

the specific complex oligosaccharides such as those found in human or bovine milk (U.S. Patent No. 8,198,872 and U.S. Pub. No. 2013/0195803, the contents of which are incorporated herein by reference). When *B. infantis* comes in contact with certain complex oligosaccharides a number of genes are specifically induced within the bacterium whose protein products as enzymes and binding proteins are responsible for the uptake and internal deconstruction of those complex oligosaccharides, and the individual sugar components are then catabolized to provide energy for the growth and reproduction of that organism (Sela et al, 2008, *PNAS*, 105(48): p. 18964-69).

SUMMARY

[0004] The instant disclosure provides compositions comprising isolated complex oligosaccharide fractions from mammalian milk sources, optionally supplemented with purified fucosylated/sialylated oligosaccharides. The mammalian milk may be from human or bovine sources, and including but not limited to, the bovine source is from bovine colostrum. The fucosylated oligosaccharide(s) may comprise synthetically produced and purified 2'-fucosyllactose, 3-fucosyllactose, difucosyllactose, or lacto-N-fucosylpentose.

[0005] In some examples, the composition further comprises bifidobacteria that internalize the complex oligosaccharides or dietary glycans prior to their hydrolysis and metabolism. The combination of the bifidobacteria with the complex oligosaccharide may result in the conversion of the bifidobacteria to an activated bifidobacteria (ABI). The bifidobacteria is preferably selected from *B. longum*, *B. breve*, *B. bifidum* or *B. pseudocatenulatum*, and more preferably, the *B. longum* is *B. longum* subsp. *infantis*.

[0006] In other examples, any of the compositions described herein provide a method of improving the health of a mammal comprising administering to a mammal a composition comprising a complex oligosaccharide from a mammalian milk source, optionally supplemented with a fucosylated and/or sialylated oligosaccharide, and a bifidobacteria that internalizes the complex oligosaccharide prior to its hydrolysis, and wherein the fucosylated oligosaccharide can be a synthetically produced and purified form of 2'-fucosyllactose, 3-fucosyllactose, difucosyllactose, or lacto-N-fucosylpentose, wherein the bifidobacteria is preferably selected from *B. longum*, *B. breve*, *B. bifidum* or *B. pseudocatenulatum*, and more

preferably, the *B. longum* is *B. longum* subsp. *infantis*. The bifidobacteria is typically provided in a daily dose of from 10 thousand to 100 billion cfu, preferably 1 billion to 50 billion, and most preferably 5 billion to 25 billion, and the oligosaccharides are provided in a daily dose of from 1 to 20 g, preferably in a daily dose of from 1 to 10 g.

[0006a] In one aspect, there is provided a composition comprising activated *Bifidobacterium*, wherein the *Bifidobacterium* is *B. longum*, *B. breve*, *B. bifidum* or *B. pseudocatenulatum*, wherein the composition is in the form of a dry powder or a dry powder suspended in an oil, and further wherein activated *Bifidobacterium* has any of the genes Blon_2334, Blon_2335, Blon_2336, Blon_2337, Blon_2338, Blon_2339, Blon_2344, Blon_2346, Blon_2347, Blon_2331, and/or Blon_0042 of *B. longum* subspecies *infantis* upregulated, or corresponding genes from other *Bifidobacterium*.

[0006b] In another aspect, there is provided use of a therapeutically effective amount of the composition as described herein in the manufacture of a medicament for treating or preventing dysbiosis in a mammalian gastrointestinal tract.

[0006c] In another aspect, there is provided use of an effective amount of the composition as described herein in the manufacture of a medicament for treating or preventing dysbiosis in the gastrointestinal tract of a mammal, wherein the medicament is to be administered to a subject in order to increase levels of said *Bifidobacterium* in the feces of that mammal to greater than 10% of the total microbiome found in that feces.

[0006d] In another aspect, there is provided a method of preparing activated *Bifidobacterium* comprising cultivating *Bifidobacterium* by incubating the bacteria under conditions wherein the activated *Bifidobacterium* has been cultured in the presence of at least one mammalian milk oligosaccharide, then recovering and drying the activated *Bifidobacterium*, whereby any of the genes Blon_2334, Blon_2335, Blon_2336, Blon_2337, Blon_2338, Blon_2339, Blon_2344, Blon_2346, Blon_2347, Blon_2331, and Blon_0042 is upregulated, gene Blon_2168 is downregulated, or combinations thereof.

[0006e] In another aspect, there is provided a method of preparing activated bifidobacteria compositions comprising (a) isolating bifidobacteria from infant feces, (b) purifying the bacteria, (c) inoculating a fermenter with a purified strain of the bacteria, (d) incubating the bacteria in the fermenter in the presence of a mammalian milk oligosaccharide isolated from a mammalian milk source, (e) harvesting the cells, (f) purifying the bacteria obtained in (e), and drying the recovered bacteria.

CLAIMS

1. A composition comprising activated *Bifidobacterium*, wherein the *Bifidobacterium* is *B. longum*, *B. breve*, *B. bifidum* or *B. pseudocatenulatum*, wherein the composition is in the form of a dry powder or a dry powder suspended in an oil, and further wherein activated *Bifidobacterium* has any of the genes Blon_2334, Blon_2335, Blon_2336, Blon_2337, Blon_2338, Blon_2339, Blon_2344, Blon_2346, Blon_2347, Blon_2331, and/or Blon_0042 of *B. longum* subspecies *infantis* upregulated, or corresponding genes from other *Bifidobacterium*.
2. The composition of claim 1, wherein the *B. longum* is *B. longum* subspecies *infantis*.
3. The composition of claim 1 or 2, wherein the activated *Bifidobacterium* has been cultured in the presence of at least one mammalian milk oligosaccharide.
4. The composition of claim 1, 2, or 3, wherein the *Bifidobacterium* expresses a gene coding for a sialidase, a sialic acid transporter, a fucose transporter, or a fucosidase.
5. The composition of any one of claims 1–4, wherein the activated *Bifidobacterium* contains a transport system capable of internalizing one or more oligosaccharide before said oligosaccharide is hydrolyzed and is further capable of hydrolyzing said internalized oligosaccharide, wherein said oligosaccharide has the structure of an oligosaccharide found in a mammalian milk.
6. The composition of any one of claims 1-5, wherein the activated *Bifidobacterium* has a higher binding affinity to mammalian mucosal cells than *Bifidobacteria* of the same species cultivated in the absence of complex mammalian milk oligosaccharides.
7. The composition of any one of claims 1-6, wherein the *Bifidobacterium* is present at a concentration of from 1 billion cfu/g to 500 billion cfu/g.
8. The composition of any one of claims 1-7, further comprising an isolated complex mammalian milk oligosaccharide.

9. The composition of any of claims 1-8, further comprising a stabilizer.
10. The composition of any one of claims 1-9, wherein the composition is in the form of a packet, sachet, orally disintegrating tablet, foodstuff, capsule, lozenge, effervescent tablet, suppository, or enema.
11. Use of a therapeutically effective amount of the composition of any one of claims 1-7 in the manufacture of a medicament for treating or preventing dysbiosis in a mammalian gastrointestinal tract.
12. Use of an effective amount of the composition of any one of claims 1-7 in the manufacture of a medicament for treating or preventing dysbiosis in the gastrointestinal tract of a mammal, wherein the medicament is to be administered to a subject in order to increase levels of said *Bifidobacterium* in the feces of that mammal to greater than 10% of the total microbiome found in that feces.
13. A method of preparing activated *Bifidobacterium* comprising cultivating *Bifidobacterium* by incubating the bacteria under conditions wherein the activated *Bifidobacterium* has been cultured in the presence of at least one mammalian milk oligosaccharide, then recovering and drying the activated *Bifidobacterium*, whereby any of the genes Blon_2334, Blon_2335, Blon_2336, Blon_2337, Blon_2338, Blon_2339, Blon_2344, Blon_2346, Blon_2347, Blon_2331, and Blon_0042 is upregulated, gene Blon_2168 is downregulated, or combinations thereof.
14. A method of preparing activated bifidobacteria compositions comprising (a) isolating bifidobacteria from infant feces, (b) purifying the bacteria, (c) inoculating a fermenter with a purified strain of the bacteria, (d) incubating the bacteria in the fermenter in the presence of a mammalian milk oligosaccharide isolated from a mammalian milk source, (e) harvesting the cells, (f) purifying the bacteria obtained in (e), and drying the recovered bacteria.