

PREBIOTIC FORMULATIONS AND METHODS OF USE

CROSS REFERENCE

- [0001] This application claims the benefit of U.S. Provisional Application Nos. 61/155,150, filed February 24, 2009 and 61/272,622, filed October 13, 2009 which are incorporated herein by reference in their entireties.

BACKGROUND OF THE INVENTION

- [0002] According to several sources, there are 30 to 50 million people in the world who are lactose intolerant. In the 1960s and 1970s, it was reported that 70% of the adults in the world had lactose intolerance. In 1995, it was reported that 75% of the adults in the world and 25% of the adults in the U.S. were categorized as being lactose intolerant. In 1994, it was reported that 75% of African Americans and Native Americans and 90% of Asian Americans had lactose intolerance. It has also been reported that 30% of adults who are mostly North American descendants of Europeans have adapted to high lactase activity into adulthood. Research concludes that this adaptation is genetically controlled, permanent, and related to a long tradition of milk and milk product consumption in these regions of the world.
- [0003] Lactose intolerance is the inability to digest significant amounts of lactose, a major natural sugar found in milk and milk products of all mammals. Lactose intolerance is caused by a shortage of the enzyme lactase, which is produced by the cells that line the small intestine and is essential to lactose digestion. Lactase breaks down lactose, a disaccharide, into two simpler forms of sugar called glucose and galactose, which are then transported across the cell membrane and absorbed into the bloodstream. If lactase is not present, or not present in sufficient levels, excess undigested lactose passes through the small intestines into the large intestine where it is fermented by bacteria in the colon ("colonic microbiota," "gut microbiota," "intestinal microbiota," or "commensal gut microbiota"). The fermentation of lactose in the large intestine produces hydrogen and methane which can lead to bloating, gas, and diarrhea. These symptoms are caused by a very low activity of lactase in the intestines and are found in subjects who are lactose intolerant. Not all subjects deficient in lactase have the symptoms commonly associated with lactose intolerance, but those who do are said to have lactose intolerance.
- [0004] If a subject suspects that he or she has lactose intolerance, it is potentially harmful for him or her to restrict his or her diet because restriction can result in a nutrition shortage or a failure to detect a more serious disease. Milk and other dairy products are major sources for nutrition in the basic American diet. The primary nutrients in milk are protein, calcium, riboflavin, vitamin A, and vitamin D. Calcium is an important part of the recommended daily allowance of vitamins and minerals and any deficiency therein can lead to health risks such as osteoporosis, hypertension, or weak bone density.

- [0005] Young children who have lactose intolerance are very rare. The amount of the enzyme lactase a body produces generally reaches a maximum immediately after birth and then decreases in the majority of people during the ages of about 3-15.
- [0006] Generally, humans develop lactose intolerance from a primary or secondary cause. The primary cause is an onset of loss of lactase that is believed to be a permanent condition. This onset can occur at a variable period after the weaning period. The primary cause is also genetically determined. The secondary cause is generally a temporary condition that occurs as a result of another disease or event that damages the lining of the small intestine where lactase is active. This temporary condition can be caused by acute diarrhea, disease, parasitic infection, Cohn's disease, celiac disease, gastrointestinal surgery, or the intake of certain medications.
- [0007] In addition to the primary and secondary causes, certain human ethnic and racial populations have more of a predisposition for lactose intolerance. In these populations, social and cultural habits and attitudes influence lactose intolerance. Lactose activity can also decrease with age in certain ethnic and racial populations, including those populations which have origins in Europe, the African plains, and the Siberian Steppes. Humans who are most likely to have or develop lactose intolerance include those of Asian, Middle Eastern, North American, African, and Latin American decent.

SUMMARY OF THE INVENTION

- [0008] In one aspect, a method for increasing lactose tolerance in a subject experiencing one or more symptoms of lactose intolerance is provided comprising administering a composition comprising GOS to the subject each day for a predetermined number of days. In one embodiment, said composition further comprises a probiotic. In another embodiment, said composition does not contain a probiotic. In another embodiment, said composition comprises a lower dosage of GOS on the first day of administration than the last day of administration. In another embodiment, said composition comprises the same dosage of GOS on the first day of administration as the last day of administration. In another embodiment, said method comprises administering said composition once a day. In another embodiment, said method comprises administering said composition twice a day. In another embodiment, said method comprises administering said composition three times a day. In another embodiment, said composition comprising GOS is provided as a powder, a tablet, or a capsule. In another embodiment, said composition comprising GOS is administered without a meal. In another embodiment, said composition comprising GOS is administered in conjunction with a meal. In another embodiment, said composition comprising GOS is administered with breakfast and dinner. In another embodiment, said composition comprising GOS is administered with breakfast, lunch, and dinner. In another embodiment, said one or more symptoms comprise flatulence, heartburn, stomach upset, nausea, bloating, flatulence, diarrhea, abdominal pain, cramping, or vomiting. In another embodiment, at least about 96% percent of the total weight of the composition is GOS.

- [0009] In another aspect, a composition for increasing lactose tolerance in a subject comprising about 0.1 to 2 grams of a GOS composition is provided, wherein said GOS composition comprises about 96% GOS by weight. In another embodiment, the composition further comprises a pharmaceutically acceptable excipient. In another embodiment, said composition is contained within a capsule. In another embodiment, said capsule is a gelatin capsule. In another embodiment, said gelatin capsule comprises a viscous syrup or liquid comprising GOS. In another embodiment, said syrup does not contain an anti-oxidant or preservative. In another embodiment, said composition is contained in a tablet. In another embodiment, the composition further comprises a probiotic. In another embodiment, said GOS composition comprises an enteric coating.
- [0010] In another aspect, an oral dosage form of GOS comprising about 0.1 to 2 grams of a GOS composition is provided wherein said GOS composition is a viscous syrup or liquid encapsulated in a gelatin capsule. In one embodiment, said GOS composition comprises about 80% GOS by weight. In another embodiment, said GOS composition comprises about 96% GOS by weight. In another embodiment, the GOS composition further comprises a probiotic. In another embodiment, the GOS composition comprises an enteric coating.
- [0011] In another aspect, an oral dosage form of GOS comprising a GOS composition in a tablet or capsule formulated for controlled release is provided. In one embodiment, said controlled release occurs in the intestines. In another embodiment, said GOS composition comprises an enteric coating. In another embodiment, said controlled release occurs in the lower intestine. In another embodiment, the composition further comprises a probiotic. In one embodiment, said GOS composition comprises an enteric coating.

INCORPORATION BY REFERENCE

- [0012] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

- [0013] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:
- [0014] Figure 1 illustrates the chemical structure of lactulose.
- [0015] Figure 2 illustrates the chemical structure of raffinose.
- [0016] Figure 3 illustrates the chemical structure of stachyose.
- [0017] Figure 4 illustrates the chemical structure of inulin.

- [0018] Figure 5 illustrates a treatment regimen with a 70% GOS composition.
- [0019] Figure 6 illustrates another treatment regimen with a 70% GOS composition.
- [0020] Figure 7 illustrates a treatment regimen with a 90% GOS composition.
- [0021] Figure 8 illustrates another treatment regimen with a 93% GOS composition.
- [0022] Figure 9 illustrates a treatment regimen with a 95% GOS composition.
- [0023] Figure 10 illustrates a non-limiting example of different GOS with a DP of 2, 3, and 4.
- [0024] Figure 11 illustrates an HPLC chromatograph of a sample containing high purity GOS.
- [0025] Figure 12 illustrates *Lactobacillus acidophilus* NCFM growth on 2% GOS1 (95%) or glucose.
- [0026] Figures 13A and 13B illustrate HPLC chromatograms of GOS compositions of the present invention before (13A) and after (13B) a purification step.
- [0027] Figure 14 illustrates comparative growth of *L. acidophilus*, *Bifidobacterium lactis*, *Bifidobacterium breve*, and *Bifidobacterium longum* on GOS1 (95%).
- [0028] Figure 15 illustrates comparative growth of *B. longum*, *Bifidobacterium pseudolongum*, *Bifidobacterium animalis*, and *Bifidobacterium adolescentis* on glucose and GOS1 (95%).
- [0029] Figure 16 illustrates comparative growth of *B. pseudolongum* NCK20383 on glucose, lactose, GOS1 (95%), and GOS2 (90%).
- [0030] Figure 17 illustrates comparative growth of four bifidobacterial strains on glucose, GOS1 (95%), GOS2 (90%), and lactose.
- [0031] Figure 18 illustrates growth of 3 *Escherichia coli* strains in media with no added carbohydrate (control), or 2% added glucose, GOS1 (95%), or GOS2 (90%).

DETAILED DESCRIPTION OF THE INVENTION

I. Overview

- [0032] Described herein are methods, compositions, kits, and business methods useful for the reduction of symptoms of lactose intolerance in a subject in need thereof, and for improving overall gastrointestinal (GI) health. Symptoms of lactose intolerance include gas, heartburn, stomach upset, bloating, flatulence, diarrhea, abdominal pain, cramping, nausea, or vomiting. Minor digestive problems related to the GI also include occasional bloating, diarrhea, constipation, gas, heartburn, or stomach upset. The methods and compositions described herein can be useful for reducing or eliminating one or more of these symptoms, for example through colonic adaptation. Fructose and sorbitol malabsorption are also common when lactose malabsorption is present. The methods and compositions described herein can also be useful for reducing or eliminating malabsorption of saccharides or carbohydrates such as lactose, fructose, or sorbitol.
- [0033] In one aspect of the methods described, the reduction or elimination of symptoms persists after treatment of a condition has concluded. Thus, the described methods need not be used on a continuous basis but rather can be utilized for a discrete time period and then discontinued. In another aspect of the methods, reduction or elimination of symptoms can be temporary, and after an amount of time has passed, treatment can be administered when symptoms reappear to

maintain the effects of the methods described herein. In yet another aspect of the methods, the methods described can be administered on a regular basis for reducing symptoms of lactose intolerance and for improving overall gastrointestinal (GI) health.

[0034] In another aspect compositions and methods comprising a prebiotic composition are provided that are useful for treatment of lactose intolerance, reduction of symptoms of lactose intolerance, and for improving overall gastrointestinal (GI) health. In one embodiment a prebiotic composition comprises one or more saccharides (herein, interchangeably also referred to as carbohydrates or sugars) which are non-digestible by a human digestive system. In another embodiment a prebiotic composition consists essentially of a saccharide which is non-digestible by a human digestive system. In one embodiment, the one or more saccharides are oligosaccharides wherein the degree of polymerization (DP) is from 2 to 20. In one embodiment the degree of polymerization can be 2 (e.g., see **Figure 10**), 3 (e.g., see **Figure 10**), 4 (e.g., see **Figure 10**), 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In another embodiment, the one or more saccharides are a polysaccharide wherein the degree of polymerization is greater than 10. In another embodiment, the saccharide comprises a mixture of non-digestible oligosaccharides or polysaccharides. In another embodiment a prebiotic composition comprises one or more digestible saccharides and one or more non-digestible oligosaccharides or polysaccharides. In one embodiment the saccharide is an oligosaccharide, such as a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, a hexasaccharide, a heptasaccharide, an octasaccharide, a nanasaccharide, or a decasaccharide. Saccharides that are not digestible by humans include, but are not limited to, transgalactooligosaccharides, galacto-oligosaccharides, lactulose (**Figure 1**), raffinose (**Figure 2**), stachyose (**Figure 3**), lactosucrose, fructo-oligosaccharides, isomalto-oligosaccharides, xylo-oligosaccharides, paratinose oligosaccharides, difructose anhydride III, sorbitol, maltitol, lactitol, reduced paratinose, cellulose, β -glucose, β -galactose, β -fructose, verbascose, galactinol, and β -glucan, guar gum, pectin, high sodium alginate, and lambda carrageenan.

[0035] In one embodiment a prebiotic composition comprises a saccharide that is inulin (**Figure 4**), fructo-oligosaccharide (FOS), lactulose, galacto-oligosaccharide (GOS), raffinose, or stachyose. In another embodiment the saccharide is an oligosaccharide that is non-digestible by a human digestive system, contains at least one beta-glycosidic (e.g., beta galactosidic or beta glucosidic) bond, and would induce lactose digestion when fed to a subject in need thereof. In one embodiment the subject in need thereof is a human. In another embodiment the saccharide is an oligosaccharide that is non-digestible by a human digestive system and contains at least one beta-glycosidic (e.g., beta galactosidic or beta glucosidic) bond that can be digested by a bacterium. In one embodiment the bacterium is a probiotic. In one embodiment the saccharide is an oligosaccharide that is non-digestible by a human digestive system and contains at least one alpha-glycosidic bond. In one embodiment the bacterium is a lactobacilli or a bifidobacteria. In one embodiment the saccharide is GOS.

- [0036] In another embodiment the saccharide is an oligosaccharide that is non-digestible by a human digestive system, contains at least one alpha-glycosidic (e.g., alpha galactosidic or alpha glucosidic) bond, and would induce lactose digestion when fed to a subject in need thereof. In one embodiment the subject in need thereof is a human. In another embodiment the saccharide is an oligosaccharide that is non-digestible by a human digestive system and contains at least one alpha-glycosidic (e.g., alpha galactosidic or alpha glucosidic) bond that can be metabolized by a bacterium. In one embodiment the bacterium is a probiotic. In one embodiment the bacterium is a lactobacilli or a bifidobacteria. In one embodiment the saccharide is GOS.
- [0037] In one embodiment, a prebiotic composition comprises at least one non-digestible saccharide and optionally contains one or more digestible saccharides or oligosaccharides. Digestible saccharides are those which are digestible by a human digestive system. In one embodiment, the one or more digestible saccharide is lactose, galactose, or glucose. In another embodiment, a prebiotic composition does not contain lactose. In one embodiment, a prebiotic composition does not contain any probiotic bacteria. In another embodiment, a prebiotic composition contains at least one strain of probiotic bacteria.
- [0038] In one embodiment, a prebiotic composition contains an oligosaccharide that increases β -galactosidase activity in the large intestine. In one embodiment, a prebiotic composition contains an oligosaccharide that increases the amount of probiotic activity in the large intestine.

II. Prebiotics

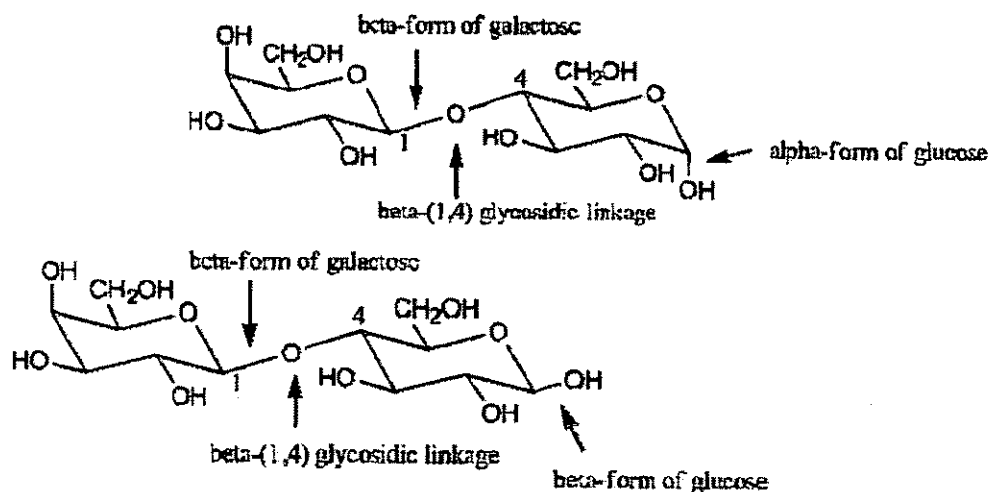
- [0039] Prebiotics are non-digestible substances that when consumed provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria (Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr. 1995 Jun;125(6):1401-12.). A prebiotic is generally a saccharide that is non-digestible or essentially non-digestible by a human and acts to encourage the growth of probiotic bacteria in the gut, increase adhesion of probiotic bacteria in the gut, displace pathogens, or provide a fermentable dose of carbohydrate to probiotic bacteria (symbiotic) or selected commensal bacteria and increase the levels of those microbial populations (notably lactobacilli and bifidobacteria) in the gastrointestinal tract. A prebiotic can be a saccharide that is non-digestible by the human host and can act as a non-digestible fiber in the diet. This non-digestibility is because humans lack the enzymes to break down some or all of the prebiotic oligosaccharide as it travels through the digestive tract. When a prebiotic reaches the small intestine and colon, bacteria encoding an enzyme or enzymes capable of digesting the prebiotic can break down the prebiotic into simple sugars that the bacteria can use. For example, bifidobacteria and lactobacilli have been reported to digest prebiotic saccharides.
- [0040] Suitable prebiotics can include one or more of a carbohydrate, carbohydrate monomer, carbohydrate oligomer, or carbohydrate polymer. In one embodiment, the prebiotics are non-non-digestible saccharides, which include non-non-digestible monosaccharides, non-digestible oligosaccharides, or non-non-digestible polysaccharides. In one embodiment, the sugar units of

- an oligosaccharide or polysaccharide can be linked in a single straight chain or can be a chain with one or more side branches. The length of the oligosaccharide or polysaccharide can vary from source to source. In one embodiment, small amounts of glucose can also be contained in the chain. In another embodiment, the prebiotic composition can be partially hydrolyzed or contain individual sugar moieties that are components of the primary oligosaccharide.
- [0041] In one embodiment, a prebiotic composition described herein consists essentially of one or more non-digestible saccharides. In another embodiment, a prebiotic composition consists essentially of one or more non-digestible oligosaccharides. In one embodiment, the non-digestible oligosaccharides are GOS. In other embodiments, a composition described herein consists essentially of non-digestible GOS and does not contain a probiotic microbe, or microbes.
- [0042] In one embodiment a prebiotic composition of the invention allows the colonic microbiota, comprising microorganisms known to increase the ability of a subject to tolerate fermentable carbohydrates, to be regularly maintained or replenished through consumption of the prebiotic composition. In one embodiment, adaptation of the intestinal and colonic microbiota increases the intestine and colon's capacity to use lactose without producing gas. Adaptive changes in microbiota of the gastrointestinal tract can be useful for the reduction of bloating, diarrhea, gastric distention, pain, or flatulence from the consumption of dairy products and other lactose containing compositions. In one embodiment, tolerance of a human subject to dairy products, in general, can be improved through regular consumption of a prebiotic composition.
- [0043] Prebiotics can promote colonic bacteria that slow fermentation. For example, FOS, neosugar, or inulin promote the growth of acid-forming bacteria in the colon such as bacteria belonging to the genera *Lactobacillus* or *Bifidobacterium*. For instance, *Lactobacillus acidophilus* and *Bifido bacterium bifidus* can play a role in reducing the number of pathogenic bacteria in the colon. Additional properties, such as the effect of prebiotics on colonic pH and stool bulking provide for their classification as dietary fibers. In experimental models, prebiotics can improve the bioavailability of essential minerals. As a fiber, prebiotics are thought to slow digestion. Other polymers, such as various galactans and carbohydrate based gums, such as psyllium, guar, carrageen, gellan, and konjac, are also known to improve gastrointestinal (GI) health. The carbohydrate lactulose can also improve GI health.
- [0044] In one embodiment a prebiotic composition comprises one or more of GOS, lactulose, raffinose, stachyose, lactosucrose, FOS (i.e. oligofructose or oligofructan), inulin, isomalto-oligosaccharide, xylo-oligosaccharide, paratinose oligosaccharide, transgalactosylated oligosaccharides (i.e. transgalacto-oligosaccharides), transgalactosylate disaccharides, soybean oligosaccharides (i.e. soyoligosaccharides), gentiooligosaccharides, glucooligosaccharides, pecticoligosaccharides, palatinose polycondensates, difructose anhydride III, sorbitol, maltitol, lactitol, polyols, polydextrose, reduced paratinose, cellulose, β -glucose, β -galactose, β -fructose, verbascose, galactinol, and β -glucan, guar gum, pectin, high, sodium alginate, and lambda carrageenan, or mixtures thereof.

- [0045] In one embodiment, a prebiotic composition comprises a mixture of one or more non-digestible oligosaccharides, non-digestible polysaccharides, free monosaccharides, non-digestible saccharides, starch, or non-starch polysaccharides. In one embodiment, a prebiotic component of a prebiotic composition is a GOS composition.
- [0046] In one embodiment a prebiotic composition reduces or eliminates one or more symptoms associated with lactose intolerance or with lactose digestive problems, including but not limited to cramps, flatulence, stomach pain, vomiting, bloating, diarrhea, nausea, gastric distention and intestinal pain, in a subject in need thereof. In one embodiment the subject is a patient. In another embodiment the subject is a human. In another embodiment the subject is a non-human animal.
- [0047] The term "about" means the referenced numeric indication plus or minus 10% of that referenced numeric indication.
- [0048] The term "percent by weight," as used in reference to the percent by weight of a component in a composition, means the percentage of the component's weight in comparison to the total dry weight of the composition.

A. Oligosaccharide structure

- [0049] Oligosaccharides are generally considered to have a reducing end and a non-reducing end, whether or not the saccharide at the reducing end is in fact a reducing sugar. In accordance with accepted nomenclature, most oligosaccharides are depicted herein with the non-reducing end on the left and the reducing end on the right. Most oligosaccharides described herein are described with the name or abbreviation for the non-reducing saccharide (e.g., Gal or D-Gal), preceded or followed by the configuration of the glycosidic bond (α or β), the ring bond, the ring position of the reducing saccharide involved in the bond, and then the name or abbreviation of the reducing saccharide (e.g., Glc or D-Glc). The linkage (e.g., glycosidic linkage, galactosidic linkage, glucosidic linkage) between two sugar units can be expressed, for example, as 1,4, 1 $\rightarrow$ 4, or (1-4). Each saccharide is in the cyclic form (i.e. pyranose or furanose form). For example, lactose is a disaccharide composed of cyclic forms of galactose and glucose joined by a beta (1-4) linkage where the acetal oxygen bridge is in the beta orientation. Lactose exists as alpha- and beta-lactose (see structures below). β -lactose can be expressed as β -D-galactopyranosyl-(1-4) β -D-glucopyranose, β -D-Gal-(1-4)- β -D-Glc or as Gal β (1-4)-Glc. α -lactose can be expressed as β -D-galactopyranosyl-(1-4) α -D-glucopyranose, β -D-Gal-(1-4)- α -D-Glc or as Gal β (1-4)-Glc.
- [0050] Both FOS and GOS are non-digestible saccharides. β glycosidic linkages of saccharides, such as those found in, but not limited to, FOS and GOS, make these prebiotics mainly non-digestible and unabsorbable in the stomach and small intestine (see below). Also, α -linked GOS (α -GOS) is not hydrolyzed by human salivary amylase, but can be used by *Bifidobacterium bifidum* and *Clostridium butyricum* (Yamashita A. et al. (2004) J. Appl. Glycosci. 51:115-122). FOS and GOS can pass through the small intestine and into the large intestine (colon) mostly intact, except where probiotic and commensal microbes are able to metabolize the oligosaccharides.



B. GOS

1. Introduction

- [0051] GOS (also known as galacto-oligosaccharides, galactooligosaccharides, *trans*-oligosaccharide (TOS), *trans*-galacto-oligosaccharide (TGOS), and *trans*-galactooligosaccharide) are oligomers or polymers of galactose molecules ending mainly with a glucose or sometimes ending with a galactose molecule and have varying degree of polymerization (generally the DP is between 2-20) and type of linkages. In one embodiment, GOS comprises galactose and glucose molecules. In another embodiment, GOS comprises only galactose molecules. In a further embodiment, GOS are galactose-containing oligosaccharides of the form of $[\beta\text{-D-Gal-(1-6)}]_n\text{-}\beta\text{-D-Gal-(1-4)-D-Glc}$ wherein n is 2-20. In another embodiment, GOS are galactose-containing oligosaccharides of the form $\text{Glc } \alpha\text{1-4-}[\beta\text{ Gal 1-6}]_n$ where $n=2\text{-}20$. In another embodiment, GOS are in the form of $\alpha\text{-D-Glc (1-4)-}[\beta\text{-D-Gal-(1-6)-}]_n$ where $n=2\text{-}20$. Gal is a galactopyranose unit and Glc (or Glu) is a glucopyranose unit.
- [0052] In one embodiment, a prebiotic composition comprises a GOS-related compound. A GOS-related compound can have the following properties: a) a "lactose" moiety; e.g., GOS with a gal-glu moiety and any polymerization value or type of linkage; or b) be stimulatory to "lactose fermenting" microbes in the human GI tract; for example, raffinose (gal-fru-glu) is a "related" GOS compound that is stimulatory to both lactobacilli and bifidobacteria.
- [0053] In one embodiment, a prebiotic composition comprises GOS with a low degree of polymerization. In one embodiment a prebiotic composition comprising GOS with a low degree of polymerization increases growth of probiotic and select commensal bacteria to a greater extent than an equivalent amount of a prebiotic composition comprising GOS with a high degree of polymerization. In one embodiment, a prebiotic composition comprising a high percentage of GOS with a low degree of polymerization increases growth of probiotic and beneficial commensal bacteria to a greater extent than an equivalent amount of a prebiotic composition comprising a low percentage of GOS with a low degree of polymerization. In one embodiment a prebiotic composition comprises GOS with a degree of polymerization less than 20, such as less than 10, less than 9, less than 8, less

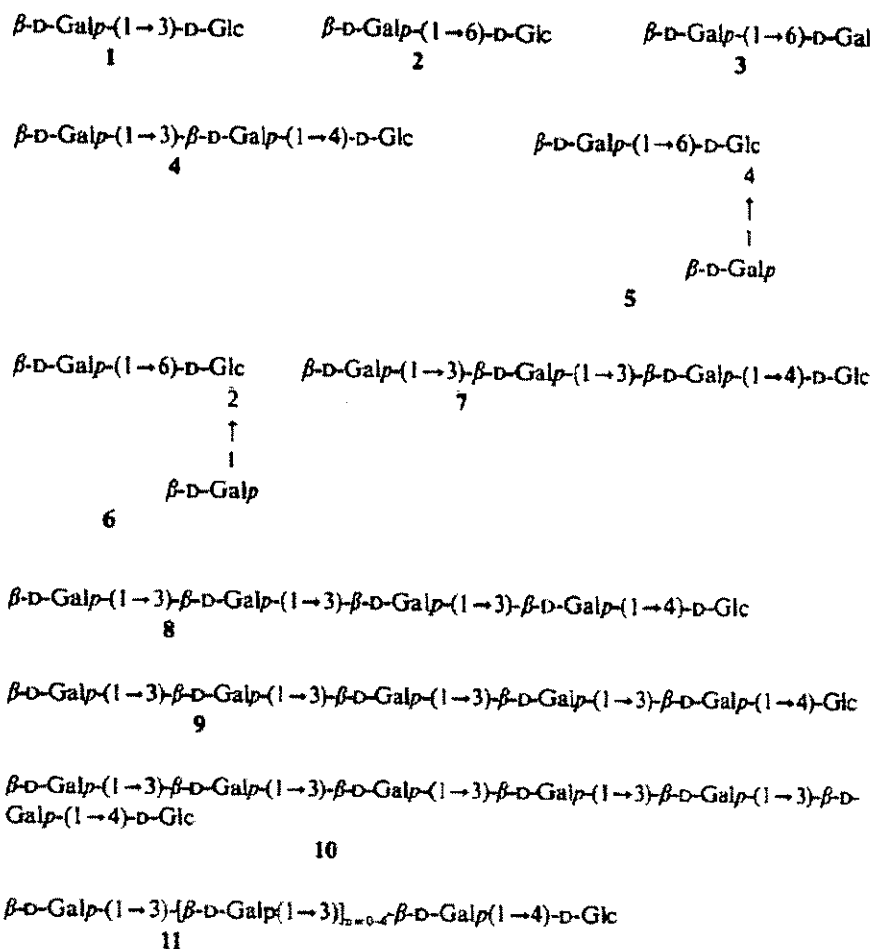
than 7, less than 6, less than 5, less than 4, or less than 3. In another embodiment a prebiotic composition comprising GOS with a low degree of polymerization increases growth of probiotic and/or beneficial commensal microbes in the GI tract of a subject.

2. GOS synthesis

[0054] GOS is found in human and bovine maternal milk. GOS can be produced from lactose syrup using the transgalactosylase activity of the enzyme β -galactosidase (Crittenden, (1999) *Probiotics: A Critical Review*. Tannock, G. (ed) Horizon Scientific Press, Wymondham, pp. 141-156). β -D-galactosidase is known to catalyze not only the hydrolysis of the β -D-galactoside linkage of lactose to give D-glucose and D-galactose but also to carry out transgalactosylation reactions where the D-galactosyl group of a β -D-galactoside is transferred onto a hydroxylated acceptor. For example, when a β -D-galactoside such as lactose or another carbohydrate is present, it is possible to obtain new glycoside linkages between the D-galactose unit and the acceptor. The starting galactoside such as lactose can also be present in a GOS mixture following the transgalactosylation reactions. As used herein, GOS comprises one or more saccharides that have been produced from a glycoside and the transgalactosylation reaction of a β -galactosidase. Thus, GOS includes saccharides such as transgalactosylated oligosaccharides (i.e. transgalacto-oligosaccharides) or transgalactosylate disaccharides. The DP of the formed oligosaccharide can vary, typically from 2-20, depending on the enzyme source. In one embodiment, a GOS composition is a blend of one or more saccharides with a DP range of 2-6 (i.e. di- through hexasaccharides). In another embodiment, a GOS composition is a blend of one or more saccharides with a DP range of 2-8 (i.e. di- through octasaccharides). In another embodiment, a GOS composition is a blend of one or more saccharides with a DP range of greater than 8. In yet another embodiment, a GOS composition is a blend of one or more saccharides with a DP range of 9-15. In another embodiment, a GOS composition is a blend of one or more saccharides with a DP of 1, a DP range of 2-6, a DP range of 6-8, and DP range of greater than 8.

3. GOS linkages

[0055] Linkages between the individual sugar units found in GOS include β -(1-6), β -(1-4), β -(1-3) and β -(1-2) linkages. β -(1-3) linkages are less common than β -(1-6) or β -(1-4) linkages. In one embodiment, GOS comprises a number of β -(1-6) linked or β -(1-4) galactopyranosyl units linked to a terminal glucopyranosyl residue through an α -(1-4) glycosidic bond. In another embodiment, GOS comprises a number of β -(1-6) linked or β -(1-4) galactopyranosyl units linked to a terminal glucopyranosyl residue through a β -(1-4) glycosidic bond. In another embodiment, GOS formed by transgalactosylation comprise β -D-galactopyranosyl-(1-3) linkages. In one embodiment, GOS are branched saccharides. Branched oligosaccharides can be formed as an artifact of the transgalactosylation reaction. In another embodiment, GOS are linear saccharides. Non-limiting GOS examples include the following shown below:



- [0056] The source of the β -galactosidase can determine the GOS end products from transgalactosylation reactions. For example, β -galactosidase from *Streptococcus thermophilus* can produce a collection of transgalactosylated disaccharides including Gal β (1-6) Glc, Gal β (1-3) Glc, Gal β (1-2) Glc, and Gal β (1-6) Gal (Matsumoto et al., (1992), Chapter 5: Galactooligosaccharides, in Japanese Technology Reviews, ed. by Karbe, I., Gordon and Breach, NY, pp. 90-160). Transgalactosylated oligosaccharides (TOS) can be produced using β -galactosidase from *Aspergillus oryzae* (Tanaka et al, (1983) *Bifidobacteria Microflora*, 2, 17-24), and consists of tri-, tetra-, penta- and hexa-GOS. In another embodiment GOS are prepared using β -galactosidase from *A. oryzae* and *Streptococcus thermophilus* (Ito et al., (1990), *Microbial Ecology in Health and Disease*, 3, 285-292) and contains 36% tri-, tetra-, penta- and hexa-GOS, 16% disaccharides galactosyl-glucose and galactosyl-galactose, 38% monosaccharides, and 10% lactose.
- [0057] In one embodiment a strain of *Bifidobacterium bifidum* (for example, accession number NCIMB 41171) produces a galactosidase activity that converts lactose to a GOS mixture comprising the disaccharide Gal α (1-6) Gal, at least one trisaccharide selected from Gal β (1-6)-Gal β (1-4)-Glc and Gal β (1-3)-Gal β (1-4)-Glc, the tetrasaccharide Gal β (1-6)-Gal β (1-6)-Gal β (1-4)-Glc and the pentasaccharide Gal β (1-6)-Gal β (1-6)-Gal β (1-6)-Gal β (1-4)-Glc. In one embodiment, a

- GOS composition is a mixture of 10 to 45% w/v of the disaccharide, 10 to 45% w/v of the trisaccharide, 10 to 45% w/v of the tetrasaccharide and 10 to 45% w/v of the pentasaccharide.
- [0058] In another embodiment, a GOS composition is a mixture of oligosaccharides comprising 20-28 % by weight of β (1-3) linkages, 20-25 % by weight of β (1-4) linkages, and 45-55 % by weight of β (1-6) linkages. In one embodiment, a GOS composition is a mixture of oligosaccharides comprising 26 % by weight of β (1-3) linkages, 23 % by weight of β (1-4) linkages, and 51 % by weight of β (1-6) linkages.
- [0059] Alpha-GOS (also called alpha-bond GOS or alpha-linked GOS) are oligosaccharides having an alpha-galactopyranosyl group. Alpha-GOS comprises at least one alpha glycosidic linkage between the saccharide units. Alpha-GOS are generally represented by α -(Gal)_n (n usually represents an integer of 2 to 10) or α -(Gal)_n Glc (n usually represents an integer of 1 to 9). Examples include a mixture of α -galactosylglucose, α -galactobiose, α -galactotriose, α -galactotetraose, and higher oligosaccharides. Additional non-limiting examples include melibiose, manninootriose, raffinose, stachyose, and the like, which can be produced from beat, soybean oligosaccharide, and the like.
- [0060] Commercially available and enzyme synthesized alpha-GOS products are also useful for the compositions described herein. Synthesis of alpha-GOS with an enzyme is conducted utilizing the dehydration condensation reaction of α -galactosidase with the use of galactose, galactose-containing substance, or glucose as a substrate. The galactose-containing substance includes hydrolysates of galactose-containing substances, for example, a mixture of galactose and glucose obtained by allowing beta-galactosidase to act on lactose, and the like. Glucose can be mixed separately with galactose and be used as a substrate with α -galactosidase (see e.g., WO 02/18614). Methods of preparing alpha-GOS have been described (see e.g., EP1514551 and EP2027863).
- [0061] In one embodiment, a GOS composition comprises a mixture of saccharides that are alpha-GOS and saccharides that are produced by transgalactosylation using β -galactosidase. In another embodiment, GOS comprises alpha-GOS. In another embodiment, alpha-GOS comprises α -(Gal)₂ from 10% to 100% by weight. In one embodiment, GOS comprises only saccharides that are produced by transgalactosylation using β -galactosidase.
- [0062] In one embodiment, a GOS composition can comprise GOS with alpha linkages and beta linkages.

4. GOS saccharide unit composition

- [0063] In one embodiment, a GOS composition is a mixture of oligosaccharides comprising 1-20 % by weight of di-saccharides, 1-20 % by weight tri-saccharides, 1-20 % by weight tetra-saccharide, and 1-20 % by weight penta-saccharides. In another embodiment, a GOS composition is a mixture of oligosaccharides consisting essentially of 1-20 % by weight of di-saccharides, 1-20 % by weight tri-saccharides, 1-20 % by weight tetra-saccharide, and 1-20 % by weight penta-saccharides. In one embodiment, a GOS composition is a mixture of oligosaccharides comprising 1-20 % by weight of saccharides with DP of 1-3, 1-20 % by weight of saccharides with DP of 4-6,

- 1-20 % by weight of saccharides with DP of 7-9, and 1-20 % by weight of saccharides with DP of 10-12, 1-20 % by weight of saccharides with DP of 13-15.
- [0064]** In another embodiment, a GOS composition is a 1:1:1:1:1 ratio of saccharides with a DP of 2:3:4:5:6. In one embodiment, a GOS composition is a 1:2:3:2:1:1 ratio of saccharides with a DP of 1:2:3:4:5:6. In another embodiment, a GOS composition is a (12 to 13):(4 to 5):1 ratio of saccharides with a DP of 3:4:5. In one embodiment, a GOS composition is a 12.3: 4.8: 1 ratio of saccharides with a DP of 3:4:5. In one embodiment, a GOS composition is a (8-10):(10-15):(4-6):(1-3) ratio of saccharides with a DP of 2:3:4:5.
- [0065]** In another embodiment, a GOS composition is a mixture of oligosaccharides comprising 50-55 % by weight of di-saccharides, 20-30 % by weight tri-saccharides, 10-20 % by weight tetra-saccharide, and 1-10 % by weight penta-saccharides. In one embodiment, a GOS composition is a mixture of oligosaccharides comprising 52 % by weight of di-saccharides, 26 % by weight tri-saccharides, 14 % by weight tetra-saccharide, and 5 % by weight penta-saccharides.
- [0066]** In another embodiment, a GOS composition is a mixture of oligosaccharides comprising 45-55 % by weight tri-saccharides, 15-25 % by weight tetra-saccharides, 1-10 % by weight penta-saccharides. In another embodiment, a GOS composition is a mixture of oligosaccharides comprising 49.3 % by weight tri-saccharides, 19 % by weight tetra-saccharides, 4 % by weight penta-saccharides.
- [0067]** In another embodiment, a GOS composition is a mixture of oligosaccharides comprising 2-5 % by weight of a mixture of tri- to hexa-saccharides, 25-35 % by weight Gal β (1-6) Glc, 5-15 % by weight Gal β (1-3) Glc, 5-15 % by weight Gal β (1-2) Glc, 25-30 % by weight Gal β (1-6) Gal, and 1-5% by weight Gal β (1-3) Gal, and optionally further contains one or more digestible saccharides or oligosaccharides. In another embodiment, a GOS composition is a mixture of oligosaccharides comprising 3.9 % by weight of a mixture of tri- to hexa-saccharides, 32.6 % by weight Gal β (1-6) Glc, 7.6 % by weight Gal β (1-3) Glc, 9.4 % by weight Gal β (1-2) Glc, 27.2 % by weight Gal β (1-6) Gal, and 2.5% Gal β (1-3) Gal, and optionally further contains one or more digestible saccharides or oligosaccharides. Digestible saccharides or oligosaccharides are carbohydrates that can be digested by the human digestive system, and include but are not limited to lactose, galactose, or glucose. In one embodiment digestible saccharides found in a GOS composition comprise lactose, galactose, or glucose. In another embodiment, a GOS composition is a mixture of non-digestible oligosaccharides and lactose, glucose or galactose. In another embodiment, a GOS composition is composed of 62 % by weight oligosaccharides and 38% digestible saccharides.

5. GOS and saccharimetric measurement

- [0068]** In another embodiment, a GOS composition comprises a mixture of oligosaccharides, wherein the composition has a saccharimetric measurement at least about 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 degrees Brix. In another embodiment, a GOS composition comprises a mixture of

oligosaccharides, wherein the composition has a saccharimetric measurement of between about 50-100, 50-80, 60-80, or 70-80 degrees Brix. In another embodiment, a GOS composition has a saccharimetric measurement of between about 72 and 78 degrees Brix. For example, a GOS composition can comprise greater than about 93% GOS and have a saccharimetric degree of 75 degrees Brix. In another embodiment, a GOS composition can comprise greater than about 93% GOS, less than about 5% digestible saccharides (such as lactose, glucose, and galactose), and have a saccharimetric degree of 75± degrees Brix. In yet another embodiment, a GOS composition can comprise greater than about 93% GOS, less than about 5% digestible saccharides, less than about 10ppm heavy metals, less than 0.1% sulphated ash, and have a saccharimetric measurement of 75 degrees Brix.

6. Percentages and amounts of GOS in prebiotic compositions

[0069] In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises 1-100% by weight GOS. The percentage by weight of GOS refers to the weight of GOS relative to the total dry weight of the GOS composition. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 1% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 5% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 10% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 20% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 30% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 40% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 50% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 60% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 70% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 72.3% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 80% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 85% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 90% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 91% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 92% by weight GOS. In another embodiment, a

prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 93% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 94% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 95% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 96% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 97% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 98% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 99% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 100% by weight GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises between 0.1% and 100% GOS. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5 %, or 100% by weight GOS. The percentage by weight of GOS refers to the weight of GOS relative to the total dry weight of the prebiotic or GOS composition.

[0070] In another embodiments, a prebiotic composition comprises a GOS composition, wherein the GOS composition comprises about 90%, 90.1%, 90.2%, 90.3%, 90.4%, 90.5%, 90.6%, 90.7%, 90.8%, 90.9%, 91%, 91.1%, 91.2%, 91.3%, 91.4%, 91.5%, 91.6%, 91.7%, 91.8%, 91.9%, 92%, 92.1%, 92.2%, 92.3%, 92.4%, 92.5%, 92.6%, 92.7%, 92.8%, 92.9%, 93 %, 93.1%, 93.2%, 93.3%, 93.4%, 93.5%, 93.6%, 93.7%, 93.8%, 93.9%, 94%, 94.1%, 94.2%, 94.3%, 94.4%, 94.5%, 94.6%, 94.7%, 94.8%, 94.9%, 95%, 95.1%, 95.2%, 95.3%, 95.4%, 95.5%, 95.6%, 95.7%, 95.8%, 95.9%, 96%, 96.1%, 96.2%, 96.3%, 96.4%, 96.5%, 96.6%, 96.7%, 96.8%, 96.9%, 97%, 97.1%, 97.2%, 97.3%, 97.4%, 97.5%, 97.6%, 97.7%, 97.8%, 97.9%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, 100% by weight GOS. The percentage by weight of GOS refers to the weight of GOS relative to the total dry weight of the prebiotic or GOS composition.

[0071] In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 1-90%, about 10-90%, about 20-90%, about 30-90%, about 40-90%, about 40-80%, about 40-70%, about 40-60%, about 40-50%, about 50-90%, about 50-80%,

about 50-70%, about 50-60%, about 60-90%, about 60-80%, about 60-70%, about 70-90%, about 70-80%, about 70-90%, about 70-80%, about 80-90%, about 90-96%, about 93-96%, about 93-95%, about 94-98%, about 93-99%, or about 90-100% by weight GOS. The percentage by weight of GOS refers to the weight of GOS relative to the total dry weight of the prebiotic or GOS composition.

[0072] In another embodiment a prebiotic composition comprises 0.01-20 g of a GOS composition, such as about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, or about 20 g of GOS composition.

In another embodiment a prebiotic composition comprises about 0.1-2g of a GOS composition.

[0073] A prebiotic product can comprise GOS for improving gut health by promoting the growth of bifidobacteria in the gut. In one embodiment, metabolism of a GOS composition by lactobacilli and bifidobacteria yields organic acids and other agents that inhibit enteric pathogens. In another embodiment, a GOS composition provides a selective advantage for organisms in the gut that can use them. In another embodiment, a GOS composition acts as anti-adhesives for bacteria in the gut. In another embodiment a mixture of oligosaccharides is useful for the preparation of a medicament for preventing the adhesion of pathogens or toxins produced by pathogens to the gut wall.

[0074] In one embodiment a composition is provided that comprises a suitable amount of a prebiotic composition that is effective for promoting the growth of probiotics such that fermentation in the gut is slowed or gastrointestinal health is improved. In one embodiment prebiotics can be administered in an amount per serving from about 1mg to about 20g, or about 1mg to about 15g, or about 1mg to about 10g, or about 1mg to about 5g, or about 2mg to about 1000mg, or about 2mg to about 500mg, or about 2mg to about 200mg, or about 2mg to about 100mg, or about 2mg to about 50mg, or about 2mg to about 20mg, or about 5mg to about 10mg, or about 5, 6, 7, 7.5, 8, 9, or 10mg or about 0.25g to about 1.7g. In another embodiment a prebiotic can be administered in an amount per serving of about 1g, about 2g, about 3g, about 4g, about 5g, about 6g, about 7g, about 8g, about 9g, about 10g, about 11g, about 12g, about 13g, about 14g, about 15g, about 16g, about 17g, about 18g, about 19g, or about 20g. In another embodiment, the prebiotic used can be from about 0.1g to about 15g, or about 0.1g to about 1g, or about 0.1g to about 0.5g or about 0.1g to about 2g, or about 0.5g to about 1g, or about 0.2g to about 1g, or about 1g to about 5g, or about 1g to about 15g per serving. In one embodiment, the smallest effective amount of prebiotic is used. The prebiotic can be about 0.5% to about 100% by weight of a prebiotic composition.

[0075] In one embodiment a prebiotic composition (e.g., GOS) can be administered in a dose from about 1 mg to about 25 g, or about 1 mg to about 5 g, or about 1 mg to about 1000 mg, or about 1 mg to about 500 mg, or about 1 mg to about 200 mg, or about 1 mg to about 100 mg, or about 1 mg to about 50 mg, or about 2 mg to about 20 mg, or about 5 mg to about 10 mg, or about 5, 6, 7, 7.5, 8, 9, or 10 mg. In another embodiment, a prebiotic composition is used in a dose of about 7.5 mg. In

one embodiment the dose of a prebiotic composition administered to a subject can be increased from about 1 g to about 10 g over time. In one embodiment an initial dose of a prebiotic composition can be 1-3 grams. This dose can be increased over time (e.g., days or a week) so that the final dose is about 10 g of GOS.

7. GOS and other components of GOS compositions

[0076] Table 1 contains data from a certificate of analysis of a 96.8 % GOS composition, illustrating other components that can be in a prebiotic composition comprising a GOS composition.

Table 1. Certificate of analysis

Test	Results
Refractometric dried substance	76.5°BxRDS
Purity GOS	96.8 %
Related substances	
Lactose	2.0 %
Glucose	<0.1 %
Galactose	1.1 %
Density	1,383 g/ml
Color (420 nm)	0.041 A.U.
Appearance of solution	Clear
pH (10% solution)	5.8
Conductance (10% solution)	22.7 μ S/cm
Viscosity	7295 cP
Organic Volatile Impurities	
Methanol	17.0 ppm
Ethanol	< 10 ppm
Heavy metals (Pb ²⁺)	< 10 ppm
Sulphated ashes	0.07 %
Specific optical rotation	+44.6 °
T.A.M.C. (total aerobic microbial count)	40 cfu/ml
T.Y.M.C. (total combined Yeasts and Molds count)	5 cfu/ml
<i>Salmonella s.</i>	Absent cfu/10 ml
<i>Escherichia coli</i>	Absent cfu/ml

[0077] In one embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 70% by weight GOS, about 3% by weight moisture, about 30% by weight other saccharides, about 0.1% by weight ash, about 1 ppm heavy metal (e.g., Pb), and

- about 1 ppm arsenic (As_2O_3). In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 70-75% by weight GOS, about 1-3% by weight moisture, about 20% by weight lactose, less than 1 % by weight glucose, less than 1 % by weight galactose, about 0.1% by weight ash, about 1 ppm heavy metal (e.g., Pb), and about 1 ppm arsenic (As_2O_3).
- [0078] In another embodiment a GOS composition comprises GOS and one or more of water or digestible saccharides. In one embodiment a GOS composition comprises less than about 10ppm of a heavy metal (such as arsenic or lead), including but not limited to less than about 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1ppm of a heavy metal. In another embodiment a GOS composition comprises less than about 0.10% sulphated ash, including but not limited to less than about 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, or 0.1% sulphated ash. In another embodiment, a GOS composition can comprise greater than about 90% GOS, less than about 5% digestible saccharides, less than about 10ppm of heavy metals, and less than about 0.10% sulphated ash. In another embodiment, a GOS composition comprises less than about 5000 ppm ethanol and less than about 3000 ppm methanol. In another embodiment, a GOS composition comprises a bacterial count of less than about 100 cfu/g, and a mold count of less than about 10 cfu/g.
- [0079] In one embodiment, a GOS composition comprises about 1-90%, about 10-90%, about 20-90%, about 30-90%, about 40-90%, about 40-80%, about 40-70%, about 40-60%, about 40-50%, about 50-90%, about 50-80%, about 50-70%, about 50-60%, about 60-90%, about 60-80%, about 60-70%, about 70-90%, about 70-80%, about 70-90%, about 70-80%, about 80-90%, about 92-100%, about 93-99%, about 94-98%, about 92-96%, about 93-96%, or about 93-95% by weight GOS and less than about 10 ppm heavy metals and less than about 0.10% sulphated ash. Standard analytical methods can be used to determine the amount of the various components in the prebiotic or GOS composition, such as but not limited to HPLC, colorimetry (e.g., sodium sulfide colorimetry), or spectrophotometry (e.g., atomic absorption spectrophotometry).
- [0080] In another embodiment, the absorbance of a GOS composition at about A_{420} can be from about 0.3 AU to about 0.6 AU. In another embodiment, the pH of a GOS composition can be from about 3 to about 7. In one embodiment, the conductance of a GOS composition can be less than about 100 $\mu\text{S}/\text{cm}$.
- [0081] Figure 11 illustrates an HPLC chromatograph of a sample of one embodiment of a high purity GOS composition.

8. GOS and digestible saccharides

- [0082] In one embodiment, a GOS composition can comprise about 1-5% digestible saccharides, such as lactose, glucose or galactose. In another embodiment, a GOS composition can comprise about 0.001 to about 1% glucose or about 0.01 to about 0.1% glucose. In another embodiment, a GOS composition can comprise about 0.1% galactose to about 2% galactose. In another embodiment, the density of a GOS composition can be about 1200 to about 1500 g/mL.

- [0083]** In one embodiment, a GOS composition comprises about 1-90%, about 1-80%, about 1-70%, about 1-60%, about 1-50%, about 1-40%, about 40-90%, about 40-80%, about 40-70%, about 40-60%, about 40-50%, about 50-90%, about 50-80%, about 50-70%, about 50-60%, about 60-90%, about 60-80%, about 60-70%, about 70-90%, about 70-80%, about 70-90%, about 70-80%, about 80-90%, about 90-96%, about 93-96%, about 93-95%, about 94-98%, about 93-99%, or about 92-100% by weight GOS and no digestible saccharides. In another embodiment, a prebiotic composition comprises a GOS composition wherein the GOS composition comprises about 1-90%, about 1-80%, about 1-70%, about 1-60%, about 1-50%, about 1-40%, about 40-90%, about 40-80%, about 40-70%, about 40-60%, about 40-50%, about 50-90%, about 50-80%, about 50-70%, about 50-60%, about 60-90%, about 60-80%, about 60-70%, about 70-90%, about 70-80%, about 70-90%, about 70-80%, about 80-90%, about 92-100%, about 93-99%, about 94-98%, about 92-96%, about 93-96%, or about 93-95% by weight GOS and less than about 6% (such as about 5, 4, 3, 2, or 1%) digestible saccharides.
- [0084]** In one embodiment a GOS composition comprises about 70% GOS and about 20% digestible saccharides. In another embodiment a GOS composition comprises about 70-75% GOS and about 5-30% digestible saccharides.
- [0085]** In another embodiment a GOS composition comprises about 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, or 95% by weight GOS and about 1-10% by weight digestible saccharides. In one embodiment these digestible saccharides are byproducts of the GOS synthesis process.
- [0086]** In one embodiment a GOS composition comprises about 92% GOS. In another embodiment a GOS composition comprises about 92% GOS and digestible saccharides. In another embodiment a GOS composition comprises about 92% GOS and about 8% digestible saccharides. In another embodiment a GOS composition comprises about 92% GOS and no digestible saccharides. In another embodiment a GOS composition comprises about 92% GOS and no lactose, glucose, or galactose. In another embodiment a GOS composition comprises about 92% GOS and about 1-8% digestible saccharides. In another embodiment a GOS composition comprises about 92% by weight GOS and about 8% by weight digestible saccharides. In another embodiment a GOS composition comprises about 92% by weight GOS and about 5% by weight digestible saccharides.
- [0087]** In one embodiment a GOS composition comprises about 93% GOS. In another embodiment a GOS composition comprises about 93% GOS and digestible saccharides. In another embodiment a GOS composition comprises about 93% GOS and about 7% digestible saccharides. In another embodiment a GOS composition comprises about 93% GOS and no lactose. In another embodiment a GOS composition comprises about 93% GOS and no lactose, glucose, or galactose. In another embodiment a GOS composition comprises about 93% GOS and about 1-7% digestible saccharides. In another embodiment a GOS composition comprises about 93% by weight GOS and about 1-7% by weight digestible saccharides. In another embodiment a GOS composition

- comprises about 93% by weight GOS and about 7% by weight digestible saccharides. In another embodiment a GOS composition comprises about 93% by weight GOS and about 5% by weight digestible saccharides.
- [0088]** In one embodiment a GOS composition comprises about 94% GOS. In another embodiment a GOS composition comprises about 94% GOS and digestible saccharides. In another embodiment a GOS composition comprises about 94% GOS and about 6% digestible saccharides. In another embodiment a GOS composition comprises about 94% GOS and no lactose. In another embodiment a GOS composition comprises about 94% GOS and no lactose, glucose, or galactose. In another embodiment a GOS composition comprises about 94% GOS and about 1-6% digestible saccharides. In another embodiment a GOS composition comprises about 94% by weight GOS and about 5% by weight digestible saccharides.
- [0089]** In one embodiment a GOS composition comprises about 95% GOS. In another embodiment a GOS composition comprises about 95% GOS and digestible saccharides. In another embodiment a GOS composition comprises about 95% by weight GOS and about 5% by weight digestible saccharides. In another embodiment a GOS composition comprises about 95% GOS and no lactose. In another embodiment a GOS composition comprises about 95% GOS and no lactose, glucose, or galactose. In another embodiment a GOS composition comprises about 95% GOS and about 1-5% digestible saccharides. In another embodiment a GOS composition comprises about 95% by weight GOS and about 1-5% by weight digestible saccharides, such as digestible saccharides.
- [0090]** In one embodiment a GOS composition comprises about 96% GOS. In another embodiment a GOS composition comprises about 96% GOS and digestible saccharides. In another embodiment a GOS composition comprises about 96% by weight GOS and about 4% by weight digestible saccharides. In another embodiment a GOS composition comprises about 96% GOS and no lactose. In another embodiment a GOS composition comprises about 96% GOS and no lactose, glucose, or galactose. In another embodiment a GOS composition comprises about 96% GOS and about 1-4% digestible saccharides. In another embodiment a GOS composition comprises about 96% by weight GOS and about 1-4% by weight digestible saccharides.
- [0091]** In one embodiment a GOS composition comprises about 97% GOS. In another embodiment a GOS composition comprises about 97% GOS and digestible saccharides. In another embodiment a GOS composition comprises about 97% GOS and about 3% digestible saccharides. In another embodiment a GOS composition comprises about 97% GOS and no lactose. In another embodiment a GOS composition comprises about 97% GOS and no lactose, glucose, or galactose. In another embodiment a GOS composition comprises about 97% GOS and about 1-3% digestible saccharides. In another embodiment a GOS composition comprises about 97% by weight GOS and about 1-3% by weight digestible saccharides. In another embodiment a GOS composition comprises about 97% by weight GOS and about 3% by weight digestible saccharides.

- [0092] In one embodiment a GOS composition comprises about 98% GOS. In another embodiment a GOS composition comprises about 98% GOS and digestible saccharides. In another embodiment a GOS composition comprises about 98% by weight GOS and about 2% by weight digestible saccharides. In another embodiment a GOS composition comprises about 98% GOS and no lactose. In another embodiment a GOS composition comprises about 98% GOS and no lactose, glucose, or galactose. In another embodiment a GOS composition comprises about 98% GOS and about 0.1-2% digestible saccharides.
- [0093] In one embodiment a GOS composition comprises about 99% GOS. In another embodiment a GOS composition comprises about 99% GOS and digestible saccharides. In another embodiment a GOS composition comprises about 99% GOS and lactose, glucose, galactose or a combination thereof. In another embodiment a GOS composition comprises about 99% by weight GOS and about 1% by weight digestible saccharides. In another embodiment a GOS composition comprises about 99% GOS and no lactose. In another embodiment a GOS composition comprises about 99% GOS and no lactose, glucose, or galactose. In another embodiment a GOS composition comprises about 99% GOS and about 0.1-1% digestible saccharides.
- [0094] In one embodiment a GOS composition comprises about 100% GOS.
- [0095] In some embodiments, a GOS composition comprises about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 15%, or about 20% by weight of digestible saccharides. In another embodiment a GOS composition comprises about 99.9%, 99.5%, 99%, 98.5%, 98%, 97.5%, 97%, 96.5%, 96%, 95.5%, 95%, 94.5%, 94%, 93.5%, 93%, 92.5%, 92%, 91.5%, 91%, 90.5%, 90%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, or 1% by weight GOS and one or more digestible saccharides.
- [0096] In one embodiment a prebiotic composition comprises GOS. In one embodiment a prebiotic composition consists essentially of GOS. In one embodiment a prebiotic composition consists essentially of GOS and is prepared or administered without any lactose. In another embodiment a prebiotic composition consists essentially of GOS and comprises one or more digestible saccharides such as lactose, galactose, or glucose. These digestible saccharides can be present in trace amounts (e.g., less than 5% by weight of the composition) and can be byproducts of the synthesis of the GOS.
- [0097] In one embodiment a prebiotic composition comprising GOS comprises about 70% GOS and about 30% digestible saccharides by weight. For example, 8g of a prebiotic composition comprising GOS can comprise 5.6g of GOS, 1.6g lactose, and 0.8g of other digestible saccharides.
- [0098] In one embodiment, a prebiotic composition comprising GOS, and optionally digestible carbohydrates, are used in a method to stimulate lactose fermenting commensal microbes of the human gastrointestinal tract in an adaptation process designed to alleviate lactose intolerance symptoms. In one embodiment, gradual feeding of a prebiotic composition comprising GOS, at

increasing doses over a defined time frame, can adapt the lactose fermenting commensal microbes to efficiently metabolize lactose in lactose-intolerant individuals. In one embodiment this adaptation is permanent.

9. GOS and non-digestible saccharides

- [0099] In one embodiment a prebiotic composition comprises an effective amount of GOS and optionally another non-digestible saccharide. In one embodiment a prebiotic composition increases Beta-galactosidase activity of species of the *Lactobacillus* and/or *Bifidobacterium* species. In another embodiment a prebiotic composition comprises an effective amount of GOS or another non-digestible saccharide to increase the lactase activity of intestinal bacteria (e.g., *Lactobacillus* and/or *Bifidobacterium*) which breaks down the lactose that is not digested by a lactose intolerant human.
- [00100] In one embodiment a method of treatment is provided for the use of GOS and optionally another non-digestible saccharide to increase Beta-galactosidase activity of lactobacilli or bifidobacteria. In another embodiment a method of treatment is provided for the use of GOS and optionally another non-digestible saccharide to increase the lactase activity of intestinal bacteria (e.g., lactobacilli or bifidobacteria). In another embodiment a method of treatment is provided for the use of GOS and optionally another non-digestible saccharide to prevent, treat, or reduce a symptom of lactose intolerance in a human. In another embodiment a symptom of lactose intolerance in a human is treated, prevented, or reduced by administration of a composition comprising GOS and optionally another non-digestible saccharide.
- [00101] In one embodiment a prebiotic composition comprises between 80-99.9% GOS and no lactose. In another embodiment, a prebiotic composition comprises between 80-99.9% GOS and 20%-0.1% digestible saccharides. In another embodiment, a prebiotic composition comprises between 80-99.9% GOS, between 0.1-20% digestible saccharides, and between 0.1-20% non-digestible saccharides other than GOS.
- [00102] In one embodiment a prebiotic composition comprising GOS comprises about 90% GOS and no lactose. For example 8g of a prebiotic composition comprising GOS can comprise about 7.2g of GOS. In another embodiment, a prebiotic composition comprises about 90% GOS and about 5% digestible saccharides. For example, 8g of a prebiotic composition comprising GOS can comprise 7.2g of GOS and about 0.4g of digestible saccharides. In another embodiment, a prebiotic composition comprises about 90% GOS, about 5% digestible saccharide, and about 2% non-digestible saccharides other than GOS. For example, 8g of a prebiotic composition comprising GOS can comprise about 7.2g of GOS, about 0.4g digestible saccharide, and about 0.16g of other non-digestible saccharides.
- [00103] In one embodiment a prebiotic composition comprising GOS comprises about 91% GOS and no lactose. For example 8g of a prebiotic composition comprising GOS can comprise about 7.28g of GOS. In another embodiment, a prebiotic composition comprises about 91% GOS and about 5% digestible saccharides. For example, 8g of a prebiotic composition comprising GOS

can comprise 7.28g of GOS and about 0.4g of digestible saccharides. In another embodiment, a prebiotic composition comprises about 91% GOS, about 5% digestible saccharides, and about 2% non-digestible saccharides other than GOS. For example, 8g of a prebiotic composition comprising GOS can comprise about 7.28g of GOS, about 0.4g of digestible saccharides, and about 0.16g of other non-digestible saccharides.

[00104] In one embodiment a prebiotic composition comprising GOS comprises about 92% GOS and no lactose. For example 8g of a prebiotic composition comprising GOS can comprise about 7.36g of GOS. In another embodiment, a prebiotic composition comprises about 92% GOS and about 5% digestible saccharides. For example, 8g of a prebiotic composition comprising GOS can comprise 7.36g of GOS and about 0.4g of digestible saccharides. In another embodiment, a prebiotic composition comprises about 92% GOS, about 5% digestible saccharides, and about 2% non-digestible saccharides other than GOS. For example, 8g of a prebiotic composition comprising GOS can comprise about 7.36g of GOS, about 0.4g of digestible saccharides, and about 0.16g of other non-digestible.

[00105] In one embodiment a prebiotic composition comprises about 93% GOS and no lactose. For example 8g of a prebiotic composition comprising GOS can comprise about 7.44g of GOS. In another embodiment, a prebiotic composition comprises about 93% GOS and about 5% digestible saccharides. For example, 8g of a prebiotic composition comprising GOS can comprise 7.44g of GOS and about 0.4g of digestible saccharides. In another embodiment, a prebiotic composition comprises about 93% GOS, about 5% digestible saccharides, and about 2% non-digestible saccharides other than GOS. For example, 8g of a prebiotic composition comprising GOS can comprise about 7.44g of GOS, about 0.4g of digestible saccharides, and about 0.16g of other non-digestible.

[00106] In one embodiment a prebiotic composition comprises about 94% GOS and no lactose. For example 8g of a prebiotic composition comprising GOS can comprise about 7.52g of GOS. In another embodiment, a prebiotic composition comprises about 94% GOS and about 5% digestible saccharides. For example, 8g of a prebiotic composition comprising GOS can comprise 7.52g of GOS and about 0.4g of digestible saccharides. In another embodiment, a prebiotic composition comprises about 94% GOS, about 5% digestible saccharides, and about 1% non-digestible saccharides other than GOS. For example, 8g of a prebiotic composition comprising GOS can comprise about 7.52g of GOS, about 0.4g of digestible saccharides, and about 0.08g of other non-digestible saccharides.

[00107] In one embodiment a prebiotic composition comprises about 95% GOS and no lactose. For example 8g of a prebiotic composition comprising GOS can comprise about 7.6g of GOS. In another embodiment, a prebiotic composition comprises about 95% GOS and about 5% digestible saccharides. For example, 8g of a prebiotic composition comprising GOS can comprise 7.6g of GOS and about 0.4g of digestible saccharides.

- [00108] In one embodiment a prebiotic composition comprises about 96% GOS and no lactose. For example 8g of a prebiotic composition comprising GOS can comprise about 7.68g of GOS. In other embodiments, a prebiotic composition comprising about 96% GOS comprises about 4% digestible saccharides. For example, 8g of a prebiotic composition comprising GOS can comprise 7.68g of GOS and about 0.32g of digestible saccharides.
- [00109] In one embodiment a prebiotic composition comprises about 97% GOS and no lactose. For example 8g of a prebiotic composition comprising GOS can comprise about 7.76g of GOS. In other embodiments, a prebiotic composition comprising about 97% GOS comprises about 3% digestible saccharides. For example, 8g of a prebiotic composition comprising GOS can comprise 7.76g of GOS and about 0.24g of digestible saccharides.
- [00110] In one embodiment a prebiotic composition comprises about 98% GOS and no lactose. For example 8g of a prebiotic composition comprising GOS can comprise about 7.84g of GOS. In other embodiments, a prebiotic composition comprising about 96% GOS comprises about 2% digestible saccharides. For example, 8g of a prebiotic composition comprising GOS can comprise 7.84g of GOS and about 0.16g of digestible saccharides.
- [00111] In one embodiment a prebiotic composition comprises about 99% GOS and no lactose. For example 8g of a prebiotic composition comprising GOS can comprise about 7.92g of GOS. In other embodiments, a prebiotic composition comprising about 99% GOS comprises about 1% digestible saccharides. For example, 8g of a prebiotic composition comprising GOS can comprise 7.92g of GOS and about 0.08g of digestible saccharides.
- [00112] In one embodiment a prebiotic composition comprises about 100% GOS and no lactose. For example 8g of a prebiotic composition comprising GOS can comprise about 8.0g of GOS. In other embodiments, a prebiotic composition comprising about 99.9% GOS comprises less than about 1% digestible saccharides. For example, 8g of a prebiotic composition comprising GOS can comprise 8.0g of GOS and about 0.1g of digestible saccharides.

10. GOS effects

- [00113] In one embodiment a GOS composition reduces or eliminates one or more symptoms associated with lactose intolerance or with lactose digestive problems, including but not limited to cramps, flatulence, stomach pain, vomiting, bloating, diarrhea, nausea, gastric distention and intestinal pain, in a subject in need thereof. In one embodiment the subject is a patient. In another embodiment the subject is a human. In another embodiment the subject is a non-human animal.

B. FOS

- [00114] FOS are chain oligomers or polymers of the sugar fructose that are found in a variety of foods. The sugar units can be linked in a single straight chain or can be a chain with side branches. In many cases small amounts of glucose are also contained in the chain. The length of the fructose chains can vary from source to source. FOS are primarily polyfructans with a degree of polymerization (DP) generally ranging from 2 to 20 (oligofructose) or greater than 20 (inulin).

Generally, the D-fructose moieties in FOS are joined by β -(2-1) linkages and the oligomers or polymers are terminated with a D-glucose molecule linked to fructose by an α -(1-2) bond.

[00115] In one embodiment a prebiotic composition comprises a FOS composition, wherein the FOS composition comprises about 1% or more of the composition by weight, such as about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% FOS. In other embodiments, the FOS composition comprises about 0.5% or more of FOS in the FOS composition by weight, such as about 0.5%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, or 35% FOS. In another embodiment the prebiotic or FOS composition comprises 0.01-20 g of FOS, such as about 0.01, 0.03, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 g of FOS. In another embodiment the prebiotic or FOS composition comprises FOS and water and one or more digestible saccharides. In one embodiment a prebiotic composition comprises less than about 10ppm of a heavy metal (such as arsenic or lead), including but not limited to less than about 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1ppm of a heavy metal.

[00116] In another embodiment, a prebiotic composition comprises a mixture of FOS and GOS. In one embodiment, about 90 % by weight of the prebiotic component is GOS and about 10 % by weight of the prebiotic component is FOS. In one embodiment, about 50 % by weight of the prebiotic component is GOS and about 50 % by weight of the prebiotic component is FOS. In one embodiment, 1-90 % by weight of the prebiotic component is GOS and 10-60 % by weight of the prebiotic component is FOS. In another embodiment, the prebiotic component of a prebiotic composition is 90-100% by weight GOS.

C. Inulin

[00117] Inulin is an example of a longer chained compound that is considered to be a FOS. The shorter (lower molecular weight) compounds tend to have a sweet taste. The size and complexity of the FOS molecules gives it desirable characteristics. Although the simple sugars fructose and glucose are quickly absorbed into the body by the intestines, FOS for the most part is non-digestible and therefore acts as a fiber in the diet. This is because humans do not have the enzymes to break down the FOS as it travels down the digestive tract. When the FOS reaches the large intestine and the colon, the bacteria that are found there start to break down the FOS. These bacteria have the enzymes needed to break down FOS. Some *Bifidobacterium* and *Lactobacillus* species have been reported to use FOS. It is believed that foods that promote the growth of bifidobacteria are beneficial for gastrointestinal health.

[00118] In one embodiment a prebiotic composition comprises inulin, wherein the inulin comprises 1% or more of the composition by weight, such as about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37,

38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% inulin. In another embodiment a prebiotic composition comprises 1-20 g of inulin, such as about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 g of inulin. In another embodiment a prebiotic composition comprises inulin, water, or one or more digestible saccharides. In one embodiment a prebiotic composition comprises less than about 10ppm of a heavy metal (such as arsenic or lead), including but not limited to less than about 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1ppm of a heavy metal.

D. Lactulose

[00119] Lactulose is a disaccharide that is formed from one molecule of fructose and galactose. It can be produced by isomerization of lactose. In one embodiment a prebiotic composition comprises lactulose (4-O- β -D-Galactopyranosyl- β -D-fructofuranose), wherein lactulose comprises about 1% or more of the composition by weight, such as about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% lactulose. In another embodiment a prebiotic composition comprises 1-20 g of lactulose, such as about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 g of lactulose. In another embodiment a prebiotic composition comprises lactulose, water, or one or more digestible saccharides. In one embodiment the composition comprises less than about 10ppm of a heavy metal (such as arsenic or lead), including but not limited to less than about 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1ppm of a heavy metal.

E. Raffinose

[00120] Raffinose (melitose, melitriose, gossypose, α -D-galactosylsucrose) is a trisaccharide composed of galactose, fructose, and glucose. The enzyme α -galactosidase, which is not found in the human digestive tract, can hydrolyze raffinose. Thus, in humans, raffinose passes through the stomach and upper intestine and is digested by bacteria that do contain α -galactosidase in the lower intestine. In one embodiment a prebiotic composition comprises raffinose, wherein the raffinose comprises 1% or more of the composition by weight, such as about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% raffinose. In another embodiment a prebiotic composition comprises 1-20 g of raffinose, such as about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 g of raffinose. In another embodiment a prebiotic composition comprises raffinose or one or more digestible saccharides. In one embodiment a prebiotic composition comprises less than about 10ppm of a heavy metal (such as arsenic or lead), including but not limited to less than about 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1ppm of a heavy metal.

F. Stachyose

[00121] Stachyose is a tetrasaccharide that consists of two α -D-galactose units, one α -D-glucose unit, and one β -D-fructose unit. It is linked as gal(α 1 $\rightarrow$ 6) gal(α 1 $\rightarrow$ 6)glc(α 1 $\leftrightarrow$ 2 β)fru. Stachyose is not completely digestible by humans. In one embodiment a prebiotic composition comprises stachyose, wherein the stachyose comprises 1% or more of the composition by weight, such as about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% stachyose. In another embodiment a prebiotic composition comprises 1-20 g of stachyose, such as about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 g of stachyose. In another embodiment a prebiotic composition comprises stachyose, water, or one or more digestible saccharides. In one embodiment a prebiotic composition comprises less than about 10ppm of a heavy metal (such as arsenic), including but not limited to less than about 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1ppm of a heavy metal.

G. GOS and inulin

[00122] In one embodiment, a prebiotic composition comprises GOS and inulin. In another embodiment, the ratio of GOS:inulin is about 99:1, about 95:1, about 90:1, about 85:1, about 80:1, about 75:1, about 70:1, about 65:1, about 60:1, about 55:1, about 50:1, about 45:1, about 40:1, about 35:1, about 30:1, about 25:1, about 20:1, about 15:1, about 10:1, about 9:1, about 8:1, about 7:1, about 6:1, about 5:1, about 4:1, about 3:1, about 2:1, or about 1:1. A prebiotic composition comprising GOS and inulin can include between 0.4 g to 20 g GOS and inulin. A prebiotic composition comprising GOS and inulin can contain about 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, or 20 g GOS and inulin.

III. Probiotics

A. Introduction

[00123] Probiotics (or probiotic bacteria) typically refer to beneficial live microorganisms, e.g., bacteria, found in the gastrointestinal tract and, when administered in adequate amounts, confer a health benefit on the host (or subject in need thereof). Reports indicate that probiotic microbes favorably alter the intestinal microbiota balance, inhibit the growth of harmful bacteria, promote good digestion, modulate immune functions, and increase resistance to both viral and bacterial infections. Bacterial cultures that are generally recognized as safe (GRAS) or known commensal or probiotic microbes could be used to assist in the reduction or elimination of lactose intolerance-

like symptoms or improving overall GI health, for example through colonic adaptation, are applicable in the methods and compositions described herein.

B. Bacteria

[00124] Examples of probiotics include, but are not limited to, those that acidify the colon such as those from the genera *Lactobacillus* or *Bifidobacterium*, which are thought to maintain a healthy balance of intestinal microbiota by producing organic acids (lactic & acetic acids), hydrogen peroxide, and bacteriocins which are documents to inhibit enteric pathogens. Bacteriocins are small antimicrobial peptides which can kill both closely-related bacteria, or exhibit a broader spectrum of activity (e.g. nisin) which includes most Gram-positive pathogens (e.g. *Listeria*, *Staphylococcus*, and *Clostridium* species).

[00125] Non-exclusive examples of probiotic bacteria that can be used in the methods and compositions described herein include *L. acidophilus*, a probiotic microbe which is an important member of the microbiota of the GI tract and has been used extensively and successfully as a probiotic cultures in dietary supplements, foods, and dairy products. These beneficial bacteria have been reported to modulate immune function, inhibit carcinogenesis, facilitate metabolism of cholesterol, and assist in digestion. Numerous reports over many *Lactobacillus* species are reported to promote a healthy microbiota, reduce putrefaction, and reduce endotoxemia. Other *Lactobacillus* bacteria which can be employed include, but are not limited to, *L. crispatus*, *L. casei*, *L. rhamnosus*, *L. reuteri*, *L. fermentum*, *L. plantarum*, *L. sporogenes*, and *L. bulgaricus*. Other probiotic bacteria suitable for the compositions include *Bifidobacterium lactis*, *B. animalis*, *B. bifidum*, *B. longum*, *B. adolescentis*, and *B. infantis*. Yeasts, such as *Saccharomyces boulardii*, are also suitable as probiotics and may act to restore the intestinal microbiota. Mixtures of one or more species or strains of bacteria can be used. For example, yogurt is a product which already contains bacteria species, such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, which are used for fermentation. Yogurt can be supplemented with prebiotics and additional bacterial species that are considered probiotic cuultures.

[00126] Other strains of probiotic bacteria that can be used in the methods and compositions described herein include, for example, *Bacillus coagulans* GBI-30, 6086; *Bifidobacterium animalis* subsp. *lactis* BB-12; *Bifidobacterium breve* Yakult; *Bifidobacterium infantis* 35624; *Bifidobacterium animalis* subsp. *lactis* HN019 (DR10); *Bifidobacterium longum* BB536; *Escherichia coli* M-17; *Escherichia coli* Nissle 1917; *Lactobacillus acidophilus* DDS-1; *Lactobacillus acidophilus* LA-5; *Lactobacillus acidophilus* NCFM; *Lactobacillus casei* DN114-001 (*Lactobacillus casei* Immunitas(s)/Defensis); *Lactobacillus casei* CRL431; *Lactobacillus casei* F19; *Lactobacillus paracasei* St11 (or NCC2461); *Lactobacillus johnsonii* La1 (*Lactobacillus* LC1, *Lactobacillus johnsonii* NCC533); *Lactococcus lactis* L1A; *Lactobacillus plantarum* 299V; *Lactobacillus reuteri* ATTC 55730 (*Lactobacillus reuteri* SD2112); *Lactobacillus rhamnosus* ATCC 53013; *Lactobacillus rhamnosus* LB21; *Saccharomyces cerevisiae* (*boulardii*) lyo; mixture of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri*

RC-14; mixture of *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* BB-12 or BL-04; mixture of *Lactobacillus acidophilus* CL1285 and *Lactobacillus casei*; and a mixture of *Lactobacillus helveticus* R0052 and *Lactobacillus rhamnosus* R0011.

[00127] In one embodiment, a composition comprises a prebiotic and probiotic. In one embodiment a prebiotic composition comprises or consists essentially of GOS. In one embodiment, a prebiotic composition is administered with increasing doses of probiotics during the period of treatment. In another embodiment, a prebiotic composition is administered with constant doses (dose amounts that do not change) of probiotics during the period of treatment. In another embodiment, a prebiotic composition is administered with both increasing doses of probiotics for a portion of the treatment and a constant dose of probiotics during another portion of the treatment period.

C. Dose timing and size of probiotics

[00128] In one embodiment, probiotic bacteria, such as *L. acidophilus*, are given prior to beginning treatment with a prebiotic. In one embodiment, probiotic bacteria, such as *L. acidophilus*, are given in conjunction with treatment with a prebiotic (e.g., comprising or consisting essentially of GOS), for part or all of the treatment with the prebiotic. Thus, in one embodiment, some or all doses of a prebiotic (e.g., comprising or consisting essentially of GOS) are accompanied by a dose of bacteria, e.g., live cultured bacteria, e.g., *L. acidophilus*. In one embodiment, bacteria, e.g., *L. acidophilus* are given initially with a prebiotic (e.g., comprising or consisting essentially of GOS), but then use of the bacteria is discontinued. For example, the initial one, two, three, four, five, six, seven, eight, nine, ten, or more than ten days of treatment with a prebiotic (e.g., comprising or consisting essentially of GOS) can include doses of bacteria, with the use of bacteria discontinued after that time. In one embodiment, bacteria, e.g., bacteria in yogurt, or bacteria by themselves, can be given for the first two days of treatment; then the administration of bacteria is discontinued. In another embodiment, probiotic bacteria, either alone or in combination with other substances or treatments are used after the treatment with a prebiotic (comprising or consisting essentially of GOS) is terminated. The bacteria can be taken for any suitable period after the termination of treatment with prebiotic and can be taken daily or at regular or irregular intervals. Doses can be as described below.

[00129] Any suitable amount of probiotic per serving can be used that allows an effective microbiota in the GI. Typically, probiotics are given as live cultured bacteria. The dose can be about 0.001mg to about 1mg, or about 0.5mg to about 5mg, or about 1mg to about 1000mg, or about 2 mg to about 200mg, or about 2 mg to about 100mg, or about 2 mg to about 50mg, or about 4 mg to about 25mg, or about 5 mg to about 20mg, or about 10 mg to about 15mg, or about 50mg to about 200mg, or about 200mg to about 1000mg, or about 10, 11, 12, 12.5, 13, 14, or 15mg per serving. In one embodiment, *L. acidophilus* is used in a dose of about 12.5mg per serving. The probiotic bacteria can also be about 0.5% w/w to about 20% w/w of the final composition. The dose of probiotics can be given in combination with one or more prebiotics.

Another common way of specifying the amount of probiotics is as a colony forming unit (cfu). A cfu is an individual cell which is able to clone itself into an entire colony of identical cells. In one embodiment, one or more strains of probiotic bacteria are ingested in an amount of about 1×10^6 to about 1×10^9 cfu's, or about 1×10^6 cfu's to about 1×10^9 cfu's, or about 10×10^6 cfu's to about 0.5×10^9 cfu's, or about 113×10^5 cfu's to about 113×10^6 cfu's, or about 240×10^5 cfu's to about 240×10^6 cfu's, or about 0.3×10^9 cfu's per serving. In another embodiment, one or more strains of probiotic bacteria are administered as part of a dairy product. In one embodiment, a typical serving size for a dairy product such as fluid milk is about 240g. In other embodiments, a serving size is about 245g, or about 240g to about 245g, or about 227 to about 300g. In one embodiment the dairy product is yogurt. Yogurt can have a serving size of about 4 oz, or about 6 oz, or about 8 oz, or about 4 oz to 10 oz, or about half cup, or about 1 cup, or about 113g, or about 170g, or about 227g, or about 245g or about 277g, or about 100g to about 350g.

[00130] In one embodiment probiotic bacteria are given as live cultured bacteria, e.g., in combination with a prebiotic (e.g., comprising or consisting essentially of GOS) and, optionally, other substances. The dose can be about 1 mg to about 1000 mg, or about 2 mg to about 200 mg, or about 2 mg to about 100 mg, or about 2 mg to about 50 mg, or about 4 mg to about 25 mg, or about 5 mg to about 20 mg, or about 10 mg to about 15 mg, or about 10, 11, 12, 12.5, 13, 14, or 15 mg of probiotic bacteria. In one embodiment, *L. acidophilus* is used in a dose of about 12.5 mg. In one embodiment, as the administration of a prebiotic (e.g., comprising or consisting essentially of GOS) dose to a subject increases, the dose of bacteria increases as well. For example, an initial dose of a prebiotic (e.g., comprising or consisting essentially of GOS) can be about 0.6 g to 1.0 g, e.g., 0.8 g, given in combination with about 10-15 mg, e.g., about 12.5 mg, of *L. acidophilus*. The dose of a prebiotic (e.g., comprising or consisting essentially of GOS) can be increased incrementally by about 0.6 g to 1.0 g, e.g., 0.8 g, and the accompanying dose of *L. acidophilus* can be increased by about 10-15 mg, e.g., about 12.5 mg, of *L. acidophilus*.

IV. GOS Formulations

A. Formulations introduction

[00131] In one aspect a prebiotic composition for the treatment of the symptoms of lactose intolerance is provided. In one embodiment a prebiotic composition comprises inulin, FOS, lactulose, GOS, raffinose, stachyose, or a combination thereof. In one embodiment a prebiotic composition comprises or consists essentially of GOS. In another embodiment a prebiotic composition comprises GOS and one or more digestible saccharides. Digestible saccharides are saccharides that are digestible by humans and include, but are not limited to lactose, glucose, and galactose. In one embodiment a prebiotic composition comprises GOS and less than 20% of one or more digestible saccharides. In one embodiment a prebiotic composition comprises GOS and less than 10% of one or more digestible saccharides. In one embodiment a prebiotic composition comprises GOS and less than 5% of one or more digestible saccharides. In another embodiment a prebiotic composition contains less than 5% lactose. In another embodiment a prebiotic

composition contains less than 4% lactose. In another embodiment a prebiotic composition contains less than 3% lactose. In another embodiment a prebiotic composition contains less than 2% lactose. In another embodiment a prebiotic composition contains less than 1% lactose. In another embodiment a prebiotic composition contains less than 0.5% lactose. In another embodiment a prebiotic composition contains less than 0.4% lactose. In another embodiment a prebiotic composition contains less than 0.3% lactose. In another embodiment a prebiotic composition contains less than 0.2% lactose. In another embodiment a prebiotic composition contains less than 0.1% lactose. In another embodiment a prebiotic composition contains less than 0.05% lactose. In another embodiment a prebiotic composition contains less than 0.01% lactose. In another embodiment a prebiotic composition contains less than 0.005% lactose. In one embodiment a prebiotic composition comprises GOS and essentially no lactose. In one embodiment a prebiotic composition does not contain any lactose. In another embodiment a prebiotic composition contains GOS and at least one probiotic bacteria strain. In another embodiment a prebiotic composition comprises GOS and optionally one or more of lactose, at least one probiotic bacteria strain, or a buffer. Additional ingredients include ingredients to improve handling, preservatives, antioxidants, flavorings and the like.

[00132] In one embodiment, a prebiotic composition comprises GOS or a probiotic. In other embodiment, a prebiotic composition is in the form of a powder, tablet, capsule, or liquid. In one embodiment, a prebiotic composition can be administered with a dairy product and is in the form of milk or other common dairy product such as a yogurt, shake, smoothie, cheese, and the like.

[00133] In embodiments where a prebiotic composition comprises less than 100% by weight of GOS the remaining ingredients can be any suitable ingredients intended for the consumption of the subject in need thereof, e.g., human, including, but not limited to, other prebiotics (e.g., FOS), a buffer, one or more digestible saccharides, ingredients intended to inhibit clumping and increase pourability, such as silicone dioxide and microcrystalline cellulose, or similar ingredients as are well-known in the art. Remaining ingredients can also include ingredients to improve handling, preservatives, antioxidants, flavorings, and the like.

B. Buffer components

[00134] One or more buffers, optionally with a calcium counterion, can also be administered in methods and compositions described herein. Any buffer suitable for consumption by the subject being treated, e.g., human, are useful for the compositions herein. The buffer neutralizes stomach acidity which can, e.g., allow live bacteria to reach the gut. Buffers include citrates, phosphates, and the like. One embodiment utilizes a buffer with a calcium counterion, such as Calcium Phosphate Tribasic. The calcium can serve to restore the calcium that many lactose intolerant subjects are missing in their diet. A recent study demonstrated the ability of calcium phosphate to protect *Lactobacillus acidophilus* from bile. Calcium phosphate can help neutralize stomach acidity.

[00135] In one embodiment, a buffer such as calcium phosphate is given prior to beginning treatment with a prebiotic composition (such as a composition comprising or consisting essentially of GOS), optionally in conjunction with administration of bacteria. In one embodiment, a buffer such as calcium phosphate is given in conjunction with treatment with a prebiotic composition (e.g., a composition comprising or consisting essentially of GOS), for part or all of the treatment with lactose. Thus, in one embodiment, some or all doses of a prebiotic composition are accompanied by a dose of a buffer such as calcium phosphate. In one embodiment, a buffer such as calcium phosphate is given initially with a prebiotic composition (such as a composition comprising or consisting essentially of GOS), but then its use is discontinued. For example, the initial one, two, three, four, five, six, seven, eight, nine, ten, or more than ten days of treatment with a prebiotic composition can include doses of a buffer such as calcium phosphate, with the use of the buffer discontinued after that time. In one embodiment, a buffer such as calcium phosphate can be given for the first two days of treatment, and then the administration of buffer is discontinued. In one embodiment, a buffer such as calcium phosphate, either alone or in combination with other substances or treatments is used after the treatment with a prebiotic composition is terminated. A buffer such as calcium phosphate can be taken for any suitable period after the termination of treatment with lactose, and can be taken daily or at regular or irregular intervals. Doses can be as described below.

[00136] Numerous buffers suitable for human consumption are known in the art, and any suitable buffer can be used in the methods and compositions described herein. Calcium triphosphate is an exemplary buffer, and its counterion supplies a nutrient that is often lacking in lactose-intolerant subjects, i.e. calcium. In one embodiment a buffer can be used in a dose from about 2 mg to about 2000 mg, or about 4 mg to about 400 mg, or about 4 mg to about 200 mg, or about 4 mg to about 100 mg, or about 8 mg to about 50 mg, or about 10 mg to about 40 mg, or about 20 mg to about 30 mg, or about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 mg. In another embodiment a prebiotic composition further comprises an amount of a buffer from 1-50 mg, such as about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 mg. In one embodiment, buffer is used in a dose of about 25 mg. In one embodiment, calcium phosphate is used in a dose of about 25 mg. The dose can be given in combination with a prebiotic composition (e.g., a composition comprising or consisting essentially of GOS). In one embodiment, as a prebiotic composition dose increases, the dose of buffer increases as well. For example, an initial dose of a prebiotic composition can be about 0.6 g to 1.0 g, e.g., 0.8 g, given in combination with about 20-30 mg, e.g., about 25 mg, of buffer, e.g., calcium phosphate. The dose of a prebiotic composition can be increased incrementally by about 0.6 g to 1.0 g, e.g., 0.8 g, and the accompanying dose of buffer, e.g., calcium phosphate, can be increased by about 20-30 mg, e.g., about 25 mg, of buffer, e.g., calcium phosphate.

C. Compositions comprising GOS and at least one probiotic bacteria strain

[00137] In one embodiment, a prebiotic composition comprises GOS and at least one probiotic bacteria strain. The GOS can comprise more than 1% of the weight of the composition while the at least one probiotic bacteria strain will typically comprise less than about 10%, 5%, 4%, 3%, or 2% by weight of the compositions (herein all percentages are weight percent unless otherwise indicated). For example, the GOS can be present at about 1-99.75% by weight and the at least one probiotic bacteria strain at about 0.25-2 % by weight, or the GOS can be present at about 89-96% by weight and the bacteria at about 1.2-3.7% by weight. In one embodiment, GOS are present at about 92% by weight and at least one probiotic bacteria strain, (e.g., *L. acidophilus* or *Bifidobacterium lactis*), is present at about 1.5% by weight. In one embodiment, GOS are present at about 92% by weight and at least one probiotic bacteria strain, (e.g., *L. acidophilus* or *Bifidobacterium lactis*), is present at about 1.5% by weight. In another embodiment, GOS are present at about 93% by weight and at least one probiotic bacteria strain, (e.g., *L. acidophilus* or *Bifidobacterium lactis*), is present at about 1.5% by weight. In another embodiment, GOS are present at about 94% by weight and at least one probiotic bacteria strain, (e.g., *L. acidophilus* or *Bifidobacterium lactis*), is present at about 1.5% by weight. In another embodiment, GOS are present at about 95% by weight and at least one probiotic bacteria strain, (e.g., *L. acidophilus* or *Bifidobacterium lactis*), is present at about 1.5% by weight. In another embodiment, GOS are present at about 96% by weight and at least one probiotic bacteria strain, (e.g., *L. acidophilus* or *Bifidobacterium lactis*), is present at about 1.5% by weight. In another embodiment, GOS are present at about 97% by weight and at least one probiotic bacteria strain, (e.g., *L. acidophilus* or *Bifidobacterium lactis*), is present at about 1.5% by weight. In another embodiment, GOS are present at about 98% by weight and at least one probiotic bacteria strain, (e.g., *L. acidophilus* or *Bifidobacterium lactis*), is present at about 1.5% by weight. In another embodiment, GOS are present at about 98.5% by weight and at least one probiotic bacteria strain, (e.g., *L. acidophilus* or *Bifidobacterium lactis*), is present at about 1.5% by weight. If the at least one probiotic bacteria strain and GOS do not make up 100% by weight of the prebiotic composition, the remaining ingredients can be any suitable ingredients intended for consumption by the subject in need thereof, e.g., human, including, but not limited to, other prebiotics (e.g., FOS), one or more buffers, digestible saccharides ingredients intended to inhibit clumping and increase pourability, such as silicone dioxide and microcrystalline cellulose, or similar ingredients as are well-known in the art. Remaining ingredients can also include ingredients to improve handling, preservatives, antioxidants, flavorings and the like.

D. Compositions comprising GOS and a buffer

[00138] In another embodiment, a prebiotic composition comprises GOS and a buffer (e.g., calcium phosphate tribasic). For example, GOS can be present at about 1-100% by weight and the buffer at about 0.50-4% by weight, or GOS can be present at about 1-96% by weight and the buffer at about 1 to about 3.75% by weight. In one embodiment, GOS are present at about 1% by weight and buffer is present at about 3% by weight. In one embodiment, GOS are present at

about 5% by weight and buffer is present at about 3% by weight. In one embodiment, GOS are present at about 10% by weight and buffer is present at about 3% by weight. In one embodiment, GOS are present at about 15% by weight and buffer is present at about 15% by weight. In one embodiment, GOS are present at about 20% by weight and buffer is present at about 3% by weight. In one embodiment, GOS are present at about 25% by weight and buffer is present at about 3% by weight. In one embodiment, GOS are present at about 30% by weight and buffer is present at about 3% by weight. In one embodiment, GOS are present at about 35% by weight and buffer is present at about 3% by weight. In one embodiment, GOS are present at about 40% by weight and buffer is present at about 3% by weight. In one embodiment, GOS are present at about 50% by weight and buffer is present at about 3% by weight. In one embodiment, GOS are present at about 60% by weight and buffer is present at about 3% by weight. In one embodiment, GOS are present at about 70% by weight and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 90% by weight and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 92% by weight and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 93% by weight and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 94% by weight and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 95% by weight and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 96% by weight and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 97% by weight and buffer is present at about 2% by weight. In another embodiment, GOS are present at about 98% by weight and buffer is present at about 1% by weight. In another embodiment, GOS are present at about 99% by weight and buffer is present at about 1% by weight. In another embodiment, GOS are present at about 100% by weight and buffer is present at less than about 1% by weight. If the buffer and GOS do not make up 100% by weight of the composition, the remaining ingredients can be any suitable ingredients intended for consumption by the subject (e.g., a human) including, but not limited to, probiotics (e.g., beneficial bacteria) or other prebiotics (e.g., FOS), but also including ingredients intended to inhibit clumping and increase pourability, such as silicone dioxide and microcrystalline cellulose, or similar ingredients as are well-known in the art. Remaining ingredients can also include ingredients to improve handling, preservatives, antioxidants, flavorings and the like.

E. Compositions comprising a digestible saccharide, a probiotic bacteria, and GOS

[00139] In one embodiment, a prebiotic composition comprises a digestible saccharide, a probiotic bacteria (e.g., *L. acidophilus* or *Bifidobacterium*), and GOS. In one embodiment, lactose can be present at about 1-20% by weight, bacteria at about 0.25-2.10% by weight, and GOS at about 1-98.75% by weight. In another embodiment lactose can be present at about 5-20% by weight, bacteria at about 0.91-1.95% by weight, and GOS at about 1 to about 96% by weight. In another embodiment, lactose is present at about 20% by weight, bacteria at about 1.5% by weight, and GOS are present at about 1% by weight. In another embodiment, lactose is present at about 20%

by weight, bacteria at about 1.5% by weight, and GOS are present at about 50% by weight. In another embodiment, lactose is present at about 20% by weight, bacteria at about 1.5% by weight, and GOS are present at about 60% by weight. In another embodiment, lactose is present at about 20% by weight, bacteria at about 1.5% by weight, and GOS are present at about 70% by weight. In another embodiment, lactose is present at about 5% by weight, bacteria at about 1.5% by weight, and GOS are present at about 90% by weight. In another embodiment, lactose is present at about 5% by weight, bacteria at about 1.5% by weight, and GOS are present at about 92% by weight. In another embodiment, lactose is present at about 5% by weight, bacteria at about 1.5% by weight, and GOS are present at about 93% by weight. In another embodiment, lactose is present at about 5% by weight, bacteria at about 1% by weight, and GOS are present at about 94% by weight. In another embodiment, lactose is present at about 4.5% by weight, bacteria at about 1.5% by weight, and GOS are present at about 94% by weight. In another embodiment, lactose is present at about 4.5% by weight, bacteria at about 0.5% by weight, and GOS are present at about 95% by weight. In another embodiment, lactose is present at about 3.5% by weight, bacteria at about 0.5% by weight, and GOS are present at about 96% by weight. In another embodiment, lactose is present at about 2.5% by weight, bacteria at about 0.5% by weight, and GOS are present at about 97% by weight. In another embodiment, lactose is present at about 1.5% by weight, bacteria at about 0.5% by weight, and GOS are present at about 98% by weight. In another embodiment, lactose is present at about 0.5% by weight, bacteria at about 0.5% by weight, and GOS are present at about 99% by weight. If the bacteria, GOS and lactose do not make up 100% of the composition, the remaining ingredients can be any suitable ingredients intended for consumption by the subject, e.g., a human, including, but not limited to a buffer, digestible saccharides (e.g., lactose, glucose, or galactose), ingredients intended to inhibit clumping and increase pourability, such as silicone dioxide and microcrystalline cellulose, or similar ingredients as are well-known in the art. Remaining ingredients can also include ingredients to improve handling, preservatives, antioxidants, flavorings and the like.

F. Compositions comprising GOS, a probiotic bacteria, and buffer

[00140] In one embodiment, a prebiotic composition comprises GOS, a probiotic bacteria strain, and buffer. In one embodiment, GOS can be present at about 1-100% by weight, a probiotic bacteria strain at about 0.25-2% by weight, and the buffer at about 0.50-4% by weight. In another embodiment, GOS can be present at about 1-95% by weight, a probiotic bacteria strain at about 0.91-1.95% by weight, and the buffer at about 1.2 - 3.75% by weight. In another embodiment, GOS are present at about 1% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 5% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 10% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 15% by weight, a probiotic bacteria strain at about 1.5%

by weight, and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 20% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 25% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 30% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 35% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 40% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 50% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 60% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 70% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 90% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 92% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 93% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 94% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 95% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 3% by weight. In another embodiment, GOS are present at about 96% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 2% by weight. In another embodiment, GOS are present at about 97% by weight, a probiotic bacteria strain at about 1.5% by weight, and buffer is present at about 1.5% by weight. In another embodiment, GOS are present at about 99% by weight, a probiotic bacteria strain at about 0.5% by weight, and buffer is present at about 0.5% by weight. In another embodiment, GOS are present at about 100% by weight, a probiotic bacteria strain at less than about 0.5% by weight, and buffer is present at less than about 0.5% by weight. If the probiotic bacteria strain, buffer, and GOS do not make up 100% of the composition, the remaining ingredients can be any suitable ingredients intended for the consumption of a subject (e.g., human) including, but not limited to, other prebiotics (e.g., FOS), digestible saccharides (e.g., lactose, glucose or galactose), ingredients intended to inhibit clumping and increase pourability, such as silicone dioxide and microcrystalline cellulose, or similar ingredients as are well-known in the art. Remaining ingredients can also include ingredients to improve handling, preservatives, antioxidants, flavorings and the like.

G. Compositions comprising a digestible saccharide, GOS, and a buffer

[00141] In one embodiment, a prebiotic composition comprises a digestible saccharide, GOS, and a buffer. For example, lactose can be present at about 1-20% by weight, GOS at about 1 -100% by weight, and the buffer at about 0.50-4% by weight, or the lactose can be present at about 5-20% by weight, GOS at about 1 - 96% by weight, and the buffer at about 1.2 - 3.75% by weight. In one embodiment, lactose is present at about 20% by weight, GOS at about 1% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 5% by weight, GOS at about 1% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, GOS at about 10% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, GOS at about 15% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, GOS at about 20% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, GOS at about 25% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, GOS at about 30% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, GOS at about 35% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, GOS at about 40% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, GOS at about 50% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, GOS at about 60% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, GOS at about 70% by weight, and buffer is present at about 3% by weight. In another embodiment, lactose is present at about 5% by weight, GOS at about 90% by weight, and buffer is present at about 3% by weight. In another embodiment, lactose is present at about 5% by weight, GOS at about 92% by weight, and buffer is present at about 3% by weight. In another embodiment, lactose is present at about 4% by weight, GOS at about 93% by weight, and buffer is present at about 3% by weight. In another embodiment, lactose is present at about 3% by weight, GOS at about 94% by weight, and buffer is present at about 3% by weight. In another embodiment, lactose is present at about 2% by weight, GOS at about 95% by weight, and buffer is present at about 3% by weight. In another embodiment, lactose is present at about 1% by weight, GOS at about 96% by weight, and buffer is present at about 3% by weight. If GOS, buffer and lactose do not make up 100% of the composition by weight, the remaining ingredients can be any suitable ingredients intended for consumption by a subject (e.g., human) including, but not limited to, bacteria, ingredients intended to inhibit clumping and increase pourability, such as silicone dioxide and microcrystalline cellulose, or similar ingredients as are well-known in the art. Remaining ingredients can also include ingredients to improve handling, preservatives, antioxidants, flavorings and the like.

H. Compositions comprising a digestible saccharide, bacteria, GOS, and a buffer

[00142] In one embodiment, a prebiotic composition comprises a digestible saccharide, bacteria, GOS, and buffer. For example, lactose can be present at about 1-20% by weight, bacteria at about 0.25 - 2.10% by weight, GOS at about 1 -100% by weight, and the buffer at about 0.50-4% by weight, or the lactose can be present at about 5-20% by weight, bacteria at about 0.91 - 1.95% by weight, GOS at about 70 -95% by weight, and the buffer at about 1.2 - 3.75% by weight. In one embodiment, lactose is present at about 20% by weight, bacteria at about 1.47% by weight, GOS at about 1% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, bacteria at about 1.47% by weight, GOS at about 10% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, bacteria at about 1.47% by weight, GOS at about 15% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, bacteria at about 1.47% by weight, GOS at about 20% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, bacteria at about 1.47% by weight, GOS at about 25% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, bacteria at about 1.47% by weight, GOS at about 30% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, bacteria at about 1.47% by weight, GOS at about 35% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, bacteria at about 1.47% by weight, GOS at about 40% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, bacteria at about 1.47% by weight, GOS at about 50% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, bacteria at about 1.47% by weight, GOS at about 60% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 20% by weight, bacteria at about 1.47% by weight, GOS at about 70% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 5% by weight, bacteria at about 1.47% by weight, GOS at about 90% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 3% by weight, bacteria at about 1.47% by weight, GOS at about 92% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 2% by weight, bacteria at about 1.47% by weight, GOS at about 93% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 1% by weight, bacteria at about 1.47% by weight, GOS at about 94% by weight, and buffer is present at about 3% by weight. In one embodiment, lactose is present at about 0.5% by weight, bacteria at about 1.47% by weight, GOS at about 95% by weight, and buffer is present at about 3% by weight. If the bacteria, GOS, buffer and lactose do not make up 100% of the composition by weight, the remaining ingredients can be any suitable ingredients intended for consumption by a subject, e.g., human, including, but not limited to, ingredients intended to inhibit clumping and increase pourability, such as silicone dioxide and microcrystalline cellulose, or similar ingredients

as are well-known in the art. Remaining ingredients can also include ingredients to improve handling, preservatives, antioxidants, flavorings and the like.

I. Additional ingredients

[00143] Additional ingredients include ingredients to improve handling, preservatives, antioxidants, flavorings and the like. For example, in one embodiment, a prebiotic composition in powdered form can include flavorings such that when mixed in a liquid (e.g., water), the powder can flavor the liquid with various flavors such as grape, strawberry, lime, lemon, chocolate, and the like. In one embodiment, the compositions include microcrystalline cellulose or silicone dioxide. Preservatives can include, for example, benzoic acid, alcohols, for example, ethyl alcohol, and hydroxybenzoates. Antioxidants can include, for example, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tocopherols (e.g., Vitamin E), and ascorbic acid (Vitamin C).

V. Dosage forms

A. General

[00144] Compositions described herein include any suitable form, including liquid or powder. Powdered compositions can be as pure powder, or can be in the form of capsules, tablets, or the like. Powder can be packaged in bulk (e.g., in a container containing sufficient prebiotic or other substances for a subject to follow for an entire course of treatment with increasing doses of prebiotic, or a portion of a course of treatment), or as individual packets (e.g., packets containing a single dose of prebiotic plus other components, or packets containing the dose of prebiotic and other components needed for a particular day of a prebiotic treatment regimen). If packaged in bulk, the powder can be in any suitable container, such as a packet, sachet, canister, ampoule, ramekin, or bottle. The container can also include one or more scoops or similar serving devices of a size or sizes appropriate to measure and serve one or more doses of prebiotic and, optionally, other ingredients included in the powder. Liquid compositions contain prebiotic and, optionally, other ingredients, in a suitable liquid, e.g., water or buffer. Liquid compositions can be provided in bulk (e.g., in a container containing sufficient prebiotic or other substances for one subject in need thereof to follow an entire course of treatment with increasing doses of prebiotic, or a portion of a course of treatment), or as individual containers, such as cans, bottles, soft packs, and the like (e.g., containers containing a single dose of prebiotic plus other components in suitable liquid, or containers containing the dose of prebiotic and other components needed for a particular day of a prebiotic treatment regimen). The container can also include one or more measuring cups or similar serving devices of a size or sizes appropriate to measure and serve one or more doses of prebiotic and, optionally, other ingredients included in the liquid.

B. Oral dosage forms and components

[00145] In one aspect provided herein are methods and compositions formulated for oral delivery to a subject in need thereof. In one embodiment a composition is formulated to deliver a

composition comprising a prebiotic to a subject in need thereof. In another embodiment a composition is formulated to deliver a composition comprising prebiotic and a probiotic to a subject in need thereof.

1. Forms

[00146] In one embodiment, a composition is administered in solid, semi-solid, micro-emulsion, gel, or liquid form. Examples of such dosage forms include tablet forms disclosed in US Patent Nos. 3048526, 3108046, 4786505, 4919939, and 4950484; gel forms disclosed in US Patent Nos. 4904479, 6482435, 6572871, and 5013726; capsule forms disclosed in US Patent Nos. 4800083, 4532126, 4935243, and 6258380; or liquid forms disclosed in US patent Nos. 4625494, 4478822, and 5610184; each of which is incorporated herein by reference in its entirety.

[00147] Forms of the compositions that can be used orally include tablets, push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. Tablets can be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets can be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with binders (e.g., povidone, gelatin, hydroxypropylmethyl cellulose), inert diluents, preservative, antioxidant, disintegrant (e.g., sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) or lubricating, surface active or dispersing agents. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets can optionally be coated or scored and can be formulated so as to provide slow or controlled release of the active ingredient therein. Tablets can optionally be provided with an enteric coating, to provide release in parts of the gut (e.g., colon, lower intestine) other than the stomach. All formulations for oral administration can be in dosages suitable for such administration. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds (prebiotics or probiotics) can be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers can be added. Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions can be used, which can optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or Dragee coatings for identification or to characterize different combinations of active compound doses.

[00148] Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water soluble carrier such as polyethylene glycol or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

[00149] Oral liquid preparations can be in the form of, for example, aqueous or oily suspensions, solutions, emulsions syrups or elixirs, or can be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations can contain conventional additives, such as suspending agents, for example sorbitol, methyl cellulose, glucose syrup, gelatin, hydroxyethyl cellulose, carboxymethyl cellulose, aluminum stearate gel or hydrogenated edible fats, emulsifying agents, for example lecithin, sorbitan monooleate, acacia; nonaqueous vehicles (which can include edible oils), for example almond oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and, if desired, conventional flavoring or coloring agents.

[00150] In one embodiment, a provided prebiotic composition includes a softgel formulation. A softgel can contain a gelatin based shell that surrounds a liquid fill. The shell can be made of gelatin, plasticiser (e.g., glycerin and/or sorbitol), modifier, water, color, antioxidant, or flavor. The shell can be made with starch or carrageenan. The outer layer can be enteric coated. In one embodiment, a softgel formulation can include a water or oil soluble fill solution, or suspension of a composition, for example, a prebiotic composition, covered by a layer of gelatin.

[00151] An enteric coating can control the location of where a prebiotic composition is absorbed in the digestive system. For example, an enteric coating can be designed such that a prebiotic composition does not dissolve in the stomach but rather travels to the small intestine, where it dissolves. An enteric coating can be stable at low pH (such as in the stomach) and can dissolve at higher pH (for example, in the small intestine). Material that can be used in enteric coatings includes, for example, alginic acid, cellulose acetate phthalate, plastics, waxes, shellac, and fatty acids (e.g., stearic acid, palmitic acid). Enteric coatings are described, for example, in US Patent Nos. 5,225,202, 5,733,575, 6,139,875, 6,420,473, 6,455,052, and 6,569,457, all of which are herein incorporated by reference in their entirety. The enteric coating can be an aqueous enteric coating. Examples of polymers that can be used in enteric coatings include, for example, shellac (trade name EmCoat 120 N, Marcoat 125); cellulose acetate phthalate (trade name aquacoat CPD®, Sepifilm™ LP, Klucel®, Aquacoat® ECD, and Metolose®); polyvinylacetate phthalate (trade name Sureteric®); and methacrylic acid (trade name Eudragit®).

[00152] In one embodiment, an enteric coated prebiotic composition is administered to a subject. In another embodiment, an enteric coated probiotic composition is administered to a subject. In another embodiment, an enteric coated probiotic and prebiotic composition is administered to a subject. In one embodiment, probiotic bacteria can be administered to a subject using an enteric coating. The stomach has an acidic environment that can kill probiotics. An enteric coating can protect probiotics as they pass through the stomach and small intestine.

[00153] Enteric coatings can be used to (1) prevent the gastric juice from reacting with or destroying the active substance, (2) prevent dilution of the active substance before it reaches the intestine, (3) ensure that the active substance is not released until after the preparation has passed

the stomach, and (4) prevent live bacteria contained in the preparation from being killed because of the low pH-value in the stomach.

[00154] Enteric coatings can also be used for avoiding irritation of or damage to the mucous membrane of the stomach caused by substances contained in the oral preparation, and for counteracting or preventing formation or release of substances having an unpleasant odor or taste in the stomach. Finally, such coatings can be used for preventing nausea or vomiting on intake of oral preparations.

[00155] In one embodiment a prebiotic composition is provided as a tablet, capsule, or caplet with an enteric coating. In one embodiment the enteric coating is designed to hold the tablet, capsule, or caplet together when in the stomach. The enteric coating is designed to hold together in acid conditions of the stomach and break down in non-acid conditions and therefore release the drug in the intestines.

[00156] Softgel delivery systems can also incorporate phospholipids or polymers or natural gums to entrap a composition, for example, a prebiotic composition, in the gelatin layer with an outer coating to give desired delayed/control release effects, such as an enteric coating.

[00157] Formulations of softgel fills can be at pH 2.5-7.5.

[00158] A softgel formulation can be sealed tightly in an automatic manner. A softgel formulation can easily be swallowed, allow for product identification using colors and several shapes, allow uniformity, precision and accuracy between dosages, be safe against adulteration, provide good availability and rapid absorption, and offer protection against contamination, light and oxidation. Furthermore, softgel formulations can avoid unpleasant flavors due to content encapsulation.

[00159] A composition comprising a softgel formulation can be in any of number of different sizes, including, for example, round, oblong, oval, tube, droplet, or suppositories.

[00160] In one embodiment a composition is provided in a dosage form which comprises an effective amount of prebiotic and one or more release controlling excipients as described herein. Suitable modified release dosage vehicles include, but are not limited to, hydrophilic or hydrophobic matrix devices, water-soluble separating layer coatings, enteric coatings, osmotic devices, multi-particulate devices, and combinations thereof. In one embodiment the dosage form is a tablet, caplet, capsule or lollipop. In another embodiment, the dosage form is a liquid, oral suspension, oral solution, or oral syrup. In yet another embodiment, the dosage form is a gel capsule, soft gelatin capsule, or hard gelatin capsule.

[00161] In another embodiment a composition comprising a prebiotic is provided in effervescent dosage forms. The compositions can also comprise non-release controlling excipients.

[00162] In another embodiment, a composition comprising a prebiotic is provided in a dosage form that has at least one component that can facilitate release of the prebiotic. In a further embodiment the dosage form can be capable of giving a discontinuous release of the compound in the form of at least two consecutive pulses separated in time from 0.1 up to 24 hours. The

compositions can comprise one or more release controlling and non-release controlling excipients, such as those excipients suitable for a disruptable semi-permeable membrane and as swellable substances.

[00163] In another embodiment a composition comprising a prebiotic is provided in an enteric coated dosage form. The composition can also comprise non-release controlling excipients.

[00164] In another embodiment a composition comprising a prebiotic is provided in a dosage form for oral administration to a subject in need thereof, which comprises one or more pharmaceutically acceptable excipients or carriers, enclosed in an intermediate reactive layer comprising a gastric juice-resistant polymeric layered material partially neutralized with alkali and having cation exchange capacity and a gastric juice-resistant outer layer.

[00165] In one embodiment a composition comprising a prebiotic is provided in the form of enteric-coated granules, for oral administration. The compositions can further comprise cellulose, disodium hydrogen phosphate, hydroxypropyl cellulose, hypromellose, lactose, mannitol, and sodium lauryl sulfate.

[00166] In another embodiment a composition comprising a prebiotic is provided in the form of enteric-coated pellets, for oral administration. The compositions can further comprise glyceryl monostearate 40-50, hydroxypropyl cellulose, hypromellose, magnesium stearate, methacrylic acid copolymer type C, polysorbate 80, sugar spheres, talc, and triethyl citrate.

[00167] In one embodiment a composition comprising a prebiotic is provided in the form of enteric-coated granules, for oral administration. The compositions can further comprise carnauba wax, crospovidone, diacetylated monoglycerides, ethylcellulose, hydroxypropyl cellulose, hypromellose phthalate, magnesium stearate, mannitol, sodium hydroxide, sodium stearyl fumarate, talc, titanium dioxide, and yellow ferric oxide.

[00168] In another embodiment a composition comprising a prebiotic can further comprise calcium stearate, crospovidone, hydroxypropyl methylcellulose, iron oxide, mannitol, methacrylic acid copolymer, polysorbate 80, povidone, propylene glycol, sodium carbonate, sodium lauryl sulfate, titanium dioxide, and triethyl citrate.

[00169] The compositions provided herein can be in unit-dosage forms or multiple-dosage forms. Unit-dosage forms, as used herein, refer to physically discrete units suitable for administration to human or non-human animal subject in need thereof and packaged individually. Each unit-dose can contain a predetermined quantity of an active ingredient(s) sufficient to produce the desired therapeutic effect, in association with other pharmaceutical carriers or excipients. Examples of unit-dosage forms include, but are not limited to, ampoules, syringes, and individually packaged tablets and capsules. Unit-dosage forms can be administered in fractions or multiples thereof. A multiple-dosage form is a plurality of identical unit-dosage forms packaged in a single container, which can be administered in segregated unit-dosage form. Examples of multiple-dosage forms include, but are not limited to, vials, bottles of tablets or capsules, or bottles of pints or gallons. In another embodiment the multiple dosage forms comprise different pharmaceutically active agents.

For example a multiple dosage form can be provided which comprises a first dosage element comprising a composition comprising a prebiotic and a second dosage element comprising lactose or a probiotic, which can be in a modified release form.

[00170] In this example a pair of dosage elements can make a single unit dosage. In one embodiment a kit is provided comprising multiple unit dosages, wherein each unit comprises a first dosage element comprising a composition comprising a prebiotic and a second dosage element comprising probiotic, lactose or both, which can be in a modified release form. In another embodiment the kit further comprises a set of instructions.

[00171] In one embodiment compositions can be formulated in various dosage forms for oral administration. The compositions can also be formulated as a modified release dosage form, including immediate-, delayed-, extended-, prolonged-, sustained-, pulsatile-, controlled-, extended, accelerated-, fast-, targeted-, programmed-release, and gastric retention dosage forms. These dosage forms can be prepared according to known methods and techniques (see, Remington: The Science and Practice of Pharmacy, supra; Modified-Release Drug Deliver Technology, Rathbone et al., Eds., Drugs and the Pharmaceutical Science, Marcel Dekker, Inc.: New York, N.Y., 2002; Vol. 126, which is herein incorporated by reference in its entirety).

[00172] In one embodiment, the compositions are in one or more dosage forms. For example, a composition can be administered in a solid or liquid form. Examples of solid dosage forms include but are not limited to discrete units in capsules or tablets, as a powder or granule, or present in a tablet conventionally formed by compression molding. Such compressed tablets can be prepared by compressing in a suitable machine the three or more agents and a pharmaceutically acceptable carrier. The molded tablets can be optionally coated or scored, having indicia inscribed thereon and can be so formulated as to cause immediate, substantially immediate, slow, controlled or extended release of a composition comprising a prebiotic. Furthermore, dosage forms of the invention can comprise acceptable carriers or salts known in the art, such as those described in the Handbook of Pharmaceutical Excipients, American Pharmaceutical Association (1986), incorporated by reference herein in its entirety.

[00173] In one embodiment, an effective amount of a composition comprising a prebiotic is mixed with a pharmaceutical excipient to form a solid preformulation composition comprising a homogeneous mixture of compounds described herein. When referring to these compositions as "homogeneous," it is meant that the agents are dispersed evenly throughout the composition so that the composition can be subdivided into unit dosage forms such as tablets, caplets, or capsules. This solid preformulation composition can then be subdivided into unit dosage forms of the type described above comprising from, for example, about 1 g to about 20 mg of a prebiotic composition. A prebiotic composition can be formulated, in the case of caplets, capsules or tablets, to be swallowed whole, for example with water.

[00174] The compositions described herein can be in liquid form. The liquid formulations can comprise, for example, an agent in water-in-solution and/or suspension form; and a vehicle

comprising polyethoxylated castor oil, alcohol, and/or a polyoxyethylated sorbitan mono-oleate with or without flavoring. Each dosage form comprises an effective amount of an active agent and can optionally comprise pharmaceutically inert agents, such as conventional excipients, vehicles, fillers, binders, disintegrants, pH adjusting substances, buffer, solvents, solubilizing agents, sweeteners, coloring agents, and any other inactive agents that can be included in pharmaceutical dosage forms for oral administration. Examples of such vehicles and additives can be found in Remington's Pharmaceutical Sciences, 17th edition (1985).

2. Manufacturing

[00175] The dosage forms described herein can be manufactured using processes that are well known to those of skill in the art. For example, for the manufacture of tablets, an effective amount of a prebiotic can be dispersed uniformly in one or more excipients, for example, using high shear granulation, low shear granulation, fluid bed granulation, or by blending for direct compression. Excipients include diluents, binders, disintegrants, dispersants, lubricants, glidants, stabilizers, surfactants and colorants. Diluents, also termed "fillers," can be used to increase the bulk of a tablet so that a practical size is provided for compression. Non-limiting examples of diluents include lactose, cellulose, microcrystalline cellulose, mannitol, dry starch, hydrolyzed starches, powdered sugar, talc, sodium chloride, silicon dioxide, titanium oxide, dicalcium phosphate dihydrate, calcium sulfate, calcium carbonate, alumina and kaolin. Binders can impart cohesive qualities to a tablet formulation and can be used to help a tablet remain intact after compression. Non-limiting examples of suitable binders include starch (including corn starch and pregelatinized starch), gelatin, sugars (e.g., glucose, dextrose, sucrose, lactose and sorbitol), celluloses, polyethylene glycol, waxes, natural and synthetic gums, e.g., acacia, tragacanth, sodium alginate, and synthetic polymers such as polymethacrylates and polyvinylpyrrolidone. Lubricants can also facilitate tablet manufacture; non-limiting examples thereof include magnesium stearate, calcium stearate, stearic acid, glyceryl behenate, and polyethylene glycol. Disintegrants can facilitate tablet disintegration after administration, and non-limiting examples thereof include starches, alginic acid, crosslinked polymers such as, e.g., crosslinked polyvinylpyrrolidone, croscarmellose sodium, potassium or sodium starch glycolate, clays, celluloses, starches, gums and the like. Non-limiting examples of suitable glidants include silicon dioxide, talc, and the like. Stabilizers can inhibit or retard drug decomposition reactions, including oxidative reactions. Surfactants can also include and can be anionic, cationic, amphoteric or nonionic. If desired, the tablets can also comprise nontoxic auxiliary substances such as pH buffering agents, preservatives, e.g., antioxidants, wetting or emulsifying agents, solubilizing agents, coating agents, flavoring agents, and the like.

[00176] In one embodiment, a softgel formulation is made with a gelatin mass for the outer shell, and a composition including one or more substances, for example prebiotics and/or probiotics, for the capsule fill can be prepared. To make the gelatin mass, gelatin powder can be mixed with water and glycerin, heated, and stirred under vacuum. Additives, for example, flavors or colors,

can be added to molten gelatin using a turbine mixer and transferred to mobile vessels. The gelatin mass can be kept in a steam-jacketed storage vessel at a constant temperature.

[00177] The encapsulation process can begin when the molten gel is pumped to a machine and two thin ribbons of gel are formed on either side of machine. These ribbons can then pass over a series of rollers and over a set of die that determine the size and shapes of capsules. A fill composition, for example a prebiotic and/or probiotic fill composition, can be fed to a positive displacement pump, which can dose the fill and inject it between two gelatin ribbons prior to sealing them together through the application of heat and pressure. To remove excess water, the capsules can pass through a conveyer into tumble dryers where a portion of the water can be removed. The capsules can then be placed on, for example, trays, which can be stacked and transferred into drying rooms. In the drying rooms, dry air can be forced over capsules to remove any excess moisture.

3. Release formulations

[00178] Immediate-release formulations of an effective amount of a prebiotic composition can comprise one or more combinations of excipients that allow for a rapid release of a pharmaceutically active agent (such as from 1 minute to 1 hour after administration). In one embodiment an excipient can be microcrystalline cellulose, sodium carboxymethyl cellulose, sodium starch glycolate, corn starch, colloidal silica, Sodium Laurel Sulphate, Magnesium Stearate, Prosolve SMCC (HD90), croscarmellose Sodium, Crospovidone NF, Avicel PH200, and combinations of such excipients.

[00179] "Controlled-release" formulations (also referred to as sustained release (SR), extended-release (ER, XR, or XL), time-release or timed-release, controlled-release (CR), or continuous-release) refer to the release of a prebiotic composition from a dosage form at a particular desired point in time after the dosage form is administered to a subject. Controlled-release formulations can include one or more excipients, including but not limited to microcrystalline cellulose, sodium carboxymethyl cellulose, sodium starch glycolate, corn starch, colloidal silica, Sodium Laurel Sulphate, Magnesium Stearate, Prosolve SMCC (HD90), croscarmellose Sodium, Crospovidone NF, or Avicel PH200. Generally, controlled-release includes sustained but otherwise complete release. A sudden and total release in the large intestine at a desired and appointed time or a release in the intestines such as through the use of an enteric coating are both considered controlled-release. Controlled-release can occur at a predetermined time or in a predetermined place within the digestive tract. It is not meant to include a passive, uncontrolled process as in swallowing a normal tablet. Examples include, but are not limited to, those described in U.S. Patent Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; 4,008,719; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; 5,733,556; 5,871,776; 5,902,632; and 5,837,284 each of which is incorporated herein by reference in its entirety.

[00180] In one embodiment a controlled release dosage form begins its release and continues that release over an extended period of time. Release can occur beginning almost immediately or can

be sustained. Release can be constant, can increase or decrease over time, can be pulsed, can be continuous or intermittent, and the like. Generally, however, the release of at least one pharmaceutically active agent from a controlled-release dosage form will exceed the amount of time of release of the drug taken as a normal, passive release tablet. Thus, for example, while all of at least one pharmaceutically active agent of an uncoated aspirin tablet should be released within, for example, four hours, a controlled-release dosage form could release a smaller amount of aspirin over a period of six hours, 12 hours, or even longer. Controlled-release in accordance with the compositions and methods described herein generally means that the release occurs for a period of six hours or more, such as 12 hours or more.

[00181] In another embodiment a controlled release dosage refers to the release of an agent, from a composition or dosage form in which the agent is released according to a desired profile over an extended period of time. In one embodiment, controlled-release results in dissolution of an agent within 20-720 minutes after entering the stomach. In another embodiment, controlled-release occurs when there is dissolution of an agent within 20-720 minutes after being swallowed. In another embodiment, controlled-release occurs when there is dissolution of an agent within 20-720 minutes after entering the intestine. In another embodiment, controlled-release results in substantially complete dissolution after at least 1 hour following administration. In another embodiment, controlled-release results in substantially complete dissolution after at least 1 hour following oral administration. For example, controlled-release compositions allow delivery of an agent to a subject in need thereof over an extended period of time according to a predetermined profile. Such release rates can provide therapeutically effective levels of agent for an extended period of time and thereby provide a longer period of pharmacologic or diagnostic response as compared with conventional rapid release dosage forms. Such longer periods of response provide for many inherent benefits that are not achieved with immediate-release dosages. When used in connection with the dissolution profiles discussed herein, the term "controlled-release" refers to wherein all or less than all of the total amount of a dosage form, made according to methods and compositions described herein, delivers an active agent over a period of time greater than 1 hour.

[00182] In one aspect, controlled-release refers to delayed release of an agent, from a composition or dosage form in which the agent is released according to a desired profile in which the release occurs after a period of time.

[00183] When present in a controlled-release oral dosage form, the compositions described herein can be administered at a substantially lower daily dosage level than immediate-release forms.

[00184] In one embodiment, the controlled-release layer is capable of releasing about 30 to about 40% of the one or more active agents (e.g., prebiotic or probiotic) contained therein in the stomach of a subject in need thereof in about 5 to about 10 minutes following oral administration. In another embodiment, the controlled-release layer is capable of releasing about 90% of the one or more active agents (e.g., prebiotic or probiotic) is released in about 40 minutes after oral administration.

- [00185] In some embodiment, the controlled-release layer comprises one or more excipients, including but not limited to silicified microcrystalline cellulose (e.g., HD90), croscarmellose sodium (AC-Di-Sol), hydroxyl methyl propyl cellulose, magnesium stearate, or stearic acid. In one embodiment, a controlled release formulation weighs between about 100 mg to 3 g.
- [00186] Pharmaceutical carriers or vehicles suitable for administration of the compounds provided herein include all such carriers known to those skilled in the art to be suitable for the particular mode of administration. In addition, the compositions can one or more components that do not impair the desired action, or with components that supplement the desired action, or have another action.
- [00187] In another embodiment, an effective amount of the prebiotic is formulated in an immediate release form. In this embodiment the immediate-release form can be included in an amount that is effective to shorten the time to its maximum concentration in the blood. By way of example, certain immediate-release pharmaceutical preparations are taught in United States Patent Publication US 2005/0147710A1 entitled, "Powder Compaction and Enrobing," which is incorporated herein in its entirety by reference.
- [00188] The dosage forms described herein can also take the form of pharmaceutical particles manufactured by a variety of methods, including but not limited to high-pressure homogenization, wet or dry ball milling, or small particle precipitation (nano spray). Other methods to make a suitable powder formulation are the preparation of a solution of active ingredients and excipients, followed by precipitation, filtration, and pulverization, or followed by removal of the solvent by freeze-drying, followed by pulverization of the powder to the desired particle size.
- [00189] In a further aspect the dosage form can be an effervescent dosage form. Effervescent means that the dosage form, when mixed with liquid, including water and saliva, evolves a gas. Some effervescent agents (or effervescent couple) evolve gas by means of a chemical reaction which takes place upon exposure of the effervescent disintegration agent to water or to saliva in the mouth. This reaction can be the result of the reaction of a soluble acid source and an alkali monocarbonate or carbonate source. The reaction of these two general compounds produces carbon dioxide gas upon contact with water or saliva. An effervescent couple (or the individual acid and base separately) can be coated with a solvent protective or enteric coating to prevent premature reaction. Such a couple can also be mixed with previously lyophilized particles (such as a prebiotic). The acid sources can be any which are safe for human consumption and can generally include food acids, acid and hydrite antacids such as, for example: citric, tartaric, malic, fumaric, adipic, and succinics. Carbonate sources include dry solid carbonate and bicarbonate salt such as, preferably, sodium bicarbonate, sodium carbonate, potassium bicarbonate and potassium carbonate, magnesium carbonate and the like. Reactants which evolve oxygen or other gasses and which are safe for human consumption are also included. In one embodiment citric acid and sodium bicarbonate are used.

- [00190]** In another aspect the dosage form can be in a candy form (e.g., matrix), such as a lollipop or lozenge. In one embodiment an effective amount of a prebiotic is dispersed within a candy matrix. In one embodiment the candy matrix comprises one or more sugars (such as dextrose or sucrose). In another embodiment the candy matrix is a sugar-free matrix. The choice of a particular candy matrix is subject to wide variation. Conventional sweeteners such as sucrose can be utilized, or sugar alcohols suitable for use with diabetic patients, such as sorbitol or mannitol can be employed. Other sweeteners, such as the aspartanes, can also be easily incorporated into a composition in accordance with compositions described herein. The candy base can be very soft and fast dissolving, or can be hard and slower dissolving. Various forms will have advantages in different situations.
- [00191]** A candy mass composition comprising an effective amount of the prebiotic can be orally administered to a subject in need thereof so that an effective amount of the prebiotic will be released into the subject's mouth as the candy mass dissolves and is swallowed. A subject in need thereof includes a human adult or child.
- [00192]** In one embodiment a candy mass is prepared that comprises one or more layers which can comprise different amounts or rates of dissolution of the prebiotic. In one embodiment a multilayer candy mass (such as a lollipop) comprises an outer layer with a concentration of the prebiotic differing from that of one or more inner layers. Such a drug delivery system has a variety of applications.
- [00193]** The choices of matrix and the concentration of the drug in the matrix can be important factors with respect to the rate of drug uptake. A matrix that dissolves quickly can deliver drug into the subject's mouth for absorption more quickly than a matrix that is slow to dissolve. Similarly, a candy matrix that contains the prebiotic in a high concentration can release more of the prebiotic in a given period of time than a candy having a low concentration. In one embodiment a candy matrix such as one disclosed in US Patent No. 4671953 or US Application Publication No.2004/0213828 (which are herein incorporated by reference in their entirety) is used to deliver the prebiotic.
- [00194]** The dosage forms described herein can also take the form of pharmaceutical particles manufactured by a variety of methods, including but not limited to high-pressure homogenization, wet or dry ball milling, or small particle precipitation (e.g., nGimat's NanoSpray). Other methods useful to make a suitable powder formulation are the preparation of a solution of active ingredients and excipients, followed by precipitation, filtration, and pulverization, or followed by removal of the solvent by freeze-drying, followed by pulverization of the powder to the desired particle size. In one embodiment the pharmaceutical particles have a final size of 3-1000 μM , such as at most 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 μM . In another embodiment the pharmaceutical particles have a final size of 10-500 μM . In another embodiment the

pharmaceutical particles have a final size of 50-600 μ M. In another embodiment the pharmaceutical particles have a final size of 100-800 μ M.

[00195] In one embodiment an oral dosage form (such as a powder, tablet, or capsule) is provided comprising a prebiotic composition comprising about 0.7 g of GOS, about 0.2 g of lactose, about 0.01 g of glucose, about 0.01 g of galactose, about 0.1-0.2 g of a binder, about 0.1-0.2 g of a dispersant, about 0.1-0.2 g of a solubilizer, wherein the GOS are composed of about 1-25 % disaccharides, about 1-25 % trisaccharides, about 1-25 % tetrasaccharides, and about 1-25 % pentasaccharides. The oral dosage form can be in the form of a powder, capsule, or tablet. Suitable amounts of binders, dispersants, and solubilizers are known in the art for preparation of oral tablets or capsules.

[00196] In another embodiment an oral dosage form (such as a powder, tablet or capsule) is provided comprising a prebiotic composition comprising about 1-99.9% by weight of GOS, about 0.5-20% by weight of lactose, about 0.1-2% by weight of glucose, about 0.1-2% by weight of galactose, about 0.05-2% by weight of a binder, about 0.05-2% by weight of a dispersant, about 0.05-2% by weight of a solubilizer, wherein the GOS are composed of about 1-25 % by weight disaccharides, about 1-25 % by weight trisaccharides, about 1-25 % by weight tetrasaccharides, and about 1-25 % by weight pentasaccharides.

[00197] In another embodiment an oral dosage form (such as a powder, tablet, or capsule) is provided comprising a prebiotic composition comprising about 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, 99.5, 100% by weight of GOS, about 0, 5, 10, 15, or 20% by weight of lactose, about 0.1, 0.5, 1, or 2% by weight of glucose, about 0.1, 0.5, 1, or 2% by weight of galactose, about 0.05, 0.1, 0.5, 1, or 2% by weight of a binder, about 0.05, 0.1, 0.5, 1, or 2% by weight of a dispersant, about 0.05, 0.1, 0.5, 1, or 2% by weight of a solubilizer, wherein the GOS are composed of about 1, 5, 10, 15, 20, or 25 % by weight disaccharides, about 1, 5, 10, 15, 20, or 25 % by weight trisaccharides, about 1, 5, 10, 15, 20, or 25 % by weight tetrasaccharides, and about 1, 5, 10, 15, 20, or 25 % by weight pentasaccharides.

[00198] In another embodiment, an oral dosage form is provided comprising a prebiotic composition, wherein the oral dosage form is a syrup. The syrup can comprise about 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 85% solid. The syrup can comprise about 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% liquid, for example, water. The solid can comprise a prebiotic composition. The solid can be, for example, about 1-96%, 10-96%, 20-96%, 30-96%, 40-96%, 50-96%, 60-96%, 70-96%, 80-96%, or 90-96% prebiotic composition. The solid can be, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, or 96% prebiotic composition. In one embodiment a prebiotic composition comprises GOS. In another embodiment a prebiotic composition comprises GOS and another

prebiotic. In another embodiment a prebiotic composition comprises GOS and inulin or GOS and FOS.

[00199] In one embodiment, the softgel capsule is about 0.25 mL, 0.5 mL, 1.0 mL, 1.25 mL, 1.5 mL, 1.75 mL, or 2.0 mL. In another embodiment, a softgel capsule comprises about 0.1 g to 2.0 g of prebiotic composition. In another embodiment, a softgel capsule comprises about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2.0 g of a prebiotic composition. In one embodiment the prebiotic composition comprises GOS. In another embodiment, a softgel capsule comprises GOS and inulin or FOS.

[00200] In another embodiment, a prebiotic composition is provided that does not contain a preservative. In another embodiment, a prebiotic composition is provided that does not contain an antioxidant. In another embodiment, a prebiotic composition is provided that does not contain a preservative or an antioxidant. In one embodiment a prebiotic composition comprising GOS does not contain a preservative or an antioxidant.

[00201] In another embodiment, a prebiotic composition is formulated as a viscous fluid. In another embodiment, a prebiotic composition is formulated such that its water content is low enough that it does not support microbial growth. In another embodiment, a prebiotic composition is formulated as a viscous fluid without a preservative in a gel capsule. In another embodiment, a prebiotic composition comprising GOS is a viscous fluid. In another embodiment, a prebiotic composition comprises a high percentage of GOS that does not support microbial growth. In another embodiment, the prebiotic composition comprises GOS and inulin or FOS.

[00202] In another embodiment, an oral dosage form is provided comprising a prebiotic composition, wherein the oral dosage form is a softgel. In one embodiment the softgel comprises a syrup. In one embodiment the syrup comprises a prebiotic composition. In one embodiment the prebiotic composition comprises GOS. In another embodiment the prebiotic composition comprises more than 80% GOS. In another embodiment the prebiotic composition comprises between 80-99.9% GOS. In another embodiment the prebiotic composition comprises more than 80% GOS. In another embodiment the prebiotic composition comprises about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 99.9% GOS.

[00203] In one embodiment a GOS composition is formulated for delivery in a soft gel capsule. In one embodiment a GOS composition formulated for delivery in a soft gel capsule is a high percentage GOS composition, such as a 90-100% GOS composition (e.g., 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% GOS composition by weight). In another embodiment a GOS composition formulated for delivery in a soft gel capsule comprises about 95% GOS. In another embodiment a GOS composition formulated for delivery in a soft gel capsule comprises about 96% GOS. In another embodiment, the GOS composition is formulated such that its water content is low enough that it does not support microbial growth. In another embodiment, the GOS composition is formulated as a viscous fluid without a preservative in a gel capsule. In another embodiment,

the GOS composition is formulated as a viscous fluid without an antioxidant in a gel capsule. In another embodiment the soft gel capsule comprises about 0.1-2 g of a GOS composition.

[00204] In another embodiment a prebiotic composition can be formulated as described, in US Patent No. 6,750,331, which is herein incorporated by reference in its entirety. A prebiotic composition can be formulated to comprise an oligosaccharide, a foaming component, a water-insoluble dietary fiber, or a neutralizing component. In one embodiment a prebiotic composition can be in the form of a chewable tablet.

[00205] In one embodiment a foaming component can be at least one member selected from the group consisting of sodium hydrogencarbonate, sodium carbonate, and calcium carbonate. In one embodiment a neutralizing component can be at least one member selected from the group consisting of citric acid, L-tartaric acid, fumaric acid, L-ascorbic acid, DL-malic acid, acetic acid, lactic acid, and anhydrous citric acid. In one embodiment a water-insoluble dietary fiber can be at least one member selected from the group consisting of crystalline cellulose, wheat bran, oat bran, cone fiber, soy fiber, and beet fiber. The formulation can contain a sucrose fatty acid ester, powder sugar, fruit juice powder, and/or flavoring material.

[00206] Formulations of the provided invention can include additive components selected from various known additives. Such additives include, for example, saccharides (excluding oligosaccharides), sugar alcohols, sweeteners and like excipients, binders, disintegrators, lubricants, thickeners, surfactants, electrolytes, flavorings, coloring agents, pH modifiers, fluidity improvers, and the like. Specific examples of the additives include wheat starch, potato starch, corn starch, dextrin and like starches; sucrose, glucose, fructose, maltose, xylose, lactose and like saccharides (excluding oligosaccharides); sorbitol, mannitol, maltitol, xylitol and like sugar alcohols; calcium phosphate, calcium sulfate and like excipients; starch, saccharides, gelatine, gum arabic, dextrin, methyl cellulose, polyvinylpyrrolidone, polyvinyl alcohol, hydroxypropylcellulose, xanthan gum, pectin, gum tragacanth, casein, alginic acid and like binders and thickeners; leucine, isoleucine, L-valine, sugar esters, hardened oils, stearic acid, magnesium stearate, talc, macrogols and like lubricants; CMC, CMC-Na, CMC-Ca and like disintegrators; polysorbate, lecithin and like surfactants; aspartame, alitame and like dipeptides; silicon dioxide and like fluidity improvers; and stevia, saccharin, and like sweeteners. The amounts of these additives can be properly selected based on their relation to other components and properties of the preparation, production method, etc.

[00207] In one embodiment, a GOS composition is a chewable oral dosage formulation. In one embodiment the chewable formulation can comprises between about 1-99.9% GOS. In one embodiment, a GOS composition comprises about 80% GOS, about 5% L-ascorbic acid, about 2% anhydrous citric acid, about 3% sodium hydrogencarbonate, about 3% calcium carbonate, about 2% sucrose fatty acid, about 3% fruit juice powder, and about 2% potassium carbonate.

- [00208] In another embodiment, a GOS composition comprises about 85% GOS, about 5% L-ascorbic acid, about 3% sodium hydrogencarbonate, about 2% sodium carbonate, about 2% sucrose fatty acid ester, about 2% fruit juice powder, and about 1% potassium carbonate.
- [00209] In another embodiment, a GOS composition comprises about 90% GOS, about 2% L-ascorbic acid, about 1% anhydrous citric acid, about 2% sodium hydrogencarbonate, about 2% sodium carbonate, about 2% sucrose fatty acid ester, and about 1% potassium carbonate.
- [00210] In another embodiment, a GOS composition comprises about 95% GOS, about 2% L-ascorbic acid, about 1% sodium hydrogencarbonate, and about 2% fruit juice powder. In another embodiment, a GOS composition comprises about 95% GOS and about 5% of L-ascorbic acid, anhydrous citric acid, sodium hydrogencarbonate, calcium carbonate, sucrose fatty acid, fruit juice powder, or potassium carbonate.
- [00211] In another embodiment, a GOS composition comprises about 95% GOS and about 5% of L-ascorbic acid, anhydrous citric acid, sodium hydrogencarbonate, calcium carbonate, sucrose fatty acid, fruit juice powder, and potassium carbonate.

VI. Treatment

A. Lactose intolerance

- [00212] The invention provides methods and prebiotic compositions useful for the reduction of symptoms of lactose intolerance and for improving overall gastrointestinal (GI) health. Symptoms of lactose intolerance include gas, bloating, diarrhea, abdominal pain, cramping, and vomiting. Minor digestive problems related to the GI also include occasional bloating, diarrhea, constipation, gas, heartburn, or stomach upset. The methods and compositions described herein can be useful for reducing or eliminating one or more of these symptoms, for example through colonic adaptation. These compositions are expected to modify the colonic microbiota, which may result in an increased tolerance to lactose and other fermentable carbohydrates. Furthermore, these compositions can allow the colonic microbiota, comprising microorganisms known to increase the ability of an individual to tolerate fermentable carbohydrates, to be regularly replenished through consumption of the compositions. Adaptation of the intestinal and colonic microbiota, improve the composition of the intestinal microbiota, and the capacity for consumption of foods comprising lactose can be increased. For example, an individual's tolerance to dairy in general can be improved through regular consumption of a prebiotic composition. This change in colonic microbiota is useful for the reduction of bloating, diarrhea, gastric distention and pain, and/or flatulence from the consumption of dairy products or other foods comprising lactose. In one embodiment, a method of treating lactose intolerance is disclosed. In another embodiment, a method of treating at least one symptom of lactose intolerance is disclosed.
- [00213] There are at least three types of lactose intolerance. Primary lactose intolerance results from a decrease in lactase production as a subject ages. Secondary lactose intolerance can result when a subject's small intestine decreases lactase production after an illness, surgery, or injury to

the small intestine. Secondary lactose intolerance can occur as a result of Crohn's disease, celiac disease, or gastroenteritis. This type of lactose intolerance can be temporary or permanent. A third type of lactose intolerance is congenital lactose intolerance, in which a subject is born with lactose intolerance. Risk factors that can make a person more prone to lactose intolerance include, for example, age (lactose intolerance usually has an onset of after age 5), ethnicity (lactose intolerance is more common in black, Asian, Hispanic, and American Indian populations), and premature birth (infants born 28 to 32 weeks of gestation).

B. Testing lactose intolerance

[00214] Lactose intolerance can be tested either indirectly or directly. Indirect testing methods include, but are not limited to: a hydrogen breath test, a stool acidity test, a blood glucose test, or milk challenge test. In the hydrogen breath test, the breath is measured to determine the amount of hydrogen produced after consuming a measured amount of lactose, typically 15g. The lactose is administered by drinking a lactose mixture, and the subject exhales into a vacuum-sealed collection tube at three one hour time intervals. A high level of hydrogen in the breath indicates an improper digestion of lactose. In a stool test, the stool is tested to determine the amount of acid. In a blood glucose test, the blood is tested to determine the amount of glucose (sugar) content after administering a predetermined amount of lactose-containing product to the subject. The direct method measures lactase activity in a mucosal biopsy specimen.

[00215] The stool acidity test is typically used to test lactose intolerance in infants and young children. The hydrogen breath test is typically not recommended for young children since dehydration can occur due to diarrhea after ingestion of the lactose-containing drink.

[00216] Effectiveness of treatment can be measured in a number of ways. Conventional measurements, such as those described, can be used before and after treatment. Alternatively, or in addition, the amount of lactose-containing product that can be administered before the onset of one or more symptoms can be measured or evaluated before and after treatment. Thus, for example, treatment can be considered fully or partially effective if, after treatment, less hydrogen is produced on average in a subject after challenge with a food comprising lactose (such as a dairy product).

[00217] More commonly, a subject can not precisely test the amount of hydrogen or use a blood glucose test to measure effectiveness. Instead, a subject can subjectively determine the quantity of lactose-containing products they can consume, and the types and degree of symptoms experienced after such consumption. "Partial" elimination of symptoms of lactose intolerance includes a subjective or measurable increase in the amount of lactose that can be consumed before the onset of symptoms. "Substantial" elimination of symptoms of lactose intolerance, as used herein, encompasses an effect where at least about twice the amount of lactose or a lactose containing food can be consumed after treatment before the onset of symptoms as could have been consumed before treatment. "Complete" or "substantially complete" elimination of symptoms of lactose intolerance, as used herein, indicates that normal amounts of lactose can be consumed after

treatment (i.e., the amount of lactose in a typical diet for the area or culture in which the subject normally lives) without symptoms, or with only the rare occurrence of symptoms.

[00218] In one embodiment a subject in need thereof can consume one half cup (4 oz.; about 120 mL) of milk with no, or minimal, symptoms of lactose intolerance. However, consumption of 1 or more cups (about 240 mL) of milk causes symptoms of lactose intolerance, such as gas or diarrhea, to occur. After treatment with a composition and/or dosing regimen disclosed herein, a subject can find that 1 and one-half cups (about 360 mL) of milk can be consumed in a single administration without causing any symptoms of lactose intolerance. The subject would experience the substantial elimination of the symptoms of lactose intolerance. In another embodiment a subject can find that after treatment with a composition and/or dosing regimen disclosed a normal diet for their geographical or cultural region can be consumed with no, or rare, symptoms of lactose intolerance.

[00219] In another embodiment effectiveness can be measured by a percentage decrease in one or more symptoms of lactose intolerance. In this measurement, the severity of a predetermined symptom, or set of symptoms is measured before and after treatment, e.g., using pre and post Likert scale. Exemplary symptoms include gas, bloating, diarrhea, cramping, abdominal pain, and vomiting. Any one or more than one, of the symptoms can be measured. For example, a subject can be asked to rate one or more symptoms on a scale of increasing severity from 1 to 5. In one embodiment, a set of symptoms is rated, and the ratings are added; for example, gas, bloating, diarrhea, abdominal pain, abdominal distension, vomiting, nausea, or cramping can be rated. In another embodiment a percentage change in one or more symptoms of lactose intolerance can be calculated based on a subject's ratings before and after treatment with a composition or method disclosed herein. In one embodiment the composition is a prebiotic composition. In one embodiment the prebiotic composition comprises GOS. In one embodiment symptoms of lactose intolerance can be considered to be reduced by the a subject's reported decrease in one or more specific symptoms after challenge with a food comprising lactose (e.g., if there is a 50% decrease in symptoms, then symptoms of lactose intolerance are reduced by 50%).

[00220] In another embodiment a milk challenge test is used to determine if a subject is lactose intolerant. In the milk challenge test, a subject fasts overnight, and then the person drinks a glass of milk in the morning. After drinking the milk, nothing else is eaten or drunk for three to five hours. If a subject experiences one or more symptoms of lactose intolerance within several hours after consuming the milk then the subject is lactose intolerant.

[00221] In another embodiment a subject is directly tested for lactose intolerance by biopsying the intestinal lining and measuring lactase levels in the lining.

C. Types of lactose intolerance and treatments

[00222] People can have different degrees of lactose intolerance. Lactose intolerance can also be psychologically induced. There are also many different variations of lactose intolerance depending on the subject. For example, some subjects cannot consume cheese, melted cheese,

plain milk, or warm dairy containing products like milk in coffee without experiencing one or more symptoms of lactose intolerance. In another embodiment a subject cannot consume any dairy products without experiencing one or more symptoms of lactose intolerance. In some embodiment a lactose intolerant subject is limited to consuming special "lactose free" foods that have been manufactured to be free of lactose. Some examples of these "lactose free" foods are: MOCHA MIX® ice cream, TOFUTTI® ice cream and ice cream sandwiches, LACTAID® brand milk, FORMAGG™ cheese, TOFUTTI® "Better than Cream Cheese", and margarine.

[00223] In one embodiment a subject consumes a lactase tablet to help digest the lactose in milk or a milk product. Each lactase tablet typically hydrolyzes up to 99% of the ingested lactose within 24 hours and is designed to be ingested with the lactose containing food. Other possible techniques for dealing with lactose maldigestion are to use microgranules containing bioactive compounds or microorganisms (see, e.g., U.S. Patent No. 5,952,021, which is herein incorporated by reference in its entirety). The use of an active lactase composition for treatment of lactase deficiency is described in U.S. Patent No. 3,718,739, which is herein incorporated by reference in its entirety. Digestive Advantage™ Lactose Intolerance Therapy, which includes probiotics and digestive enzymes, can also be used for dietary management of lactose maldigestion.

D. Administration of prebiotic compositions

[00224] In one embodiment a prebiotic composition is used in a method by administering increasing doses of the composition to a subject who is suffering from lactose intolerance, experiencing symptoms of lactose intolerance, or is in need of improving overall gastrointestinal (GI) health. In one embodiment the subject experiences a reduction or elimination of one or more symptoms of lactose intolerance or an improvement in overall gastrointestinal health after administration of the prebiotic composition. In one embodiment the prebiotic composition comprises GOS. In one embodiment a GOS composition can optionally comprise digestible saccharides. In one embodiment, a GOS composition is administered in about equal doses over a period of time to a subject with lactose intolerance or symptoms of lactose intolerance, or to a subject in need of improved gastrointestinal health. In one embodiment a GOS composition is administered in increasing doses, for a period of time, to a subject with lactose intolerance or symptoms of lactose intolerance, or to a subject in need of improved gastrointestinal health. In one embodiment a GOS composition is provided in any form suitable for oral consumption, such as by a liquid, tablet, capsule, or powdered form. In one embodiment a subject is treated with just a GOS composition, without supplementation with a probiotic.

[00225] In another embodiment, other substances can be administered in combination with a GOS composition. In one embodiment lactose is simultaneously administered with a GOS composition. In one embodiment lactose is administered before a GOS composition (e.g., before a regimen of increasing doses of a GOS composition begins, or before a dose of a GOS composition during such a regimen). In another embodiment a digestible saccharide is administered after a dose of GOS composition (e.g., after a regimen of increasing doses of GOS compositions begins, or after

a dose of GOS compositions during such a regimen). In another embodiment, a digestible saccharide can be administered simultaneously with, before, or after the administration of the GOS composition or any combination thereof.

[00226] In another embodiment a GOS composition is supplemented with one or more other non-digestible saccharides, such as inulin, FOS, lactulose, raffinose, stachyose, or a combination thereof. In another embodiment the GOS composition is supplemented with one or more strains of probiotic bacteria. In another embodiment the GOS composition is supplemented with one or more digestible saccharides, salts, or buffers, e.g., phosphates.

[00227] In another embodiment a GOS composition is administered in combination with lactase, or with a product containing pre-digested lactose. In another embodiment a GOS composition is administered in an increasing dose, in combination with lactase or with a product containing pre-digested lactose. In another embodiment a GOS composition is administered in an about equal doses over time, in combination with lactase or with a product containing pre-digested lactose.

[00228] In another embodiment, one or more symptoms of lactose intolerance in a subject exhibiting symptoms of lactose intolerance are decreased or eliminated by administering to the subject a GOS composition for a period of time. In one embodiment the administration comprises increasing the amounts of a GOS composition administered to a subject over time. In another embodiment the administration comprises administering about equal amounts of a GOS composition to a subject over time. In one embodiment, a symptom of lactose intolerance remains partially, substantially, or completely eliminated or decreased in severity in a subject for at least about 1 day, 1 week, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 9 months, one year, 18 months, two years, three years, four years, or five years after the termination of treatment. In another embodiment a symptom of lactose intolerance remains partially, substantially, or completely eliminated or decreased in severity in a subject for more than 5 years. In another embodiment a symptom of lactose intolerance is permanently eliminated or decreased in severity in a subject after the termination of treatment. In another embodiment, the methods herein decrease symptoms of lactose intolerance in a subject exhibiting symptoms of lactose intolerance by administering to the subject increasing amounts of a GOS composition for a period of time, wherein symptoms of lactose intolerance are substantially eliminated for at least about one month after treatment is terminated.

[00229] In another embodiment, a symptom of lactose intolerance in a subject exhibiting symptoms of lactose intolerance is decreased or eliminated by administering to the subject increasing amounts of a prebiotic composition for a period of time, wherein the symptoms of lactose intolerance, measured as described herein, are decreased by an average of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or about 100% when compared to symptoms prior to the administration of a prebiotic composition. An "average" decrease is a decrease as measured in a group of subjects exhibiting symptoms of lactose intolerance, such as more than about 2, 3, 4, 5, 10, 20, or 30 subjects. In one embodiment, the decrease in or elimination of a

symptom of lactose intolerance persists for at least about 1 day, 1 week, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 9 months, one year, 18 months, two years, three years, four years, or five years. In another embodiment a symptom of lactose intolerance remains partially, substantially, or completely eliminated or decreased in severity in a subject for more than 5 years after the termination of treatment. In one embodiment, the decrease or elimination of a symptom is permanent. In another embodiment, the invention provides a method of decreasing symptoms of lactose intolerance in a subject exhibiting symptoms of lactose intolerance by administering to the subject increasing amounts of a prebiotic composition for a period of time, wherein one or more symptoms of lactose intolerance, measured as described herein, are decreased by an average of at least about 20% and remain decreased by at least about 20% for at least about one month after treatment is terminated.

[00230] In another embodiment, the methods herein decrease symptoms of lactose intolerance in a subject exhibiting symptoms of lactose intolerance by administering to the subject increasing amounts of a prebiotic composition for a period of time, wherein one or more symptoms of lactose intolerance, measured as described herein, are decreased by an average of about least about 50% and remain decreased by at least about 50% for at least about one month after treatment is terminated.

[00231] In another embodiment, the methods herein decrease symptoms of lactose intolerance in a subject exhibiting symptoms of lactose intolerance by administering to the subject increasing amounts of a prebiotic composition for a period of time, wherein one or more symptoms of lactose intolerance, measured as described herein, are decreased by an average of about least about 75% and remain decreased by at least about 75% for at least about one month after treatment is terminated.

[00232] In one embodiment the total duration of treatment of lactose intolerance can be from about one week to about 12 weeks, or about four weeks to about ten weeks, or about four weeks to about eight weeks, or about six weeks. During this period of time, the subject is started on a program of taking increasing amounts of a prebiotic composition described herein (such as a composition comprising or consisting essentially of GOS), optionally along with ingestion of lactose containing food products. In one embodiment a prebiotic composition can also be administered in combination with another substance (such as a probiotic), as described herein. In one embodiment, the total duration of treatment is about 5 days to about 35 days. In one embodiment, the total duration of treatment is about 7 days to about 90 days, or about 7 days to about 60 days, or about 14 days to about 50 days, or about 14 days to about 40 days, or about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 days. In another embodiment, the total duration of treatment is about 30 days. In another embodiment, the total duration of treatment is about 34 days. In another embodiment, the total duration of treatment is about 36 days. In another embodiment, the total duration of treatment is

about 38 days. In another embodiment, the total duration of treatment is about 42 days. In another embodiment, the total duration of treatment is about 60 days. In another embodiment, the total duration of treatment is about 90 days.

[00233] In another embodiment, the total duration of treatment is based on a subject's response to the treatment. For example, an individual can experience a reduction in lactose intolerance symptoms after 14 days of treatment with a prebiotic composition. In another example an individual can experience a reduction in lactose intolerance symptoms after 30 days of treatment with a prebiotic composition. Thus, the duration of treatment is determined by an individual subject's response to a prebiotic composition and the onset of relief from one or more lactose intolerance symptoms.

[00234] In one embodiment the treatment is continuous. In one embodiment, the duration of the treatment is based on a subject's symptoms of lactose intolerance. Thus, a subject can experience symptoms at a given dose of a prebiotic composition (e.g., a composition comprising or consisting essentially of GOS), and can require that the subject stay at that dose, or a lower dose, until symptoms subside. Thus, in one embodiment, the duration of the treatment is not determined at the outset, but continues until the maximum dose of a prebiotic composition (such as a composition comprising or consisting essentially of GOS), is achieved per day, or until the desired level of lactose tolerance is achieved. In one embodiment the maximum amount of prebiotic composition (e.g., a composition comprising or consisting essentially of GOS), administered per day is between 0.4 g and 20 g, such as about 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, or 20 g per day. In another embodiment, a dose can be about 0.4 g to 6 g.

[00235] In one embodiment, a subject can be given one dose for a period of time during a treatment regimen and a second dose during a second period of time during the treatment regimen. For example, a subject can be administered one dose of prebiotic composition for a one or two week period and a second dose for a subsequent one or two week period. In one embodiment the prebiotic composition comprises GOS.

[00236] In one embodiment an increasing dosage of a prebiotic composition (e.g., a composition comprising or consisting essentially of GOS), can be achieved by increasing the number of doses per day of the composition administered, increasing the amount of a prebiotic composition administered per dose, or both. In one embodiment, both strategies are used. Thus, in one embodiment, a prebiotic composition (e.g., a composition comprising or consisting essentially of GOS), is initially administered once per day, at increasing doses, for a pre-determined number of days. This can be followed by a period of time when a prebiotic composition is administered twice per day as a first and second dose. The first dose of a prebiotic composition can be administered at a constant dose while the second dose can be administered in increasing doses, for a pre-determined number of days. In one embodiment the prebiotic composition comprises GOS.

In one embodiment, the dose can be administered to a subject at a frequency of once per day, twice per day, or three times per day. The number of days of administration can last for a period of about 1 to 90 days, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90 days.

[00237] In another embodiment, a prebiotic composition can be administered twice per day. The first dose of the prebiotic composition (e.g., a composition comprising or consisting essentially of GOS), can remain constant while the second dose increases over time. In another embodiment, the prebiotic composition (e.g., a composition comprising or consisting essentially of GOS), can be administered an average of about once per day, twice per day, three, four, five, six, or more than six times per day, or any combination thereof. The prebiotic composition can be administered for a period of about 1 to 90 days, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90 days.

[00238] In another embodiment the prebiotic composition is administered at the same dosage level at each administration. Thus, in one embodiment, a prebiotic composition (e.g., a composition comprising or consisting essentially of GOS), is initially administered once to six times per day at the same dosage level. The prebiotic composition can be administered for a period of about 1 to 90 days, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90 days.

[00239] In one embodiment, a subject who has completed a treatment regimen consumes dairy products at least once every 4-5 days in order to maintain the reduction in symptoms of lactose intolerance.

[00240] In another embodiment, a subject self-administers a prebiotic composition (e.g., a composition comprising or consisting essentially of GOS). In one embodiment, the prebiotic composition (e.g., a composition comprising or consisting essentially of GOS), composition is supplied or recommended by a health professional, e.g., a dietician, nutritionist, nurse, physician, or other qualified health professional. In another embodiment, the prebiotic composition (e.g., a composition comprising or consisting essentially of GOS), is administered by a health professional or results of the program are monitored by a health professional. In one embodiment, a prebiotic composition (e.g., a composition comprising or consisting essentially of GOS), is labeled as a medical food.

[00241] In one embodiment a subject in need thereof can repeat courses of treatment with a prebiotic composition. The course of treatment can be repeated when symptoms of lactose

intolerance reappear or increase to an undesirable level. Alternatively, the course of treatment can be repeated at regular or predetermined intervals. Thus, treatment can be repeated after about one month, two months, three months, four months, six months, eight months, ten months, one year, 18 months, two years, three years, four years, five years, or more than five years, or any combination thereof (e.g., treatment can be repeated after one year, then every two to five years thereafter). The treatment can be repeated in the same form (e.g., duration, dosage, timing of dosage, additional substances, etc.) as used in the first treatment or it can be modified. For example, treatment duration can be shortened or lengthened, dosage can be increased more quickly or slowly or a higher or lower starting dose of a prebiotic composition, a different prebiotic composition (such as a composition comprising inulin, FOS, lactulose, raffinose, stachyose or combinations thereof) can be used (e.g., containing more or less of other substances, or fewer or more substances in addition to GOS or digestible saccharides), and the like.

[00242] In one embodiment an initial dose of a prebiotic composition is administered to a subject in need thereof as part of a dosing regime with incremental increases in the dosage of the prebiotic composition over time. The incremental increases in a prebiotic composition dosage can be any suitable dose size. In one embodiment, the starting dose of a prebiotic composition is about 0.05 g to 4.0 g, or about 0.1 g to about 3 g, or about 0.2 g to about 3.0 g, or about 0.2 g to about 2 g, or about 0.4 g to about 1.6 g, or about 0.4 g to about 1.4 g, or about 0.6 g to about 1.2 g, or about 0.6 g to about 1.0 g, or about 0.7 g to about 0.9 g, or about 0.8 g. In another embodiment, the starting dose of a prebiotic composition is about 0.2 g to about 4.7 g, about 0.5 g to about 8.0 g, or about 0.4 g to about 6.8 g. In one embodiment, the incremental increase in prebiotic or GOS composition dosage can vary, or each increase can be the same, or any combination thereof. In another embodiment, an amount of a prebiotic composition administered to a subject in need thereof can be increased incrementally by about 0.05 g to 4.0 g, or about 0.1 g to about 3 g, or about 0.2 g to about 3.0 g, or about 0.2 g to about 2 g, or about 0.4 g to about 1.6 g, or about 0.4 g to about 1.4 g, or about 0.6 g to about 1.2 g, or about 0.6 g to about 1.0 g, or about 0.7 g to about 0.9 g, or about 0.8 g. In another embodiment, an amount of a prebiotic composition administered to a subject in need thereof can be increased incrementally by about 0.5 g, about 0.29 g, about 0.30 g, or about 0.42 g, about 0.43 g. In another embodiment, an amount of a prebiotic composition administered to a subject in need thereof can be increased incrementally by 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1 g per dose. The maximum dose reached in treatment can be any suitable dose size, depending on the subject being treated and the outcome desired. In one embodiment the maximum dose of a prebiotic composition administered in a single dose can be about 1 g to about 2 g, about 3 g to about 4 g, about 5 g to about 6 g, about 6 g to about 60 g, or about 12 g to about 48 g, or about 14g to about 36 g, or about 16g to about 36 g, or about 18g to about 34 g, or about 20 g to about 32 g, or about 22 g to about 30 g, or about 23 g to about 29 g, or about 24 g to about 28 g, or about 25 to about 27 g, or about 25.5 g to about 26.5 g, or about

25.5 g, 25.6 g, or 25.7 g per dose. In one embodiment the maximum dose of prebiotic composition administered is about 12 g per dose.

[00243] In one embodiment of the invention, an initial dose of prebiotic composition is about 0.4 g, and the dose is increased by 0.4 g over time, for example, daily, until a maximum dose of 20 g to 25 g of a prebiotic composition is reached. In another embodiment, the initial dose of a prebiotic composition is about 0.5 g, and the dose is increased by 0.5 g over time, for example, daily, until a maximum of 8.0 g to 15 g of prebiotic composition per day is reached.

[00244] A prebiotic composition can be administered in any suitable form, such as a powder, capsules, tablets, a powder that can be dissolved in a liquid prior to consumption, or in liquid form, (e.g., GOS pre-dissolved in a liquid). Any grade or form of prebiotics that is suitable for consumption by the subject being treated, e.g., by a human, can be used.

[00245] Additional substances can be given in conjunction with a prebiotic composition or GOS composition. These substances can enhance the action of the increasing doses of prebiotic by, e.g., encouraging the growth of bacteria in the gut that alleviate symptoms of lactose intolerance, increasing adhesion of probiotic or beneficial commensal bacteria, or allowing doses of probiotic bacteria to more readily pass through the stomach without being destroyed. These substances can be given prior to treatment with prebiotic, during treatment with prebiotic, after treatment with prebiotic, or any combination thereof. If administered during prebiotic treatment, they can be administered with the dose of prebiotic being given, or before or after the dose of prebiotic, or any combination thereof.

[00246] In one embodiment substances of use in the invention in conjunction with a prebiotic composition include a probiotic microbe(s), lactase or other lactose digestive enzymes, or buffers (such as phosphates). One or more of these substances can be used in combination with prebiotic composition at any suitable time before, during, after treatment, or some combination thereof. In one embodiment, during some or all of the treatment, a prebiotic composition is administered in conjunction with live bacteria. In another embodiment, during some or all of the treatment, a prebiotic composition is administered in conjunction with lactase or other lactose digestive enzymes. In another embodiment, during some or all of the treatment, a prebiotic composition is administered in conjunction with a buffer (e.g., phosphates). In another embodiment, during some or all of the treatment, a prebiotic composition (e.g., GOS) comprises trace amounts of digestible saccharides, such as lactose, glucose or galactose. In one embodiment the trace amounts of digestible saccharides make up 5% by weight (such as 4%, 3%, 2%, 1%, 0.5%, or 0.1%) or less of the prebiotic composition. In another embodiment the trace amounts of digestible saccharides make up about 20% by weight (such as about 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1%) or less of the prebiotic composition.

E. Treatment regimens

[00247] In one embodiment, treatment with a prebiotic composition (e.g., a composition comprising or consisting essentially of GOS), optionally in conjunction with a probiotic composition, one or more digestible saccharides, a buffer, or a combination thereof, is used in combination with other treatments to reduce the symptoms of lactose intolerance. Any suitable treatment for the reduction of symptoms of lactose intolerance can be used, e.g., the use of lactase. In another embodiment lactase can be administered before, during, or after treatment with a prebiotic composition, or any combination thereof. In one embodiment, when symptoms of lactose intolerance are not completely or substantially completely eliminated by treatment with a prebiotic composition, lactase can be administered after prebiotic treatment is terminated. The lactase can be used on an as-needed basis.

[00248] A subject to be treated can include, for example, a human, for example, a preterm newborn, a full term newborn, an infant up to one year of age, young children (e.g., 1 yr to 12 yrs), teenagers, (e.g., 13-19 yrs), adults (e.g., 20-64 yrs), pregnant women, and elderly adults (65 yrs and older).

[00249] Treatment regimens can last, for example, about 1-20 days, about 1-25 days, about 1-30 days, about 1-35 days, about 1-40 days, about 1-45 days, about 1-50 days, about 5-30 days, about 5-35 days, about 5-40 days, about 5-45 days, about 5-50 days, about 5-55 days, about 5-60 days, or about 5-90 days. Treatment regimens can last exactly or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90 days. The amount of each dose in a treatment regimen can be constant. For example, a constant dose of prebiotics can be administered each day to a subject for the duration of the treatment regimens described above. The dosing regimen can be, for example, a constant 0.1-20 g of prebiotic per day, or the dosing regimen can be an escalating regimen, for example, 2 g of prebiotic on day 1 and 20 g of prebiotic on day 20. The dose can escalate by, for example, about 0.1 g, 0.2 g, 0.3 g, 0.4 g, 0.5 g, 0.6 g, 0.7 g, 0.8 g, 0.9g, 1.0g, 1.1g, 1.2 g, 1.3 g, 1.4 g, 1.5 g, 1.6 g, 1.7 g, 1.8 g, 1.9 g, 2.0 g, 2.1 g, 2.2 g, 2.3 g, 2.4 g, 2.5 g, 2.6 g, 2.7 g, 2.8 g, 2.9 g, 3.0 g, 3.1 g, 3.2 g, 3.3 g, 3.4 g, 3.5 g, 3.6 g, 3.7 g, 3.8 g, 3.9 g, 4.0 g, 4.1 g, 4.2 g, 4.3 g, 4.4 g, 4.5 g, 4.6 g, 4.7 g, 4.8 g, 4.9 g, or 5.0 g per day. The dosing regimen can include between 0.1 and 20 g of prebiotic per day. The regimen can also include escalating the number of doses per day, for example, 1 dose per day, 2 doses per day, 3 doses per day, 4 doses per day, 5 doses per day, 6 doses per day, 7 doses per day, 8 doses per day, 9 doses per day, or 10 doses per day. For example, 1 dose per day can be administered on day 1, 2 doses per day on day 10, and 3 doses per day on day 20 of a treatment regimen.

[00250] In one embodiment, the treatment occurs in phases. One phase utilizes a single administration of a prebiotic composition per day, generally though not necessarily with food, e.g., dinner. The dose of a prebiotic composition increases over time. For example, the dose of a

prebiotic composition can increase each day. Another phase, generally following the first phase, utilizes two administrations of a prebiotic composition per day, again, generally with food, e.g., with breakfast and dinner. Again, during this phase the dose of a composition comprising a prebiotic increases over time, e.g., increasing each day. In one embodiment, the treatment includes one phase in which a composition comprising a prebiotic composition is administered once per day in conjunction with a probiotic (e.g., live bacteria). This phase, if used, is generally the first phase of the method.

[00251] Optionally a probiotic microbe(s) can be administered during some or all of the entire period of treatment. For example, in one embodiment, a probiotic can be included in a prebiotic-containing product that is administered to a subject. Typically, during the preceding phases no dairy products are consumed. A final phase of the protocol can involve the gradual reintroduction of dairy into the diet, either with or without the continuing use of the prebiotic composition used in the first phases of treatment. Finally, treatment is concluded and no further ingestion of a prebiotic composition is required.

[00252] In another embodiment the dosing regimen comprises five phases. The first phase comprises administration of a prebiotic composition for two days, optionally with a probiotic. In the second phase, a prebiotic composition is taken with food once a day (e.g., breakfast, lunch, or dinner) for a period of about 10 to 30 days, or about 14 to 24 days, or about 16 to 20 days, or about 18 days. In the third phase, a prebiotic composition is taken twice a day with food (e.g., both breakfast and dinner) for another period of about 6 to 18 days, or about 8 to 16 days, or about 10 to 14 days, or about 12 days. For the fourth phase lasting another 2, 3, 4, 5, or 6 days (e.g., about 4 days) thereafter, a prebiotic composition is administered with both dinner and breakfast, along with the addition of a lactose containing product (e.g., a dairy product). Prior to this time, dairy products are not administered during the first phases, e.g., the first about 30-34 days of the regimen. This total period, e.g., of approximately 38 days, can constitute the full period in which a prebiotic composition is administered, but more importantly administered essentially in these time periods. In one embodiment, following the actual administration of a prebiotic composition, the regimen optionally includes a fifth phase: the actual ingestion of dairy products every few days to maintain and build up tolerance to lactose, but without the administration of a prebiotic composition (to test the establishment of lactose tolerance). If lactose tolerance is not established, the regimen can be repeated. In the first period of time, through the first, roughly 18 days, the amount of a prebiotic composition administered at dinner time increases regularly each day. Thereafter, and in the third period, a prebiotic composition is administered regularly each day in combination with a breakfast meal. Moreover, and for the final days, e.g., the final four days, a lactose containing food item, such as milk, also is regularly increased for those 4 days.

[00253] If an initial treatment regimen is successful in generating lactose tolerance in a lactose intolerant person, and the lactose intolerance recurs, one or more treatment regimens can be repeated.

- [00254] In one embodiment, a first dose of a prebiotic composition is administered in increasing amounts for a 6-week period. On the first and second days of this period, probiotic bacteria comprising one or more strains of bacteria (e.g., in a food containing product also having a live culture bacteria) is administered with the prebiotic composition. One such food item containing live cultured bacteria is yogurt. Further, during the third phase during this 6-week period, a second dose of a prebiotic composition (such as a composition comprising or consisting essentially of GOS) is administered, typically at breakfast time.
- [00255] In one embodiment a prebiotic composition and a probiotic composition are administered to a subject in need thereof. In one embodiment, in the first day of the regimen, a subject ingests 8 ounces (about 226.4 g) or less of a probiotic composition along with 1 tablespoon (about 14.8 mL) of a prebiotic composition, at the dinner meal. In one embodiment, a subject in need thereof will ingest 8 ounces (about 226.4 g) or less of a probiotic composition on the first day, along with 1 tablespoon (about 14.8 mL) of a prebiotic composition with dinner. On the second day, the amount of the yogurt ingested is reduced by half to 4 ounces (about 113.2 g) or less of a probiotic composition, although the administration of the a prebiotic composition remains the same. On the third day, administration of the probiotic composition is stopped, but administration of a prebiotic composition remains at 1 tablespoon (about 14.8 mL). During the 4th through the 18th days, the amount of a prebiotic composition (e.g., a composition comprising or consisting essentially of GOS) ingested with dinner is increased by 1 tablespoon (about 14.8 mL) each day until 16 tablespoons (about 237 mL) are reached on the day 18.
- [00256] In the third phase of the regimen, both 1 tablespoon (about 14.8 mL) of a prebiotic composition (such as a composition comprising or consisting essentially of GOS) is ingested in the morning, with breakfast, and 16 tablespoons (about 237 mL) of a prebiotic composition (e.g., a composition comprising or consisting essentially of GOS) are ingested with dinner. From day 16 until day 34, the same ratio of a prebiotic composition (e.g., a composition comprising or consisting essentially of GOS) with dinner is maintained, but the morning dose increases daily at a rate of a tablespoon (about 14.8 mL) per day. In this way, by day 34, the subject in need thereof is ingesting 32 tablespoons (about 474 mL) of a prebiotic composition (such as a composition comprising or consisting essentially of GOS).
- [00257] On day 35, ingestion of the a prebiotic composition (such as a composition comprising or consisting essentially of GOS) is discontinued and in place thereof, a dairy product such as milk (without prebiotic composition) is ingested, with 9 ounces (about 255 g) of milk in the morning and an additional 9 ounces (about 255 g) in the evening. The milk amounts are increased incrementally at a rate of an ounce (about 28.3 g) per day, such that, by day 38, the subject is ingesting 12 ounces (about 340 g) of milk with breakfast and an additional 12 ounces (about 340 g) of milk at dinner. Optionally, on days 39 through 42, cheese is substituted for milk.
- [00258] In another embodiment the number of days in which a prebiotic or probiotic composition is administered can vary, and the quantity of the dosages can similarly be modified according to

the needs of a particular subject and the symptoms of the subject. Even though there can be variations in both the time period and the dosage rates, the concept of increasing the dosages of a prebiotic composition for specific time periods is maintained and encompassed by the methods herein.

[00259] In another embodiment a subject in need thereof can ingest more than 5 tablespoons (about 74 mL) of a prebiotic composition by day 7. As a result, the amount of a prebiotic composition ingested by day 7 can be increased to 6 tablespoons (about 89 mL) on day 8. Determination of whether or not the subject is capable of increasing the dosage or the time period depends on whether or not the subject encounters any adverse affects.

[00260] The same alterations can be made in the time intervals between the administration of a prebiotic composition and a lactose containing food item. Thus, if desired, the subject in need thereof could potentially alter the amount of a prebiotic composition every 12 hours. In like manner, that time period could vary to 36 or even 48 hours. As indicated previously, a prebiotic composition of the invention can be administered in a powder formulation of a prebiotic composition (e.g., a composition comprising or consisting essentially of GOS), the latter of which can be mixed with water and administered much in the same manner as a soft drink. A prebiotic composition can also be incorporated in one or more capsules, capsules, or gels, as indicated. Further, a prebiotic composition can be supplied in a liquid formulation for oral administration.

[00261] In one embodiment a subject in need thereof is treated with a regimen using a powdered prebiotic composition using a dosing schedule as set forth in Figures 5, 6, or 7. For Figures 5 and 6, 70% GOS refers to a GOS composition comprising 70% by weight GOS, about 20% by weight lactose, and 10 % by weight digestible saccharides. In Figure 5, a prebiotic composition contains a GOS composition (starting at 0.5 g and increased to 8.00 g over 34 days) with 0 % by weight additional lactose. For example, the amount of 70% GOS composition administered can be about 0.5 g, 1 g, 1.5 g, 2 g, 2.5 g, 3 g, 3.5 g, 4 g, 4.5 g, 5 g, 5.5 g, 6 g, 6.5 g, 7 g, 7.5 g, or 8.0 g. In Figure 6, a prebiotic composition contains a 70% GOS composition (starting at 0.29 g and increased to 4.69 g over 34 days) with additional lactose (starting at 0.33 g and increased to 5.3 g over 34 days). For example, the amount of 70% GOS composition administered can be about 0.29 g, 0.59 g, 0.88 g, 1.17 g, 1.46 g, 1.76 g, 2.05 g, 2.34 g, 2.64 g, 2.93 g, 3.22 g, 3.52 g, 3.81 g, 4.10 g, 4.39 g, or 4.69 g. In Figure 7, the 90% GOS are a GOS composition comprising 90% by weight GOS and 10 % by weight digestible saccharides. In this figure, a prebiotic composition contains a GOS composition (starting at 0.42 g and increased to 6.74 g over 34 days) with 0 % by weight additional lactose. For example, the amount of 90% GOS composition administered can be about 0.42 g, 0.84 g, 1.26 g, 1.68 g, 2.11 g, 2.53 g, 2.95 g, 3.37 g, 3.79 g, 4.21 g, 4.63 g, 5.05 g, 5.47 g, 5.89 g, 6.32 g, or 6.74 g. In Figure 8, the 93% GOS composition is a GOS composition comprising 90% by weight GOS (starting at 0.42 g and increased to 6.74 g over 34 days). For example, the amount of 93% GOS composition administered can be about 0.42 g, 0.84 g, 1.26 g, 1.68 g, 2.11 g, 2.53 g, 2.95 g, 3.37 g, 3.79 g, 4.21 g, 4.63 g, 5.05 g, 5.47 g, 5.89 g, 6.32 g, or 6.74 g.

g. In Figure 9, the 95% GOS composition is a GOS composition comprising 95% by weight GOS. For example, the amount of 95% GOS composition administered can be about 0.42 g, 0.84 g, 1.26 g, 1.68 g, 2.11 g, 2.53 g, 2.95 g, 3.37 g, 3.79 g, 4.21 g, 4.63 g, 5.05 g, 5.47 g, 5.89 g, 6.32 g, or 6.74 g. In another embodiment, a prebiotic composition contains a GOS composition (starting at a certain amount and increasing to a maximum amount over 34 days) with additional lactose (starting at a certain amount and increasing to a maximum amount over 34 days). In one embodiment a capsule containing GOS composition powder, is administered to a subject in need thereof. At day 34, the subject in need thereof has completed the protocol and can now enjoy dairy products pain-free. In one embodiment, no future protocol, supplements, or medication is needed for these subjects in need thereof to consume dairy products. In another embodiment, the protocol can be re-administered as needed.

[00262] In one embodiment, a prebiotic composition can be administered in a 16 day program. Examples of 16 day programs are shown in Tables 2, 3, and 4. Milk can be provided to the subject after completion of the 16 day program.

Table 2. Two examples of 16 day treatment programs.

Low			High		
	PM dose (g of GOS)	AM dose (g of GOS)		PM dose (g of GOS)	AM dose (g of GOS)
Day 1	1.50	1.50	Day 1	1.50	1.50
Day 2	1.50	1.50	Day 2	1.50	1.50
Day 3	1.50	1.50	Day 3	1.50	1.50
Day 4	1.50	1.50	Day 4	1.50	1.50
Day 5	1.50	1.50	Day 5	3.00	3.00
Day 6	1.50	1.50	Day 6	3.00	3.00
Day 7	1.50	1.50	Day 7	3.00	3.00
Day 8	1.50	1.50	Day 8	3.00	3.00
Day 9	3.00	3.00	Day 9	4.50	4.50
Day 10	3.00	3.00	Day 10	4.50	4.50
Day 11	3.00	3.00	Day 11	4.50	4.50
Day 12	3.00	3.00	Day 12	4.50	4.50
Day 13	3.00	3.00	Day 13	6.00	6.00
Day 14	3.00	3.00	Day 14	6.00	6.00
Day 15	3.00	3.00	Day 15	6.00	6.00
Day 16	3.00	3.00	Day 16	6.00	6.00
	8 oz Milk	8 oz Milk		8 oz Milk	8 oz Milk
	10 oz Milk	10 oz Milk		10 oz Milk	10 oz Milk
	12 oz Milk	12 oz Milk		12 oz Milk	12 oz Milk

Table 3. Two examples of 16 day treatment programs.

	PM dose (g of GOS)	AM dose (g of GOS)		PM dose (g of GOS)	AM dose (g of GOS)
Day 1	0.40	0.40	Day 1	1.20	1.20
Day 2	0.80	0.80	Day 2	1.20	1.20
Day 3	1.20	1.20	Day 3	1.20	1.20
Day 4	1.60	1.60	Day 4	3.00	3.00

Day 5	2.00	2.00	Day 5	3.00	3.00
Day 6	2.40	2.40	Day 6	3.00	3.00
Day 7	2.80	2.80	Day 7	3.00	3.00
Day 8	3.20	3.20	Day 8	4.60	4.60
Day 9	3.60	3.60	Day 9	4.60	4.60
Day 10	4.00	4.00	Day 10	4.60	4.60
Day 11	4.40	4.40	Day 11	4.60	4.60
Day 12	4.80	4.80	Day 12	6.10	6.10
Day 13	5.20	5.20	Day 13	6.10	6.10
Day 14	5.60	5.60	Day 14	6.10	6.10
Day 15	6.00	6.00	Day 15	6.10	6.10
Day 16	6.40	6.40	Day 16	6.10	6.10
	8 oz Milk	8 oz Milk		8 oz Milk	8 oz Milk
	10 oz Milk	10 oz Milk		10 oz Milk	10 oz Milk
	12 oz Milk	12 oz Milk		12 oz Milk	12 oz Milk

Table 4. Example of a 16 day treatment program

	PM dose (g of GOS)	AM dose (g of GOS)
Day 1	3.00	
Day 2	3.00	
Day 3	3.00	
Day 4	3.00	3.00
Day 5	3.00	3.00
Day 6	3.00	3.00
Day 7	3.00	3.00
Day 8	4.60	4.60
Day 9	4.60	4.60
Day 10	4.60	4.60
Day 11	4.60	4.60
Day 12	6.10	6.10
Day 13	6.10	6.10
Day 14	6.10	6.10
Day 15	6.10	6.10
Day 16	6.10	6.10
	8 oz Milk	8 oz Milk
	10 oz Milk	10 oz Milk
	12 oz Milk	12 oz Milk

[00263] In another embodiment, a prebiotic composition can be administered during a 30 or 34 day treatment program. Examples of 30 and 34 day treatment programs are shown in Tables 5, 6, and 7. Milk can be provided after the treatment program.

Table 5. Two examples of 30 day treatment programs.

Low			High		
	PM dose (g of GOS)	AM dose (g of GOS)		PM dose (g of GOS)	AM dose (g of GOS)
Day 1	1.50	1.50	Day 1	3.00	
Day 2	1.50	1.50	Day 2	3.00	
Day 3	1.50	1.50	Day 3	3.00	
Day 4	1.50	1.50	Day 4	3.00	3.00
Day 5	1.50	1.50	Day 5	3.00	3.00
Day 6	1.50	1.50	Day 6	3.00	3.00
Day 7	1.50	1.50	Day 7	3.00	3.00
Day 8	1.50	1.50	Day 8	3.00	3.00
Day 9	1.50	1.50	Day 9	3.00	3.00

Day 10	1.50	1.50	Day 10	3.00	3.00
Day 11	1.50	1.50	Day 11	4.60	4.60
Day 12	1.50	1.50	Day 12	4.60	4.60
Day 13	1.50	1.50	Day 13	4.60	4.60
Day 14	1.50	1.50	Day 14	4.60	4.60
Day 15	1.50	1.50	Day 15	4.60	4.60
Day 16	3.00	3.00	Day 16	4.60	4.60
Day 17	3.00	3.00	Day 17	4.60	4.60
Day 18	3.00	3.00	Day 18	4.60	4.60
Day 19	3.00	3.00	Day 19	4.60	4.60
Day 20	3.00	3.00	Day 20	4.60	4.60
Day 21	3.00	3.00	Day 21	6.10	6.10
Day 22	3.00	3.00	Day 22	6.10	6.10
Day 23	3.00	3.00	Day 23	6.10	6.10
Day 24	3.00	3.00	Day 24	6.10	6.10
Day 25	3.00	3.00	Day 25	6.10	6.10
Day 26	3.00	3.00	Day 26	6.10	6.10
Day 27	3.00	3.00	Day 27	6.10	6.10
Day 28	3.00	3.00	Day 28	6.10	6.10
Day 29	3.00	3.00	Day 29	6.10	6.10
Day 30	3.00	3.00	Day 30	6.10	6.10
	8 oz Milk	8 oz Milk		8 oz Milk	8 oz Milk
	10 oz Milk	10 oz Milk		10 oz Milk	10 oz Milk
	12 oz Milk	12 oz Milk		12 oz Milk	12 oz Milk

Table 6. Examples of a 30 and 34 day treatment program.

	PM dose (g of GOS)	AM dose (g of GOS)		PM dose (g of GOS)	AM dose (g of GOS)
Day 1	1.20		Day 1	0.40	
Day 2	1.20		Day 2	0.40	
Day 3	1.20		Day 3	0.40	
Day 4	1.20		Day 4	0.80	
Day 5	3.00		Day 5	1.20	
Day 6	3.00		Day 6	1.60	
Day 7	3.00		Day 7	2.00	
Day 8	3.00		Day 8	2.40	
Day 9	4.60		Day 9	2.80	
Day 10	4.60		Day 10	3.20	
Day 11	4.60		Day 11	3.60	
Day 12	4.60		Day 12	4.00	
Day 13	6.10		Day 13	4.40	
Day 14	6.10	1.20	Day 14	4.80	
Day 15	6.10	1.20	Day 15	5.20	
Day 16	6.10	1.20	Day 16	5.60	
Day 17	6.10	1.20	Day 17	6.00	
Day 18	6.10	3.00	Day 18	6.40	
Day 19	6.10	3.00	Day 19	6.40	0.40
Day 20	6.10	3.00	Day 20	6.40	0.80
Day 21	6.10	3.00	Day 21	6.40	1.20
Day 22	6.10	4.60	Day 22	6.40	1.60
Day 23	6.10	4.60	Day 23	6.40	2.00
Day 24	6.10	4.60	Day 24	6.40	2.40
Day 25	6.10	4.60	Day 25	6.40	2.80
Day 26	6.10	6.10	Day 26	6.40	3.20
Day 27	6.10	6.10	Day 27	6.40	3.60

Day 28	6.10	6.10	Day 28	6.40	4.00
Day 29	6.10	6.10	Day 29	6.40	4.40
Day 30	6.10	6.10	Day 30	6.40	4.80
8 oz Milk	8 oz Milk		Day 31	6.40	5.20
10 oz Milk	10 oz Milk		Day 32	6.40	5.60
12 oz Milk	12 oz Milk		Day 33	6.40	6.00
			Day 34	6.40	6.40
			Day 35	8 oz Milk	8 oz Milk
			Day 36	10 oz Milk	10 oz Milk
			Day 37	12 oz Milk	12 oz Milk

Table 7. Examples of 30 day treatment programs.

	PM dose (g of GOS)	AM dose (g of GOS)		PM dose (g of GOS)	AM dose (g of GOS)
Day 1	1.20	1.20	Day 1	3.00	
Day 2	1.20	1.20	Day 2	3.00	
Day 3	1.20	1.20	Day 3	3.00	
Day 4	1.20	1.20	Day 4	3.00	3.00
Day 5	1.20	1.20	Day 5	3.00	3.00
Day 6	1.20	1.20	Day 6	3.00	3.00
Day 7	1.20	1.20	Day 7	3.00	3.00
Day 8	3.00	3.00	Day 8	3.00	3.00
Day 9	3.00	3.00	Day 9	3.00	3.00
Day 10	3.00	3.00	Day 10	3.00	3.00
Day 11	3.00	3.00	Day 11	4.60	4.60
Day 12	3.00	3.00	Day 12	4.60	4.60
Day 13	3.00	3.00	Day 13	4.60	4.60
Day 14	3.00	3.00	Day 14	4.60	4.60
Day 15	4.60	4.60	Day 15	4.60	4.60
Day 16	4.60	4.60	Day 16	4.60	4.60
Day 17	4.60	4.60	Day 17	4.60	4.60
Day 18	4.60	4.60	Day 18	4.60	4.60
Day 19	4.60	4.60	Day 19	4.60	4.60
Day 20	4.60	4.60	Day 20	4.60	4.60
Day 21	4.60	4.60	Day 21	6.10	6.10
Day 22	6.10	6.10	Day 22	6.10	6.10
Day 23	6.10	6.10	Day 23	6.10	6.10
Day 24	6.10	6.10	Day 24	6.10	6.10
Day 25	6.10	6.10	Day 25	6.10	6.10
Day 26	6.10	6.10	Day 26	6.10	6.10
Day 27	6.10	6.10	Day 27	6.10	6.10
Day 28	6.10	6.10	Day 28	6.10	6.10
Day 29	6.10	6.10	Day 29	6.10	6.10
Day 30	6.10	6.10	Day 30	6.10	6.10
8 oz Milk	8 oz Milk		8 oz Milk	8 oz Milk	
10 oz Milk	10 oz Milk		10 oz Milk	10 oz Milk	
12 oz Milk	12 oz Milk		12 oz Milk	12 oz Milk	

[00264] Dosages of prebiotics can be administered to a subject in gelatin caps "00", which can hold between 0.546 – 1.092 g (e.g., of powder); gelatin caps "0", which can hold between 0.408-0.816 g (e.g., of powder), and gelatin caps "#1", which can hold between 0.300 and 0.600 g (e.g. of powder). For example, approximately 3 g of prebiotic composition can be administered to a subject in three gelatin cap 00 pills. Approximately 1.5 g of prebiotic composition can be

administered two gelatin caps "00" or two gelatin caps "0." A prebiotic composition can be measured using a scoop.

[00265] Variations in the doses and timing in which the prebiotic compositions are administered can result in an effective treatment for increasing tolerance for lactose containing product. For example, the presented doses will be tested on subjects in need thereof. Thus, when applying the protocol of the present invention to younger subjects in need thereof, the weight of the subject might be a consideration. For example, a subject weighing 50 pounds (about 22.5 kg) can be administered lower dosages of a prebiotic composition than an adult. In another embodiment the timing of administration of a prebiotic composition to a pediatric subject can be different (e.g., once per day for 4 weeks) or the duration of administration can be shorter or longer than the duration of administration to an adult. In one embodiment the duration of administration of a prebiotic composition to a pediatric subject is shorter than the duration of administration to an adult. In one embodiment the duration of administration of a prebiotic composition to a pediatric subject is longer than the duration of administration to an adult.

[00266] In one embodiment the amount of a prebiotic composition administered to a subject can be proportionally adjusted based on the subject's weight. Although the doses are disclosed as being administered with breakfast and dinner, alternatively the order of the doses can be switched, or can be administered at other times of the day with meals such as lunch or snacks (or conceivably with no meals). The program can also be reduced into a shortened or lengthened program. In one embodiment a program of administration of a prebiotic composition to a subject in need thereof can be an abbreviated 1 week program or it can be lengthened up to a 10 week program. Although the methods and compositions herein have been described for use in humans, they are also capable of being administered to other mammals.

VI. Kits

[00267] In another aspect, the invention provides kits for the treatment of the symptoms of lactose intolerance. The kits include a prebiotic composition in suitable packaging for use by a subject in need thereof in the treatment of one or more symptoms of lactose intolerance. Any of the compositions described herein can be packaged in the form of a kit. A kit can contain an amount of a prebiotic composition and, optionally, other ingredients as described herein, sufficient for an entire course of treatment, or for a portion of a course of treatment. Thus, in one embodiment, a kit can include sufficient prebiotic composition for the first, second, third, fourth, fifth, and sixth weeks of treatment, or additional weeks of treatment if used, or any combination thereof. Doses of a prebiotic composition can be individually packaged, or the prebiotic composition can be provided in bulk, or combinations thereof. In one embodiment the individually packaged prebiotic composition is provided as a tablet, caplet, capsule or container of powder. In another embodiment the prebiotic composition is provided in a controlled release formulation. In another embodiment the prebiotic composition is provided as a formulation with an enteric coating. Thus, in one embodiment, a kit provides, in suitable packaging, individual doses of a prebiotic

composition that correspond to dosing points in a treatment regimen, wherein the doses are packaged in one or more packages intended for use in the treatment of symptoms of lactose intolerance. For example, a kit can contain doses of a prebiotic composition, as described herein, for a treatment program, where the prebiotic composition is taken in increasing doses, so that individual packets of a prebiotic composition are increasing in amount of a prebiotic composition contained in the packet, from lower doses intended for use at the start of the program to higher doses as the program progresses. As doses are provided for later points in the program, two or more doses per day can be provided, each in its individual packet. Each packet can be labeled to indicate the day and time of day that it is intended to be taken, or the packaging containing the packets can be so labeled, or both. A "packet," as used in this context, is any individual container that contains a prebiotic composition, whether the prebiotic composition is in solid or liquid form, and can include a packet that contains powder, tablets, or pills, or a packet that contains a liquid.

[00268] In one embodiment, the prebiotic composition can be provided in bulk in a single container, or in two, three, four, five, or more than five containers (e.g., where each container contains enough of a prebiotic composition for a particular week of a treatment program). If more than one bulk container is provided, the bulk containers can be suitably packaged together to provide sufficient prebiotic composition for all or a portion of a treatment protocol. The container or containers can be labeled with a label indicating information useful to the subject in need thereof performing the treatment protocol, such as dosing schedules.

[00269] The prebiotic composition can be packaged with other suitable substances, such as probiotic bacteria, FOS, or buffer, as described herein. The other substance or substances can be packaged separately from the prebiotic composition, or mixed with the prebiotic composition, or combinations thereof. Thus, in one embodiment, kits of the invention include a powder or liquid containing all the ingredients intended to be used in a course of treatment or a portion of a course of treatment, e.g., a prebiotic composition and optionally a probiotic, FOS, or a buffer. In one embodiment, a prebiotic composition is packaged in one package or set of packages, and additional components, such as bacteria, FOS, or buffer, are packaged separately from the prebiotic composition.

[00270] Kits can further include written materials, such as instructions, expected results, testimonials, explanations, warnings, clinical data, information for health professionals, and the like. In one embodiment, the kits contain a label or other information indicating that the kit is only for use under the direction of a health professional, such as a dietician, nutritionist, nurse, physician, or other appropriate health professional. In another embodiment, the kits contain or include information, such as a label, designating the material within as a medical food.

[00271] In one embodiment, the invention provides a kit that includes a container of powder, where the powder includes a prebiotic composition, and optionally FOS, bacteria, or buffer, and a label on the container that indicates proper dosage and schedule of use for the powder. The container can further include scoops or other measuring or serving devices. In one embodiment,

the invention provides a kit that includes a container of liquid, where the liquid includes a prebiotic composition and additionally FOS, bacteria, or buffer, and a label on the container that indicates proper dosage and schedule of use for the liquid. The container can further include measuring or serving devices.

VII. Business Methods

[00272] The invention also provides business methods for marketing compositions and methods for the treatment of the symptoms of lactose intolerance or for overall improvement in gastrointestinal health. In one embodiment, the invention provides a method of doing business that includes marketing a composition for the treatment of symptoms of lactose intolerance wherein the treatment is by administering increasing doses of a prebiotic composition according to any of the methods described herein, optionally in combination with other substances such as FOS, lactose, bacteria, and buffers. In one embodiment, the composition is part of a kit, as described herein. The methods can further include producing such compositions or kits. The marketing can be directly to the consumer, or to suitable health professionals, or combinations thereof. The methods of marketing used in these embodiments of the invention include, but are not limited to, print, television, or radio commercials, infomercials, internet advertising, testimonials, word of mouth, telemarketing, and the like.

[00273] Also provided herein is a method of doing business such as providing a prebiotic composition as described herein to another entity that manufactures an already existing brand or product (such as a drink or dairy product) already available to the public. Methods encompass a method of doing business comprising marketing a prebiotic composition for use with an existing brand or product (drink or dairy product), wherein the prebiotic composition, when combined with the existing brand or product, causes the existing brand or product to have the added beneficial effects of lactose intolerance treatment or improving overall GI health.

EXAMPLES

Example 1: Clinical Trial Synopsis

[00274] A Phase 2b, multicenter, randomized, placebo-controlled trial will be conducted of RP-G28—(a 96% GOS composition, by weight) versus placebo in subjects with moderate to severe symptoms on a hydrogen breath test, milk challenge, and stool bacterial analysis that are associated with lactose intolerance.

[00275] There will be two primary study objectives of the clinical trial:

[00276] 1. The first primary objective will be to assess the ability of a 30 day treatment with RP-G28 to improve lactose digestion and tolerance in 60 subjects in comparison with placebo (n=30), and to determine if their symptoms caused by a lactose challenge during their Hydrogen Breath Test (HBT) are reduced 90 days after the end of treatment, and to demonstrate that any effect observed at the end of the 30 days persists for at least 90 days after treatment is completed.

[00277] 2. The second primary objective will be to assess the safety of RP-G28 in this population by assessing adverse events throughout the period on RP-G28 or placebo and the ability to

tolerate the drug. This will be assessed by data collected at weekly telephone calls during the 30 days of treatment, and every other week calls for the next three months after the treatment is completed.

[00278] There will be five secondary study objectives:

[00279] 1. Patient compliance will be measured by subject responses at weekly telephone calls and by assessing the amount of RP-G28 or placebo the subject returns at the end of treatment when they visit the clinic for their HBT.

[00280] 2. RP-G28 and placebo groups will be compared in terms of their symptom scores in the presence of dairy intake (Days 35 to 90) versus their original baseline (historical) scores.

[00281] 3. The duration of any improvement in symptoms reported during the HBT observed at the end of treatment (Day 30) will be evaluated by comparing symptom scores at the end of treatment with those obtained during the HBT three months later for the active treatment and placebo groups.

[00282] 4. The effect observed at the end of treatment (Day 30) versus baseline will be assessed by comparing the decreased scores of those on treatment versus those on placebo, using scores obtained on a lactose challenge in the two HBT.

[00283] 5. The trial will compare the amount of dairy products ingested during days 35 to 90 versus baseline in the two groups, as measured from a dietary sheet completed by subjects.

[00284] **Study Design:** This will be a parallel group trial of a 30-day course on RP-G28 (total n=60 subjects) or placebo (n=30 subjects) following a dosing schedule to be provided to each subject. Subjects will be enrolled who exceed a pre-specified level of symptoms on a lactose challenge test during the HBT at baseline. This test will be repeated at the end of treatment and approximately three months later. Each of three symptoms of lactose intolerance (see next paragraph) will be assessed hourly as 0 (no symptoms); 1 (mild symptoms); 2 (moderate symptoms); 3 (strong symptoms); and 4 (severe symptoms). Adverse events will be collected at weekly telephone calls for six weeks and calls every other week for the next three months, as well as during each visit to the clinic where the HBT is conducted. Patients will be instructed to eat a fixed amount of dairy portions during days 35 to 90 (3 to 7 dairy servings per week, where the definition of a serving is defined on an instruction sheet to be given to each subject).

[00285] **Rationale for the doses to be used:** The goal of this study will be to develop tolerance in subjects who are lactose intolerant, and a primary principle of developing tolerance is to gradually increase the dose of the drug. The doses of RP-G28 (given once daily in sachets) will be gradually increased over Days 1 to 19 to reach the level of lactose equivalent to that in an 8 ounce (about 226 g) glass of milk (one serving of dairy). During the second half of the dose titration, the sachets will contain the equivalence of 8 ounces (about 226 g) of milk in the PM, while a second set of the same incremental doses used over Days 3-19 will be repeated in the AM. Once subjects receive the equivalence of 8 ounces (about 226 g) of milk twice daily, dosages will be further increased to reach the equivalence of 12 ounces (about 340 g) of milk in both the morning and

afternoon. This level of 12 ounces (about 340 g) of milk is chosen to develop tolerance to a total of three servings of dairy per day, the recommended level in the US Dietary Guidelines to meet calcium and other nutrient needs. This approach has previously been used successfully in many thousands of patients. In this clinical trial each day's dosage will be individually labeled in a sachet and printed with instructions for how to take it with water.

[00286] Primary Clinical Endpoint: The severity of three symptoms of lactose intolerance: gas, diarrhea, and "stomach pain" (any bloating, cramps or stomach pain) on ingesting 20 grams of lactose in solution will be assessed during the HBT.

[00287] To be enrolled subjects can experience one of the three following scores during baseline testing:

[00288] a. at least one strong or severe score (i.e., 3 or 4) on a single symptom on at least two time points during the six hour HBT;

[00289] b. at least two moderate scores (i.e., 2 each) on a single symptom on at least two time points during the six hour HBT; or

[00290] c. at least one moderate score or greater (i.e., a 2 or more) on each of two symptoms on at least two time points during the six hour HBT.

[00291] However, the efficacy of RP-G28 will be assessed by calculating the average score for subjects on RP-G28 versus those on placebo. Scores will be based on the following rating system: 0 (no symptoms); 1 (mild symptoms); 2 (moderate symptoms); 3 (strong symptoms) and 4 (severe symptoms). Therefore the maximal score is 3 (symptoms) times 6 hourly reports times 4 points maximally or 72. This score will be assessed during each of the three HBT evaluations, at baseline, within one week of completing the 30 day program and three months later. No distinction will be made between the importance of the three symptoms, and there will not be any weighting of the scores based on the specific symptom. The primary assessment of efficacy will be determined by comparing the decrease in the average score (baseline score minus the score at 90 days after the end of treatment) for those receiving RP-G28 versus those receiving placebo. (See the statistical section for additional details). The baseline score minus the score at the end of treatment will be a secondary measure of efficacy.

[00292] Secondary Clinical Endpoints:

[00293] 1. Symptom score of lactose intolerance collected on questionnaires presented to subjects at baseline and read over the telephone every other week during Days 38 to 90. (Note that these scores will not be obtained during the HBT).

[00294] 2. Score of the amount of hydrogen on the HBT in parts per million at the end of the trial and three months later, as compared with the amount at baseline. Hydrogen will be measured hourly during the six hour test and the sum of the six hour production will be compared to the baseline test.

[00295] 3. Symptom scores after the lactose challenge during the HBT at the end of the 30-day treatment versus the baseline score.

[00296] 4. Symptom scores after the lactose challenge during the HBT at three months after the treatment is completed versus the symptoms score at the end of the 30-day treatment.

[00297] **Number of Subjects:** A total of 90 subjects will be enrolled, 60 subjects in the active treatment group and 30 in the placebo group. It is anticipated that three clinical trial sites will be used. The unbalanced number of patients per group will be used to stimulate recruitment and to encourage subjects to enroll in the trial. The power of this trial is discussed in the statistical section. Subjects who do not complete the initial post-treatment HBT will be replaced to obtain 60 and 30 completers in the two groups.

[00298] **Diagnosis of Lactose Intolerance:** Patients can have symptoms of lactose intolerance with a total score of at least 8 for any one of the three symptoms or a total score of 16 for the three symptoms evaluated, on being challenged with 14 g of lactose solution during the HBT, whether or not the HBT data for hydrogen are positive. The HBT will be conducted in the investigator's clinic or other facility and will consist of 14g of lactose in solution, with a positive score defined at 10 parts per million of hydrogen above the subject's baseline at anytime during the six hour treatment. Patients with a positive HBT will be defined as lactose maldigesters. Because of the relatively large number of both false positives and false negatives reported in the literature with the HBT (although it is a diagnostic tool available for lactose intolerance) it is not scientifically appropriate to only enroll patients with a positive HBT and also symptoms of lactose intolerance. Therefore, the HBT is primarily used to evaluate symptoms of lactose intolerance and not the amount of hydrogen produced, although the latter will be used as a secondary endpoint.

[00299] **Major Inclusion Criteria:**

[00300] 1. Subjects of either sex aged 12 years and above.

[00301] 2. History of intolerance to milk and other dairy products of at least three months duration.

[00302] 3. During the lactose challenge (during the HBT) subjects can have one of the following ratings of their symptoms:

[00303] a. At least one score of strong or severe on any one symptom.

[00304] b. At least two scores of moderate on one symptom.

[00305] c. At least one score of moderate on each of two symptoms.

[00306] 4. An HBT will also be administered to assess the amount of hydrogen produced.

Note that there is no specific score on the amount of hydrogen measured in the HBT required for entry, but 10 parts per million of hydrogen above baseline is an amount that can be used to classify the patient as a lactose maldigester.

[00307] 5. Subjects can agree to refrain from all other treatments and products used for lactose intolerance during the trial.

[00308] **Duration of Treatment:** 30-days on investigational treatment, followed by a HBT and a three month period to evaluate the duration of any benefit.

- [00309] **Dropouts:** Subjects who drop out or are discontinued will not be replaced.
- [00310] **Study Drug Dose and Mode of Administration:** RP-G28 will be self-administered by subjects on an out-patient basis using a dosing schedule to be provided. RP-G28 will be packaged in individual sachet packs for dilution in water. Each pack will be labeled with the study day and time (i.e., am or pm).
- [00311] **Comparator Therapy:** Placebo (dextrose) will be given in equal amounts and using the same dosing schedule and packaging as RP-G28.
- [00312] Subjects will be asked about symptoms and also about the amount of dairy intake at their weekly or bi-weekly telephone calls.

Criteria for Evaluation:

- [00313] **Efficacy Measures:**
- [00314] A five point Likert scale (severe (4), strong (3), moderate (2), mild (1) or none (0)) will be used to score each of the three cardinal signs of lactose intolerance (i.e., gas, diarrhea, and cramps). The scale will be used following the HBT and during the bi-weekly telephone calls for the following three months after treatment is completed (Days 35 to 90). Note that the form for cramps will indicate to the subjects that it includes abdominal pain and bloating, which maintains the three cardinal signs. Experts in this field state that many, if not most, patients cannot separate these three overlapping symptoms (i.e. cramps, bloating, and abdominal pain), and that it makes most sense to refer to three cardinal signs (i.e. gas, diarrhea, and cramps).
- [00315] **Safety Assessments:**
- [00316] The incidence and severity of adverse events, blood pressure, and heart rate will be assessed during the three HBTs, and adverse events will be solicited during the weekly (Days 1-30) and the bi-weekly (Days 35-90) telephone calls to subjects using a standard script that will be read to them.
- [00317] **Statistical Plan:**
- [00318] Hydrogen production during the HBT and symptom comparisons will be evaluated according to the method of Hertzler and Savaiano (1996).
- [00319] **Efficacy Measures:**
- [00320] The primary efficacy assessment will be made by comparing changes in symptoms reported by each group during the HBT after the lactose challenge. Secondary efficacy will be assessed by responses to symptom questionnaires administered by telephone at bi-weekly intervals during the three month follow-up period. Finally, the reported amount of dairy portions ingested by subjects in each group will be compared for Days 35-90.
- [00321] The primary efficacy measure for this study will be the total symptom score during the HBT lactose challenges (maximum score = 72) at baseline, following treatment, and 90 days following treatment. Primary efficacy will be analyzed through a 2 group (Treatment and Control) X 2 time points (baseline and 90 days after 30 days of treatment) analysis of variance (ANOVA)

for alpha at .05. Using the results of Landon, et al. (2006), the power for this study (with 60 Treatment vs. 30 Control subjects) is 95%. Note that the primary assessment of efficacy is 90 days after the end of the 30-day treatment, which will demonstrate that the effect persists for at least three months past the time of treatment.

[00322] The secondary efficacy measure is to compare the scores of lactose intolerance symptoms obtained during the HBT challenge conducted three months after treatment with scores obtained at baseline and at the end of treatment. This analysis will be conducted employing a 2 group X 3 time points ANOVA.

[00323] Another secondary efficacy measure of bi-weekly subject reports will be computed and charted on patient listings, but not subjected to analysis since they are based on self-reporting of symptoms under non-standard conditions.

[00324] Finally, breath hydrogen concentrations will be summed for hours 1 to 6 after lactose challenge during the HBT at each of the three HBT evaluations. These scores will also be compared using a 2 group X 3 time points ANOVA for significant differences in mean breath hydrogen concentrations.

Safety Measures:

[00325] Summary statistics will be calculated for subject disposition, demographics, and baseline characteristics, patient compliance, blood pressure and heart rate. All adverse events will be recorded and reported for subjects in both Treatment and Control groups. These will be presented as lists, appropriate figures and in summary tables. All Serious Adverse Events will be reported to regulatory agencies per regulations and guidelines.

[00326] Subjects will visit the clinic once at baseline for screening and baseline assessments. Those who sign an informed consent and pass the lactose challenge given during HBT, and also the other screening evaluations will be randomized and given sachets of RP-G28 or placebo to take according to an attached sheet that labeled with each day and time of treatment. A page of instructions will also be provided. Subjects will return to clinic (with their unused medication) for a follow-up HBT within a week of completing the 30-day treatment period. A follow-up visit will take place approximately three months after the second HBT, for a lactose challenge and final HBT.

[00327] **Time and Events Chart:**

[00328] Subjects will be evaluated for adverse events, blood pressure and heart rate at each clinic visit. Safety with taking RP-G28 or placebo will be evaluated weekly during the 30-day treatment period and adverse events will be assessed during biweekly telephone calls from Day 35 to 90.

[00329] **Dosage Schedule:**

[00330] RP-G28 is a powder that will be ingested orally for 30 days using the regimen that follows the schedule to be provided at baseline after subjects are enrolled.

[00331] Subjects will not use any dairy products from Days 1 to 30 apart from what is listed in their instructions. From Days 35 to Day 90 subjects will be instructed to take from 3 to 7 dairy servings per week. All of the specific details will be presented in the full protocol.

Example 2: Study of GOS treatment of subjects

[00332] Subjects will take a 90% purity level GOS compound according to the schedule in Table 8. The subjects will be instructed to daily measure the exact dosage amount in Table 8 with the scoops provided and mix the powder in 6 to 8 ounces (about 170 to 226 g) of room temperature water. The mixtures will be stirred for 2 minutes before drinking. Alternatively, subjects will take gel capsules containing GOS. Subjects will be instructed not to skip any doses; if dosing for a day is forgotten, subjects will be instructed to back up a day in the routine and not to double on doses. The subjects will conduct self reported symptom scoring before, after, and 30 days thereafter program. A likert scale scoring system will be used: 1-5 symptom rating [(1) no symptoms, (2) minor symptoms, (3) moderate symptoms, (4) strong symptoms, (5) severe symptoms)] of subject's reported gas, cramps, bloating and/or diarrhea from dairy consumption.

[00333] Table 8. Dosing schedule for small study of GOS treatment of subjects.

	PM - Dosages	AM- Dosage
Day 1	0.40	
Day 2	0.40	
Day 3	0.40	
Day 4	0.80	
Day 5	1.20	
Day 6	1.60	
Day 7	2.00	
Day 8	2.40	
Day 9	2.80	
Day 10	3.20	
Day 11	3.60	
Day 12	4.00	
Day 13	4.40	
Day 14	4.80	
Day 15	5.20	
Day 16	5.60	
Day 17	6.00	
Day 18	6.40	
Day 19	6.40	0.40
Day 20	6.40	0.80
Day 21	6.40	1.20
Day 22	6.40	1.60
Day 23	6.40	2.00
Day 24	6.40	2.40
Day 25	6.40	2.80
Day 26	6.40	3.20
Day 27	6.40	3.60
Day 28	6.40	4.00
Day 29	6.40	4.40

Day 30	6.40	4.80
Day 31	6.40	5.20
Day 32	6.40	5.60
Day 33	6.40	6.00
Day 34	6.40	6.40

[00334] Dosages are in grams; PM-take with evening meal; AM-take with morning meal

Example 3: Growth of *Lactobacillus* and *Bifidobacterium* strains in a GOS solution

[00335] The growth of *Lactobacillus* and *Bifidobacterium* strains was evaluated in scratch MRS (Table 9) supplemented with either 2% glucose or 2% GOS and automatically monitored by determining the change in absorbance (A600) as a function of the time using a FLUOStar OPTIMA microtiter plate reader. The strains were incubated at 37°C aerobically. Results are shown in Figure 12. Some strains were grown under anaerobic conditions at 37°C and OD's were read manually over time, when indicated.

Table 9: Scratch MRS formula:

Reagents	Amount (g) per liter
Proteose peptone N3	10.0
Beef extract	10.0
Yeast extract	5.0
Polysorbate 80	1.0
Ammonium citrate	2.0
Sodium acetate	5.0
Magnesium sulfate	0.1
Manganese sulfate	0.05
Dipotassium phosphate	2.0
Glucose or GOS	20

Example 4: Purification of a GOS composition

[00336] Figures 13A and B illustrate HPLC chromatograms of GOS compositions of the present invention before (13A) and after (13B) a purification procedure.

Example 5: Comparative growth of *Bifidobacterium* species on galactooligosaccharides.

[00337] The objective of the study was to determine the ability of various *Bifidobacterium* species and strains to grow on galactooligosaccharides

[00338] The growth of *Lactobacillus* and *Bifidobacterium* strains was evaluated in scratch MRS (Table 10) supplemented with 2% of a carbohydrate solution. The carbohydrates used in the experiments were: Glucose – Fisher; Lactose – Fisher; GOS1 – 95% GOS purity from Inalco SPA – Provided by Ritter Pharmaceuticals; GOS2 – 90% GOS purity from GTC – provided by Ritter Pharmaceuticals.

[00339] Carbohydrate stock solutions were filter sterilized and then added to either a scratch MRS formulation (table 1), or a semisynthetic medium Table 11.

Table 10: Scratch MRS composition

Reagents	Amount (g) per liter
Proteose peptone N3	10.0
Beef extract	10.0
Yeast extract	5.0
Polysorbate 80	1.0
Ammonium citrate	2.0
Sodium acetate	5.0
Magnesium sulfate	0.1
Manganese sulfate	0.05
Dipotassium phosphate	2.0
Carbohydrates	20

[00340] Table 11: Semisynthetic medium for *Escherichia coli* (Barrangou, R., E. Altermann, R. Hutkins, R. Cano, and T. Klaenhammer. 2003. Functional and comparative genomic analyses of an operon involved in fructooligosaccharide utilization by *Lactobacillus acidophilus*. *Proc. Nat. Acad. Sci. USA*. 100:8957-8962).

1% bactopectone (w/v) (Difco),
0.5% yeast extract (w/v) (Difco),
0.2% dipotassium phosphate (w/v) (Fisher),
0.5% sodium acetate (w/v) (Fisher),
0.2% ammonium citrate (w/v) (Sigma),
0.02% magnesium sulfate (w/v) (Fisher),
0.005% manganese sulfate (w/v) (Fisher),
0.1% Tween 80 (v/v) (Sigma).
Carbohydrates were added at 2%.

[00341] Culture Methods:

[00342] *Lactobacillus* and *Bifidobacterium* cultures were propagated in MRS broth overnight, and then transferred once through the test medium. For growth experiments, cultures were inoculated into the MRS scratch medium containing one of the 4 carbohydrates to be examined. Growth was monitored either: **automatically, using a FLUOStar OPTIMA microtiter plate reader to monitor the change in absorbance (A600) as a function of the time. The strains were incubated at 37C aerobically for these experiments; or ** manually, using a standard spectrophotometer to monitor the change in OD600nm over time, in 5 ml liquid culture tubes. These cultures were incubated anaerobically at 37°C in a COY anaerobic chamber, flushed with anaerobic gas.

[00343] Species Identification:

[00344] All bifidobacterial cultures used in these experiments were confirmed by taxonomic identification using 16S rRNA sequencing, via standard methods (Kullen, M.J., R.B. Sanzky Dawes, D.C. Crowell and T.R. Klaenhammer (2000) Use of DNA sequence of variable regions of the 16SrRNA gene for rapid and accurate identification of bacteria in the *Lactobacillus acidophilus* complex. *J. Appl. Microbiol.* 89:511-518.

[00345] Results:

[00346] Figures 14-19 illustrate the growth of *Lactobacillus acidophilus* NCFM and various strains of *Bifidobacterium* and *Escherichia coli* over time. Key conclusions from these data are detailed below.

[00347] First, *Lactobacillus acidophilus* NCFM grows equally well on GOS1 (95%) as on glucose, indicating that the microbe efficiently metabolizes GOS1 (95%) (Figure 14).

[00348] Second, six different species of *Bifidobacterium* were examined for their ability to grow on GOS1 (95%) (Figures 15 & 16). The results showed that most strains grew on GOS1 (95%), but at rates that were slower than when growing on glucose. The exceptions were *B. pseudolongum* which grew equally well on GOS1 (95%) and glucose (Figure 15), and *B. adolescentis*, which grew better on GOS1 (95%) than on glucose. The species of *B. adolescentis* and *B. longum* predominate in the feces of adult humans. (Hoover, DG. 2000. *Bifidobacterium*. Pp 210-217. In The Encyclopedia of Food Microbiology. Carl Batt and P.D. Patel (Eds). Academic Press, San Diego.

[00349] Third, five different species of *Bifidobacterium* were also examined for their comparative growth on four different carbohydrate sources; glucose, lactose, GOS1 (95%) and GOS2 (90%) (Figures 17 & 18). Notable in these results was that all four of the species grew reasonably well on GOS, but in each case slightly better on GOS2 (90%), than on GOS 1 (95%). This difference was attributed to the larger percentage of simple carbohydrates present in the GOS2 sample. These contaminating carbohydrates would be expected to be galactose, lactose and glucose, all of which could stimulate slightly more growth from the GOS2 substrate. The *B. bifidum* strain used in the experiments grew poorly on all carbohydrates. Surprisingly, none of the *Bifidobacterium* strains used in these experiments grew on lactose, except for *B. pseudolongum*. It is speculated that contaminating glucose carried over from the initial propagation cultures in standard MRS broth may have been sufficient to elicit catabolite repression of the lactose metabolic pathways during these experiments.

[00350] Fourth, three different strains of *Escherichia coli* were examined for their ability to grow on GOS1 (95%) and GOS2 (90%) (Figure 19). The results show that the *E. coli* strains could not grow on GOS1 (95%), or in the absence of added carbohydrate (control). In contrast, all three strains grew well on GOS2 (90%) at rates that were comparable to growth on glucose. The results indicate that the 10% contaminating carbohydrates (e.g. glucose, galactose, lactose) in the GOS2 (90%) sample were sufficient to stimulate growth of *E. coli* strains to levels equal to free glucose.

These results argue for the importance of the purity of the GOS compound in order to promote growth of the targeted beneficial microbes in the GI tract (e.g. lactobacilli and bifidobacteria), rather than stimulate *E. coli* and potentially other coliform bacteria in the GI tract.

[00351] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein can be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for increasing lactose tolerance in a subject experiencing one or more symptoms of lactose intolerance comprising administering a composition comprising GOS to the subject each day for a predetermined number of days.
2. The method of claim 1, wherein said composition further comprises a probiotic.
3. The method of claim 1, wherein said composition does not contain a probiotic.
4. The method of claim 1, wherein said composition comprises a lower dosage of GOS on the first day of administration than the last day of administration.
5. The method of claim 1, wherein said composition comprises the same dosage of GOS on the first day of administration as the last day of administration.
6. The method of claim 1, wherein said method comprises administering said composition once a day.
7. The method of claim 1, wherein said method comprises administering said composition twice a day.
8. The method of claim 1, wherein said method comprises administering said composition three times a day.
9. The method of claim 1, wherein said composition comprising GOS is provided as a powder, a tablet, or a capsule.
10. The method of claim 1, wherein said composition comprising GOS is administered without a meal.
11. The method of claim 1, wherein said composition comprising GOS is administered in conjunction with a meal.
12. The method of claim 11, wherein said composition comprising GOS is administered with breakfast and dinner.
13. The method of claim 11, wherein said composition comprising GOS is administered with breakfast, lunch, and dinner.
14. The method of claim 1, wherein said one or more symptoms comprise flatulence, heartburn, stomach upset, nausea, bloating, flatulence, diarrhea, abdominal pain, cramping, or vomiting.
15. The method of claim 1, wherein at least about 96% percent of the total weight of the composition is GOS.
16. A composition for increasing lactose tolerance in a subject comprising about 0.1 to 2 grams of a GOS composition, wherein said GOS composition comprises about 96% GOS by weight.
17. The composition of claim 16 further comprising a pharmaceutically acceptable excipient.
18. The composition of claim 16, wherein said composition is contained within a capsule.
19. The composition of claim 18, wherein said capsule is a gelatin capsule.
20. The composition of claim 19, wherein said gelatin capsule comprises a viscous syrup or liquid comprising GOS.

21. The composition of claim 20, wherein said syrup does not contain an anti-oxidant or preservative.
22. The composition of claim 16, wherein said composition is contained in a tablet.
23. An oral dosage form of GOS comprising about 0.1 to 2 grams of a GOS composition wherein said GOS composition is a viscous syrup or liquid encapsulated in a gelatin capsule.
24. The oral dosage form of claim 23, wherein said GOS composition comprises about 80% GOS by weight.
25. The oral dosage form of claim 23, wherein said GOS composition comprises about 96% GOS by weight.
26. An oral dosage form of GOS comprising a GOS composition in a tablet or capsule formulated for controlled release.
27. The oral dosage form of claim 26, wherein said controlled release occurs in the intestines.
28. The oral dosage form of claim 26, wherein said GOS composition comprises an enteric coating.
29. The oral dosage form of claim 26, wherein said controlled release occurs in the lower intestine.
30. The composition of claims 16, 23 or 26, further comprising a probiotic.
31. The composition of claim 30, wherein said GOS composition comprises an enteric coating.