydrate of interest such as porphyrin). Thus, in some cases the metric is reached within 5 days of introduction of the resource (e.g., carbohydrate of interest, rare carbohydrate of interest such as porphyrin). In

some case, some cases the metric is reached within 6 days of introduction of the resource (e.g., carbohydrate of interest, rare carbohydrate of interest such as porphyran). In some case, the metric is reached within 7 days of introduction of the resource (e.g., carbohydrate of interest, rare carbohydrate of interest such as porphyran).

5 In some embodiments a method of the disclosure includes a step of detecting the presence of (e.g., measure the abundance of) the genetically modified bacterial cell after it has been introduced into an individual (e.g., 3 days, 4 days, 5 days, 6 days, and/or 7 days after introduction; and/or 3 days, 4 days, 5 days, 6 days, and/or 7 days after providing the carbohydrate of interest). In some such cases, the step of detecting includes measuring the
10 colony forming units (e.g., CFU/ μ l) of the genetically modified bacterial cell present in the organ (e.g., gut) of the individual. In some such cases the detecting further includes measuring the colony forming units (e.g., CFU/ μ l) of other bacteria present in the organ.

In some cases, the term “controlled” is used in front of “colonization” and/or “entrenchment” (e.g., “controlled colonization” ; “controlled entrenchment”). The term
15 “controlled” is used as such to refer to a situation in which the colonization and/or entrenchment of a bacteria is controllable by a user. For example, if the growth and/or survival of a given bacterial cell (e.g., a genetically modified bacterial cell) can be modulated by administering an energy and/or carbon source (e.g., carbohydrate of interest) to a host (e.g., a human) and/or a host organ, then the colonization and/or entrenchment of the bacterial is
20 controllable. For example, in such a situation the population size and/or duration of colonization can depend on the amount and/or frequency with which the energy and/or carbon source (e.g., carbohydrate) is administered.

iii. Carbohydrate of interest

25 In some embodiments the carbohydrate of interest (e.g., rare carbohydrate of interest) is a polysaccharide. In some cases the carbohydrate of interest (e.g., rare carbohydrate of interest) is a sulfated carbohydrate. In some cases the carbohydrate of interest (e.g., rare carbohydrate of interest) is selected from the group consisting of porphyran, agarose, carrageenan, and any combination thereof. In some cases the carbohydrate of interest (e.g.,
30 rare carbohydrate of interest) is a carbohydrate cleaved by a glycoside hydrolase belonging to glycoside hydrolase family GH86. In some cases the carbohydrate of interest (e.g., rare carbohydrate of interest) is a marine carbohydrate. Examples of marine carbohydrates include but are not limited to: porphyran, agarose, agaropectin, carrageenan, and marine microbe exopolysaccharides. In some cases the carbohydrate of interest (e.g., rare carbohydrate of
35 interest) is selected from porphyran and agarose. In some cases the carbohydrate of interest (e.g., rare carbohydrate of interest) is porphyran. In some cases the carbohydrate of interest (e.g., rare carbohydrate of interest) is agarose.

In some cases the carbohydrate of interest (e.g., rare carbohydrate of interest) is a carbohydrate that contains a glycosidic linkage selected from the group consisting of β -d-galactopyranose to α -l-galactopyranose-6-sulphate, β -d-galactopyranose to 3,6-anhydro- α -l-galactopyranose.

5 In some cases the carbohydrate of interest (e.g., rare carbohydrate of interest) is a sulfated polygalactan. In some such cases, one or more of the galactose residues of the sulfated polygalactan can be a 3,6-anhydro-galactose (e.g., in some cases joined by alternating α -1,3 and β -1,4-glycosidic linkage). In some cases, one or more of the galactopyranose residues of the sulfated polygalactan can be modified by one or more ester
10 sulfates. In some cases, one or more of the galactose residues of the sulfated polygalactan is a 3,6-anhydro-galactose (e.g., in some cases joined by alternating α -1,3 and β -1,4-glycosidic linkage); and one or more of the galactopyranose residues of the sulfated polygalactan is modified by one or more ester sulfates.

15 In some cases, the carbohydrate of interest is administered to an individual and in some such cases the carbohydrate of interest is isolated. The term "isolated" refers to the state in which a molecule (e.g., a carbohydrate) can be. In such a case, the carbohydrate will be free or substantially free of material with which it is naturally associated such as other carbohydrates with which it is found in the natural environment, or the environment in which it is prepared.

20 In some cases, less than 50% (e.g., less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.2%, less than 0.1%, or none; e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of other bacterial cells present in the organ utilize
25 the carbohydrate of interest (e.g., rare carbohydrate of interest) as a carbon source. In some cases, the carbohydrate of interest (e.g., rare carbohydrate of interest) cannot be utilized or digested by mammalian (e.g., human) cells.

In some cases, the carbohydrate of interest is administered at a sufficient frequency and/or at a sufficient amount for controlled colonization and/or controlled entrenchment. For
30 example, the carbohydrate of interest can be administered 1, 2, 3, 4, 5, 6, or more times per day, week, month, or year. The carbohydrate of interest can be administered over a period of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more days, weeks, months, or years. In some cases, the carbohydrate of interest can be administered by oral administration. Any convenient type of oral administration can be used. For example, oral administration can include delivery via
35 eating (e.g., incorporated into food), drinking (e.g., incorporated into a solution such as drinking water), oral gavage (e.g., using a stomach tube), aerosol spray, tablets, capsules, pills, powders, and the like.

iv. Genetically modified bacteria

In some cases a genetically modified bacterial cell includes a heterologous carbohydrate-utilization gene or gene set. "Heterologous," as used herein, means a nucleic acid and/or polypeptide that is not found in the native bacteria. For example, in a case where a bacterial cell includes a gene set (e.g., a nucleic acid encoding a set of proteins) that provides the cell with the ability to utilize a carbon source that the cell otherwise cannot use, and where that gene set is not naturally found in that bacteria, the gene set is 'heterologous' to the bacterial cell.

In some cases a subject method is a method of genetically modifying a bacterial cell to utilize as a carbon source a rare carbohydrate of interest. Any convenient method can be used to introduce a nucleic acid into a prokaryotic cell, e.g., by electroporation (e.g., using electro-competent cells), by conjugation, by chemical methods (e.g., using chemically competent cells), and the like.

In some embodiments, a genetically modified bacterial cell utilizes as a carbon source a rare carbohydrate of interest (as described elsewhere herein, e.g., wherein less than 50% of other bacterial cells present in the organ utilize the rare carbohydrate of interest as a carbon source). This is due to the fact that a subject genetically modified bacterial cell includes a carbohydrate-utilization gene or gene set that provides the genetically modified bacterial cell with the ability to use a carbohydrate of interest as a carbon source. In some cases, the carbohydrate-utilization gene or gene set is native to the cell. In some case, the carbohydrate-utilization gene or gene set is heterologous to the cell (e.g., can be from a different species of bacteria). Thus, in some cases, the carbohydrate of interest cannot be utilized as a carbon source by the genetically modified bacterial cell in the absence of a heterologous carbohydrate-utilization gene or gene set. In some cases, the genetically modified bacterial cell is capable of utilizing the carbohydrate of interest as a carbon source in the absence of a heterologous carbohydrate-utilization gene or gene set, but the presence of the heterologous carbohydrate-utilization gene or gene set enhances that capability.

In some cases, a carbohydrate-utilization gene or gene set includes a porphyranase (e.g., one from GH family 86 (GH86)). In some cases, a carbohydrate-utilization gene or gene set includes an agarase (e.g., one from GH family 86 (GH86)).

In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding BACPLE_1683-1706 from the *B. plebeius* genome (or homologs thereof) (see, e.g., Table 6). In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding BACPLE_1683-1687 from the *B. plebeius* genome (or homologs thereof). In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding BACPLE_1700-1706 from the *B.*

plebeius genome (or homologs thereof). In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding BACPLE_1683-1687 from the *B. plebeius* genome (or homologs thereof) and BACPLE_1700-1706 from the *B. plebeius* genome (or homologs thereof). In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding BACPLE_1683-1699 from the *B. plebeius* genome (or homologs thereof). In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding BACPLE_1688-1706 from the *B. plebeius* genome (or homologs thereof). In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding BACPLE_1683-1687 from the *B. plebeius* genome (or homologs thereof) as well as both porphyranases from within BACPLE_1688-1699 from the *B. plebeius* genome (or homologs thereof). In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding BACPLE_1700-1706 from the *B. plebeius* genome (or homologs thereof) as well as both porphyranases from within BACPLE_1688-1699 from the *B. plebeius* genome (or homologs thereof). In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding BACPLE_1683-1687 and BACPLE_1700-1706 from the *B. plebeius* genome (or homologs thereof) as well as both porphyranases from within BACPLE_1688-1699 from the *B. plebeius* genome (or homologs thereof).

As such, in some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with BACPLE_1683-1706 from the *B. plebeius* genome (see, e.g., Table 6). In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with BACPLE_1683-1687 from the *B. plebeius* genome. In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with BACPLE_1700-1706 from the *B. plebeius* genome. In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with BACPLE_1683-1687 from the *B. plebeius* genome (or homologs thereof) and BACPLE_1700-1706 from the *B. plebeius* genome. In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with BACPLE_1683-1699 from the *B. plebeius* genome. In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic

acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with BACPLE_1688-1706 from the *B. plebeius* genome. In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with BACPLE_1683-1687 from the *B. plebeius* genome (or homologs thereof) as well as both porphyrases from within BACPLE_1688-1699 from the *B. plebeius* genome. In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with BACPLE_1700-1706 from the *B. plebeius* genome (or homologs thereof) as well as both porphyrases from within BACPLE_1688-1699 from the *B. plebeius* genome. In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with BACPLE_1683-1687 and BACPLE_1700-1706 from the *B. plebeius* genome (or homologs thereof) as well as both porphyrases from within BACPLE_1688-1699 from the *B. plebeius* genome.

Table 6. SEQ ID NOs. and annotations for proteins encoded by *B. plebeius* genome (BACPLE_1669-1706).

B. Plebeius Homolog ID	SEQ ID NO	Annotation
BACPLE_01669	1	histidine kinase CDS
BACPLE_01670	2	beta-agarase CDS
BACPLE_01671	3	glycosylhydrolase CDS
BACPLE_01672	4	threonine synthase CDS
BACPLE_01673	5	hypothetical protein CDS
BACPLE_01674	6	altronate hydrolase CDS
BACPLE_01675	7	altronate oxidoreductase CDS
BACPLE_01676	8	sorbitol dehydrogenase CDS
BACPLE_01677	9	L-fucose:H ⁺ symporter permease CDS
BACPLE_01678	10	amidohydrolase CDS
BACPLE_01679	11	aldo/keto reductase CDS
BACPLE_01680	12	hypothetical protein CDS
BACPLE_01682	13	hypothetical protein CDS
BACPLE_01683	14	hypothetical protein CDS
BACPLE_01684	15	glycosylhydrolase CDS

B. Plebeius Homolog ID	SEQ ID NO	Annotation
BACPLE_01685	16	hypothetical protein CDS
BACPLE_01686	17	hypothetical protein CDS
BACPLE_01688	18	hypothetical protein CDS
BACPLE_01689	19	beta-porphyrinase B CDS
BACPLE_01692	20	hypothetical protein CDS
BACPLE_01693	21	beta-porphyrinase A CDS
BACPLE_01694	22	hypothetical protein CDS
BACPLE_01695	23	hypothetical protein CDS
BACPLE_01696	24	hypothetical protein CDS
BACPLE_01697	25	hypothetical protein CDS
BACPLE_01698	26	SusC/RagA family TonB-linked outer membrane protein CDS
BACPLE_01699	27	hybrid two component system
BACPLE_01700	28	alcohol dehydrogenase CDS
BACPLE_01701	29	acetylglucosamine-6-sulfatase CDS
BACPLE_01702	30	hypothetical protein CDS
BACPLE_01703	31	glycoside hydrolase CDS
BACPLE_01704	32	hypothetical protein CDS
BACPLE_01705	33	hypothetical protein CDS
BACPLE_01706	34	beta-galactosidase CDS

In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding SEQ ID NOS.: 14-34 (or homologs thereof) (see, e.g., Table 6). In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding SEQ ID NOS.: 14-17 (or homologs thereof). In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding SEQ ID NOS.: 28-34 (or homologs thereof). In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding SEQ ID NOS.: 14-17 (or homologs thereof) and SEQ ID NOS.: 28-34 (or homologs thereof). In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding SEQ ID NOS.: 14-27 (or homologs thereof). In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding SEQ ID NOS.: 18-34 (or homologs thereof). In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding SEQ ID NOS.: 14-17 (or homologs thereof) as well as both porphyrinases from within SEQ ID NOS.: 18-27 (or homologs thereof). In some cases, a

subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding SEQ ID NOS.: 28-34 (or homologs thereof) as well as both porphyrases from within SEQ ID NOS.: 18-27 (or homologs thereof). In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding SEQ ID NOS.: 14-17 and SEQ ID NOS.: 28-34 (or homologs thereof) as well as both porphyrases from within SEQ ID NOS.: 18-27 (or homologs thereof).

As such, in some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with SEQ ID NOS.: 14-34 (see, e.g., Table 6). In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with SEQ ID NOS.: 14-17. In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with SEQ ID NOS.: 28-34. In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with SEQ ID NOS.: 14-17 and SEQ ID NOS.: 28-34. In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with SEQ ID NOS.: 14-27. In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with SEQ ID NOS.: 18-34. In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with SEQ ID NOS.: 14-17 as well as both porphyrases set forth as SEQ ID NOS.: 19 and 21. In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with SEQ ID NOS.: 28-34 as well as both porphyrases set forth as SEQ ID NOS.: 19 and 21. In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins that have 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with SEQ ID NOS.: 14-17 and SEQ ID NOS.: 28-34 as well as both porphyrases set forth as SEQ ID NOS.: 19 and 21.

In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding (i) at least one of SEQ ID NOs.: 19, 21, and 22 (or a homolog(s) thereof); (ii) at least one of SEQ ID NOs.: 26 and 33 (or a homolog(s) thereof); and (iii) at least one of SEQ ID NOs.: 25 and 32 (or a homolog(s) thereof). In some cases, a subject
5 carbohydrate-utilization gene or gene set encodes SEQ ID NOs.: 19, 21-22, 25, 26, and 32-33 (or a homologs thereof).

In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding (i) at least one protein that has 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with any one of
10 SEQ ID NOs.: 19, 21, and 22; (ii) at least one protein that has 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with any one of SEQ ID NOs.: 26 and 33; and (iii) at least one protein that has 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with any one of
15 SEQ ID NOs.: 25 and 32. In some cases, a subject carbohydrate-utilization gene or gene set includes one or more nucleic acids encoding proteins having 80% or more sequence identity (e.g., 85% or more, 90% or more, 95% or more, or 100% sequence identity) with SEQ ID
NOs.: 19, 21-22, 25, 26, and 32-33.

In some embodiments, the carbohydrate-utilization gene set includes at least 3 genes (e.g., at least 4, at least 5, at least 6, at least 8 genes, at least 10 genes, at least 12 genes, at
20 least 15 genes, or at least 20 genes). In some cases, the carbohydrate-utilization gene set includes from 3-30 genes (e.g., from 5-30, 3-25, 3-20, 3-15, 3-10, 3-8, 5-25, 5-20, 5-15, 5-10, 5-8, 8-30, 8-25, 8-20, 8-15, 10-30, 10-25, 10-20, 10-15, 12-30, 12-25, 12-20, 15-30, 15-25, 20-30, or 20-25 genes). In some cases, the carbohydrate-utilization gene set includes from
3-10 genes.

A nucleic acid that includes the carbohydrate-utilization gene or gene set (e.g., a
25 heterologous carbohydrate-utilization gene or gene set) may or may not be integrated (covalently linked) into the genome of the cell. For example, in some cases, the nucleic acid that includes the carbohydrate-utilization gene or gene set is integrated into the genome of the cell (as a chromosomal integrant). In some cases, the nucleic acid that includes the
30 carbohydrate-utilization gene or gene set is maintained on an episomal element (extra chromosomal element) such as a plasmid.

In some cases the expression of genes from the carbohydrate-utilization gene or gene set is controlled by a high expression promoter. In some cases the expression of genes from the carbohydrate-utilization gene or gene set is controlled by an inducible promoter,
35 constitutive promoter, native promoter (e.g., native to the bacterial cell), heterologous promoter, or a promoter associated with the carbohydrate-utilization gene or gene set. Thus, for example, methods of the disclosure can be controlled by the type of promoter that is

present. As an illustrative example, in some cases, entrenchment (e.g., of a subject genetically modified bacterial cell into an organ of an individual, e.g., into the individual's gut) is controlled by a high expression promoter. Likewise, in some cases entrenchment (e.g., of a subject genetically modified bacterial cell into an organ of an individual, e.g., into the individual's gut) is controlled by an inducible promoter, constitutive promoter, native promoter (e.g., native to the bacterial cell), heterologous promoter, or a promoter associated with the carbohydrate-utilization gene or gene set.

Therapy etc.

The strategy disclosed here for facilitating robust colonization can facilitate a range of therapeutic applications, e.g., where it can be important to establish a high and/or predictable number of natural or engineered therapeutic microbes. For example, it may be important to colonize an organ (e.g., gut) at a high enough level to ensure that therapeutic activities may overcome competing activities of other microbes. As an example, this may be important when attempting to, for instance, change the short chain fatty acid profile or reduce the accumulation of harmful chemicals that are produced in the gut. Achieving predictable colonization can also be important for therapeutics that must be carefully dosed, such as bacteria with pro- or anti-inflammatory activities which could be used as an adjuvant for cancer immunotherapy or in the treatment of Inflammatory Bowel Diseases. The methods of the disclosure could also be used to, for example, ensure robust colonization to displace a harmful species by establishing a non-harmful version that can effectively compete for limiting resources with the harmful strain. As such, and as noted elsewhere, in some cases a subject method can result in displacement of bacteria present in the gut of an individual.

A subject genetically modified bacterial cell can be introduced into a variety of individuals with a variety of ailments. Diseases that can be treated with a subject genetically modified bacterial cell include but are not limited to diseases that are impacted by the gut microbiota, which include obesity, diabetes, heart disease, central nervous system diseases, rheumatoid arthritis, metabolic disorders, and cancer. For example, in some cases, the individual has gut inflammation, and in some such cases the individual has an inflammatory disease (e.g., Crohn's disease, ulcerative colitis, and the like), and in some cases gut inflammation can indirectly impact the disease, such as colorectal cancer or obesity.

In some cases, a subject bacterial cell is a therapeutic cell (has a positive impact on a clinical parameter of the individual). A therapeutic cell is not necessarily a genetically modified cell. For example, a cell that has not been genetically modified may serve a therapeutic purpose to an individual. In some cases, a therapeutic cell is genetically modified. For example, in some cases a therapeutic cell includes a transgene that encodes a therapeutic peptide (e.g., a peptide that when expressed by - and in some cases secreted by - a cell can

have a positive impact on the health of an individual). For example, a therapeutic cell and/or therapeutic peptide can have antimicrobial (antibiotic) activity (e.g., against one or more gut microbes), can function to change gut environmental parameters (e.g., pH control), can affect inflammation, can provide an enzymatic activity, and the like.

5 In some cases, a genetically modified bacterial cell includes a transgene that is an enzyme (e.g., a metabolic enzyme). For example, there are many small molecules produced in the gut (e.g., produced by microbes) that accumulate in the blood and cause or exacerbate diseases. Expressing an enzyme or a pathway (as a transgene) in a *Bacteroides* cell (or population of cells) to break down these products can be used in methods of treatment. For
10 example, a *Bacteroides* cell expressing such a transgene can be introduced into the gut of an individual (e.g., in order to break down small molecules, which in some cases may be produced by microbes, to reduce or even eliminate the amount absorbed by the gut of the individual, reducing the accumulation of the molecules in the blood of the individual).

In some cases, a genetically modified bacterial cell is tagged (e.g., to aid in tracking).
15 For example, in some cases, a genetically modified bacterial cell of the disclosure includes a detectable label (e.g., a nucleic acid that results in the presence of a detectable signal). As such, in some cases, a subject genetically modified cell (or population of cells) that is introduced into an individual (e.g., the gut), includes a transgene whose expression detectably labels the cell. The phrase "detectably label" as used herein refers to any expression product
20 (RNA, protein) that is detectable. The expression product (the label) can itself be detectable (directly detectable label) (e.g., a fluorescent protein), or the label can be indirectly detectable, e.g., in the case of an enzymatic label, the enzyme (e.g., luciferase or recombinase) may catalyze a chemical alteration of a substrate compound, composition or nucleic acid sequence and the product of the reaction is detectable (e.g. by fluorescence, chemiluminescence,
25 sequencing or presence and/or size of a PCR product). Thus in some cases, any of the methods of the disclosure can include a step of detecting (e.g., measuring) a detectable signal produced by a subject genetically modified bacterial cell. As an illustrative example, in some cases a genetically modified bacterial cell includes a transgene that encodes a fluorescent protein (e.g., green fluorescent protein (GFP) or any of a number of derivatives of GFP such
30 as YFP, CFP, RFP, etc.) or an enzyme that produces a fluorescent signal (e.g., luciferase), and a subject method includes a step of measuring the signal to detect the presence of (e.g., measure the abundance of) the genetically modified bacterial cell after it has been introduced into an individual (e.g., 3 days, 4 days, 5 days, 6 days, and/or 7 days after introduction; and/or 3 days, 4 days, 5 days, 6 days, and/or 7 days after providing the carbohydrate of interest).

35 In some cases, a genetically modified bacterial cell includes a transgene that is a "marker" or "marker gene" or "marker protein." A marker is an expression product (e.g., mRNA, protein, non-coding RNA) that marks a host cell such that the host cell is detectable

(e.g., detectably labeled). In some cases, the host cell is detectable by virtue of survival (e.g., the marker can be a “selectable marker”). In some cases, the host cell is detectable by observation (e.g., by direct visualization, by performing an assay, by performing a measurement step, and the like) and the marker can be referred to as a “reporter” or “reporter gene” or “reporter protein.”

As noted above, some markers are “selectable markers.” Selectable markers (a “selectable marker gene” can encode a “selectable marker protein”) provide for selection, i.e., for selective retention of cells (e.g., prokaryotic cells) that comprise the selectable marker gene, during culturing and propagation of the cells. An example of a selectable marker is a transgene that encodes a drug selectable marker protein that provides drug resistance for prokaryotic cells (e.g., Bacteroides cells). Such a selectable marker encodes a drug selectable marker protein that provides resistance for prokaryotic cells to one or more drugs (e.g., kanamycin, neomycin, ampicillin, carbenicillin, chloramphenicol, gentamicin, tetracycline, rifampin, trimethoprim, hygromycin B, spectinomycin, and the like). In some cases, a subject genetically modified bacteria can include an erythromycin resistance cassette. Proteins that provide drug resistance to cells (e.g., prokaryotic cells) in which they are expressed are known in the art. For example, wild type genes/proteins are known that provide resistance (e.g., for prokaryotic cells) to the above drugs. For example, aminoglycoside 3'-phosphotransferase (APH), is a wild type protein that provides for resistance to the drugs Kanamycin, Neomycin and Geneticin (G418); while beta-lactamase is a wild type protein that provides for resistance to the drugs ampicillin and carbenecillin. Chloramphenicol acetyltransferase (cat) confers resistance to chloramphenicol. Genes conferring resistance to aminoglycosides include aac, aad, aph and strA/B. Genes conferring resistance to β -lactams include ampC, cmv, tem and vim. Genes conferring resistance to sulfonamides include sull and sullI. Genes conferring resistance to tetracycline include tet(A), tet(B), tet(C), tet(D) and regulator, and tetR. Selectable markers can also be those useful in balanced lethal systems, e.g., in which an essential gene is maintained on a plasmid with a corresponding chromosomal deletion or suppressible mutation on the host cell genome, e.g. a tRNA selectable marker that suppresses a host chromosomal gene mutation; those useful in repressor titration systems, in which an operator sequences, e.g. the lac operator or tet operator, placed on a plasmid, derepresses a chromosomal gene; antidote/poison selection schemes, in which an antidote to a poison expressed from the host chromosome (e.g. the ccdB gene) is maintained on the plasmid; and those useful in RNA-based selection schemes, e.g. antisense regulators, or antisense regulators that inhibit the translation of a gene transcribed from the host chromosome that would otherwise promote cell death.

Bacteroides cells

In any of the embodiments of the disclosure, the bacterial cell of interest (e.g., the genetically modified cell, the cell being modified or introduced, or whose growth is being modulated, as part of a subject composition or method) can be a Bacteroides cell. In some cases, the bacterial cell of interest is not a Bacteroides cell. For example, the bacterial cell of interest can be any desired species, e.g., when the target organ is a gut the bacterial cell can be any species that can colonize a gut. In some cases the bacterial cell of interest is a Clostridium species (i.e., is a cell of the genus *Clostridium*).

The term "Bacteroides cell" is used herein to refer to a cell of the genus *Bacteroides*. As such, in some cases, a subject cell is a Bacteroides cell. Examples of species within the genus *Bacteroides* include but are not limited to: *B. fragilis* (Bf), *B. distasonis* (Bd), *B. thetaiotaomicron* (Bt), *B. vulgatus* (Bv), *B. ovatus* (Bo), *B. eggerrthii* (Be), *B. merdae* (Bm), *B. stercoris* (Bs), *B. uniformis* (Bu), and *B. caccae* (Bc). In some cases, a Bacteroides cell is a species selected from: *B. acidifaciens*, *B. amylophilus*, *B. asaccharolyticus*, *B. barnesiae*, *B. bivia*, *B. buccae*, *B. buccalis*, *B. caccae*, *B. capillosus*, *B. capillus*, *B. cellulolyticus*, *B. cellulosolvens*, *B. chinchilla*, *B. clarus*, *B. coagulans*, *B. coprocola*, *B. coprophilus*, *B. coprosuis*, *B. corporis*, *B. denticola*, *B. disiens*, *B. distasonis*, *B. dorei*, *B. eggerrthii*, *B. endodontalis*, *B. faecichinchillae*, *B. faecis*, *B. finegoldii*, *B. fluxus*, *B. forsythus*, *B. fragilis*, *B. furcosus*, *B. galacturonicus*, *B. gallinarum*, *B. gingivalis*, *B. goldsteinii*, *B. gracilis*, *B. graminisolvens*, *B. helcogenes*, *B. heparinolyticus*, *B. hypermegas*, *B. intermedius*, *B. intestinalis*, *B. johnsonii*, *B. levvi*, *B. loescheii*, *B. macacae*, *B. massiliensis*, *B. melaninogenicus*, *B. merdae*, *B. microfus*, *B. multiacidus*, *B. nodosus*, *B. nordii*, *B. ochraceus*, *B. oleiciplenus*, *B. oralis*, *B. oris*, *B. oulorum*, *B. ovatus*, *B. paurosaccharolyticus*, *B. pectinophilus*, *B. pentosaceus*, *B. plebeius*, *B. pneumosintes*, *B. polypragmatus*, *B. praeacutus*, *B. propionificaciens*, *B. putredinis*, *B. pyogenes*, *B. reticulotermitis*, *B. rodentium*, *B. ruminicola*, *B. salanitronis*, *B. salivorus*, *B. salyersiae*, *B. sartorii*, *B. splanchnicus*, *B. stercorisoris*, *B. stercoris*, *B. succinogenes*, *B. suis*, *B. tectus*, *B. termitidis*, *B. thetaiotaomicron*, *B. uniformis*, *B. ureolyticus*, *B. veroralis*, *B. vulgatus*, *B. xylanisolvens*, *B. xylanolyticus*, and *B. zoogloformans*. In some cases, a subject Bacteroides cell is a species selected from: *B. fragilis* (Bf), *B. thetaiotaomicron* (Bt), *B. vulgatus* (Bv), *B. ovatus* (Bo), and *B. uniformis* (Bu).

In some cases, the Bacteroides cell is a species selected from: *B. fragilis* (Bf), *B. distasonis* (Bd), *B. thetaiotaomicron* (Bt), *B. vulgatus* (Bv), *B. ovatus* (Bo), *B. eggerrthii* (Be), *B. merdae* (Bm), *B. stercoris* (Bs), *B. uniformis* (Bu), and *B. caccae* (Bc). In some cases, the Bacteroides cell is a species selected from: *B. fragilis* (Bf), *B. thetaiotaomicron* (Bt), *B. vulgatus* (Bv), *B. ovatus* (Bo), and *B. uniformis* (Bu). In some cases, the Bacteroides cell is a

species selected from: *B. thetaiotaomicron* (Bt), *B. vulgatus* (Bv), *B. ovatus* (Bo), and *B. uniformis* (Bu). In some cases, the Bacteroides cell is *B. thetaiotaomicron* (Bt).

KITS

5 Also provided are kits, e.g., for practicing any of the above methods. The contents of the subject kits may vary greatly. A kit can include, for example: (i) a subject genetically modified bacterial cell, and (ii) a rare carbohydrate of interest that can be utilized by the genetically modified bacterial cell. In some case, the genetically modified bacterial cell is a therapeutic cell.

10 In addition to the above components, the subject kits can further include instructions for practicing the subject methods. These instructions may be present in the subject kits in a variety of forms, one or more of which may be present in the kit. One form in which these instructions may be present is as printed information on a suitable medium or substrate, e.g., a piece or pieces of paper on which the information is printed, in the packaging of the kit, in a package insert, etc. Yet another means would be a computer readable medium, e.g., diskette, 15 CD, flash drive, etc., on which the information has been recorded. Yet another means that may be present is a website address which may be used via the internet to access the information at a removed site. Any convenient means may be present in the kits.

20 *Examples of Non-Limiting Aspects of the Disclosure*

Aspects, including embodiments, of the present subject matter described above may be beneficial alone or in combination, with one or more other aspects or embodiments. Without limiting the foregoing description, certain non-limiting aspects of the disclosure numbered 1-98 are provided below. As will be apparent to those of skill in the art upon reading 25 this disclosure, each of the individually numbered aspects may be used or combined with any of the preceding or following individually numbered aspects. This is intended to provide support for all such combinations of aspects and is not limited to combinations of aspects explicitly provided below:

1. A method, comprising: introducing to a human organ a genetically modified bacterial cell capable of controlled entrenchment and/or controlled colonization.

2. A method for modulating growth of a genetically modified bacterial cell present in a human organ, comprising: administering to a human a rare carbohydrate of interest that is utilized as a carbon source by the genetically modified bacterial cell, wherein less than 50% (e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of other bacterial cells present in the human organ utilize the rare

carbohydrate of interest as a carbon source.

3. A method for modulating growth of a genetically modified bacterial cell in an organ, comprising: introducing the genetically modified bacterial cell to the organ *in vivo*, wherein the genetically modified bacterial cell utilizes as a carbon source a rare carbohydrate of interest, wherein less than 50% (e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of other bacterial cells present in the organ utilize the rare carbohydrate of interest as a carbon source.

4. A method for displacing at least a portion of a population of bacterial cells in an organ, comprising: introducing to the organ a genetically modified bacterial cell capable of controlled entrenchment and/or controlled colonization, and displacing at least a portion of the population of bacterial cells with the genetically modified bacterial cell.

5. The method of 4, wherein the genetically modified bacterial cell and the bacterial cells in the population of bacterial cells are the same species.

6. The method of 4 or 5, further comprising providing a bacterial cell from the population of bacterial cells and removing or inactivating one or more genes in the bacterial cell, thereby generating the genetically modified bacterial cell.

7. The method of 3 or 4, wherein the organ is a human organ.

8. The method of any one of the preceding, wherein the organ is a gut.

9. The method of 1 or 4, wherein the controlled entrenchment and/or controlled colonization is controlled by a high expression promoter.

10. The method of 1 or 4, wherein the controlled entrenchment and/or controlled colonization is controlled by an inducible promoter.

11. The method of 1 or 4, wherein the controlled entrenchment and/or controlled colonization is controlled by delivering a rare carbohydrate of interest to the organ.

12. The method of 11, wherein the rare carbohydrate of interest is a carbohydrate that can be utilized as a carbon source by less than 50% (e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of other bacterial cells present in the organ, thereby generating a privileged niche.

13. The method of any one of 2-3 and 11-12, wherein the rare carbohydrate of interest is an isolated carbohydrate.

14. The method of any one of 2-3 and 11-13, wherein the rare carbohydrate of interest is a polysaccharide.

15. The method of any one of 2-3 and 11-14, wherein the rare carbohydrate of interest is a sulfated carbohydrate.

16. The method of any one of 2-3 and 11-14, wherein the rare carbohydrate of interest is

selected from the group consisting of porphyran, agarose, carrageenan, and any combination thereof.

17. The method of any one of 2-3 and 11-16, wherein the rare carbohydrate of interest is a carbohydrate cleaved by a glycoside hydrolase belonging to glycoside hydrolase family GH86.

18. The method of any one of 2-3 and 11-17, wherein the rare carbohydrate of interest is a sulfated polygalactan.

19. The method of any one of the preceding, wherein the genetically modified bacterial cell is a genetically modified gut resident bacterial cell.

20. The method of any one of the preceding, wherein the genetically modified bacterial cell is in the genus *Bacteroides*.

21. The method of any one of the preceding, wherein the genetically modified bacterial cell is selected from *B. acidifaciens*, *B. amylophilus*, *B. asaccharolyticus*, *B. barnesiae*, *B. bivius*, *B. buccae*, *B. buccalis*, *B. caccae*, *B. capillosus*, *B. capillus*, *B. cellulosilyticus*, *B. cellulosolvens*, *B. chinchilla*, *B. clarus*, *B. coagulans*, *B. coprocola*, *B. coprophilus*, *B. coprosuis*, *B. corporis*, *B. denticola*, *B. disiens*, *B. distasonis*, *B. dorei*, *B. eggerthii*, *B. endodontalis*, *B. faecichinchillae*, *B. faecis*, *B. finegoldii*, *B. fluxus*, *B. forsythus*, *B. fragilis*, *B. furcosus*, *B. galacturonicus*, *B. gallinarum*, *B. gingivalis*, *B. goldsteinii*, *B. gracilis*, *B. graminisolvens*, *B. helcogenes*, *B. heparinolyticus*, *B. hypermegas*, *B. intermedius*, *B. intestinalis*, *B. johnsonii*, *B. levvi*, *B. loescheii*, *B. macacae*, *B. massiliensis*, *B. melaninogenicus*, *B. merdae*, *B. microfus*, *B. multiacidus*, *B. nodosus*, *B. nordii*, *B. ochraceus*, *B. oleiciplenus*, *B. oralis*, *B. oris*, *B. oulorum*, *B. ovatus*, *B. paurosaccharolyticus*, *B. pectinophilus*, *B. pentosaceus*, *B. plebeius*, *B. pneumosintes*, *B. polypragmatus*, *B. praeacutus*, *B. propionificiens*, *B. putredinis*, *B. pyogenes*, *B. reticulotermitis*, *B. rodentium*, *B. ruminicola*, *B. salanitronis*, *B. salivorus*, *B. salyersiae*, *B. sartorii*, *B. splanchnicus*, *B. stercorisoris*, *B. stercoris*, *B. succinogenes*, *B. suis*, *B. tectus*, *B. termitidis*, *B. thetaiotaomicron*, *B. uniformis*, *B. ureolyticus*, *B. veroralis*, *B. vulgatus*, *B. xylanisolvens*, *B. xylanolyticus*, *B. zoogloformans*, and any combination thereof.

22. The method of any one of the preceding, wherein the genetically modified bacterial cell comprises a carbohydrate-utilization gene or gene set.

23. The method of 22, wherein the carbohydrate-utilization gene or gene set comprises one or more genes selected from the group consisting of porphyranase, glycoside hydrolase, sulfatase, galactosidase, and any combination thereof.

24. The method of 22, wherein the carbohydrate-utilization gene or gene set comprises one or more nucleic acids encoding proteins that have 80% or more sequence identity with BACPLE_1683-1706 from the *B. plebeius* genome.

25. The method of any one of 22-24, wherein the carbohydrate-utilization gene set comprises at least six genes.

26. The method of any one of 22-25, wherein the carbohydrate of interest cannot be utilized as a carbon source by the genetically modified bacterial cell in the absence of the carbohydrate-utilization gene or gene set.
27. The method of any one of 22-26, wherein the carbohydrate-utilization gene or gene set is heterologous.
28. The method of 27, wherein the carbohydrate-utilization gene or gene set is encoded on a plasmid, encoded on a bacterial artificial chromosome, or genomically integrated.
29. The method of any one of the preceding, wherein the genetically modified bacterial cell is capable of treating a metabolic disease or disorder.
30. The method of any one of the preceding, wherein the genetically modified bacterial cell comprises one or more therapeutic transgenes.
31. The method of 30, wherein the one or more therapeutic transgenes comprise one or more enzymes.
32. The method of any one of 2-3 and 11-18, further comprising administering the rare carbohydrate of interest for a sufficient amount of time and at a sufficient frequency to establish a population of the genetically modified bacterial cell.
33. The method of 32, further comprising maintaining the population of the genetically modified bacterial cell in the organ for at least 5 days.
34. The method of any one of 2-3 and 11-18, further comprising administering the rare carbohydrate of interest for at least 3 days.
35. The method of any one of the preceding, wherein the genetically modified bacterial cell is introduced orally.
36. The method of any one of the preceding, wherein the organ comprises at least 5 other bacterial species.
37. The method of any one of the preceding, further comprising colonizing the organ with the genetically modified bacterial cell at a level of at least 1% of total bacterial cells in the organ.
38. A genetically modified bacterial cell that utilizes as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% (e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of bacterial cells present in a human microbiota.
39. A genetically modified bacterial cell, comprising:
 - a heterologous carbohydrate-utilization gene or gene set that provides the genetically modified bacterial cell with an ability to utilize as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% (e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than

0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of bacterial cells present in a human microbiota.

40. A genetically modified bacterial cell, comprising:

(i) a heterologous therapeutic transgene; and

(ii) a carbohydrate-utilization gene or gene set that provides the genetically modified bacterial cell with an ability to utilize as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% (e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of bacterial cells present in a human microbiota.

41. The genetically modified bacterial cell of any one of 38-40, wherein the rare carbohydrate of interest is an isolated carbohydrate.

42. The genetically modified bacterial cell of any one of 38-41, wherein the rare carbohydrate of interest is a polysaccharide.

43. The genetically modified bacterial cell of any one of 38-42, wherein the rare carbohydrate of interest is a sulfated carbohydrate.

44. The genetically modified bacterial cell of any one of 38-42, wherein the rare carbohydrate of interest is selected from the group consisting of porphyran, agarose, carrageenan, and any combination thereof.

45. The genetically modified bacterial cell of any one of 38-44, wherein the rare carbohydrate of interest is a carbohydrate cleaved by a glycoside hydrolase belonging to glycoside hydrolase family GH86.

46. The genetically modified bacterial cell of any one of 38-45, wherein the rare carbohydrate of interest is a sulfated polygalactan.

47. The genetically modified bacterial cell of any one of 38-46, wherein the genetically modified bacterial cell is a genetically modified gut resident bacterial cell.

48. The genetically modified bacterial cell of any one of 38-47, wherein the genetically modified bacterial cell is in the genus *Bacteroides*.

49. The genetically modified bacterial cell of any one of 38-48, wherein the genetically modified bacterial cell is selected from *B. acidifaciens*, *B. amylophilus*, *B. asaccharolyticus*, *B. barnesi*, *B. bivia*, *B. buccae*, *B. buccalis*, *B. caccae*, *B. capillosus*, *B. capillus*, *B. cellulosilyticus*, *B. cellulosolvens*, *B. chinchilla*, *B. clarus*, *B. coagulans*, *B. coprocola*, *B. coprophilus*, *B. coprosuis*, *B. corporis*, *B. denticola*, *B. disiens*, *B. distasonis*, *B. dorei*, *B. eggerthii*, *B. endodontalis*, *B. faecichinchillae*, *B. faecis*, *B. finegoldii*, *B. fluxus*, *B. forsythus*, *B. fragilis*, *B. furcosus*, *B. galacturonicus*, *B. gallinarum*, *B. gingivalis*, *B. goldsteinii*, *B. gracilis*, *B. graminisolvens*, *B. helcogenes*, *B. heparinolyticus*, *B. hypermegas*, *B. intermedius*, *B. intestinalis*, *B. johnsonii*, *B. levvi*, *B. loescheii*, *B. macacae*, *B. massiliensis*, *B. melaninogenicus*, *B. merdae*, *B. microfus*, *B. multiacidus*, *B. nodosus*, *B. nordii*, *B.*

ochraceus, *B. oleiciplenus*, *B. oralis*, *B. oris*, *B. oulorum*, *B. ovatus*, *B. paurosaccharolyticus*, *B. pectinophilus*, *B. pentosaceus*, *B. plebeius*, *B. pneumosintes*, *B. polypragmatus*, *B. praeacutus*, *B. propionificiens*, *B. putredinis*, *B. pyogenes*, *B. reticulotermis*, *B. rodentium*, *B. ruminicola*, *B. salanitronis*, *B. salivosus*, *B. salyersiae*, *B. sartorii*, *B. splanchnicus*, *B. stercorisoris*, *B. stercoris*, *B. succinogenes*, *B. suis*, *B. tectus*, *B. termitidis*, *B. thetaiotaomicron*, *B. uniformis*, *B. ureolyticus*, *B. veroralis*, *B. vulgatus*, *B. xylanisolvans*, *B. xylanolyticus*, *B. zoogloformans*, and any combination thereof.

50. The genetically modified bacterial cell of 38, wherein the genetically modified bacterial cell comprises a carbohydrate-utilization gene or gene set.

51. The genetically modified bacterial cell of 39, 40, or 50, wherein the carbohydrate-utilization gene or gene set comprises one or more genes selected from the group consisting of porphyranase, glycoside hydrolase, sulfatase, galactosidase, and any combination thereof.

52. The genetically modified bacterial cell of any one of 39-40 and 50-51, wherein the carbohydrate-utilization gene or gene set comprises one or more nucleic acids encoding proteins that have 80% or more sequence identity with BACPLE_1683-1706 from the *B. plebeius* genome.

53. The genetically modified bacterial cell of any one of 39-40 and 50-52, wherein the carbohydrate-utilization gene set comprises at least six genes.

54. The genetically modified bacterial cell of any one of 39-40 and 50-53, wherein the carbohydrate of interest cannot be utilized as a carbon source by the genetically modified bacterial cell in the absence of the carbohydrate-utilization gene or gene set.

55. The genetically modified bacterial cell of 40 or 50, wherein the carbohydrate-utilization gene or gene set is heterologous.

56. The genetically modified bacterial cell of 39 or 55, wherein the carbohydrate-utilization gene or gene set is encoded on a plasmid, encoded on a bacterial artificial chromosome, or genomically integrated.

57. The genetically modified bacterial cell of any one of 38-56, wherein the genetically modified bacterial cell is capable of treating a metabolic disease or disorder.

58. The genetically modified bacterial cell of any one of 38-57, wherein the genetically modified bacterial cell comprises one or more therapeutic transgenes.

59. The genetically modified bacterial cell of 58, wherein the one or more therapeutic transgenes comprise one or more enzymes.

60. A method comprising:

providing a bacterial cell; and

genetically modifying the bacterial cell to utilize as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% (e.g., less than

30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of bacterial cells present in a human microbiome.

61. A method comprising:

providing a bacterial cell that utilizes as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% (e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of bacterial cells present in a human microbiome; and

genetically modifying the bacterial cell to express one or more therapeutic transgenes.

62. The method of 60 or 61, wherein the rare carbohydrate of interest is an isolated carbohydrate.

63. The method of any one of 60-62, wherein the rare carbohydrate of interest is a polysaccharide.

64. The method of any one of 60-63, wherein the rare carbohydrate of interest is a sulfated carbohydrate.

65. The method of any one of 60-63, wherein the rare carbohydrate of interest is selected from the group consisting of porphyran, agarose, carrageenan, and any combination thereof.

66. The method of any one of 60-65, wherein the rare carbohydrate of interest is a carbohydrate cleaved by a glycoside hydrolase belonging to glycoside hydrolase family GH86.

67. The method of any one of 60-66, wherein the rare carbohydrate of interest is a sulfated polygalactan.

68. The method of any one of 60-67, wherein the genetically modified bacterial cell is a genetically modified gut resident bacterial cell.

69. The method of any one of 60-68, wherein the genetically modified bacterial cell is in the genus *Bacteroides*.

70. The method of any one of 60-69, wherein the genetically modified bacterial cell is selected from *B. acidifaciens*, *B. amylophilus*, *B. asaccharolyticus*, *B. barnesiae*, *B. bivius*, *B. buccae*, *B. buccalis*, *B. caccae*, *B. capillosus*, *B. capillus*, *B. cellulosilyticus*, *B. cellulosolvens*, *B. chinchilla*, *B. clarus*, *B. coagulans*, *B. coprocola*, *B. coprophilus*, *B. coprosuis*, *B. corporis*, *B. denticola*, *B. disiens*, *B. distasonis*, *B. dorei*, *B. eggerthii*, *B. endodontalis*, *B. faecichinchillae*, *B. faecis*, *B. finegoldii*, *B. fluxus*, *B. forsythus*, *B. fragilis*, *B. furcosus*, *B. galacturonicus*, *B. gallinarum*, *B. gingivalis*, *B. goldsteinii*, *B. gracilis*, *B. graminisolvens*, *B. helcogenes*, *B. heparinolyticus*, *B. hypermegas*, *B. intermedius*, *B. intestinalis*, *B. johnsonii*, *B. levvi*, *B. loescheii*, *B. macacae*, *B. massiliensis*, *B. melaninogenicus*, *B. merdae*, *B. microfus*, *B. multiacidus*, *B. nodosus*, *B. nordii*, *B. ochraceus*, *B. oleiciplenus*, *B. oralis*, *B. oris*, *B. oulorum*, *B. ovatus*, *B. paurosaccharolyticus*, *B. pectinophilus*, *B. pentosaceus*, *B.*

plebeius, *B. pneumosintes*, *B. polypragmatus*, *B. praeacutus*, *B. propionicifaciens*, *B. putredinis*, *B. pyogenes*, *B. reticulotermis*, *B. rodentium*, *B. ruminicola*, *B. salanitronis*, *B. salivorus*, *B. salyersiae*, *B. sartorii*, *B. splanchnicus*, *B. stercorisoris*, *B. stercoris*, *B. succinogenes*, *B. suis*, *B. tectus*, *B. termitidis*, *B. thetaiotaomicron*, *B. uniformis*, *B. ureolyticus*, *B. veroralis*, *B. vulgatus*, *B. xylanisolvans*, *B. xylanolyticus*, *B. zoogloformans*, and any combination thereof.

71. The method of any one of 60-70, wherein the genetically modified bacterial cell comprises a carbohydrate-utilization gene or gene set.
72. The method of 71, wherein the carbohydrate-utilization gene or gene set comprises one or more genes selected from the group consisting of porphyranase, glycoside hydrolase, sulfatase, galactosidase, and any combination thereof.
73. The method of 71 or 72, wherein the carbohydrate-utilization gene or gene set comprises one or more nucleic acids encoding proteins that have 80% or more sequence identity with BACPLE_1683-1706 from the *B. plebeius* genome.
74. The method of any one of 71-73, wherein the carbohydrate-utilization gene set comprises at least six genes.
75. The method of any one of 71-74, wherein the carbohydrate of interest cannot be utilized as a carbon source by the genetically modified bacterial cell in the absence of the carbohydrate-utilization gene or gene set.
76. The method of any one of 71-75, wherein the carbohydrate-utilization gene or gene set is heterologous.
77. The method of any one of 71-76, wherein the carbohydrate-utilization gene or gene set is encoded on a plasmid, encoded on a bacterial artificial chromosome, or genomically integrated.
78. The method of any one of 60-77, wherein the genetically modified bacterial cell is capable of treating a metabolic disease or disorder.
79. The method of 61, wherein the one or more therapeutic transgenes comprise one or more enzymes.
80. A method of facilitating colonization by a genetically modified bacterial cell, the method comprising:
 - (a) introducing a genetically modified bacterial cell into a gut of an individual, wherein the genetically modified bacterial cell comprises a heterologous carbohydrate-utilization gene or gene set that provides the genetically modified bacterial cell with the ability to use a carbohydrate of interest as a carbon source; and
 - (b) administering the carbohydrate of interest to the individual, thereby providing the genetically modified bacterial cell with the carbon source.
81. The method of 80, wherein the genetically modified bacterial cell is a genetically

modified gut resident bacterial cell.

82. The method of 80 or 81, wherein the genetically modified bacterial cell is a *Bacteroides* cell.

83. The method of 82, wherein the *Bacteroides* cell is a *B. fragilis* (Bf), *B. distasonis* (Bd), *B. thetaiotaomicron* (Bt), *B. vulgatus* (Bv), *B. ovatus* (Bo), *B. eggerrthii* (Be), *B. merdae* (Bm), *B. stercoris* (Bs), *B. uniformis* (Bu), or *B. caccae* (Bc) cell.

84. The method of any one of 80-83, wherein the carbohydrate of interest cannot be utilized as a carbon source by the genetically modified bacterial cell in the absence of the heterologous carbohydrate-utilization gene or gene set.

85. The method of any one of 80-84, wherein the carbohydrate of interest is utilized as a carbon source by less than 50% (e.g., less than 30%, less than 10%, less than 3%, less than 1%, less than 0.3%, less than 0.1%, less than 0.03%, less than 0.01%, less than 0.003%, less than 0.001%, less than 0.0001%, or none) of other gut bacterial cells present in the gut of the individual.

86. The method of any one of 80-85, wherein the carbohydrate of interest is porphyran.

87. The method of 86, wherein the heterologous carbohydrate-utilization gene or gene set comprises one or more nucleic acids encoding the proteins that have 80% or more sequence identity with BACPLE_1683-1706 from the *B. plebeius* genome.

88. The method of any one of 80-87, wherein the genetically modified bacterial cell is a therapeutic bacterial cell.

89. The method of any one of 80-88, wherein the genetically modified bacterial cell comprises a nucleic acid molecule comprising a heterologous nucleic acid sequence that encodes a therapeutic polypeptide.

90. A genetically modified bacterial cell, comprising:

a heterologous carbohydrate-utilization gene or gene set that provides the genetically modified bacterial cell with the ability to use a rare carbohydrate of interest as a carbon source.

91. The genetically modified bacterial cell of 90, wherein the genetically modified bacterial cell is a genetically modified gut resident bacterial cell.

92. The genetically modified bacterial cell of 90 or 91, wherein the genetically modified bacterial cell is a *Bacteroides* cell.

93. The genetically modified bacterial cell of 92, wherein the *Bacteroides* cell is a *B. fragilis* (Bf), *B. distasonis* (Bd), *B. thetaiotaomicron* (Bt), *B. vulgatus* (Bv), *B. ovatus* (Bo), *B. eggerrthii* (Be), *B. merdae* (Bm), *B. stercoris* (Bs), *B. uniformis* (Bu), or *B. caccae* (Bc) cell.

94. The genetically modified bacterial cell of any one of 90-93, wherein the carbohydrate of interest cannot be utilized as a carbon source by the genetically modified bacterial cell in the absence of the heterologous carbohydrate-utilization gene or gene set.

95. The genetically modified bacterial cell of any one of 90-94, wherein the carbohydrate of

interest is porphyran.

96. The genetically modified bacterial cell of 95, wherein the heterologous carbohydrate-utilization gene or gene set comprises one or more nucleic acids encoding the proteins that have 80% or more sequence identity with BACPLE_1683-1706 from the *B. plebeius* genome.

97. The genetically modified bacterial cell of any of 90-96, wherein the genetically modified bacterial cell is a therapeutic bacterial cell.

98. The genetically modified bacterial cell of any of 90-97, wherein the genetically modified bacterial cell comprises a nucleic acid molecule comprising a heterologous nucleic acid sequence that encodes a therapeutic polypeptide.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1

To facilitate robust colonization, a privileged niche was created by introducing a carbohydrate into the diet that can only be utilized by the target bacteria. Remarkably, a single substrate was sufficient for boosting the colonization level of an introduced strain to more than half of the total microbes in the gut of conventional mice, which are expected to harbor hundreds of distinct bacterial strains. In addition to boosting total colonization levels, the data presented herein show that this privileged niche results in more predictable levels of target bacteria in different microbial communities. Finally, the data presented herein demonstrate that the ability to utilize the carbohydrate can be transferred to a naïve species.

Results

Selection of polysaccharides suitable for establishing a privileged niche

It was hypothesized that establishing a privileged niche that is inaccessible to other gut

microbes would enable a predictably high level of colonization in the gut by an introduced bacteria capable of occupying the privileged niche. Most substrates that are known to be safely consumable by humans and to be capable of supporting growth of bacteria are readily consumed by the host or native bacterial species in the gut and thus do not produce a privileged niche. Due to the wide diversity in carbohydrate utilization capabilities amongst gut bacteria, carbohydrates were chosen as the substrate for creating a privileged niche. It was reasoned that carbohydrates that are uncommon in the diet and are either rarely or not consumed by the gut bacteria in the human population of interest would be suitable substrates for creating a privileged niche. The marine carbohydrates porphyran and agarose are both known to be safe to consume yet are rare in Western diets. The glycoside hydrolase family containing agarases and porphyranases, GH86, was found to be underrepresented in deeply-sequenced metagenomic stool samples from healthy adult Americans in the Human Microbiome Project (Figure 1). Thus, these carbohydrates are likely to not be consumed by common American gut bacteria. In contrast to GH86, which was completely absent in 44% of samples and has a mean abundance of 0.00015, GH32, which hydrolyzes commonly consumed substrates such as the fructan polysaccharide inulin, was present in all samples and had a mean abundance of 0.0049.

Isolation of strains capable of consuming privileged substrates

To isolate a strain of gut bacteria from the environment that was capable of growing on a carbohydrate of interest, a minimal media (see Salyers et al., Appl. Environ. Microbiol. 33, 319–322 (1977)) was designed for *Bacteroides*, the most abundant bacterial genus present in the Western gut, in which the carbohydrate of interest was the sole carbon source. Strains capable of utilizing porphyran, NB001, (Figure 2, panel A) or agarose, NB002 and NB003, were isolated from the environment and the genomes of these strains were sequenced (Table 1). The porphyran utilizing strain NB001 contained a putative polysaccharide utilization locus (PUL) (SEQ ID NO: 67) on a mobile element that shares 71% identity with a previously identified porphyran-utilizing strain (Hehemann et al., Nature 464, 908–912 (2010); Hehemann et al., Proc. Natl. Acad. Sci. U. S. A. 109, 19786–19791 (2012)). The two agarose utilizers were found to have a putative PUL (SEQ ID NO: 68 and 69) sharing over 99% identity between the two strains and include genes with high homology to agarases of marine bacteria.

Table 1. Description of bacterial strains.

Name	Description	Species	Source
NB001	Porphyran consuming <i>Bacteroides</i>	<i>Bacteroides ovatus</i>	environmental isolate

NB002	Agarose consuming Bacteroides	<i>Bacteroides dorei</i>	environmental isolate
NB003	Agarose consuming Bacteroides	<i>Bacteroides uniformis</i>	environmental isolate
NB004	Porphyran naïve Bacteroides	<i>Bacteroides vulgatus</i>	environmental isolate
NB006	NB001 with GFP/Erm marker	<i>Bacteroides ovatus</i>	NB001 + pWW124 (SEQ ID NO: 60)
NB007	Porphyran strain with tdk deletion	<i>Bacteroides ovatus</i>	NB001 + UV mut.
NB008	Knockout of porphyran utilization	<i>Bacteroides ovatus</i>	NB001 +/- pWD034 (SEQ ID NO: 61)
NB009	NB004 + Short Porphyran PUL	<i>Bacteroides vulgatus</i>	NB004 + pWD037 (SEQ ID NO: 62)
NB010	NB004 + Medium Porphyran PUL	<i>Bacteroides vulgatus</i>	NB004 + pWD036 (SEQ ID NO: 63)

Porphyran establishes a privileged niche resulting in robust colonization

An *in vitro* experiment was performed to test the hypothesis that addition of porphyran can increase the ability of an introduced porphyran utilizing bacteria to colonize a complex community at a high (and predictable) level. The strain NB006 was created by modifying NB001 to contain an erythromycin resistance cassette expressing GFP to facilitate monitoring abundance via plating. NB006 was added to the complex communities, which were then grown and serially passaged in rich media either in the presence or absence of porphyran (Figure 2, panel B). In the absence of porphyran, NB006 colonization levels reached widely variable abundances across the 8 complex communities, spanning a 760-fold range by the seventh day. In the presence of porphyran, not only did the abundance of NB006 increase for all eight communities, but the deviation in abundance between the different communities also decreased considerably, spanning only a 3-fold range by the seventh day.

Next tested was the ability of porphyran to increase the abundance of NB006 in mice harboring a conventional complex microbiota (Figure 2). One group of mice was given the standard (STD) rodent diet, while a second group was fed a polysaccharide deficient (PD) diet. After three days on each diet, both groups of mice received NB006. When both groups received 1% porphyran in the drinking water, abundance of NB006 increased by almost 3 logs in mice on the STD diet, and almost 4 logs in mice on the PD diet. Based on monitoring total anaerobic colony forming units, this represents an increase in NB006 from roughly 0.1% of

total anaerobic CFU in both groups, to approximately 25% of total in mice fed the STD diet and 48% in mice fed the PD diet.

Additionally tested was the ability of porphyran to allow NB006 to stably colonize a microbiota that resists colonization. Mice were administered NB008, an isogenic strain to NB006 that cannot utilize porphyran, and this rendered them resistant to colonization by NB006 as this strain was cleared from the mice within five days after introduction (Figure 2, panel D). When mice were administered porphyran for three days simultaneous to introduction of NB006, NB008 decreased in abundance by three logs while NB006 remained constant and highly abundant (roughly 67% of total anaerobic CFU). After removal of porphyran, NB008 increased back to pre-challenge levels while NB006 decreased to one log lower abundance than NB008. After re-introduction of porphyran for seven days, NB006 displaced NB008 in a stable fashion, maintaining colonization of the mice in the absence of porphyran for thirty days. Additionally, NB008 was now excluded from the mice when re-introduced (Figure 2, panel E).

Transferring the key porphyran utilization genes is sufficient for conferring porphyran utilization to a naïve species

To confirm that the mobile element of interest in NB001 (see SEQ ID NO: 67) was necessary for the ability to grow on porphyran as a sole carbon source, an 8-gene operon was deleted that contained key glycoside hydrolases predicted to act on porphyran and constituted 16% of the mobile element. This deletion was sufficient to eliminate the ability to grow on porphyran (Figure 3).

The porphyran mobile element from NB001 contains an approximately 30kb deletion relative to the homologous mobile element from *B. plebeius*, suggesting many of the genes in the region are likely not to be involved in porphyran utilization (Figure. 4). To identify a minimal gene set necessary for porphyran utilization, portions of the PUL were synthesized of varying sizes and were integrated into the genome of a *Bacteroides* strain not capable of growth on porphyran (NB004). Initially protocols used previously to transfer a five gene PUL into a *Bacteroides* were followed, but it was found that the much larger porphyran PUL (up to 34 genes and 60kb) was not compatible with previously described techniques. Instead, the different sized porphyran PULs (Figure 4) were assembled into a plasmid with components necessary for yeast assembly, *E. coli* conjugation and *Bacteroides* genomic integration (Figure 5), which was sufficient to integrate correctly assembled porphyran PULs into naive *Bacteroides*. The shortest PUL candidate, containing 10 genes including both of the predicted porphyranases in the PUL, was not sufficient to impart porphyran utilization. However, when expanded to 24 of the 34 total genes, integration of this medium-sized PUL was sufficient to confer growth on porphyran (Figure 6).

DISCUSSION

Using a privileged niche strategy, an unprecedentedly high colonization level of an introduced strain was obtained in a complex community. Combining two or more privileged niches, potentially using dietary substrates from distinct geographic regions, could further
5 broaden the effectiveness of this strategy to allow robust colonization in the event that one of the two rare substrates is utilized by an individual's endogenous microbiota. Marine carbohydrates (e.g. porphyran, agarose, agarpectin, carrageenan, and marine microbe exopolysaccharides) offer promising substrates to establish a synthetic niche, as they are not typically found in human diets and thus are less likely to be utilized by human gut microbes. As
10 demonstrated in this disclosure, marine bacteria possess utilization genes that can be transferred to gut adapted species. For example, we were able to demonstrate this with the porphyran PUL (SEQ ID NO: 67) and with putative agarose utilization PULs (SEQ ID NO: 68 and 69), which conferred growth on agarose when present natively or when transferred to a naïve strain. Marine carbohydrates are typically easy to extract and generally safe to
15 consume.

This strategy for facilitating robust colonization can facilitate a range of therapeutic applications where it is important to establish a high or a predictable number of natural or engineered therapeutic microbes. For example, it may be important to colonize the gut at a high enough level to ensure that therapeutic activities may overcome competing activities of
20 other microbes. As an example, this may be important when attempting to, for instance, change the short chain fatty acid profile or reduce the accumulation of harmful chemicals that are produced in the gut. Achieving predictable colonization can also be important for therapeutics that must be carefully dosed, such as bacteria with pro- or anti-inflammatory activities which could be used as an adjuvant for cancer immunotherapy or in the treatment of
25 Inflammatory Bowel Diseases. This method of ensuring robust colonization could also be used to displace a harmful species by establishing a non-harmful version that can effectively compete for limiting resources with the harmful strain.

Materials and Methods

30 Identifying rare glycoside hydrolase subfamilies

87 fecal metagenomes sequenced by the HMP were downloaded and reprocessed (quality filtered and dynamically trimmed). These metagenomic sequences were then translated to their respective coding sequences and Hidden Markov Models were applied to identify all CAZymes. The data were normalized to the total count of assigned CAZyme
35 coding sequences per sample.

Porphyran extraction

Culinary Nori was added to ten times the weight of water and autoclaved for approximately 3 hours. The autoclaved Nori was then strained to remove particulates and then diluted five fold into 100% ethanol and allowed to settle for six hours. This was then
5 centrifuged for 15 minutes at 30,000 g to pellet the precipitate, which was then dried at 80°C.

Isolation of porphyran and agarose utilizing strains

A bacteroides minimal media was prepared as described by Salyers *et al* (see Salyers et al., Appl. Environ. Microbiol. 33, 319–322 (1977)) with addition of 200 µg/ml Gentamycin
10 and porphyran as 0.8% Nori extract added as the sole carbon source. Primary sewage effluent was collected, settled for approximately two hours and diluted ten-fold into the media, which was then incubated anaerobically for 24 hours at 37°C. The culture was then further diluted 200-fold into the fresh media and incubated another 24 hours anaerobically at 37°C. The saturated culture was then plated as serial dilutions onto Blood-Heart-Infusion media + 10%
15 horse blood agar plates and incubated 24 hours anaerobically at 37°C. Colonies were then picked into fresh media, incubated 24 hours anaerobically at 37°C and prepared for analysis and cryogenic storage.

Assaying abundance of porphyran utilizer in complex cultures

20 An erythromycin resistance cassette was genomically integrated into NB001. The abundance of this strain, NB006, within each complex community could then be easily assessed via selective plating. The eight communities and NB006 were separately grown in rich media, and then NB006 was added to each community at a 1:20 dilution. The complex communities containing NB006 were subsequently grown and diluted 1:1000 daily in rich
25 media either with or without a supplementation of 0.4% Nori extract, a culturing strategy that mimics the requirement for ongoing division necessary to sustain colonization of the gut. To determine the CFU/µL of NB006, 10 µL of each saturated culture was diluted into 200 µL of PBS, further successively diluted 1:10 in PBS seven times, 4 µL of each dilution was spotted onto a plate containing Brain Heart Infusion Blood agar with 200 µg/mL gentamycin and 10
30 µg/mL erythromycin, and after 24 hours of anaerobic growth at 37°C colony counts were enumerated. Both significantly increased abundance and reduced variance of NB006 across the eight communities was observed over the course of a week.

Assaying abundance of porphyran utilizer in mice

35 To test ability of porphyran to toggle abundance of NB006 *in vivo*, one group of four male Restricted Flora Swiss-Webster mice (Taconic) were maintained on standard rodent

chow (STD diet), while an identical group was given a polysaccharide deficient diet (Bio-Serv, AIN-93G 68% glucose). Both groups were administered approximately 10^8 CFU of NB006. Abundance of NB006 in the feces was monitored by selective plating on Brain Heart Infusion Blood agar with 200 µg/mL gentamicin and 10 µg/mL erythromycin. Both groups received
 5 regular water for five days, 1% porphyrin in the drinking water for five days, and then were switched back to regular water for the duration of the experiment.

To test ability of porphyrin to allow for stable colonization of a porphyrin utilizer into a resistant microbiota, two groups of three male Restricted Flora Swiss-Webster mice (Taconic) were administered NB008, an isogenic strain to NB006 that cannot use porphyrin.

10 Abundance of NB008 was monitored by introducing a tetracycline-resistance cassette and selectively plating on Brain Heart Infusion Blood agar with 200 µg/mL gentamicin and 2µg/mL tetracycline. After one week, both groups of mice received NB006 and one group received 1% porphyrin in the drinking water for three days, regular water for seven days, and then 1% porphyrin in the drinking water for seven days, until being switched back to and maintained on
 15 regular water for the duration of the experiment. Abundance of NB006 was monitored by selectively plating as above with erythromycin selection. Finally, NB008 was re-introduced to mice at day 48 and monitored in the feces.

Genome sequencing and analysis

20 Genomic DNA was isolated from *Bacteroides* strains capable of growth on porphyrin (NB001) (SEQ ID NO: 67) or agarose (NB002, NB003) (SEQ ID NO: 68 and 69, respectively) using a PureLink Genomic DNA Mini Kit (Invitrogen). Samples were prepared for multiplexed Illumina sequencing using a Nextera XT DNA Library Preparation Kit (Illumina) and run on an Illumina MiSeq using a 2 x 150bp paired-end kit. Approximately 10 million sequencing reads
 25 were obtained for each sample. De novo assembly of the reads was performed with the Geneious De Novo Assembler (Biomatters), yielding an average coverage of ~100 reads/bp. Gene annotation and alignment was also performed using Geneious.

Cloning and transferring the porphyrin utilization genes.

30 Candidate porphyrin PUL regions from the conserved mobile element of NB001 were transferred to *Bacteroides vulgatus* (NB004) via a three-step process, which is summarized here and expanded on in the sections below. First, the PUL genes of interest were amplified via PCR and assembled via yeast assembly into a custom shuttle vector capable of propagation in *S. cerevisiae* and *E. coli* as well as conjugation and genomic integration into
 35 *Bacteroides* species. Next, correctly assembled plasmids were transferred from yeast to the S17-1 conjugation strain of *E. coli* (see Simon et al., Nat. Biotechnol. 1, 784–791 (1983)) by electroporation. Finally, the plasmids were integrated into the genome of *B. vulgatus* via

conjugation and antibiotic selection, followed by sequence-verification using whole genome sequencing.

Design of porphyran PUL plasmids

A homolog (71% identity) of a porphyran mobile element was identified in the NB001 genome using the Mauve genome aligner (see e.g., Hehemann et al., Proc. Natl. Acad. Sci. U. S. A. 109, 19786–19791 (2012)). Based on gene annotations and the sequence alignment between these two regions (Figure 4), three candidate regions of varying sizes (20kb, 40kb, and 60kb) were selected that were predicted to potentially be sufficient to confer growth on porphyran to a naive *Bacteroides* strain. The shortest of these candidate PULs contained homologs of BACPLE_1688-1699 from the *B. plebeius* genome, the medium-sized PUL contained homologs of BACPLE_1683-1706, and the longest PUL contained homologs of BACPLE_1669-1706 (see, e.g., Table 6).

Previously-described techniques for PUL transfer (Sonnenburg et al., Cell 141, 1241–1252 (2010)) were insufficient to move PULs of this size between species. Therefore, a novel PUL transfer strategy was designed that utilized a custom shuttle vector containing a yeast selectable marker and origin of replication, a bacterial artificial chromosome origin and selectable marker for plasmid-based propagation of large DNAs in *E. coli*, a conjugative origin of transfer to enable conjugation from *E. coli* to *Bacteroides* species, as well as an integrase and selectable marker to enable integration into the *Bacteroides* genome. This vector was divided into three precursor fragments with flanking homology such that only upon proper assembly would the vector be capable of propagation in yeast (Tables 3 and 4). The first fragment (Vector_01) was an *Ascl* digestion of pWD011, which was custom built to contain all *Bacteroides*-specific machinery as well all yeast vector components aside from a portion of the KanMX selectable marker. The remainder of the KanMX marker was supplied via the PCR product Vector_02, which also served to bridge Vector_01 and Vector_03. Vector_03 was an *Ascl* digest of pWD012, which was derived from a blunt religation of the commercially-available pEZ-BAC vector (Lucigen). The PUL candidates were divided into multiple 6kb fragments to be used in a yeast assembly reaction (Tables 3 and 5), and PCR primers were designed such that neighboring fragments would share ~200bps of homology (Table 2).

Table 2: List of oligonucleotides used to build the porphyran PUL plasmid

ID	Sequence	SEQ ID NO:
AA34	GGGTACAGAAAATCTCGGTC	35

ID	Sequence	SEQ ID NO:
AA35	TTCATCATGTCGTACGAAGG	36
AA36	TACTTCCATTTGGGGTGAAG	37
AA37	TACAGTCCCTTTGGACAATG	38
AA38	CATACTTTAGCATCGTCGAAAAG	39
AA39	TTTCCATTTCCAGGATTCCC	40
AA40	GTAGCTCCGGTGACATTTAC	41
AA41	TCTTTAGCTGAAGAAACGGC	42
AA42	AAGCTTGCGTATGTCGATAG	43
AA43	TTATCGCCATTCTTCAGCAG	44
AA44	TGGCATCCGACGAATATAAG	45
AA45	TTTGAATAGGCCAGTATGC	46
AA46	AGGTAAAGGCACTGTTTTCC	47
AA47	ATATAGCCGGAGATTCTCCG	48
AA48	CATCTACATCATGTCGGACG	49
AA49	CTGTCCGGTCATGATACATG	50
AA50	GATTCTCTTGGGGACAGAAC	51
AA51	AGTTTCCCATTTCACGTCTG	52
AE30	ATTTATCTATCCATTTCAGTTTGATTCTCAGG ACTTTACATCGTCCTGAAAGTATTTGTTtttggg tgttgatatggcag	53
AE31	ATTTATCTATCCATTTCAGTTTGATTCTCAGG ACTTTACATCGTCCTGAAAGTATTTGTTaatcc caatacagtctgttactg	54
AE45	GTGAGTTGATTGCTACGTAAATAACTTCGTA TAGCATACATTATACGAAGTTATGGACTAcgc aggccaatatccggaa	55
AE46	GTGAGTTGATTGCTACGTAAATAACTTCGTA TAGCATACATTATACGAAGTTATGGACTAggta aacctccccgatgg	56
AE47	GTGAGTTGATTGCTACGTAAATAACTTCGTA TAGCATACATTATACGAAGTTATGGACTAttag aacatattttccgattgccag	57
AE49	AAACAGCATTCCAGGTATTAGAAG	58

ID	Sequence	SEQ ID NO:
AE50	CACTGCCCGCTTTCCAGTCGGGAAACCTGT GCGGCCGCTTTCCTTCTTCTCTCTTCTGGc agtatagcgaccagcattc	59

Table 3: List of PCR products used to build the porphyran PUL plasmids.

ID	Length (bps)	Primer 1	Primer 2	Template	SEQ ID NO:
PCR_01	6,220	AA35	AA36	NB001 Genomic DNA	71
PCR_02	6,280	AA37	AA38	NB001 Genomic DNA	72
PCR_03	6,247	AA39	AA40	NB001 Genomic DNA	73
PCR_04	6,169	AA41	AA42	NB001 Genomic DNA	74
PCR_05	6,232	AA43	AA44	NB001 Genomic DNA	75
PCR_06	6,187	AA45	AA46	NB001 Genomic DNA	76
PCR_07	6,185	AA47	AA48	NB001 Genomic DNA	77
PCR_08	6,178	AA49	AA50	NB001 Genomic DNA	78
PCR_09	6,162	AE45	AA34	NB001 Genomic DNA	79
PCR_10	6,138	AA51	AE30	NB001 Genomic DNA	80
PCR_11	5,359	AE46	AA40	NB001 Genomic DNA	81
PCR_12	4,638	AE47	AA42	NB001 Genomic DNA	82
PCR_13	3,524	AA47	AE31	NB001 Genomic DNA	83
Vector_02	769	AE49	AE50	KanMX (SEQ ID NO: 70)	84

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Table 4: List of digests used to build the porphyran PUL plasmids.

ID	Length (bps)	Plasmid	Restriction Enzyme
Vector_01	4,730	pWD011 (SEQ ID NO: 64)	Ascl
Vector_03	7,234	pWD012 (pEZ-BAC) (SEQ ID NO: 65)	Ascl

Table 5: List of porphyran PUL plasmids.

Plasmid Name	Description	Length (bps)	Parts used in yeast assembly
pWD035 (SEQ ID NO: 66)	Long Porphyrin PUL	72,558	PCR_01, PCR_02, PCR_03, PCR_04, PCR_05, PCR_06, PCR_07, PCR_08, PCR_09, PCR_10, Vector_01, Vector_02, Vector_03
pWD036 (SEQ ID NO: 63)	Medium Porphyrin PUL	53,695	PCR_04, PCR_05, PCR_06, PCR_07, PCR_08, PCR_10, PCR_11, Vector_01, Vector_02, Vector_03
pWD037 (SEQ ID NO: 62)	Short Porphyrin PUL	32,364	PCR_05, PCR_06, PCR_12, PCR_13, Vector_01, Vector_02, Vector_03

Yeast assembly of porphyrin PUL plasmids

All fragments were prepared for yeast assembly via PCR with NEB Q5 polymerase (Table 3) or restriction digest with *Ascl* from NEB (Table 4). Cleanup reactions were performed with a Zymo Clean and Concentrator kit, and the resulting DNA concentrations were measured using a Nanodrop 1000 (Thermo Scientific). Yeast assembly of plasmids pWD035-37 (Table 5) was carried as described (see, e.g., Chandran et al., *Methods Mol. Biol.* 1472, 187–192 (2017)), using 100 fmols of each part in a lithium acetate transformation of the *S. cerevisiae* strain BY4741. After rescuing the transformed cells for 1 hour at 30°C in YPD media, cells were plated on YPD plates supplemented with G418 (200µg/ml). After incubation for 48 hours at 30°C, colonies were grown up in YPD+G418 and screened for successful plasmid assembly using colony PCR. Approximately 90% of screened clones appeared to contain a correctly assembled plasmid.

Transfer of porphyrin PUL plasmids from yeast to E. coli

Yeast strains harboring properly assembled plasmids were back diluted 1:100 into YPD+G418 and grown at 30°C for 6 hours to an OD of 2. 500µl of each culture was transferred to a microcentrifuge tube, centrifuged, and resuspended in 100µl of PBS buffer. The cell mixture was then vortexed alongside ~50 0.5mm glass disruptor beads (USA Scientific) for 5 minutes to lyse the cells. 5µl of the lysate was used in an electroporation of 100µl of electrocompetent S17-1 *E. coli*. Transformations were rescued for 1 hour in LB media with shaking at 37°C and then plated onto agar plates with LB+ampicillin (100µg/ml). For large plasmids, fewer than 20 colonies were often obtained from a single electroporation. Colonies were grown up in LB+ampicillin and screened for successful plasmid transfer via colony PCR.

Conjugation of porphyran PUL plasmids into B. vulgatus

The PUL plasmids were then transferred from S17-1 *E. coli* into the genome of an environmental isolate of *B. vulgatus* (NB004). It was observed that larger plasmids resulted in fewer conjugants on erythromycin-selective plates, with the largest plasmid (pWD035) not yielding any successful plasmid integrants. Despite this size bias, both pWD036 and pWD037 were successfully integrated into NB004 as verified by whole genome sequencing of the resulting strains.

***In vitro* growth assays**

Strains were grown overnight anaerobically at 37°C in a tryptone-yeast extract-glucose (TYG) medium to saturation. Saturated cultures were diluted 1:50 into Salyer's Minimal Media supplemented with either .2% glucose or .8% porphyran extract. The diluted cultures were grown anaerobically at 37°C for 12 hours, and 100µL of each culture was transferred to a 96-well microtiter plate. The optical density at 600nm of each culture was measured using a Wallac Victor 2 1420 Multilabel Counter. OD600 readings were normalized by subtracting out the background detected when media lacking cells was measured.

Example 2

Synthetic niche generation enables strain integration in the gut microbiota

The dense microbial ecosystem in the gut is intimately connected to numerous facets of human biology, and manipulation of the gut microbiota has broad implications for human health. In the absence of profound perturbation, the abundance of bacterial strains that reside within an individual is largely stable over time. In contrast, the fate of exogenous commensal and probiotic strains applied to an established microbiota is variable, largely unpredictable, and greatly influenced by the background microbiota.

In this example, a synthetic metabolic niche was generated via administration of a privileged nutrient source, and reliable integration of an exogenous *Bacteroides* strain at predictable abundances into mice harboring diverse communities of gut microbes was demonstrated. Dietary marine polysaccharides not accessible to other members of the gut community, but utilized by the introduced strain facilitated predictable engraftment and boosted abundance by more than three orders of magnitude, independent of background microbiota. This targeted dietary support was sufficient to overcome priority exclusion by an isogenic strain, and facilitated strain replacement. Transfer of this privileged nutrient-utilization system into a naïve strain of *Bacteroides* is demonstrated, and finely tuned control of strain abundance in the gut by varying polysaccharide input is shown. The data presented here highlight the influence of nutrient availability in shaping microbiota membership, expand the ability to perform a broad spectrum of methods in the context of a complex microbiota, and

facilitate cell-based therapeutic strategies in the gut.

Results and Discussion

Changes to the microbial membership of the highly competitive and dynamic gut microbiota can impact numerous aspects of host biology. Despite the importance of gut microbe composition in human health, the rules governing invasion of commensal strains into an existing complex community are not well understood. Resident strains often appear to exclude similar invading strains although in some cases, the opposite is true: the niche occupied by existing strains can be exploited by a similar invading strain. The inability to predict or control the outcome of fecal microbiota transplants illustrates the need for basic insight into the factors that influence whether new strains of bacteria can integrate into a pre-existing, complex microbiota.

To characterize the extent to which incoming exogenous bacteria variably colonize hosts with distinct microbiotas, mice harboring a conventional mouse microbiota were used as were two groups of ex-germ free mice, each colonized with the gut microbiota from two different healthy human donors from the United States ("humanized") as model hosts. These three groups of mice were administered a strain of *Bacteroides ovatus* (NB001), a prominent gut commensal species, which was modified genetically, adding erythromycin resistance and GFP-expression cassettes for tracking purposes. NB001 was monitored by colony forming units (c.f.u.) in feces via selective plating, and colonies were verified by GFP-fluorescence (see Methods) over the course of seven days (Figure 7a). The different communities (Figure 10) varied in their ability to integrate NB001 (Figure 7b), and one human microbiota was resistant to colonization, with all mice in the group exhibiting decreased abundance of the challenge strain over the seven day period.

Inulin was then administered to the three aforementioned groups of mice seven days after inoculation with NB001, which grows at a similar rate *in vitro* in a minimal medium containing either glucose or inulin as the sole carbohydrates (MM-glucose doubling time = 157 min; MM-inulin doubling time = 127 min; Figure 11b). Over seven days of feeding an inulin-based diet (10% inulin w/v) to these three groups of mice, NB001 exhibited highly variable responses across the background microbiotas (range of mean c.f.u. per ml in feces $<4 \times 10^4$ to 2×10^{10} ; Figure 7c). This variability may be due to several factors that differentiate the three microbiotas including the varying degrees of competition from other community members for this MAC, common in the Western diet.

In contrast, microbiota utilization of the non-ubiquitous seaweed polysaccharide porphyran, found in the seaweed *Porphyra yezoensis* used to prepare culinary nori, is much less common in US microbiotas. The consumption of porphyran by some *Bacteroides* species can be facilitated by a horizontally-transferred PUL, which originated in marine bacteria. We

hypothesized that this exotic dietary MAC would create a privileged niche within the gut and promote integration of an exogenous strain competent in its use. NB001 was capable of growth on porphyran *in vitro* (doubling time = 98 min; Figure 11c), and so a custom nori diet (10% dried *Porphyra yezoensis* w/v) was administered to the three groups of mice seven days after inoculation with NB001. Indeed, in response to nori in the diet a robust increase in abundance of NB001 was observed, irrespective of background microbiota. Additionally, the variability in colonization levels across communities was eliminated (range of mean c.f.u. per ml in feces 3×10^{10} to 6×10^{10} ; Figure 7d), indicating specificity of an exotic nutrient source and cognate utilization system to promote bacterial growth *in vivo*. Access to nori substrates rescued NB001 from below the limits of detection in the most resistant microbiota and boosted its abundance to levels indistinguishable from those achieved in the other two microbiotas (Figure 7d). Together these data suggest a powerful approach to reproducibly control strain integration independent of the background microbiota, and implicate nutrient availability as a key modulator of strain integration into the gut community.

Given the context independence of strain integration via access to nori polysaccharides, it was next tested whether the population size could be toggled by addition and removal of the substrate *in vivo*. Conventional mice were colonized with NB001 and c.f.u. in feces was tracked. The mice were switched in five day intervals between regular water, water with 1% porphyran extract, and a regular water washout. To test the influence of competing polysaccharides in the diet, this experiment was performed on both standard, MAC-rich chow and a MAC-deficient chow that provides no exogenous polysaccharides to the microbiota. In both dietary contexts, with or without other competing dietary MACs, NB001 responded robustly to introduction of marine polysaccharides, showing a large and highly reproducible increase in abundance of four orders of magnitude in the absence of dietary MACs (Figure 8a) and three orders of magnitude in the presence of diverse dietary MACs (Figure 8b). This response was contingent upon access to porphyran, as deletion of eight genes required for its metabolism abolished the effect (Figure 8c, Figure 9a, 9b). Additionally, the extract alone did not significantly affect the composition of the background microbiota (Figure 12), further supporting the lack of porphyran use by members of the background community.

Bacteroides species engage in interesting colonization behavior in which an early colonizer will exclude a challenging isogenic strain, the phenomenon known as priority effects in the ecological literature. We hypothesized that this behavior, though previously demonstrated in gnotobiotic mice, would also occur in the context of a complex community, and that it could be overcome by providing a privileged nutrient to the challenging strain to create a distinct metabolic niche. Indeed, an early-colonizing NB001 strain excluded an NB001 challenge strain in conventional mice (Figure 8d). Supplementing porphyran in the

diet, accessible only to the challenging strain, allowed the challenging strain to overcome the priority effect, and resulted in displacement of the early colonizer by the challenging strain (Figure 8e). Replacement of the early-colonizing strain required that the challenging strain have access to porphyrin (Figure 13), and displacement was robust to subsequent challenge by the original colonizer (Figure 13). Interestingly, when porphyrin was supplemented for shorter periods of time, replacement of the original strain was incomplete: the early-colonizer recovered from below the levels of detection and co-existed with the secondary colonizer after cessation of porphyrin administration (Figure 8f).

The genome of NB001 was sequenced and the polysaccharide utilization locus (PUL) was identified (Figure 9a). Three different length PULs were constructed, ranging from 10 to 34 of the genes (20-60kb) from the full-length PUL (Figure 9a). Previous methods to transfer a five gene PUL were not sufficient for these much larger constructs, and so the constructs were assembled in yeast before transferring to conjugative *E. coli*, and the constructs were integrated into the chromosome of two target strains unable to utilize porphyrin for growth, *Bacteroides stercoris* and *Bacteroides thetaiotaomicron*. Transfer of the shortest PUL was insufficient to impart growth on porphyrin to either naïve species of *Bacteroides* tested (Figure 9b), but the medium PUL allowed for growth *in vitro* (Figure 9b). A significant drop-off in conjugation efficiency was observed with the long PUL, and only *B. stercoris* yielded transconjugants, suggesting inter-species differences in amenability to accepting large pieces of foreign DNA. When colonized into conventional mice, abundance of *B. thetaiotaomicron* harboring the medium length PUL could be toggled up and down through addition of porphyrin extract in the water (Figure 9c).

Finally, the tunability of strain abundance was assessed by varying the porphyrin supplemented in diet. Conventional mice were colonized with a GFP fluorescent NB001 strain and c.f.u. in the feces was tracked for five days. 1%, 0.1%, or 0.01% weight by volume porphyrin extract was then administered in the drinking water for five days, followed by a subsequent five-day washout with regular water. A ten-fold decrease in abundance of NB001 in the feces was observed with each ten-fold dilution of extract administered in the water (Figure 9d), indicating fine control over strain abundance through modulation of the porphyrin concentration. To visually assess the spatial distribution and abundance of NB001 *in vivo* in the presence of different amounts of porphyrin, frozen tissue sections were prepared and endogenous GFP of the NB001 strain was imaged via confocal microscopy. A substantial increase in GFP-positive cells was seen when comparing mice given 0.01% vs. 1% extract (Figure 9e).

Predicting amenability of a given community to the introduction of a new strain is a challenge given the diverse and divergent features of different individuals' pre-existing microbiotas, including whether or not it is already inhabited by a strain capable of excluding

the one being introduced. The results presented in this example demonstrate that privileged nutrient sources such as porphyran can modify resource partitioning in the gut microbiota, and systems such as those presented here can be deployed to enhance human health.

5 Methods

Bacterial Culture and Strain Isolation

All bacterial growth was performed at 37° C under anaerobic conditions. Growth for introduction into mice was performed in rich media (tryptone-yeast-glucose) with no antibiotics added. Growth curves and selective growth on nori extract were performed in Salyers Minimal Media (SMM 100mL in dH₂O: 0.1g (NH₄)₂SO₄, 0.1g Na₂CO₃, 0.05g L-cysteine, 10mL 1M KPO₄ pH7.2, 5mL Mineral Salts (1L in dH₂O: 18g NaCl, 0.53g CaCl₂*2H₂O, 0.4g MgCl₂*6H₂O, 0.2g MnCl₂*4H₂O, 0.2g CoCl₂*6H₂O), 1mL 0.4mg/mL FeSO₄, 0.1mL 1mg/mL Vitamin K₃, 0.1mL Histidine/Hematin*, 0.05mL 0.01mg/mL Vitamin B₁₂) with carbon source added to a final concentration of either 0.2% (Figure 9b, glucose), 0.5% (Figure 11), or 0.8% (Figure 9b, nori extract) weight by volume and filter sterilized. SMM was either made fresh the day of the experiment, or prepared without histidine/hematin or L-cysteine and stored at 4° C for up to four weeks, with the missing components added the day of the experiment.

C.f.u. were cultivated on brain-heart infusion blood agar (BHI-BA) with appropriate selective antibiotics (200 µg/mL gentamycin, and 25 µg/mL erythromycin or 2 µg/mL tetracycline), or on SMM agar plates (for GFP visualization, 2x SMM tempered to 50° C + equal volume 3% agar tempered to 50° C) with selective antibiotics.

NB001 (porphyran MAC utilizing *B. ovatus*) and NB004 (naïve *B. stercoris*) were isolated from primary waste effluent at the San Jose Wastewater Treatment Facility via selection in liquid culture (SMM) with 200ug/mL gentamycin (as *Bacteroides* are naturally gentamycin resistant*), and for NB001, growth on 0.8% nori extract. Briefly, settled primary effluent was diluted ten-fold into SMM and grown as above for 24 h, subcultured at 1:200 into fresh media and grown for 24 h, and plated in serial dilutions onto BHI-BA. Single colonies were picked into SMM for growth confirmation, cryogenic storage, and downstream analysis. A GFP-expressing, erythromycin-resistant variant of NB001 was generated as described previously.

Genome Sequencing and Analysis

Genomic DNA was isolated from NB001 and NB004 using a PureLink Genomic DNA Mini Kit (Invitrogen). Samples were prepared for multiplexed Illumina sequencing using a Nextera XT DNA Library Preparation Kit (Illumina) and run on an Illumina MiSeq using a 2x150bp paired-end kit. Approximately 10 million sequencing reads were obtained for each sample. De novo assembly of the reads was performed with the Geneious De Novo

Assembler (Biomatters), yielding an average coverage of ~100 reads/bp. Gene annotation and alignment was also performed using Geneious.

Porphyran PUL Transfer and Knockout

- 5 Generation of the porphyran utilization deficient mutant was performed using *tdk* counterselection as described previously. A thymidine kinase (*tdk*) deficient mutant (NB007) of NB001 was generated by exposing NB001 to ultraviolet light for 60 seconds and plating on BHI-BA supplemented with 200ug/mL of 5-fluoro-2'-deoxyuridine (FUdR). Eight genes predicted to be essential for growth on porphyran (homologous to BACPLE_1692-1699) were
- 10 knocked out using pWD034 (Table 7), a plasmid with 1.5kb of homology upstream and downstream of the target region assembled via Golden Gate Assembly into an erythromycin-resistant, *tdk*-containing vector.

Table 7: List of plasmids used in Example 2

Plasmid Name	Description	Length (bps)	Parts used in yeast assembly	Relevant Figure
pVW3452	High GFP, erythromycin resistance	6,031	N/A	Figure 7, Figure 9e
pNBU2-ermGb	Erythromycin resistance	4,909	N/A	Fig. 2, Figure 12
pNBU2-tetQb	Tetracycline resistance	6,662	N/A	Figure 8, Figure 12
pWD034	Porphyran PUL Deletion (main operon)	7,148	N/A	Figure 8c
pWD035	Long Porphyran PUL	72,558	PCR_01, PCR_02, PCR_03, PCR_04, PCR_05, PCR_06, PCR_07, PCR_08, PCR_09, PCR_10, Vector_01, Vector_02, Vector_03	Figure 9a, b
pWD036	Medium Porphyran PUL	53,695	PCR_04, PCR_05, PCR_06, PCR_07, PCR_08, PCR_10, PCR_11, Vector_01, Vector_02, Vector_03	Figure 9a, b, c
pWD037	Short Porphyran PUL	32,364	PCR_05, PCR_06, PCR_12, PCR_13, Vector_01, Vector_02, Vector_03	Figure 9a, b

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Generation of the porphyran PUL knock-in strains required expansion of previous

knock-in methods due to the large size of the PULs (20-60kb). Based on gene annotations and sequence alignment to a previously published mobile element conferring porphyran polysaccharide utilization capabilities, we designed three minimal PULs of varying sizes (20kb, 40kb, and 60kb, Figure 9a). To propagate such large pieces of DNA and integrate them into the *Bacteroides* genome we used a three-step process: performing yeast assembly into a custom shuttle vector, propagating it in *S. cerevisiae* and *E. coli*, and then performing conjugation and genomic integration into *Bacteroides* species. The minimal PULs were each divided into multiple 6kb fragments with 200bp homology between pieces and assembled in yeast with fragments containing the KanMX selectable marker and CEN6/ARS4 origin for selection and growth in yeast, the bacterial artificial chromosome origin and chloramphenicol selectable marker (from pEZ-BAC vector, Lucigen) for selection and growth in *E. coli*, and the conjugative origin and parts for integration and selection in *Bacteroides* (Tables 2, 3, 4 – see above). Yeast cells with successfully assembled constructs were lysed by mechanical disruption with 0.5mm glass disruptor beads (USA Scientific), and lysates mixed 1:20 with electrocompetent *E. coli* S17-1 cells and electroporated. Successfully transformed *E. coli* were then grown and conjugated with NB004 and *B. thetaiotaomicron* VPI-5482 (*Bt*) as previously described. NB004 successfully integrated all PULs, but *Bt* was initially unable to integrate any PUL constructs. To improve rates of genomic integration, we pre-integrated an NBU2 integrase-expressing plasmid with tetracycline resistance into *Bt*, and this improved efficiency such that *Bt* conjugants were obtained for both the short and medium length PUL constructs.

Nori Extract Preparation

Raw culinary nori derived from *Porphyra yezoensis* (<https://www.rawnori.com>) was added at 10% weight by volume to distilled water and subjected to hot water extraction by autoclaving for three hours. The mixture was then cooled and centrifuged at 11,000xg. Resulting supernatant was ethanol precipitated by combining with 200-proof ethanol to a final concentration of 80% ethanol, 20% supernatant and incubating at 4° C for 24-72 h. Precipitate was recovered by centrifugation at 26,000xg and dried for 24 h before manual grinding to generate a measurable powder.

Mice

Ex-germ free or restricted flora (RF) conventional Swiss-Webster mice (Taconic) were housed in gnotobiotic isolators and fed either an autoclaved standard diet (LabDiet 5K67) or a custom diet as indicated below (Bio-Serv) in strict accordance with a Protocol for Care and Use of Laboratory Animals approved by the Stanford University Administrative Panel of Laboratory Animal Care. Introduction of all *Bacteroides* strains into either ex-germ free or RF

mice was performed by oral gavage of 10^8 c.f.u. of the given strain in culture media.

Mice were humanized (Figure 7) with fecal samples from healthy human donors were stored at -80°C , thawed and resuspended in pre-reduced PBS in anaerobic conditions at a 1:1 dilution, and 0.2 mL gavaged orally into germ free mice. The mice were allowed to equilibrate the human microbiota for four weeks while consuming the standard lab diet. One week before introduction of NB001, mice were switched to MAC-deficient chow (AIN-93G, 68% glucose). Seven days after introduction of NB001, mice were switched to custom diets with inulin or nori as the only available MACs (AIN-93G, 10% unique polysaccharide, 58% glucose). RF mice (Figure 8, Figure 9) were administered nori extract in the water at the percent indicated, weight by volume.

16S rRNA Analysis

DNA was extracted from fecal samples using the PowerSoil 96-htp kit (MoBio), and amplified at the 16S v4 region (515F, 806R). Qiime 1.9 was used to analyze the resulting Illumina-generated sequencing reads as previously described*. Data were rarified to the sample with the lowest number of reads (16384), and open-reference OTU picking via UCLUST and taxonomy assignment through the Greengenes 13.8 database was performed.

Microscopy

Tissue was harvested and immediately fixed in 4% paraformaldehyde in PBS for 48 hours at 4°C . Cassettes were transferred to 20% sucrose for 24 h, and then samples were embedded in OCT Compound (Tissue-Tek) before sectioning at 30um on a Leica CM3050 S cryostat. Sections were stained for 30 minutes with 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI; Sigma-Aldrich) and Alexa Fluor 594 Phalloidin (Life Technologies). Images were taken on a Zeiss LSM 700 confocal microscope.

Example 3

Figure 14 (panels A-C). Access to porphyrin allowed for crypt invasion and stable maintenance in the presence of an exclusionary primary colonizing strain. Germ-free mice were colonized with *B. thetaiotaomicron* strain VPI-5482 that was unable to utilize porphyrin. One week later, the mice were challenged with an isogenic strain harboring the medium-length porphyrin PUL, and continued drinking normal water (panel A) or were administered 1% porphyrin in the drinking water for four days (panel B, brackets). After four days, mice in panel B were switched to regular water, and two mice were sacrificed and images of the proximal colon were captured, demonstrating the challenge strain (panel C, green) colonizing the colonic crypts with the primary strain (panel C, red). Colony forming units were tracked in the feces for the duration of the experiment, demonstrating that without access

to 1% porphyran in the water (panel A) the challenge strain clears, but with short-term access the challenge strain maintains stable co-colonization with the primary colonizing strain. These data demonstrate that with access to porphyran, an incoming strain can enter the colonic crypts.

5

CLAIMS

That which is claimed is:

1. A method, comprising: introducing to a human organ a genetically modified bacterial cell capable of controlled entrenchment and/or controlled colonization.
2. A method for modulating growth of a genetically modified bacterial cell present in a human organ, comprising: administering to a human a rare carbohydrate of interest that is utilized as a carbon source by the genetically modified bacterial cell, wherein less than 50% of other bacterial cells present in the human organ utilize the rare carbohydrate of interest as a carbon source.
3. A method for modulating growth of a genetically modified bacterial cell in an organ, comprising: introducing the genetically modified bacterial cell to the organ *in vivo*, wherein the genetically modified bacterial cell utilizes as a carbon source a rare carbohydrate of interest, wherein less than 50% of other bacterial cells present in the organ utilize the rare carbohydrate of interest as a carbon source.
4. A method for displacing at least a portion of a population of bacterial cells in an organ, comprising: introducing to the organ a genetically modified bacterial cell capable of controlled entrenchment and/or controlled colonization, and displacing at least a portion of the population of bacterial cells with the genetically modified bacterial cell.
5. The method of claim 4, wherein the genetically modified bacterial cell and the bacterial cells in the population of bacterial cells are the same species.
6. The method of claim 4 or 5, further comprising providing a bacterial cell from the population of bacterial cells and removing or inactivating one or more genes in the bacterial cell, thereby generating the genetically modified bacterial cell.
7. The method of claim 3 or 4, wherein the organ is a human organ.
8. The method of any one of the preceding claims, wherein the organ is a gut.
9. The method of claim 1 or 4, wherein the controlled entrenchment and/or controlled colonization is controlled by a high expression promoter.

10. The method of claim 1 or 4, wherein the controlled entrenchment and/or controlled colonization is controlled by an inducible promoter.
11. The method of claim 1 or 4, wherein the controlled entrenchment and/or controlled colonization is controlled by delivering a rare carbohydrate of interest to the organ.
12. The method of claim 11, wherein the rare carbohydrate of interest is a carbohydrate that can be utilized as a carbon source by less than 50% of other bacterial cells present in the organ, thereby generating a privileged niche.
13. The method of any one of claims 2-3 and 11-12, wherein the rare carbohydrate of interest is an isolated carbohydrate.
14. The method of any one of claims 2-3 and 11-13, wherein the rare carbohydrate of interest is a polysaccharide.
15. The method of any one of claims 2-3 and 11-14, wherein the rare carbohydrate of interest is a sulfated carbohydrate.
16. The method of any one of claims 2-3 and 11-14, wherein the rare carbohydrate of interest is selected from the group consisting of porphyran, agarose, carrageenan, and any combination thereof.
17. The method of any one of claims 2-3 and 11-16, wherein the rare carbohydrate of interest is a carbohydrate cleaved by a glycoside hydrolase belonging to glycoside hydrolase family GH86.
18. The method of any one of claims 2-3 and 11-17, wherein the rare carbohydrate of interest is a sulfated polygalactan.
19. The method of any one of the preceding claims, wherein the genetically modified bacterial cell is a genetically modified gut resident bacterial cell.
20. The method of any one of the preceding claims, wherein the genetically modified bacterial cell is in the genus *Bacteroides*.

21. The method of any one of the preceding claims, wherein the genetically modified bacterial cell is selected from *B. acidifaciens*, *B. amylophilus*, *B. asaccharolyticus*, *B. barnesiae*, *B. bivius*, *B. buccae*, *B. buccalis*, *B. caccae*, *B. capillosus*, *B. capillus*, *B. cellulosilyticus*, *B. cellulosolvens*, *B. chinchilla*, *B. clarus*, *B. coagulans*, *B. coprocola*, *B. coprophilus*, *B. coprosuis*, *B. corporis*, *B. denticola*, *B. disiens*, *B. distasonis*, *B. dorei*, *B. eggerthii*, *B. endodontalis*, *B. faecichinchillae*, *B. faecis*, *B. finegoldii*, *B. fluxus*, *B. forsythus*, *B. fragilis*, *B. furcosus*, *B. galacturonicus*, *B. gallinarum*, *B. gingivalis*, *B. goldsteinii*, *B. gracilis*, *B. graminisolvens*, *B. helcogenes*, *B. heparinolyticus*, *B. hypermegas*, *B. intermedius*, *B. intestinalis*, *B. johnsonii*, *B. levvi*, *B. loescheii*, *B. macacae*, *B. massiliensis*, *B. melaninogenicus*, *B. merdae*, *B. microfus*, *B. multiacidus*, *B. nodosus*, *B. nordii*, *B. ochraceus*, *B. oleiciplenus*, *B. oralis*, *B. oris*, *B. oulorum*, *B. ovatus*, *B. paurosaccharolyticus*, *B. pectinophilus*, *B. pentosaceus*, *B. plebeius*, *B. pneumosintes*, *B. polypragmatus*, *B. praeacutus*, *B. propionificaciens*, *B. putredinis*, *B. pyogenes*, *B. reticulotermitis*, *B. rodentium*, *B. ruminicola*, *B. salanitronis*, *B. salivosus*, *B. salyersiae*, *B. sartorii*, *B. splanchnicus*, *B. stercorisoris*, *B. stercoris*, *B. succinogenes*, *B. suis*, *B. tectus*, *B. termitidis*, *B. thetaiotaomicron*, *B. uniformis*, *B. ureolyticus*, *B. veroralis*, *B. vulgatus*, *B. xylanisolvens*, *B. xylanolyticus*, *B. zoogloformans*, and any combination thereof.
22. The method of any one of the preceding claims, wherein the genetically modified bacterial cell comprises a carbohydrate-utilization gene or gene set.
23. The method of claim 22, wherein the carbohydrate-utilization gene or gene set comprises one or more genes selected from the group consisting of porphyranase, glycoside hydrolase, sulfatase, galactosidase, and any combination thereof.
24. The method of claim 22, wherein the carbohydrate-utilization gene or gene set comprises one or more nucleic acids encoding proteins that have 80% or more sequence identity with BACPLE_1683-1706 from the *B. plebeius* genome.
25. The method of any one of claims 22-24, wherein the carbohydrate-utilization gene set comprises at least six genes.
26. The method of any one of claims 22-25, wherein the carbohydrate of interest cannot be utilized as a carbon source by the genetically modified bacterial cell in the absence of the carbohydrate-utilization gene or gene set.
27. The method of any one of claims 22-26, wherein the carbohydrate-utilization gene or

gene set is heterologous.

28. The method of claim 27, wherein the carbohydrate-utilization gene or gene set is encoded on a plasmid, encoded on a bacterial artificial chromosome, or genomically integrated.

29. The method of any one of the preceding claims, wherein the genetically modified bacterial cell is capable of treating a metabolic disease or disorder.

30. The method of any one of the preceding claims, wherein the genetically modified bacterial cell comprises one or more therapeutic transgenes.

31. The method of claim 30, wherein the one or more therapeutic transgenes comprise one or more enzymes.

32. The method of any one of claims 2-3 and 11-18, further comprising administering the rare carbohydrate of interest for a sufficient amount of time and at a sufficient frequency to establish a population of the genetically modified bacterial cell.

33. The method of claim 32, further comprising maintaining the population of the genetically modified bacterial cell in the organ for at least 5 days.

34. The method of any one of claims 2-3 and 11-18, further comprising administering the rare carbohydrate of interest for at least 3 days.

35. The method of any one of the preceding claims, wherein the genetically modified bacterial cell is introduced orally.

36. The method of any one of the preceding claims, wherein the organ comprises at least 5 other bacterial species.

37. The method of any one of the preceding claims, further comprising colonizing the organ with the genetically modified bacterial cell at a level of at least 1% of total bacterial cells in the organ.

38. A genetically modified bacterial cell that utilizes as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% of bacterial cells

present in a human microbiota.

39. A genetically modified bacterial cell, comprising:

a heterologous carbohydrate-utilization gene or gene set that provides the genetically modified bacterial cell with an ability to utilize as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% of bacterial cells present in a human microbiota.

40. A genetically modified bacterial cell, comprising:

(i) a heterologous therapeutic transgene; and

(ii) a carbohydrate-utilization gene or gene set that provides the genetically modified bacterial cell with an ability to utilize as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% of bacterial cells present in a human microbiota.

41. The genetically modified bacterial cell of any one of claims 38-40, wherein the rare carbohydrate of interest is an isolated carbohydrate.

42. The genetically modified bacterial cell of any one of claims 38-41, wherein the rare carbohydrate of interest is a polysaccharide.

43. The genetically modified bacterial cell of any one of claims 38-42, wherein the rare carbohydrate of interest is a sulfated carbohydrate.

44. The genetically modified bacterial cell of any one of claims 38-42, wherein the rare carbohydrate of interest is selected from the group consisting of porphyran, agarose, carrageenan, and any combination thereof.

45. The genetically modified bacterial cell of any one of claims 38-44, wherein the rare carbohydrate of interest is a carbohydrate cleaved by a glycoside hydrolase belonging to glycoside hydrolase family GH86.

46. The genetically modified bacterial cell of any one of claims 38-45, wherein the rare carbohydrate of interest is a sulfated polygalactan.

47. The genetically modified bacterial cell of any one of claims 38-46, wherein the genetically modified bacterial cell is a genetically modified gut resident bacterial cell.

48. The genetically modified bacterial cell of any one of claims 38-47, wherein the genetically modified bacterial cell is in the genus *Bacteroides*.

49. The genetically modified bacterial cell of any one of claims 38-48, wherein the genetically modified bacterial cell is selected from *B. acidifaciens*, *B. amylophilus*, *B. asaccharolyticus*, *B. barnesiae*, *B. bivius*, *B. buccae*, *B. buccalis*, *B. caccae*, *B. capillosus*, *B. capillus*, *B. cellulosilyticus*, *B. cellulosolvens*, *B. chinchilla*, *B. clarus*, *B. coagulans*, *B. coprocola*, *B. coprophilus*, *B. coprosuis*, *B. corporis*, *B. denticola*, *B. disiens*, *B. distasonis*, *B. dorei*, *B. eggerthii*, *B. endodontalis*, *B. faecichinchillae*, *B. faecis*, *B. finegoldii*, *B. fluxus*, *B. forsythus*, *B. fragilis*, *B. furcosus*, *B. galacturonicus*, *B. gallinarum*, *B. gingivalis*, *B. goldsteinii*, *B. gracilis*, *B. graminisolvens*, *B. helcogenes*, *B. heparinolyticus*, *B. hypermegas*, *B. intermedius*, *B. intestinalis*, *B. johnsonii*, *B. levvi*, *B. loescheii*, *B. macacae*, *B. massiliensis*, *B. melaninogenicus*, *B. merdae*, *B. microfus*, *B. multiacidus*, *B. nodosus*, *B. nordii*, *B. ochraceus*, *B. oleiciplenus*, *B. oralis*, *B. oris*, *B. oulorum*, *B. ovatus*, *B. paurosaccharolyticus*, *B. pectinophilus*, *B. pentosaceus*, *B. plebeius*, *B. pneumosintes*, *B. polypragmatus*, *B. praeacutus*, *B. propionificiens*, *B. putredinis*, *B. pyogenes*, *B. reticulotermitis*, *B. rodentium*, *B. ruminicola*, *B. salanitronis*, *B. salivosus*, *B. salyersiae*, *B. sartorii*, *B. splanchnicus*, *B. stercorisoris*, *B. stercoris*, *B. succinogenes*, *B. suis*, *B. tectus*, *B. termitidis*, *B. thetaiotaomicron*, *B. uniformis*, *B. ureolyticus*, *B. veroralis*, *B. vulgatus*, *B. xylanisolvens*, *B. xylanolyticus*, *B. zoogloformans*, and any combination thereof.

50. The genetically modified bacterial cell of claim 38, wherein the genetically modified bacterial cell comprises a carbohydrate-utilization gene or gene set.

51. The genetically modified bacterial cell of claim 39, 40, or 50, wherein the carbohydrate-utilization gene or gene set comprises one or more genes selected from the group consisting of porphyranase, glycoside hydrolase, sulfatase, galactosidase, and any combination thereof.

52. The genetically modified bacterial cell of any one of claims 39-40 and 50-51, wherein the carbohydrate-utilization gene or gene set comprises one or more nucleic acids encoding proteins that have 80% or more sequence identity with BACPLE_1683-1706 from the *B. plebeius* genome.

53. The genetically modified bacterial cell of any one of claims 39-40 and 50-52, wherein the carbohydrate-utilization gene set comprises at least six genes.

54. The genetically modified bacterial cell of any one of claims 39-40 and 50-53, wherein the carbohydrate of interest cannot be utilized as a carbon source by the genetically modified bacterial cell in the absence of the carbohydrate-utilization gene or gene set.

55. The genetically modified bacterial cell of claim 40 or 50, wherein the carbohydrate-utilization gene or gene set is heterologous.

56. The genetically modified bacterial cell of claim 39 or 55, wherein the carbohydrate-utilization gene or gene set is encoded on a plasmid, encoded on a bacterial artificial chromosome, or genomically integrated.

57. The genetically modified bacterial cell of any one of claims 38-56, wherein the genetically modified bacterial cell is capable of treating a metabolic disease or disorder.

58. The genetically modified bacterial cell of any one of claims 38-57, wherein the genetically modified bacterial cell comprises one or more therapeutic transgenes.

59. The genetically modified bacterial cell of claim 58, wherein the one or more therapeutic transgenes comprise one or more enzymes.

60. A method comprising:
providing a bacterial cell; and
genetically modifying the bacterial cell to utilize as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% of bacterial cells present in a human microbiome.

61. A method comprising:
providing a bacterial cell that utilizes as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% of bacterial cells present in a human microbiome; and
genetically modifying the bacterial cell to express one or more therapeutic transgenes.

62. The method of claim 60 or 61, wherein the rare carbohydrate of interest is an isolated carbohydrate.

63. The method of any one of claims 60-62, wherein the rare carbohydrate of interest is a polysaccharide.

64. The method of any one of claims 60-63, wherein the rare carbohydrate of interest is a sulfated carbohydrate.
65. The method of any one of claims 60-63, wherein the rare carbohydrate of interest is selected from the group consisting of porphyran, agarose, carrageenan, and any combination thereof.
66. The method of any one of claims 60-65, wherein the rare carbohydrate of interest is a carbohydrate cleaved by a glycoside hydrolase belonging to glycoside hydrolase family GH86.
67. The method of any one of claims 60-66, wherein the rare carbohydrate of interest is a sulfated polygalactan.
68. The method of any one of claims 60-67, wherein the genetically modified bacterial cell is a genetically modified gut resident bacterial cell.
69. The method of any one of claims 60-68, wherein the genetically modified bacterial cell is in the genus *Bacteroides*.
70. The method of any one of claims 60-69, wherein the genetically modified bacterial cell is selected from *B. acidifaciens*, *B. amylophilus*, *B. asaccharolyticus*, *B. barnesiae*, *B. bivius*, *B. buccae*, *B. buccalis*, *B. caccae*, *B. capillosus*, *B. capillus*, *B. cellulosilyticus*, *B. cellulosolvens*, *B. chinchilla*, *B. clarus*, *B. coagulans*, *B. coprocola*, *B. coprophilus*, *B. coprosuis*, *B. corporis*, *B. denticola*, *B. disiens*, *B. distasonis*, *B. dorei*, *B. eggerthii*, *B. endodontalis*, *B. faecichinchillae*, *B. faecis*, *B. finegoldii*, *B. fluxus*, *B. forsythus*, *B. fragilis*, *B. furcosus*, *B. galacturonicus*, *B. gallinarum*, *B. gingivalis*, *B. goldsteinii*, *B. gracilis*, *B. graminisolvens*, *B. helcogenes*, *B. heparinolyticus*, *B. hypermegas*, *B. intermedius*, *B. intestinalis*, *B. johnsonii*, *B. levvi*, *B. loescheii*, *B. macacae*, *B. massiliensis*, *B. melaninogenicus*, *B. merdae*, *B. microfus*, *B. multiacidus*, *B. nodosus*, *B. nordii*, *B. ochraceus*, *B. oleiciplenus*, *B. oralis*, *B. oris*, *B. oulorum*, *B. ovatus*, *B. paurosaccharolyticus*, *B. pectinophilus*, *B. pentosaceus*, *B. plebeius*, *B. pneumosintes*, *B. polypragmatus*, *B. praeacutus*, *B. propionificaciens*, *B. putredinis*, *B. pyogenes*, *B. reticulotermis*, *B. rodentium*, *B. ruminicola*, *B. salanitronis*, *B. salivorus*, *B. salyersiae*, *B. sartorii*, *B. splanchnicus*, *B. stercorisoris*, *B. stercoris*, *B. succinogenes*, *B. suis*, *B. tectus*, *B. termitidis*, *B. thetaiotaomicron*, *B. uniformis*, *B. ureolyticus*, *B. veroralis*, *B. vulgatus*, *B. xylanisolvens*, *B. xylanolyticus*, *B. zoogloformans*, and any combination thereof.

71. The method of any one of claims 60-70, wherein the genetically modified bacterial cell comprises a carbohydrate-utilization gene or gene set.
72. The method of claim 71, wherein the carbohydrate-utilization gene or gene set comprises one or more genes selected from the group consisting of porphyranase, glycoside hydrolase, sulfatase, galactosidase, and any combination thereof.
73. The method of claim 71 or 72, wherein the carbohydrate-utilization gene or gene set comprises one or more nucleic acids encoding proteins that have 80% or more sequence identity with BACPLE_1683-1706 from the *B. plebeius* genome.
74. The method of any one of claims 71-73, wherein the carbohydrate-utilization gene set comprises at least six genes.
75. The method of any one of claims 71-74, wherein the carbohydrate of interest cannot be utilized as a carbon source by the genetically modified bacterial cell in the absence of the carbohydrate-utilization gene or gene set.
76. The method of any one of claims 71-75, wherein the carbohydrate-utilization gene or gene set is heterologous.
77. The method of any one of claims 71-76, wherein the carbohydrate-utilization gene or gene set is encoded on a plasmid, encoded on a bacterial artificial chromosome, or genomically integrated.
78. The method of any one of claims 60-77, wherein the genetically modified bacterial cell is capable of treating a metabolic disease or disorder.
79. The method of claim 61, wherein the one or more therapeutic transgenes comprise one or more enzymes.
80. A method of facilitating colonization by a genetically modified bacterial cell, the method comprising:
- (a) introducing a genetically modified bacterial cell into a gut of an individual, wherein the genetically modified bacterial cell comprises a heterologous carbohydrate-utilization gene or gene set that provides the genetically modified bacterial cell with the ability to use a

carbohydrate of interest as a carbon source; and

(b) administering the carbohydrate of interest to the individual, thereby providing the genetically modified bacterial cell with the carbon source.

81. The method of claim 80, wherein the genetically modified bacterial cell is a genetically modified gut resident bacterial cell.

82. The method of claim 80 or claim 81, wherein the genetically modified bacterial cell is a *Bacteroides* cell.

83. The method of claim 82, wherein the *Bacteroides* cell is a *B. fragilis* (*Bf*), *B. distasonis* (*Bd*), *B. thetaiotaomicron* (*Bt*), *B. vulgatus* (*Bv*), *B. ovatus* (*Bo*), *B. eggerrthii* (*Be*), *B. merdae* (*Bm*), *B. stercoris* (*Bs*), *B. uniformis* (*Bu*), or *B. caccae* (*Bc*) cell.

84. The method of any one of claims 80-83, wherein the carbohydrate of interest cannot be utilized as a carbon source by the genetically modified bacterial cell in the absence of the heterologous carbohydrate-utilization gene or gene set.

85. The method of any one of claims 80-84, wherein the carbohydrate of interest is utilized as a carbon source by less than 50% of other gut bacterial cells present in the gut of the individual.

86. The method of any one of claims 80-85, wherein the carbohydrate of interest is porphyrin.

87. The method of claim 86, wherein the heterologous carbohydrate-utilization gene or gene set comprises one or more nucleic acids encoding the proteins that have 80% or more sequence identity with BACPLE_1683-1706 from the *B. plebeius* genome.

88. The method of any one of claims 80-87, wherein the genetically modified bacterial cell is a therapeutic bacterial cell.

89. The method of any one of claims 80-88, wherein the genetically modified bacterial cell comprises a nucleic acid molecule comprising a heterologous nucleic acid sequence that encodes a therapeutic polypeptide.

90. A genetically modified bacterial cell, comprising:

a heterologous carbohydrate-utilization gene or gene set that provides the genetically modified bacterial cell with the ability to use a rare carbohydrate of interest as a carbon source.

91. The genetically modified bacterial cell of claim 90, wherein the genetically modified bacterial cell is a genetically modified gut resident bacterial cell.

92. The genetically modified bacterial cell of claim 90 or claim 91, wherein the genetically modified bacterial cell is a *Bacteroides* cell.

93. The genetically modified bacterial cell of claim 92, wherein the *Bacteroides* cell is a *B. fragilis* (Bf), *B. distasonis* (Bd), *B. thetaiotaomicron* (Bt), *B. vulgatus* (Bv), *B. ovatus* (Bo), *B. eggerrthii* (Be), *B. merdae* (Bm), *B. stercoris* (Bs), *B. uniformis* (Bu), or *B. caccae* (Bc) cell.

94. The genetically modified bacterial cell of any one of claims 90-93, wherein the carbohydrate of interest cannot be utilized as a carbon source by the genetically modified bacterial cell in the absence of the heterologous carbohydrate-utilization gene or gene set.

95. The genetically modified bacterial cell of any one of claims 90-94, wherein the carbohydrate of interest is porphyran.

96. The genetically modified bacterial cell of claim 95, wherein the heterologous carbohydrate-utilization gene or gene set comprises one or more nucleic acids encoding the proteins that have 80% or more sequence identity with BACPLE_1683-1706 from the *B. plebeius* genome.

97. The genetically modified bacterial cell of any of claims 90-96, wherein the genetically modified bacterial cell is a therapeutic bacterial cell.

98. The genetically modified bacterial cell of any of claims 90-97, wherein the genetically modified bacterial cell comprises a nucleic acid molecule comprising a heterologous nucleic acid sequence that encodes a therapeutic polypeptide.

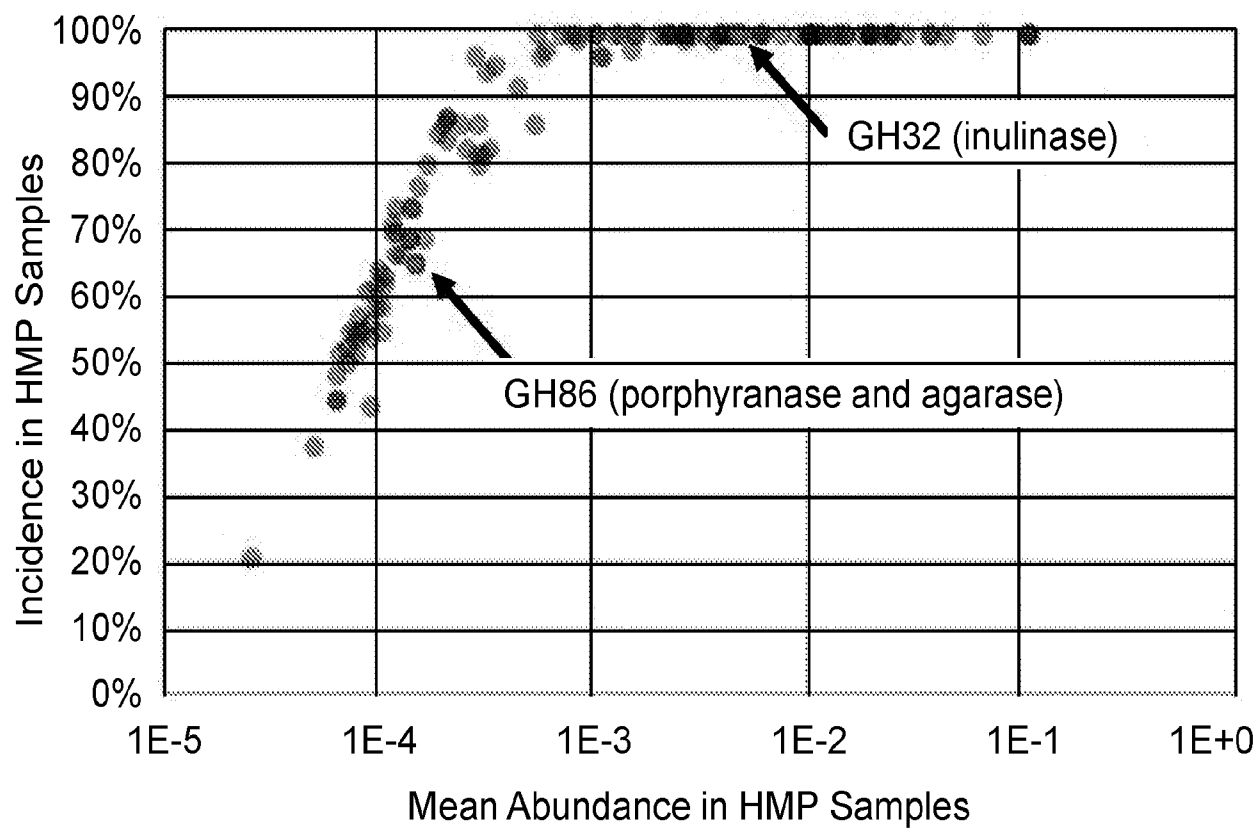
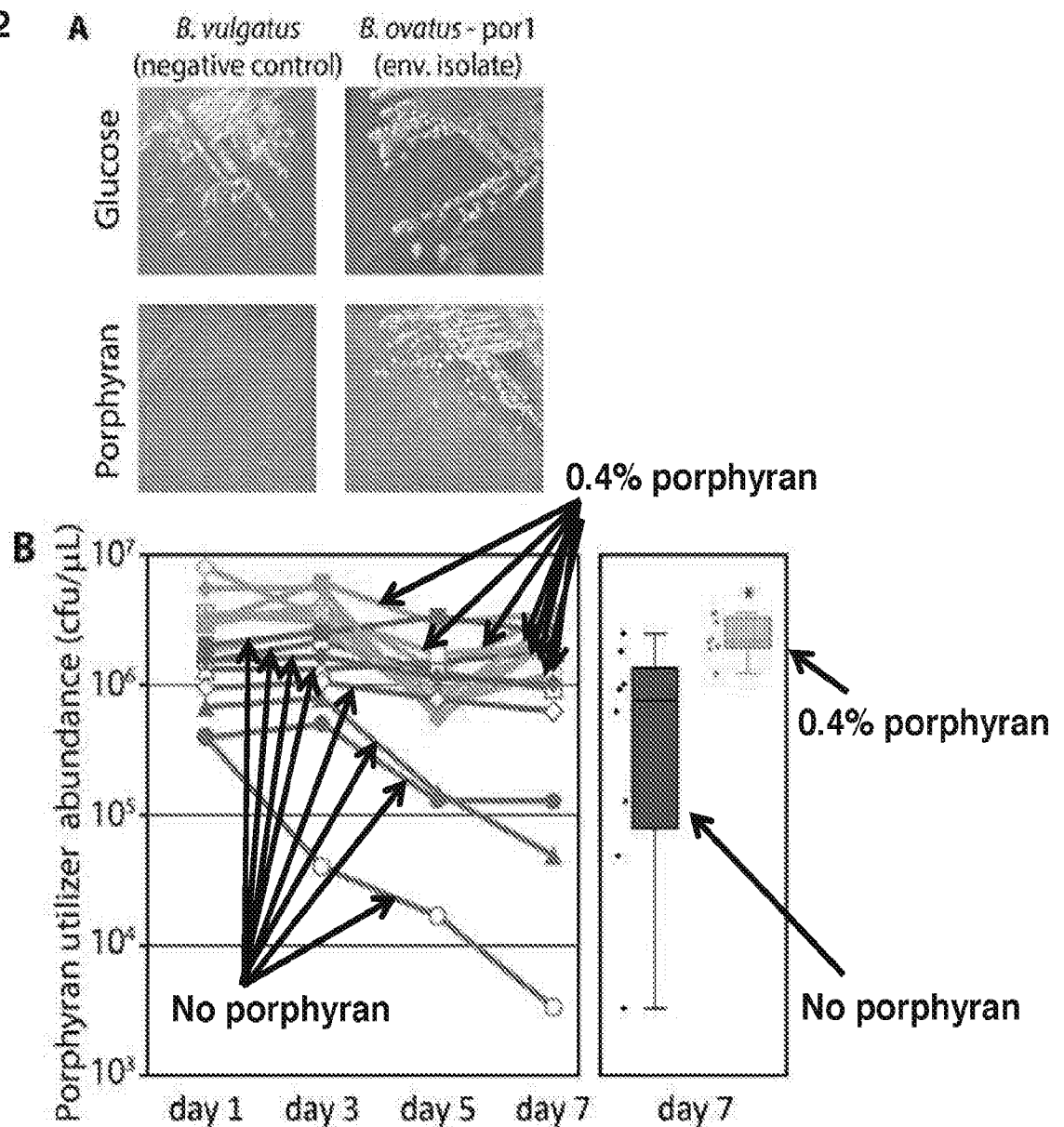
Figure 1

Figure 2

* Higher abundance ($P = 0.02$)
and
Lower deviation ($P < 0.01$)

No Por.	0.4% Por.	Community	Location	Species
		1	Site 1 (Palo Alto, CA)	<i>Mus musculus</i>
		2	Site 1 (Palo Alto, CA)	<i>Mus musculus</i>
		3	Site 2 (Palo Alto, CA)	<i>Mus musculus</i>
		4	Site 2 (Palo Alto, CA)	<i>Mus musculus</i>
		5	Site 3 (Daly City, CA)	<i>Mus musculus</i>
		6	Site 3 (Daly City, CA)	<i>Cavia porcellus</i>
		7	Site 3 (Daly City, CA)	<i>Phodopus sungorus</i>
		8	Site 3 (Daly City, CA)	<i>Chinchilla lanigera</i>

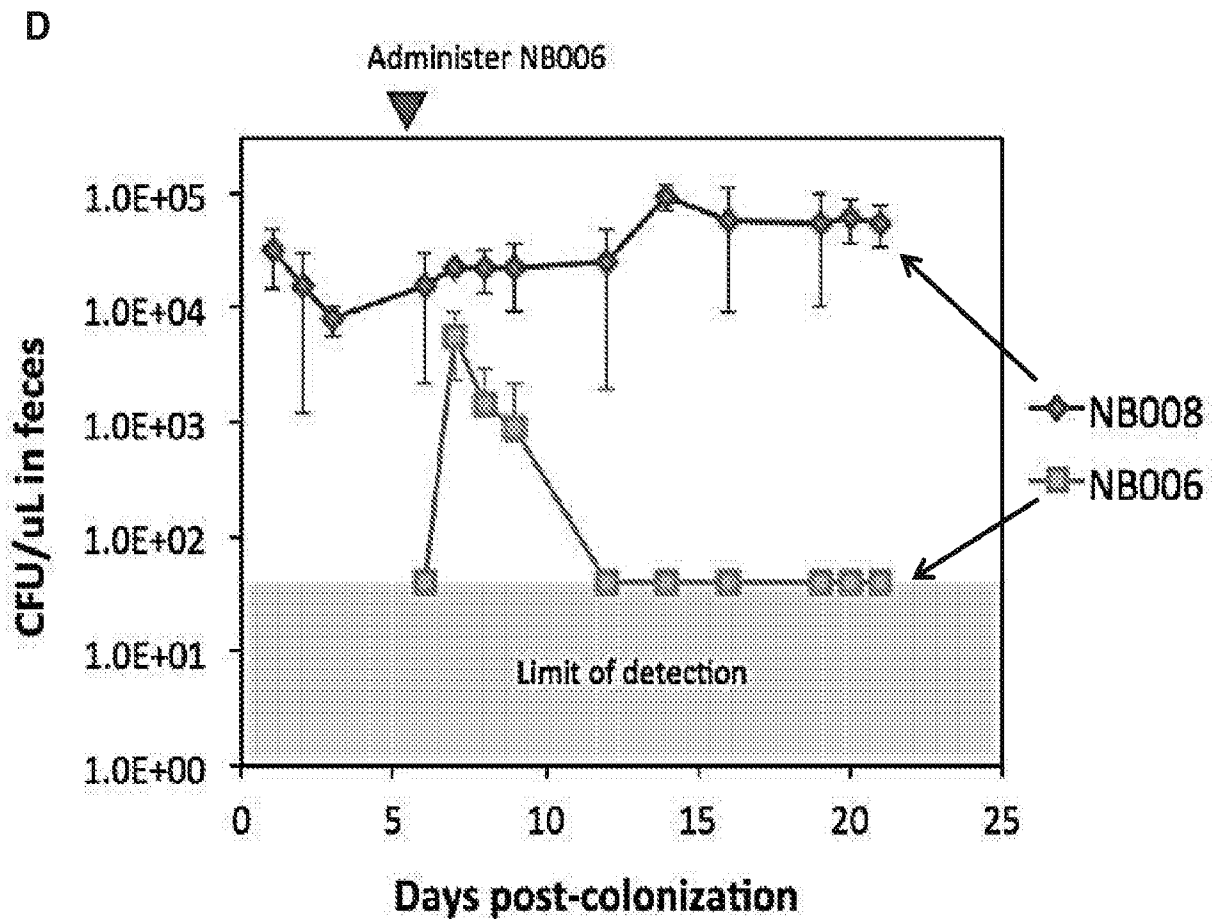
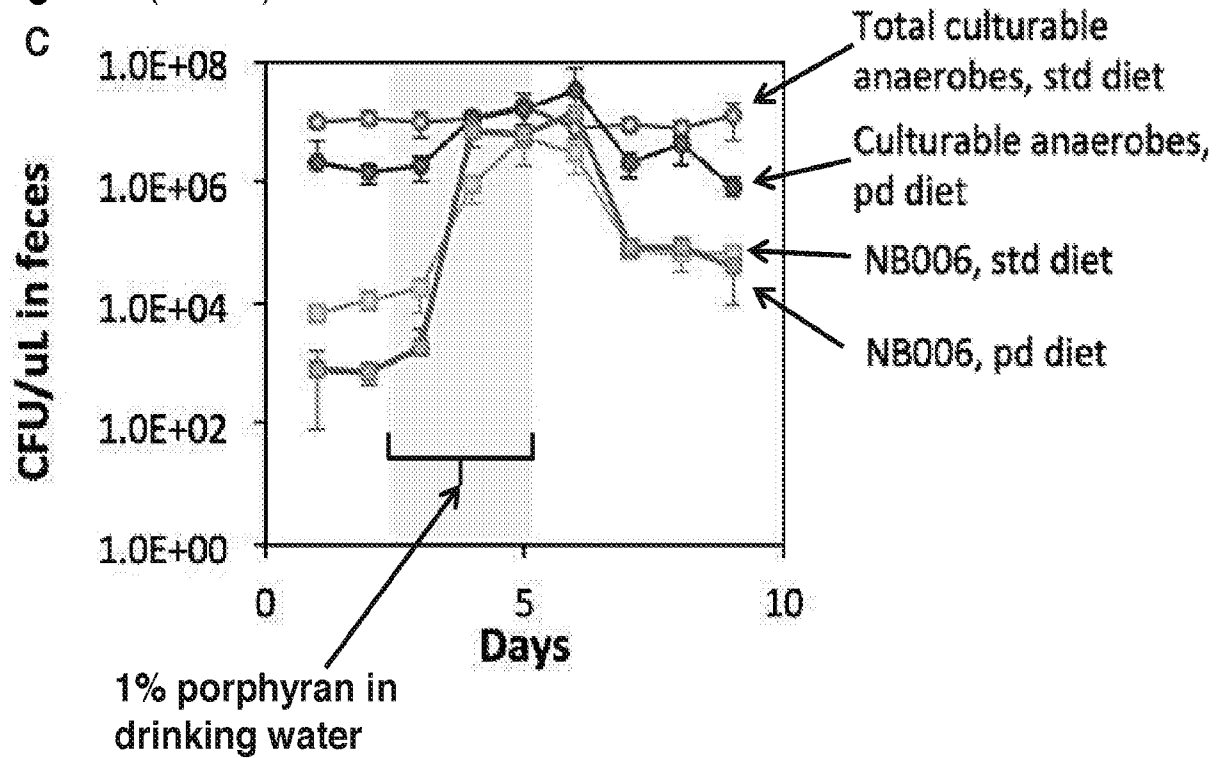
Figure 2 (Cont'd)

Figure 2 (Cont'd)

E

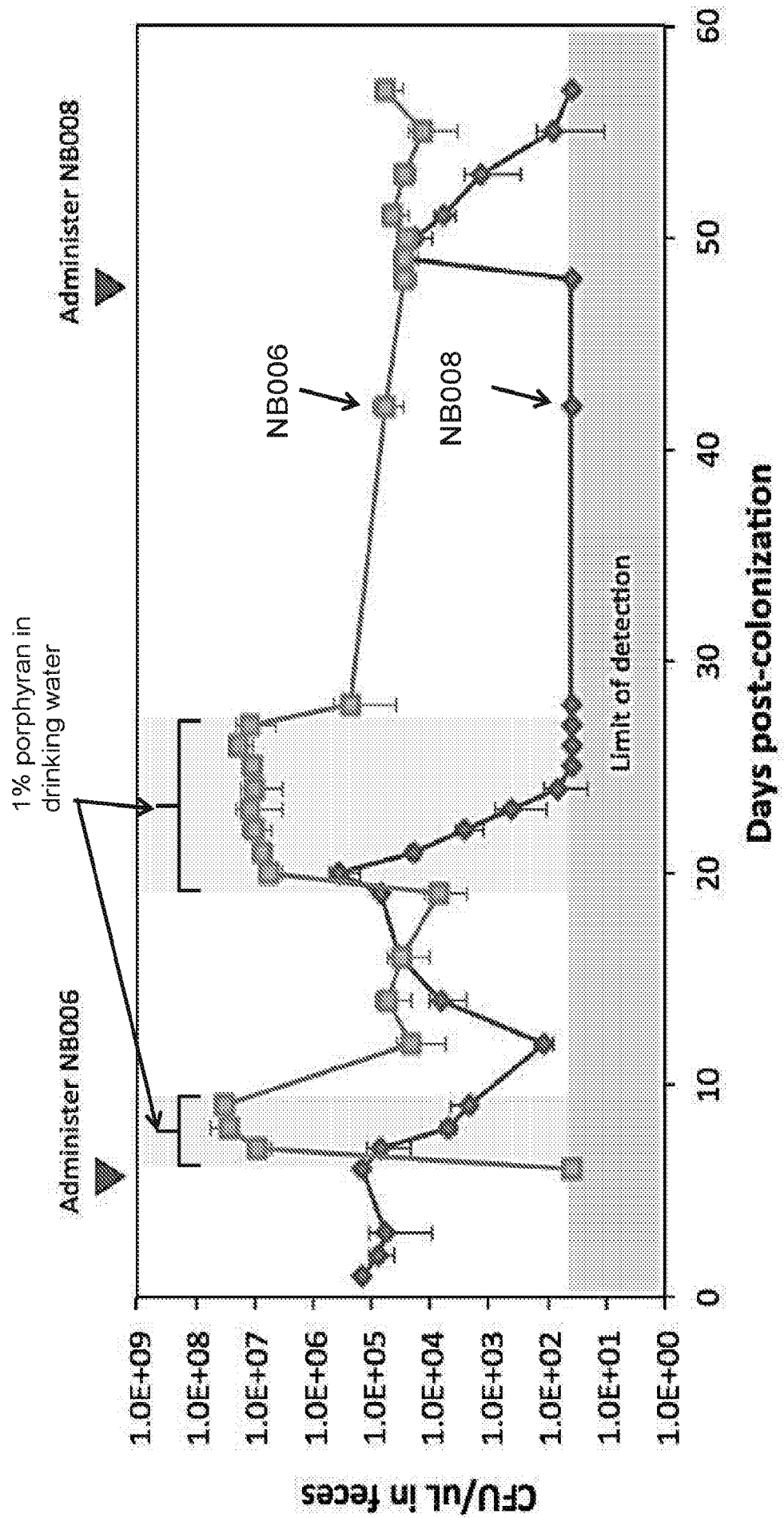


Figure 3

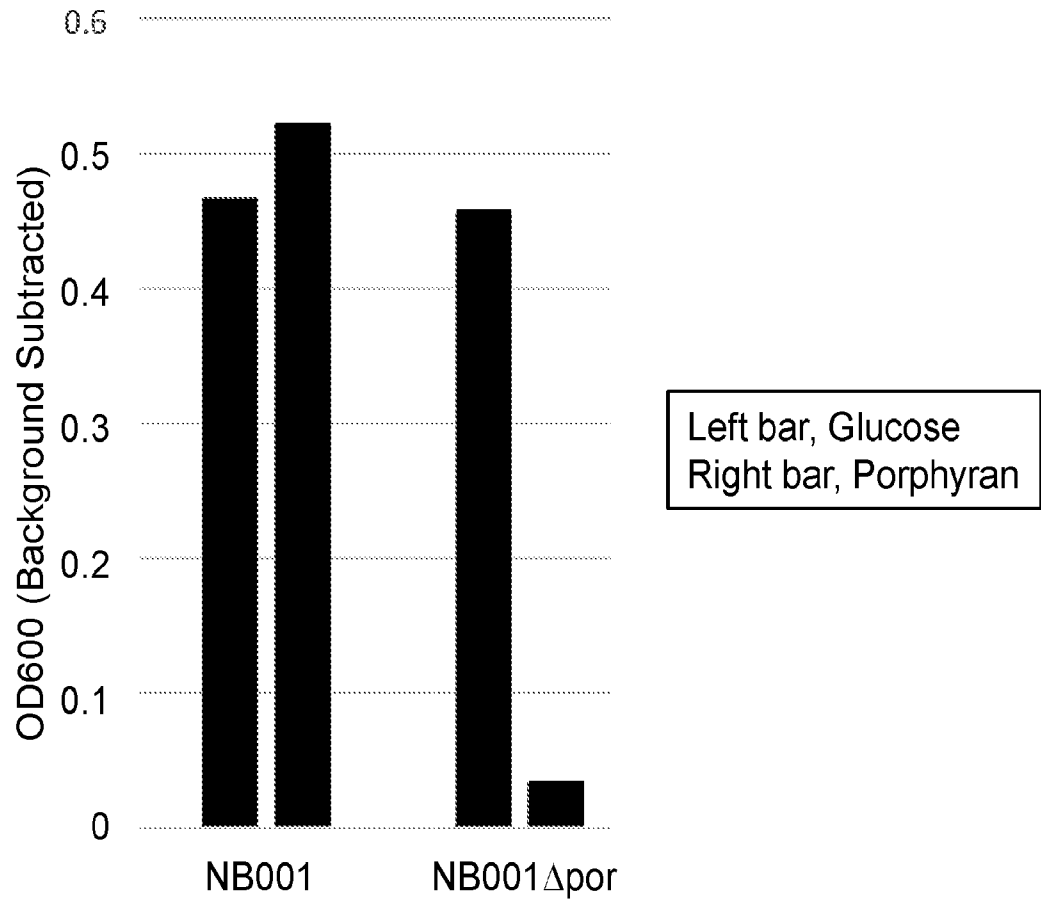
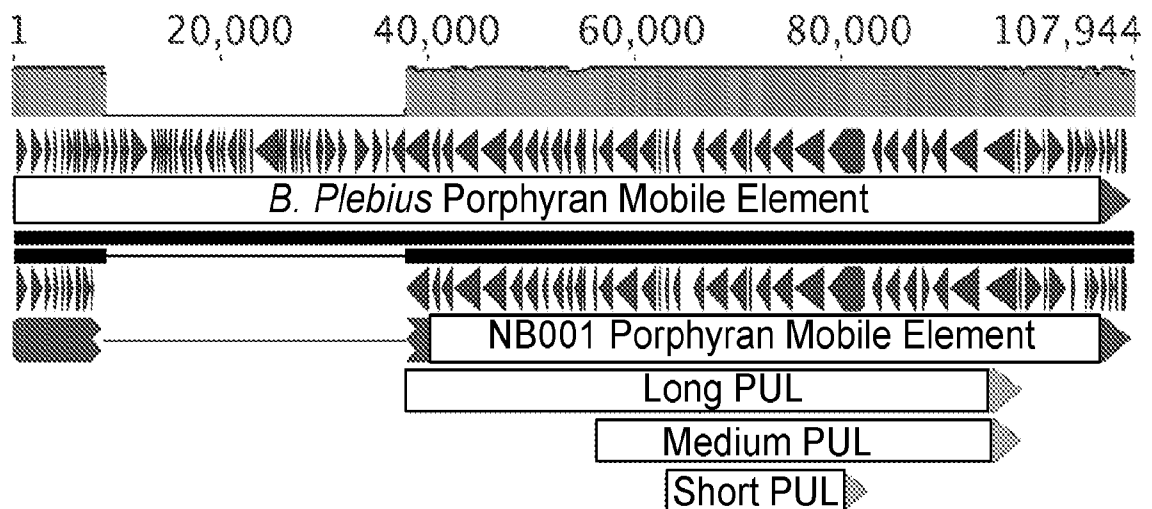


Figure 4



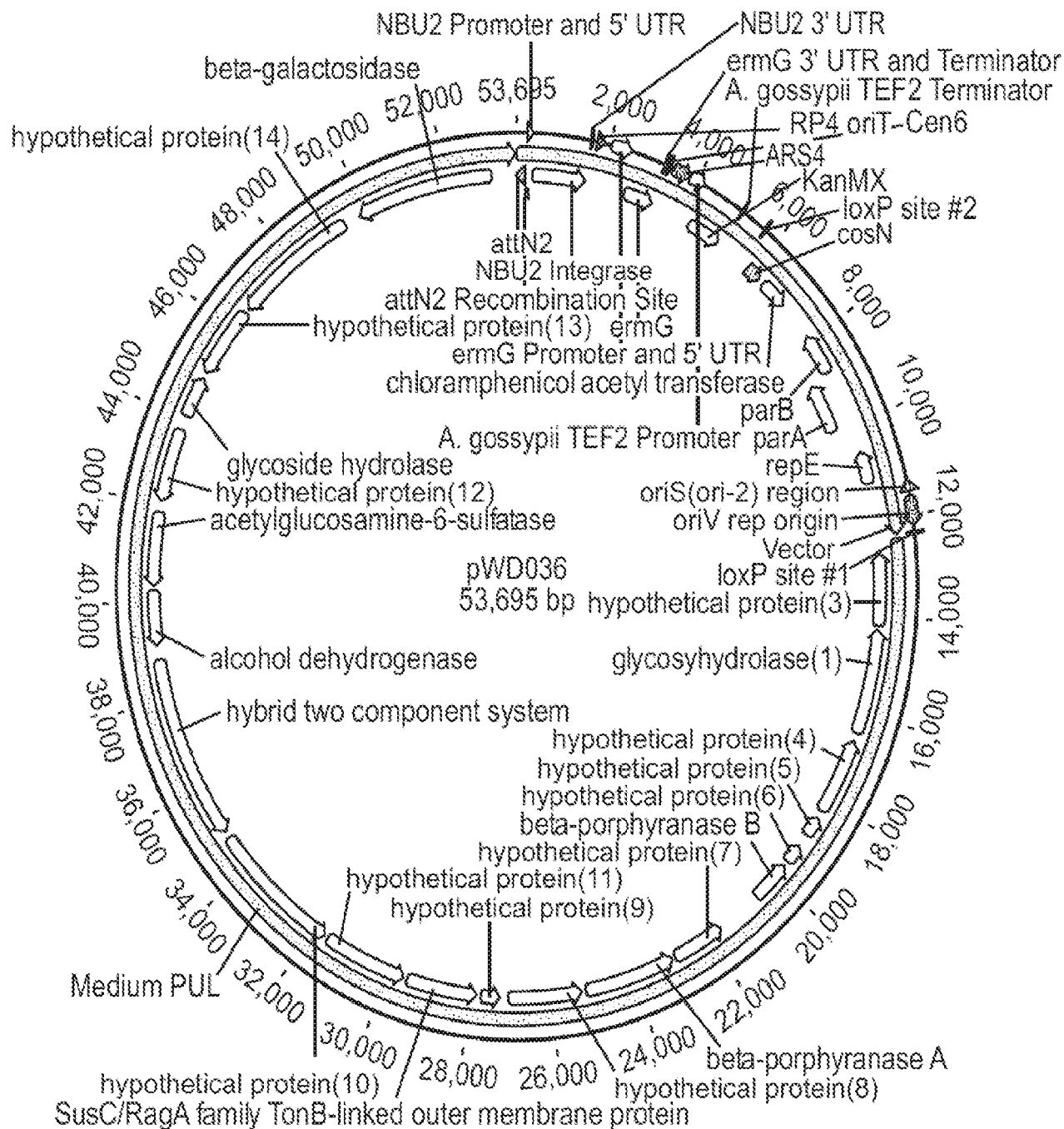


Figure 5

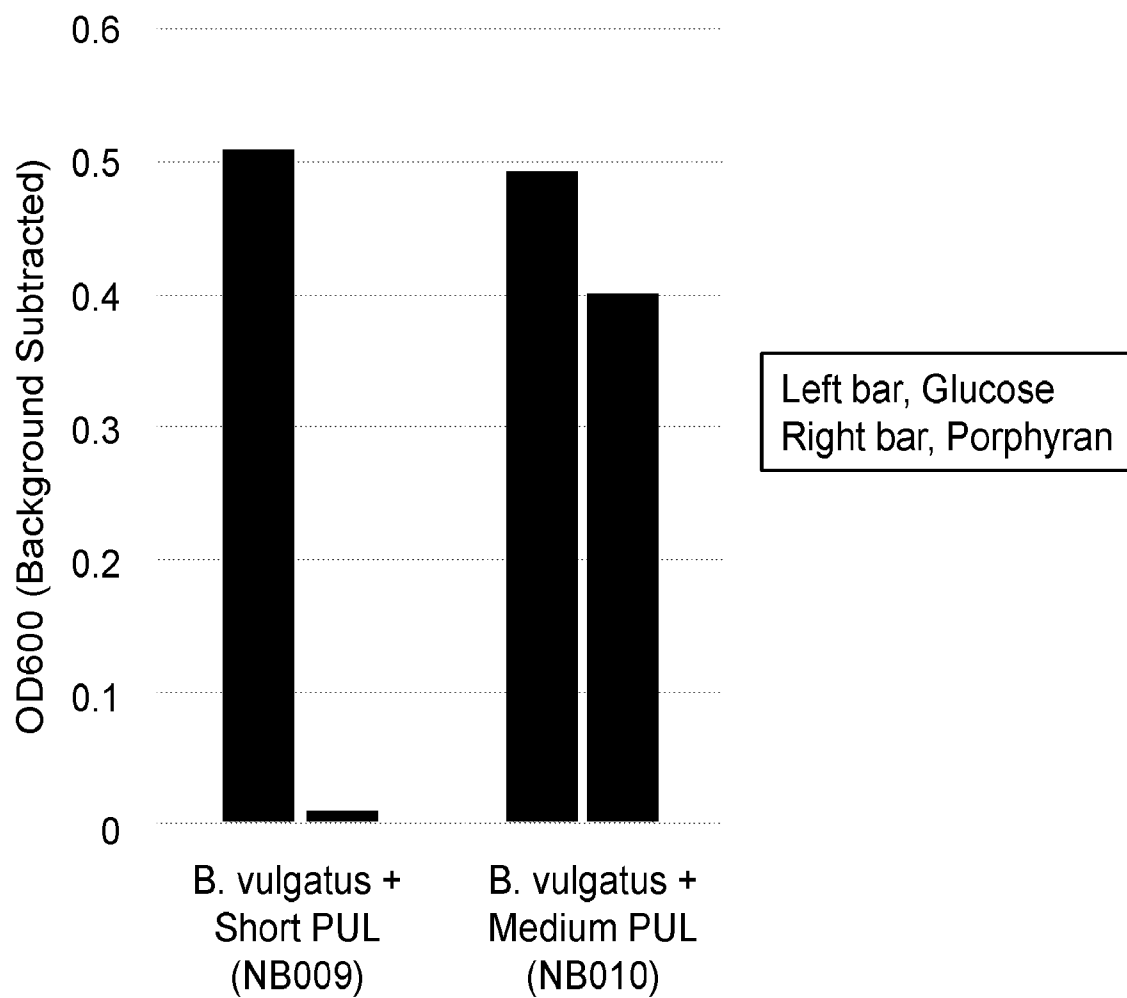
Figure 6

Figure 7

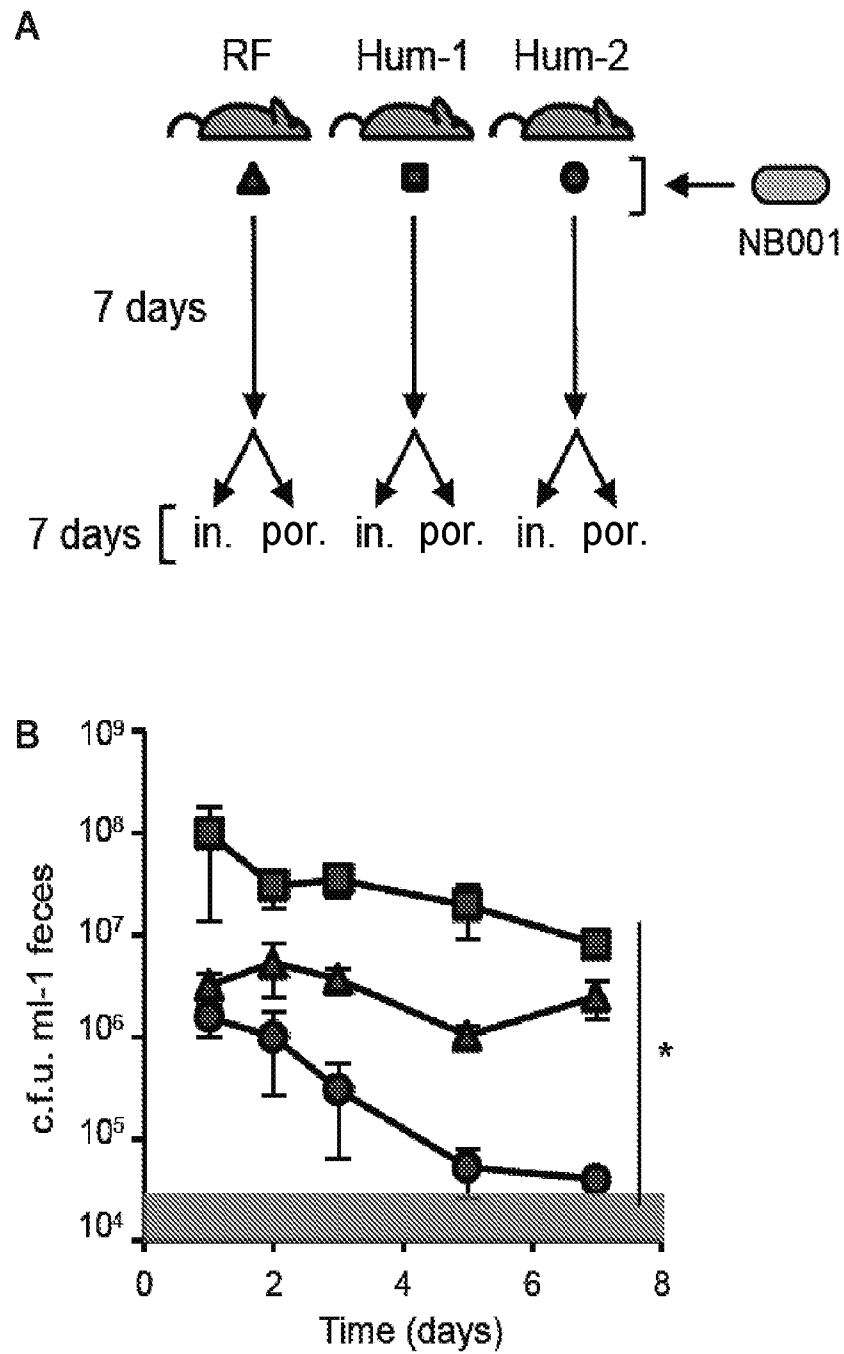


Figure 7 (Cont'd)

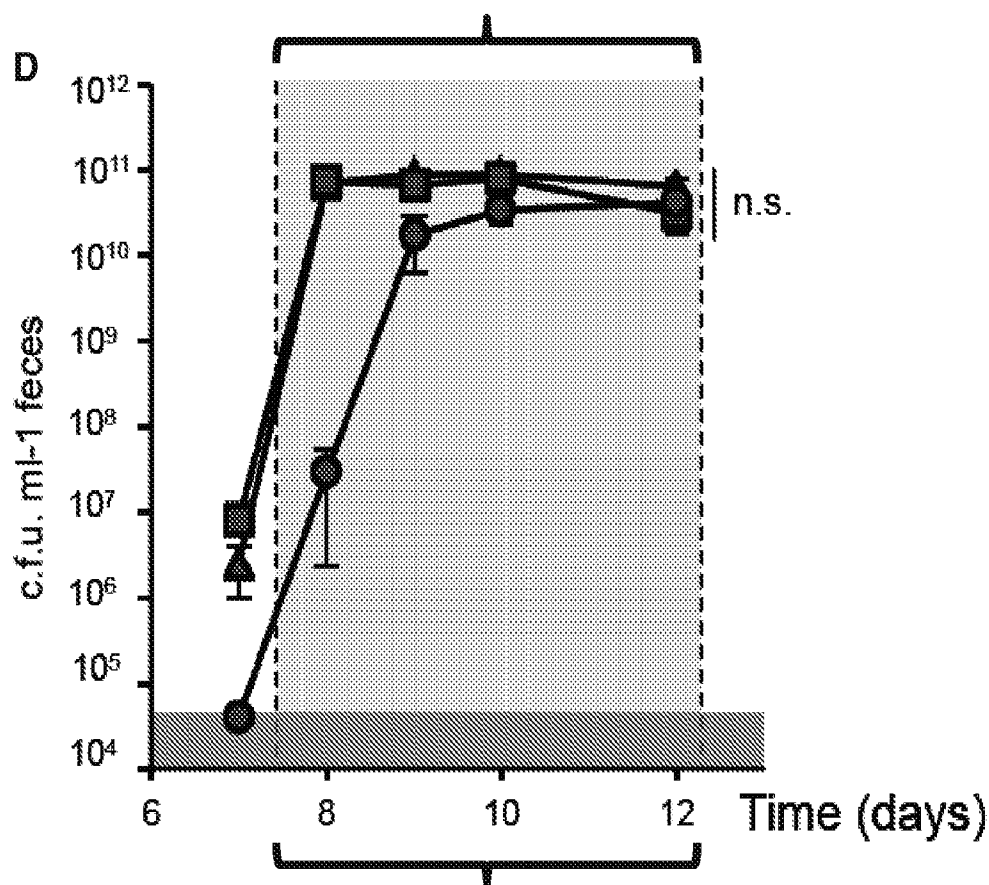
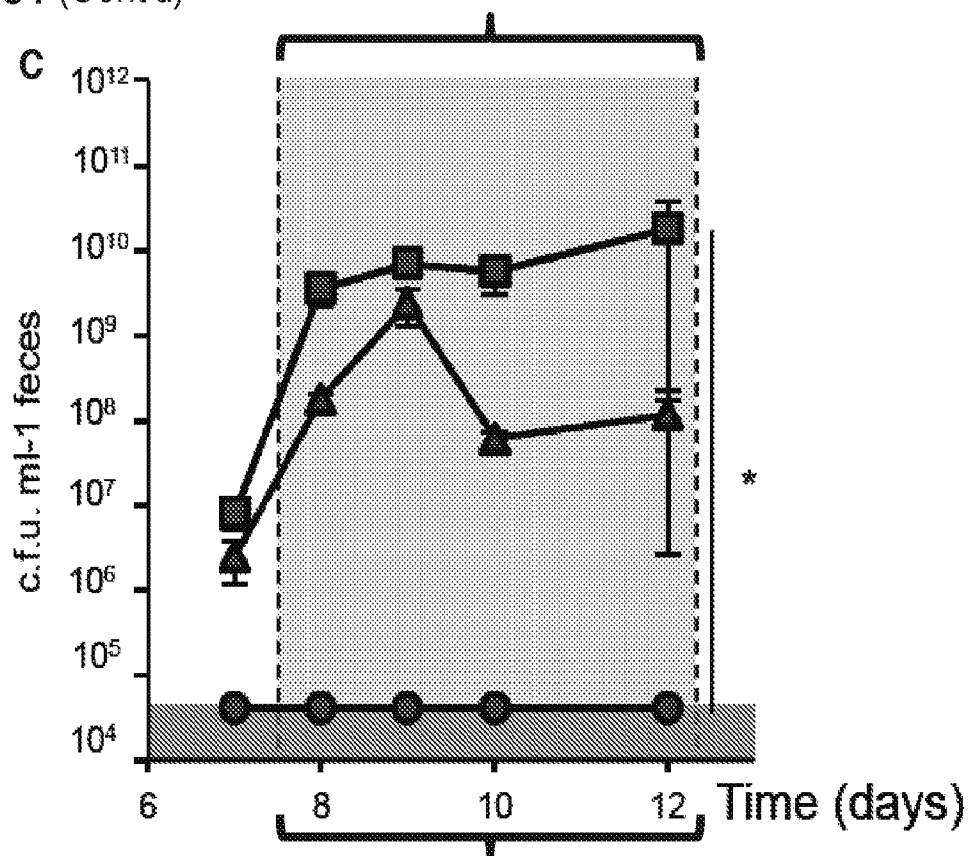
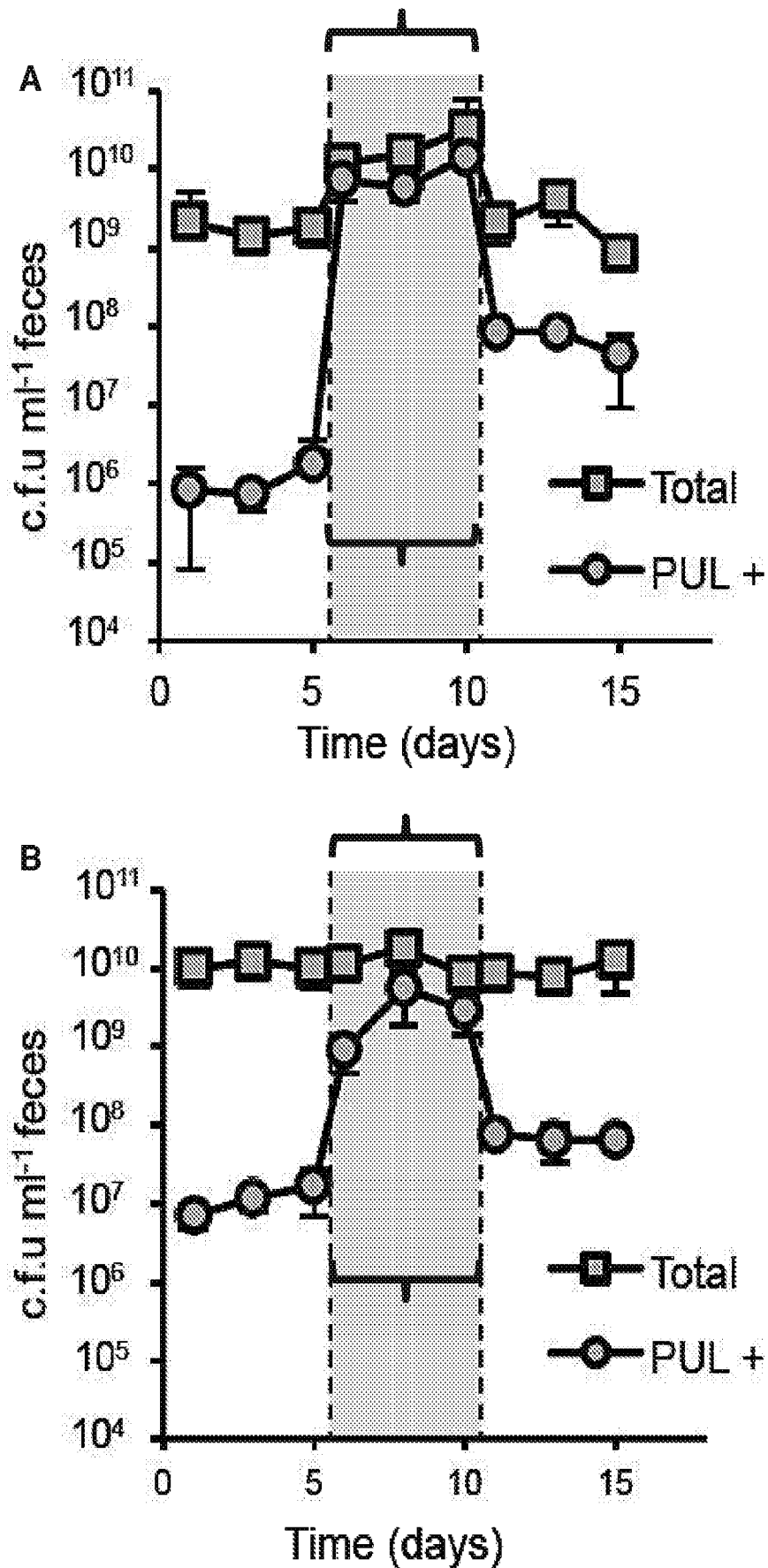


Figure 8



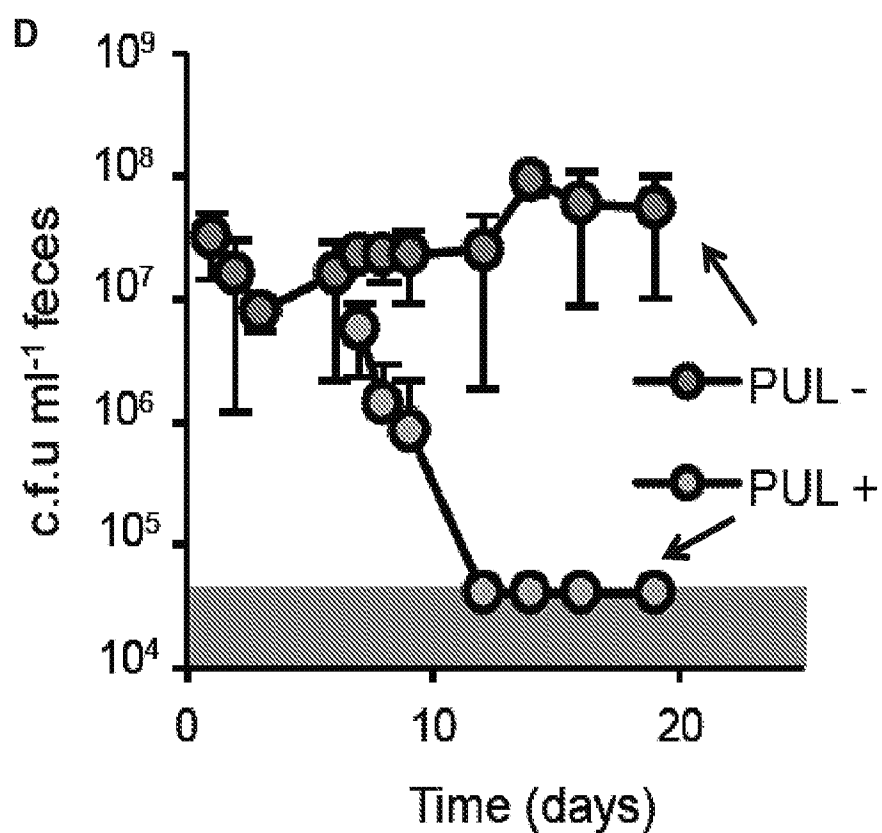
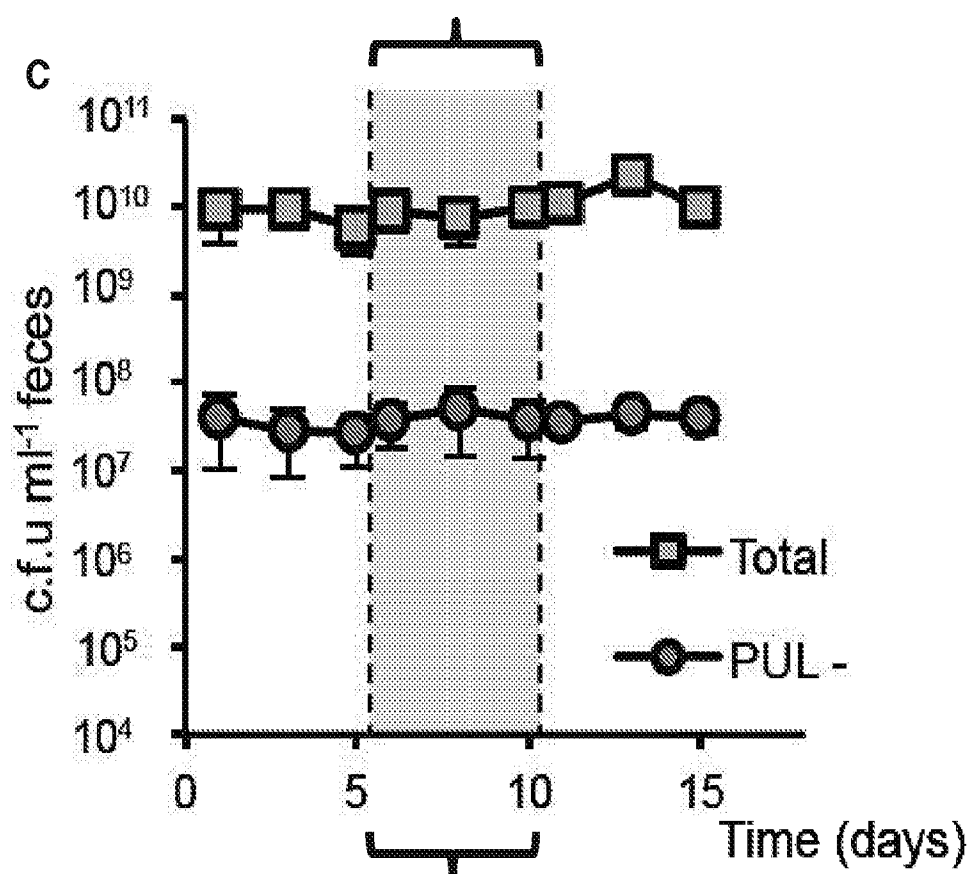
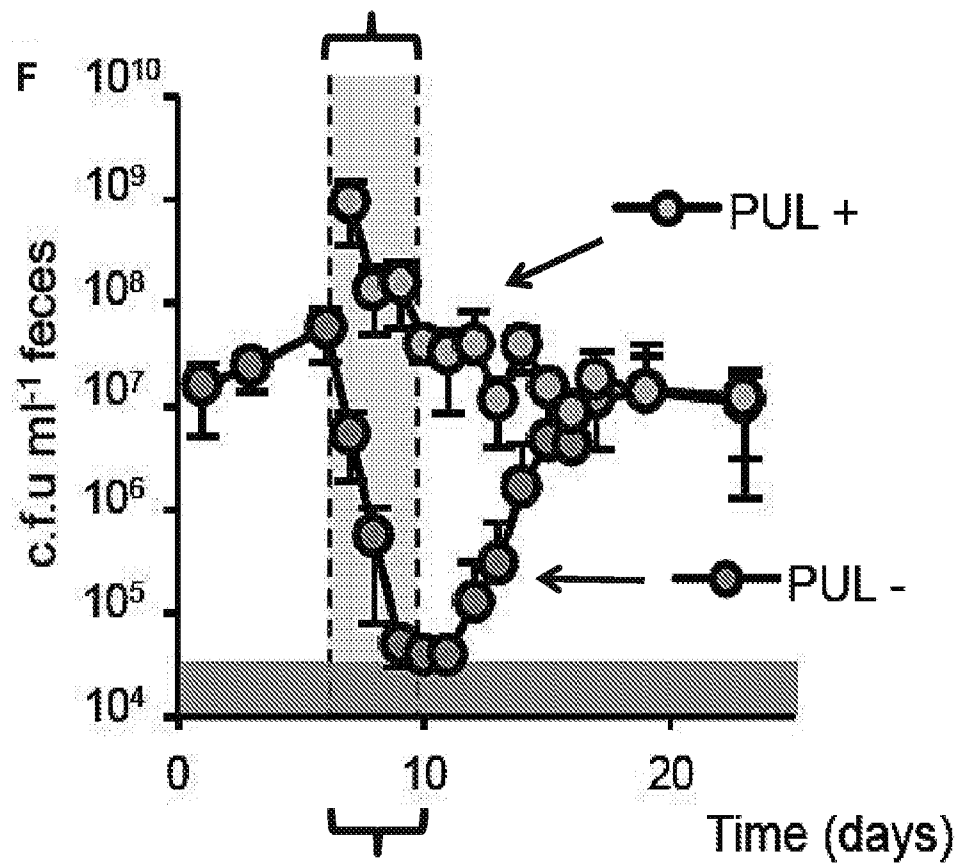
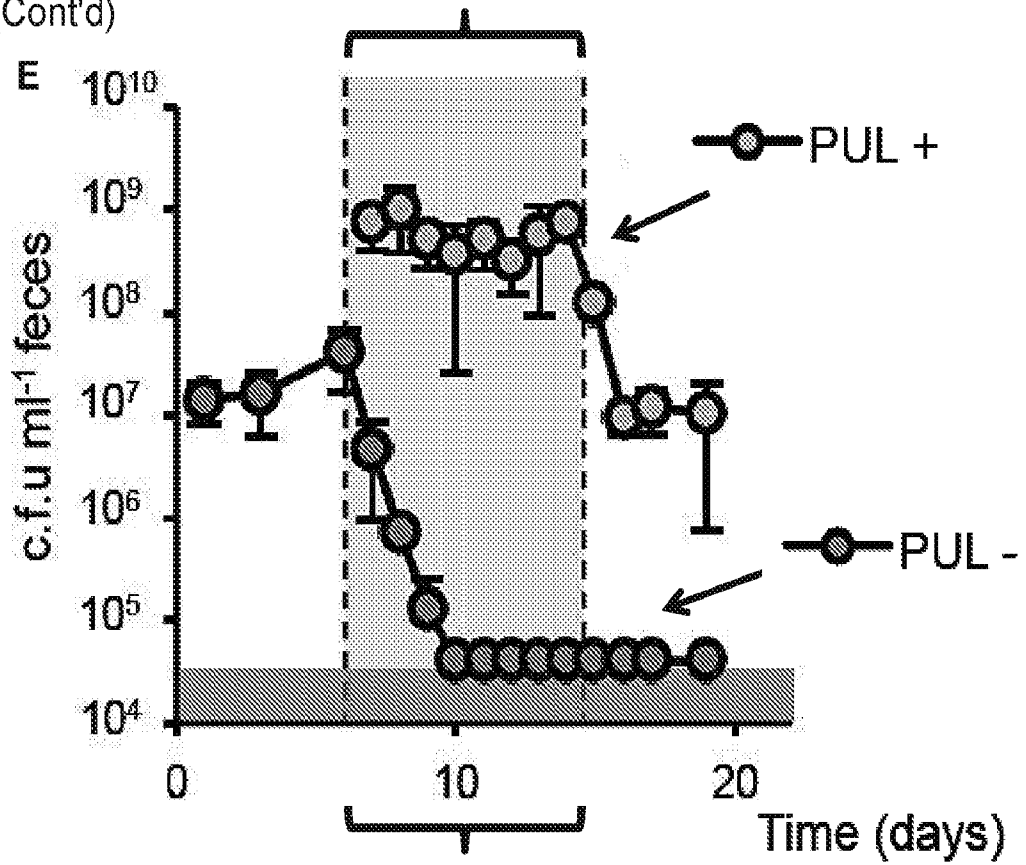


Figure 8 (Cont'd)



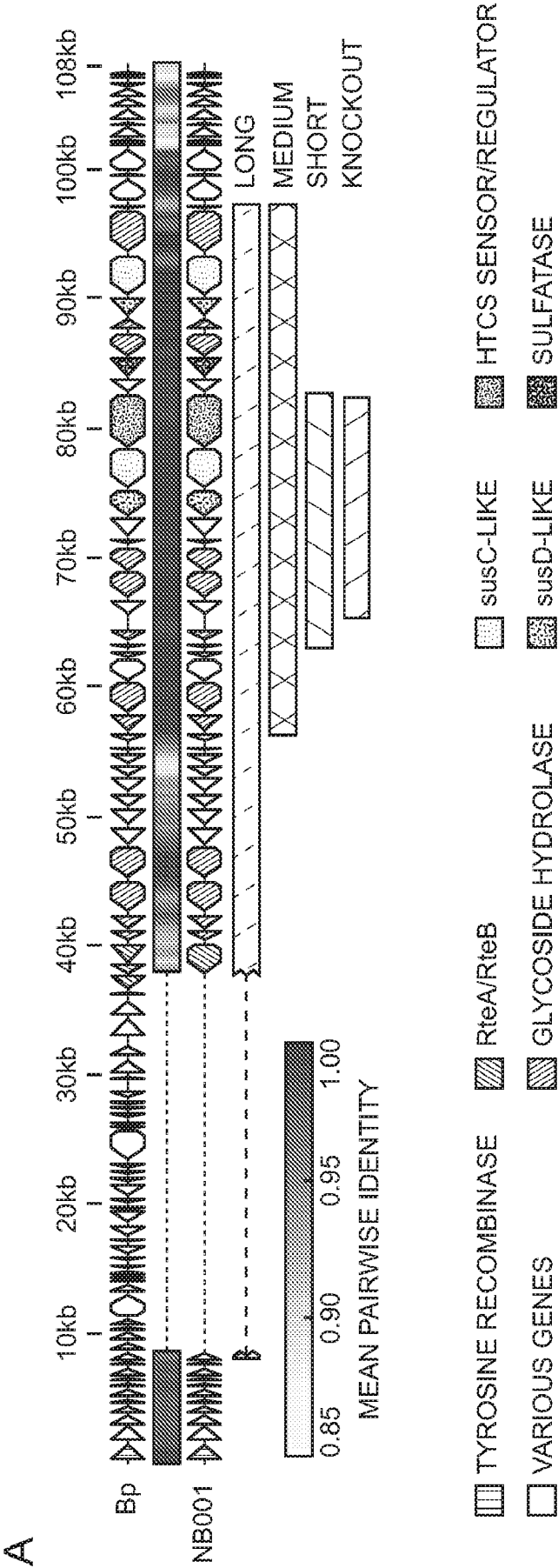


Figure 9

B

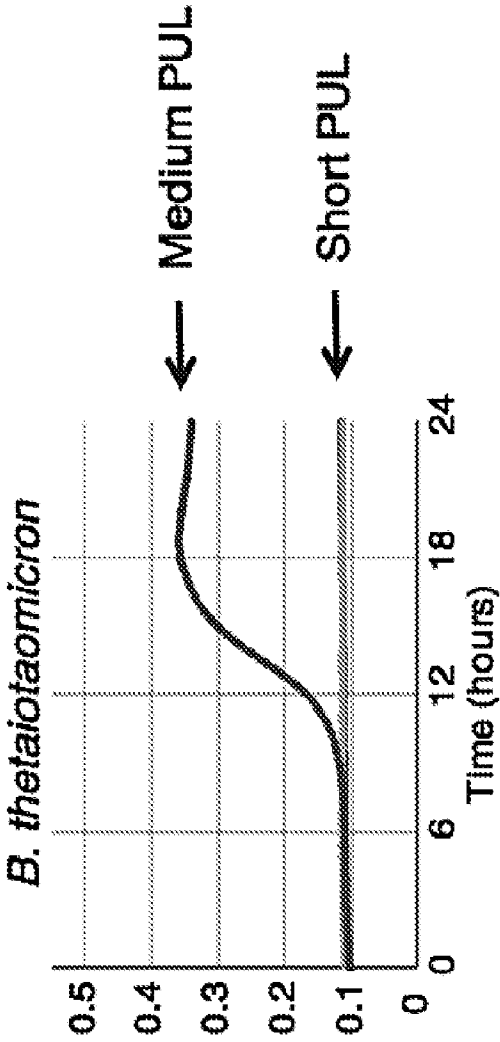
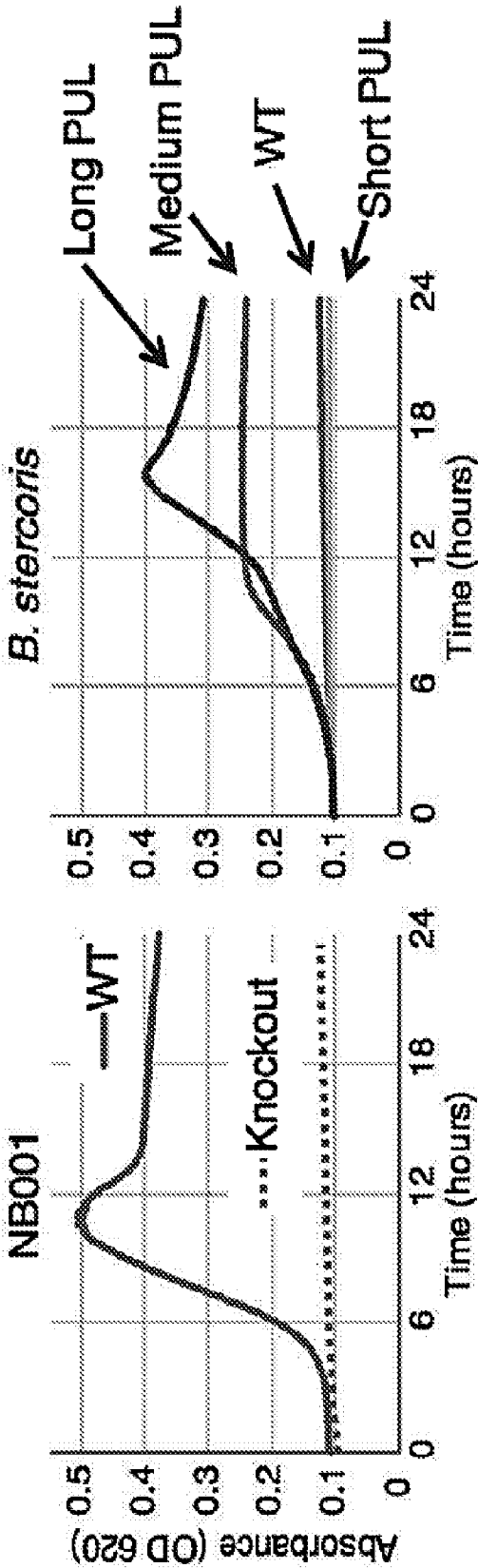


Figure 9 (Cont'd)

Figure 9 (Cont'd)

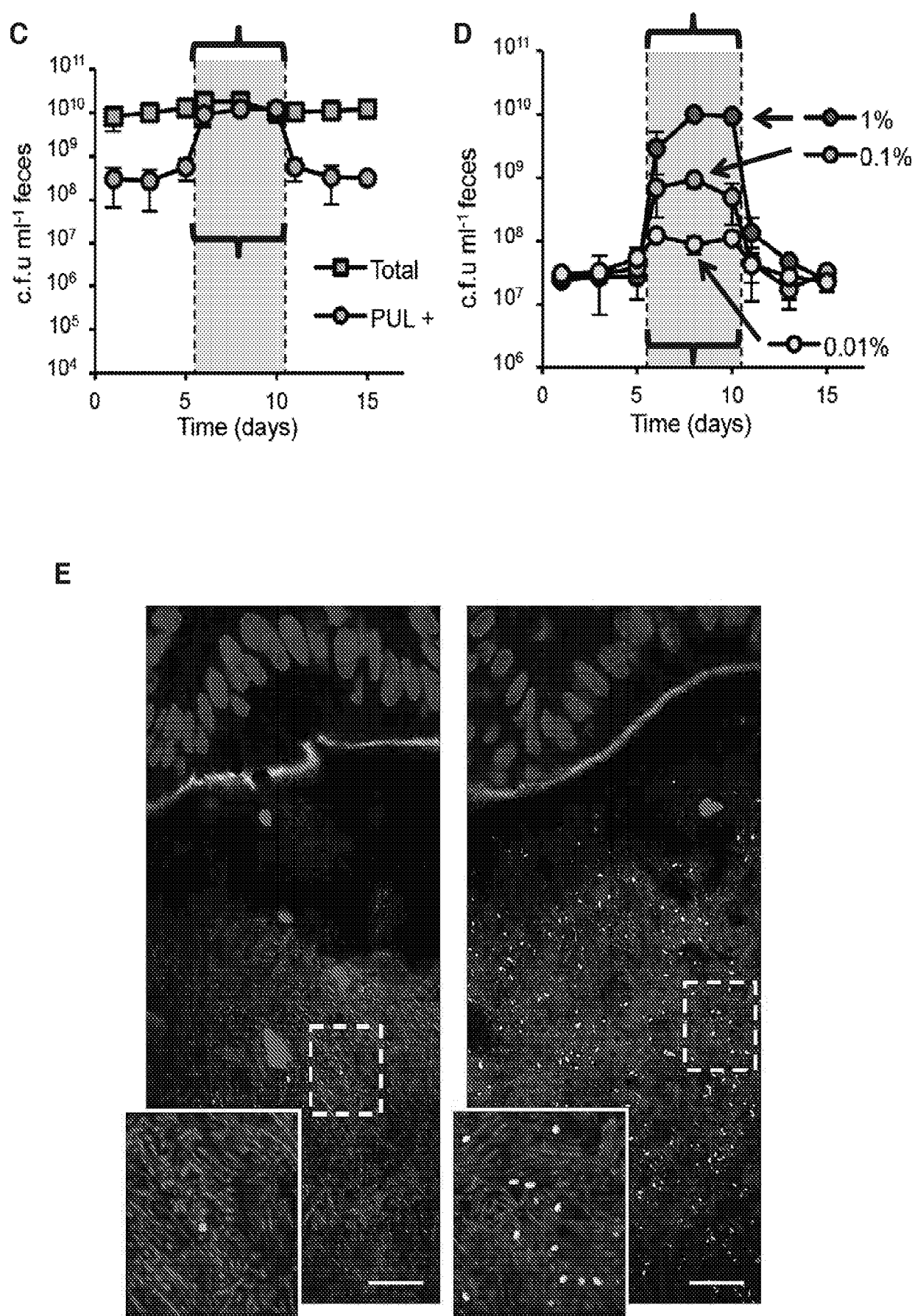
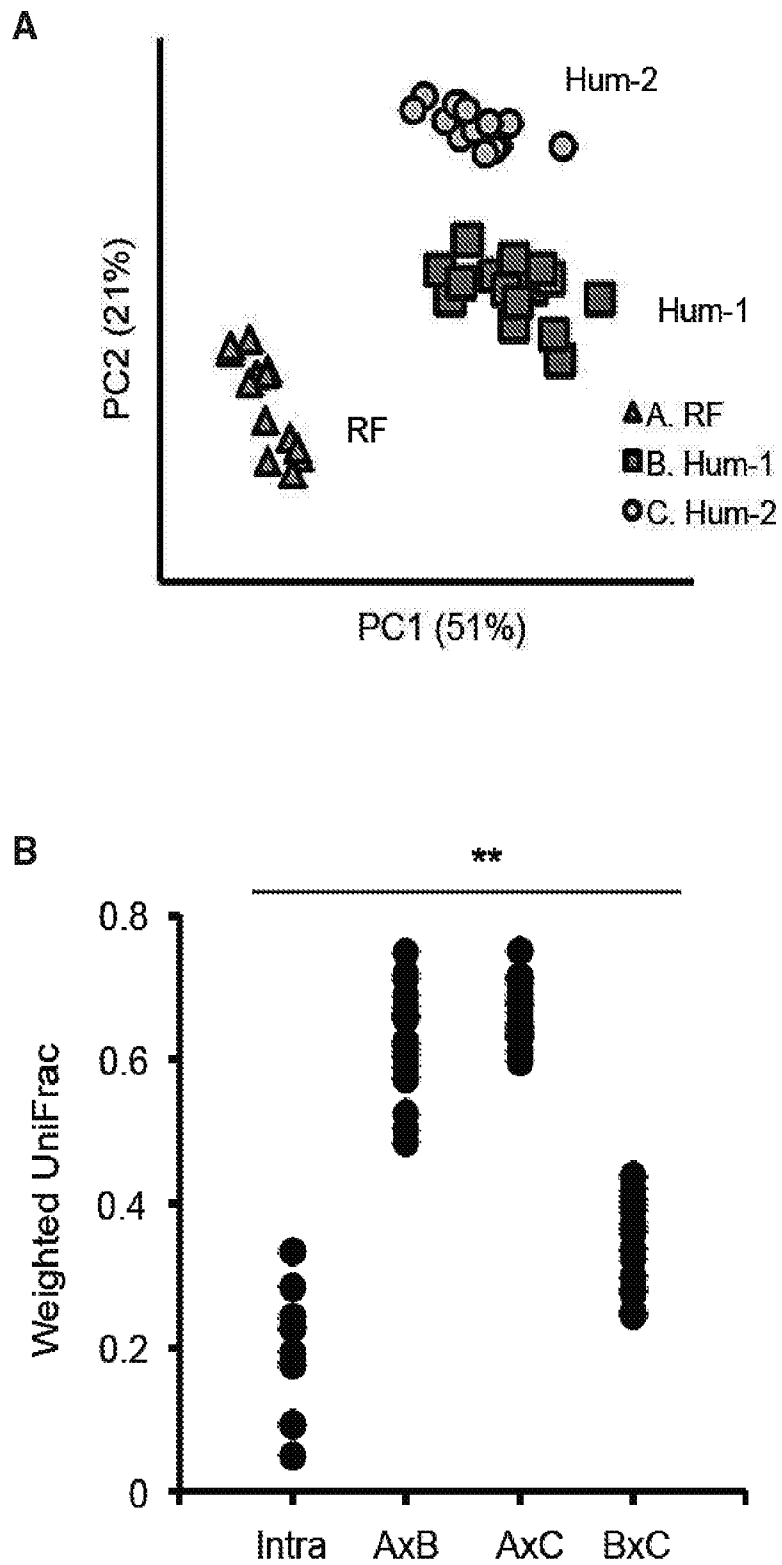


Figure 10



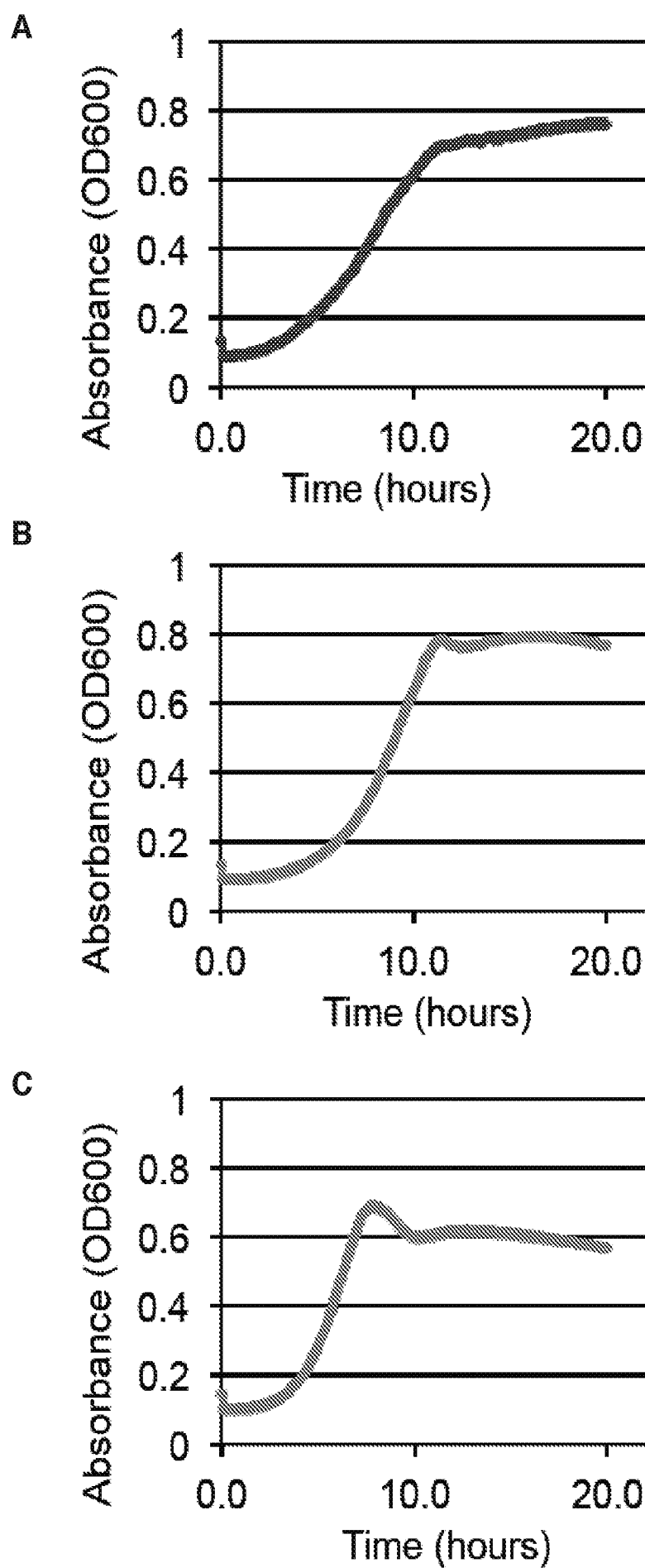


Figure 12

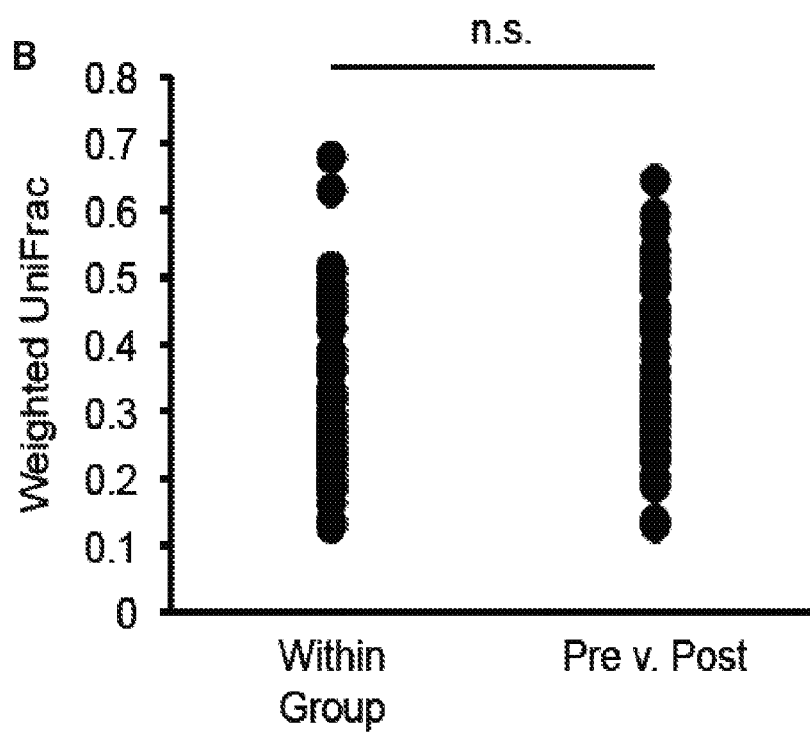
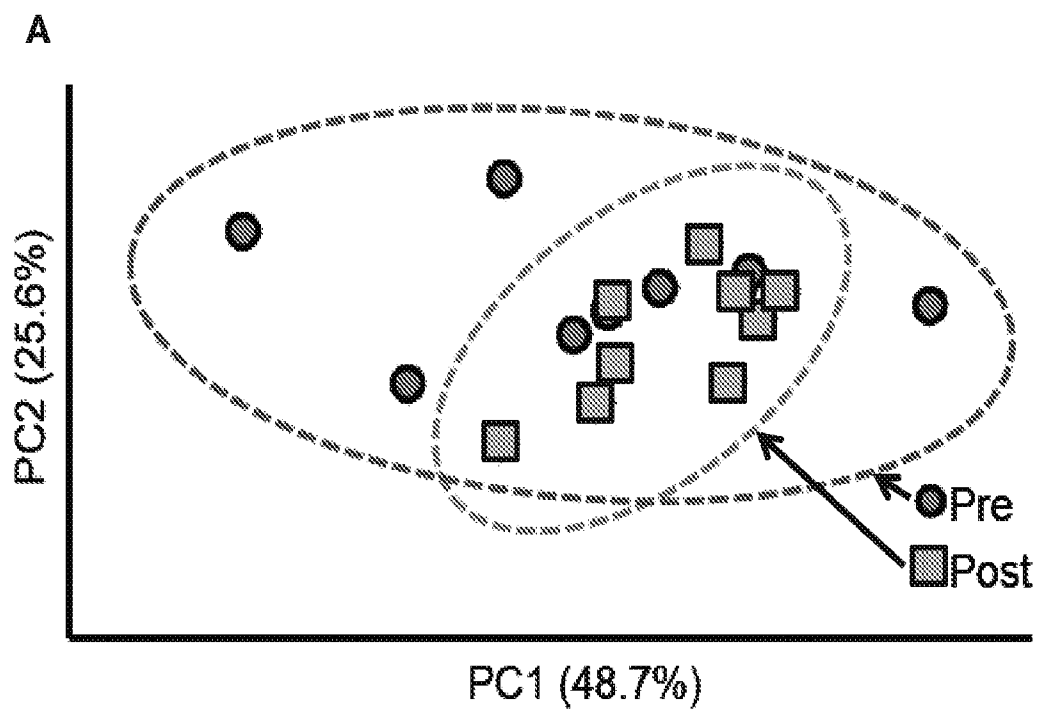


Figure 13

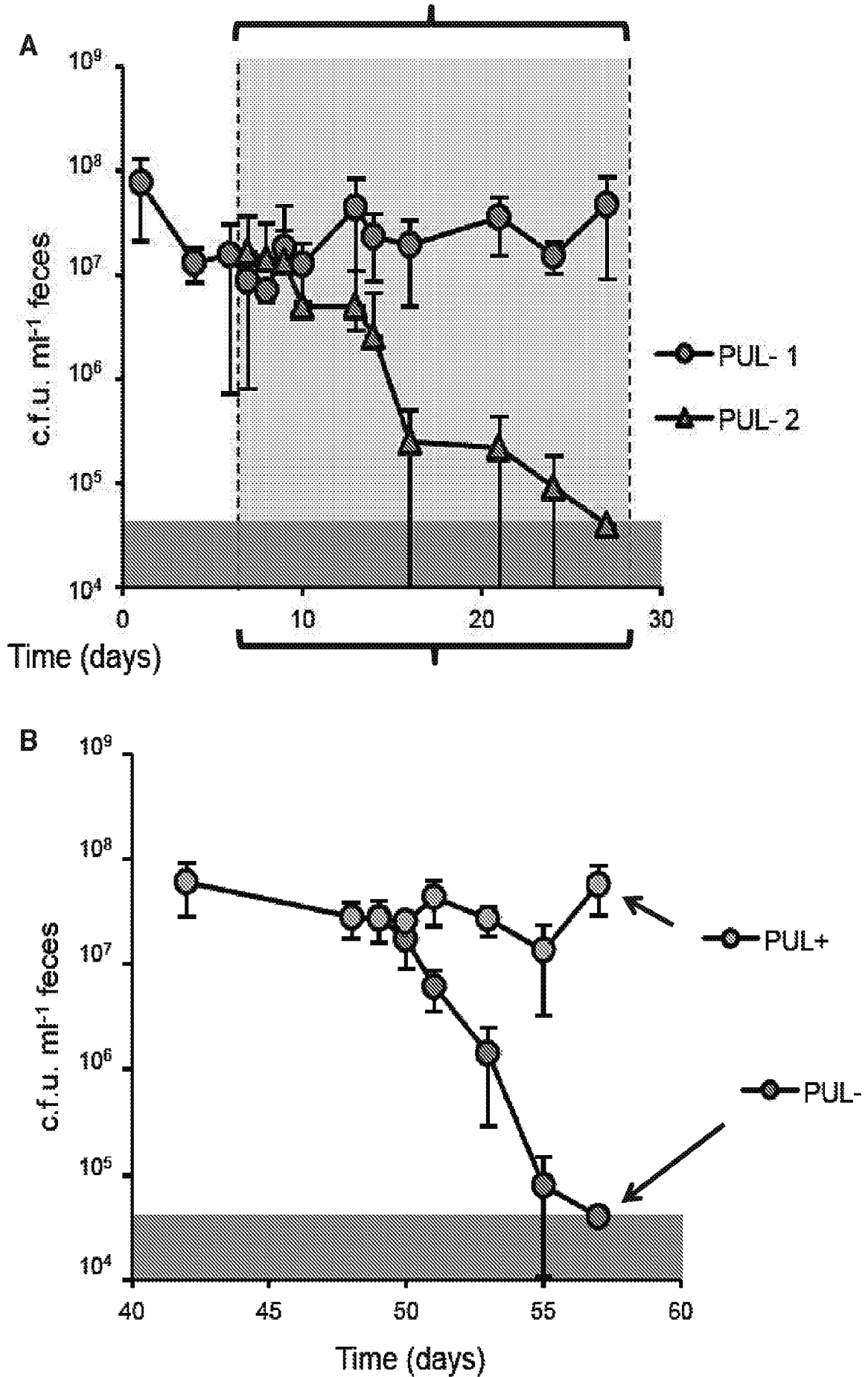


Figure 14

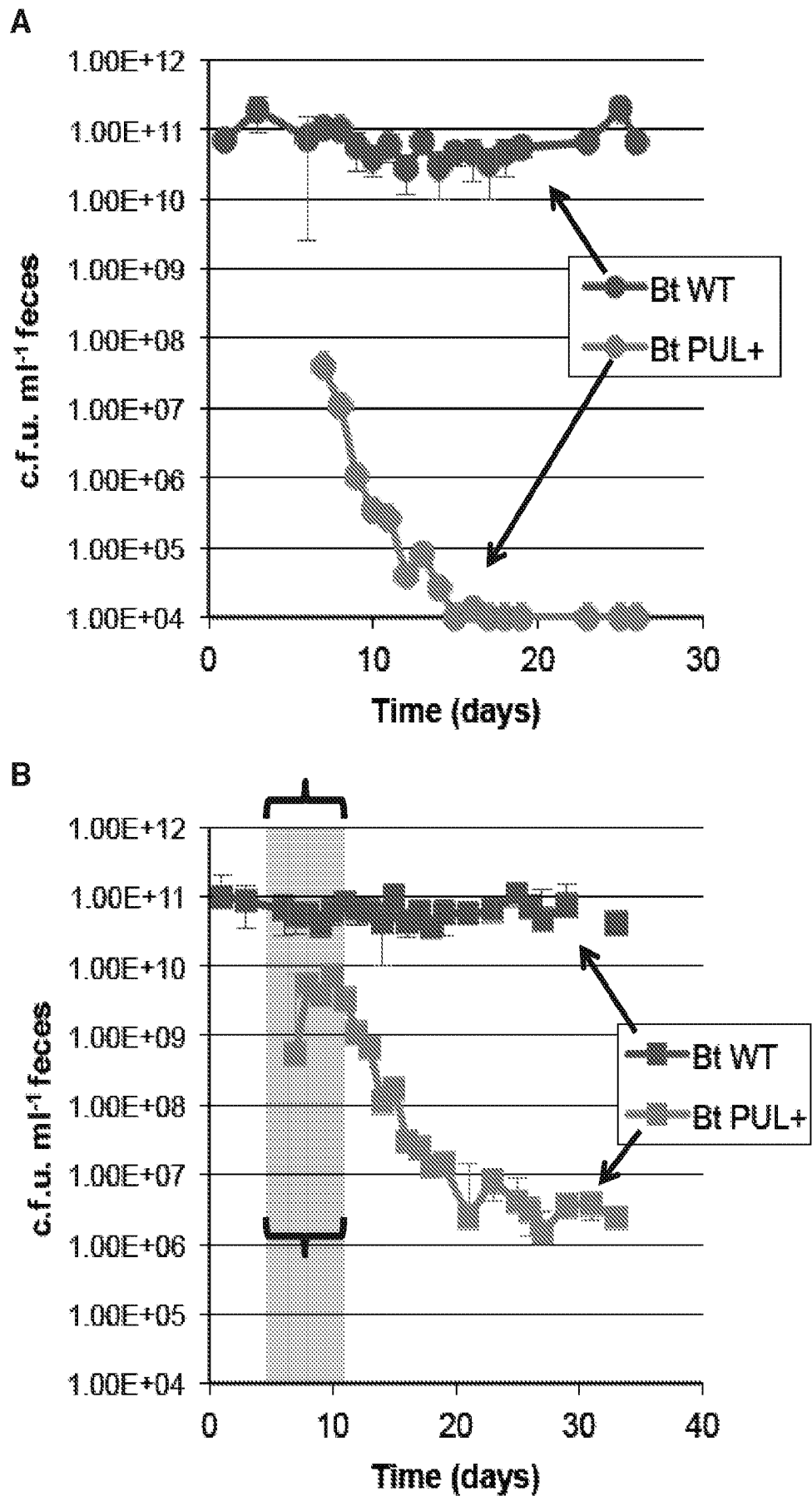


Figure 14 (Cont'd)

C



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/66408

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 35/741, A61P 1/00, C12N 1/21 (2018.01)

CPC - A61K 35/741, A61K 2035/115, A61K 35/742, A23L 29/065

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.
X	WO 2015/148680 A1 (GINKGO BIOWORKS INC.) 1 October 2015 (01.10.2015) para [0014], [0026], [0027], [0029], [0043], [0062], [0067], [0078], [0097], [0098], [00122]	1-7, 9-12, 80-83

☐ 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

"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

3 April 2018

Date of mailing of the international search report

25 APR 2018

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/66408

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.: 8, 13-37, 42-49, 52-54, 56-59, 63-78, 84-89, 94-98
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:

----- please see extra sheet -----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-7, 9-12, 80-83

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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/66408

Continuation of Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-7, 9-12, 80-83, drawn to methods of introducing to an organ a genetically modified bacterial cell, of modulating growth of a genetically modified bacterial cell present in an organ, and of facilitating colonization of an organ by a genetically modified bacterial cell

Group II: Claims 38-41, 50-51, 55, 90-93, drawn to a genetically modified bacterial cell

Group III: Claims 60-62, 79, drawn to methods of genetically modifying a bacterial cell

The inventions listed as Groups I, II, III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I requires method steps for introducing a genetically modified bacterial cell to an organ and for modulating growth and colonization of a genetically modified bacterial cell in an organ, not required by Groups II and III.

Group II requires compositions of matter comprising a genetically modified bacterial cell, not required by Groups I and III.

Group III requires method steps for producing a genetically modified bacterial cell, not required by Groups I and II.

Common Technical Features

The feature shared by Groups I, II, and III is a genetically modified bacterial cell that has the ability to utilize as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% of bacterial cells present in a human microbiota.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by WO 2015/148680 A1 to Ginkgo Bioworks Inc. (hereinafter 'Ginkgo Bioworks').

Ginkgo Bioworks discloses a genetically modified bacterial cell (para [0026] - "an engineered probiotic comprising the engineered organism described herein is provided. The engineered probiotic can be an oral probiotic for use in the gastrointestinal tract"; para [0027] - "In various embodiments, the engineered probiotic for use in the gastrointestinal tract and/or in the urinary tract can be based on a host selected from Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Verrucomicrobia or Fusobacteria divisions of Bacteria. For example, the host may be selected from Bacteroides species") that has the ability to utilize as a carbon source a rare carbohydrate of interest that is utilized as a carbon source by less than 50% of bacterial cells present in a human microbiota (para [0098] - "In some embodiments, the nucleic acid encodes one or more genes that confer the ability to utilize particular carbon source(s) not used by the parent, wildtype host cell or organism from which the engineered probiotic is derived. The inclusion of these carbon source utilization gene(s) confers a selective advantage to any cells carrying the heterologous genetic system when grown in the presence of the corresponding carbon source . . . Co-administration of the carbon source with the probiotic can be used as a means to control the propagation and duration of the probiotic treatment").

As the technical feature was known in the art at the time of the invention, it cannot be considered a special technical feature that would otherwise unify the groups.

Groups I, II, III therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.