glycans of mammalian milk become susceptible to hydrolysis by other bacteria. This releases Free Sugar Monomers (FSMs), which are capable of enabling the growth of opportunistic or highly destructive pathogens that would not have flourished otherwise. The term "dietary glycans", as used herein, refers to those indigestible glycans, sometimes referred to as "dietary fiber", or the carbohydrate polymers which are not hydrolyzed by the endogenous enzymes in the digestive tract (e.g., the small intestine) of the mammal.

[0010] Some embodiments of the invention involve compositions and methods of delivering to the gut of a dysbiotic mammal, compositions that include components capable of consuming the dietary glycans. Such compositions can reduce the concentration of FSMs in the mammal. The reduction of FSMs and dietary glycans can minimize the likelihood of an overpopulation of pathogenic bacteria that can harm that mammal. In some embodiments the compositions comprise certain bacteria (alive or dead) or other orally provided compounds that bind and/or metabolize the FSMs, thereby preventing them from being used as an energy source by the pathogenic bacteria.

[0011] Some of the embodiments of the present invention provide diagnostics for the presence of substrates enabling growth of pathogenic bacteria within mammalian neonates. Specifically, some embodiments provide diagnostics to determine the presence of FSMs.

[0012] Some of the embodiments of the present invention deliver a suite of a) microorganisms (*e.g.*, bacteria or yeast) that act as probiotics to actively remove substrates including intact dietary glycans and FSMs; b) enzymes capable of inactivating or eliminating dietary glycans and/or sugars; and/or c) binding agents that physically bind and render free sugars monomers unavailable as substrates supporting the growth of pathogenic microorganisms.

[0013] Some embodiments of the invention provide a composition administered to reduce the concentration of FSMs that may be the consequence of the use of antibiotics to treat the pathogenic bacterial overgrowth in mammals including humans.

[0013a] In one aspect, there is provided a composition comprising at least two non-pathogenic microbes, wherein one of the at least two non-pathogenic microbes is from a first species capable of internalizing and/or metabolizing dietary glycans, and wherein one of the at least two non-pathogenic microbes is from a second species capable of consuming and metabolizing free sugar monomers.

[0013b] In one aspect, there is provided the use of at least two non-pathogenic microbes in the manufacture of a medicament for treating or improving the health of an infant mammal, wherein one of the at least two non-pathogenic microbes is from a first species capable of internalizing and/or metabolizing dietary glycans, and wherein one of the at least two non-pathogenic microbes is from a second species capable of consuming and metabolizing free sugar monomers, is accomplished.

[0013c] In one aspect, there is provided a method of detecting a dysbiotic subject comprising determining a presence of free sugar monomers in the subject's feces and reporting the presence of free sugar monomers.

[0013d] In one aspect, there is provided the use of non-pathogenic microbes in the manufacture of a medicament for treating a dysbiotic subject, wherein the non-pathogenic microbes are capable of metabolizing and/or sequestering the free sugar monomers detected by the method as disclosed herein.

Brief Description of the Drawings

[0014] FIG. 1: Graph showing typical piglet E. coli isolate on pig milk sugar constituent sugars vs. conjugated glycans.

Lactobacillus (Figure 9), populations in the low-Enterobacteriaceae animals were largely unaffected. These "at risk" animals had significantly lower populations of starting Lactobacillaceae populations ($p < 0.05$), which may help explain why higher populations of Enterobacteriaceae could thrive, and why supplementation with Lactobacillus led to a reduction where populations of Enterobacteriaceae were not significantly different from low-Enterobacteriaceae animals. Figure 9 shows "At risk" (AR) or high-Enterobacteriaceae pigs could be rescued by gavage with Lactobacillus to resemble No Risk or Non Responder animals. Letters denote significance groups (a, b; $p < 0.05$). Figure 10 shows a model for PMO consumption.

[0070] All experiments involving animals were reviewed and approved by the University of California Davis Institutional Animal Care and Use Committee prior to experimentation (Approval # 17776, #18279). Throughout the study, all animals were housed in a controlled-access specific pathogen free facility at the University of California Davis dedicated to the rearing of pigs. Three healthy adult pregnant sows from the University of California herd were selected for this study. Upon delivery, the infant pigs were cohoused with sows and ear tagged for identification, following standard practices. The piglets were allowed to nurse freely until weaning after 21 days of age. Piglets were removed from the sow and transferred to separate housing and fed a standard starter feed (Hubbard Feeds Mankato, MN USA) after 21 days of age. Animals were given ad libitum access to water and feed. Milk was collected from sows while nursing their respective litters and stored at -80C.

[0071] Fecal samples were collected using a sterile cotton swab (Puritan Medical, Guilford, ME USA) rectally from each piglet after 1, 3, 5, 7, 14, 21, 28, 35, and 42 days after birth. Swabs were also used to collect fecal samples from mother sows and ~4 cm2 sites within the enclosure throughout the study.

[0072] Sequencing Library Construction. DNA was extracted from swabs using the Zymo Research Fecal DNA kit (Zymo Research Irvine, CA USA) according to the manufacturer's instructions. Extracted DNA was used as a template for PCR using barcoded primers to amplify the V4 region of the 16S rRNA gene as previously described for bacteria and the internal transcribed spacer region (ITS) to assess fungal communities.

[0073] Briefly, the V4 domain of the 16S rRNA gene was amplified using primers F515 (5'-NNNNNNNNGTGTGCCAGCMGCCGCGGTAA-3') (SEQ ID NO: 1) and R806 (5'-GGACTACHVGGGTWTCTAAT-3') (SEQ ID NO: 2), where the

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<120> COMPOSITIONS THAT METABOLIZE OR SEQUESTER FREE SUGAR MONOMERS AND USES THEREOF

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CLAIMS

1. A composition comprising at least two non-pathogenic microbes, wherein one of the at least two non-pathogenic microbes is from a first species capable of internalizing and/or metabolizing dietary glycans, and wherein one of the at least two non-pathogenic microbes is from a second species capable of consuming and metabolizing free sugar monomers.

2. The composition of claim 1, wherein the free sugar monomers include fucose, sialic acid, N-acetylglucosamine, N-acetylgalactosamine, gluconate, glucosinate, glucose, galactose, lactose, sialyllactose, fucosyllactose, lacto-N-biose, or mixtures thereof.

3. The composition of claim 1, wherein the first species of non-pathogenic microbe is a member of the genus *Bifidobacterium,* and the *Bifidobacterium* is optionally *B. longum*, *B. breve*, *B. longum* subsp. *infantis*, or *B. pseudocatenulatum*.

4. The composition of claim 3, wherein the *B. longum* subsp. *infantis* is activated.

5. The composition of claim 1, wherein the second species of non-pathogenic microbe is a member of the genus *Bifidobacterium*, *Lactobacillus*, and/or *Pediococcus*, further wherein the *Bifidobacterium* optionally is *B. infantis, B. breve, B. bifidum, B. longum, B. adolescentis, B. animalis, or B. pseudocatenulatum*, the *Lactobacillus* optionally is *L. planatarum, L. casei, L. rhamnosus, L. brevis, L. fermentum, L. crispatus, L. johnsonii, L. gasseri, L. mucosae,* or *L. salivarius*, and/or the *Pediococcus* optionally is *P. acidilacti, P. pentosaceus, P. stilesii, P. argentinicus,* or *P. claussenii*.

6. The composition of claim 1, wherein the first species of non-pathogenic microbe is present in an amount of about 10 % to about 90 % or about 20% to about 80% of total amount of the non-pathogenic microbes and/or wherein the second species of non-pathogenic microbe is present in an amount of about 10 % to about 90 % or about 20% to about 80% of total amount of non-pathogenic microbes.

7. The composition of claim 1, wherein the second species is selected based on the free sugar monomers that are present or predicted to be present in an infant mammal or wherein the second species is selected based on the free sugar monomers that are preferred by a pathogen, and

wherein the infant mammal has an increased likelihood of a pathogenic overpopulation of the pathogen.

8. The composition of claim 7, wherein the free sugar monomers are present in the infant mammal, and wherein the presence is determined by measuring the free sugar monomers in a fecal sample of the infant mammal.

9. The composition of claim 7, wherein the free sugar monomers are predicted to be present in an infant mammal, and wherein the presence is predicted based on a pathogenic bloom of the infant mammal, wherein the pathogen is optionally a member of the Class Clostridium or Phylum Proteobacteria, and further wherein the Clostridia is optionally *C. difficile* or *C. perfringens*.

10. The composition of claim 7, wherein the infant mammal has an increased likelihood of a pathogenic overpopulation of the pathogen and wherein the increased likelihood of a pathogenic overpopulation of the pathogen is due to an outbreak in the surroundings of the infant mammal.

11. The composition of claim 10, wherein the infant mammal is a human infant, infant horse or an infant pig.

12. The composition of claim 1, wherein the composition is in the form of a dry powder or a dry powder suspended in an oil, optionally wherein the dry powder further comprises cryoprotectant.

13. The composition of claim 1, further comprising about 5 to 90 % by weight dietary glycans, said dietary glycans optionally derived from a human, swine, equine, or bovine source.

14. The composition of claim 1, wherein the composition is capable of growing on dietary glycans, wherein the dietary glycans comprise sialic acid and fucose, and less than 1 % of sialic acid and less than 1 % of the fucose remains as free sugar monomers after a culture of the composition has ceased to grow.

15. The composition of claim 1, wherein at least one of the non-pathogenic microbes comprises a gene coding for a complex oligosaccharide transporter.

16. The use of at least two non-pathogenic microbes in the manufacture of a medicament for treating or improving the health of an infant mammal, wherein one of the at least two non-pathogenic microbes is from a first species capable of internalizing and/or metabolizing dietary glycans,

and wherein one of the at least two non-pathogenic microbes is from a second species capable of consuming and metabolizing free sugar monomers, is accomplished.

17. The use according to claim 16, wherein the free sugar monomers consumed by the second species are those present as a consequence of prior antibiotic administration.

18. The use according to any one of claim 16 or 17, wherein the mammal is an infant that is receiving dietary glycans contemporaneously with the administration of the medicament.

19. The use according to claim 18, wherein the infant mammal is a nursing infant mammal.

20. The use according to any one of claims 16–19, wherein the medicament is to be first administered at a period of within 96 hours of the birth of the infant mammal.

21. A method of detecting a dysbiotic subject comprising determining a presence of free sugar monomers in the subject's feces and reporting the presence of free sugar monomers.

22. The use of non-pathogenic microbes in the manufacture of a medicament for treating a dysbiotic subject, wherein the non-pathogenic microbes are capable of metabolizing and/or sequestering the free sugar monomers detected by the method of claim 21.

23. The use according to claim 22, wherein the medicament further comprises a non-pathogenic microbe, capable of internalizing and/or metabolizing dietary glycans.

24. The use according to any one of claims 22 or 23, wherein the medicament comprising non-pathogenic microbes is a composition according to any one of claims 1–15.

25. The use according to any one of claims 21–24, wherein the subject is a mammal.

26. The use according to claim 25, wherein the mammal is a human, a cow, a pig, a rabbit, a goat, a sheep, a cat, a dog, a horse, or a camel.

27. The use according to claim 26, wherein the mammal is an infant.

28. The use according to claim 27, wherein the mammal is an infant that is receiving dietary glycans contemporaneously with the administration of the composition.

29. The use according to claim 28, wherein the mammal is a nursing infant mammal.

30. The use according to any one of claims 22-24 or 25-29, wherein the medicament is formulated to be first administered at a period of within 96 hours of the birth of the mammal.