## SUMMARY

**[0003a]** In one aspect, the present disclosure provides a composition comprising a *Bifidobacterium longum* subsp. *infantis* comprising a functional H5 gene cluster, wherein the *Bifidobacterium longum* subsp. *infantis* is *Bifidobacterium longum* subsp. *infantis* EVC001 deposited under ATCC Accession No. PTA-125180; wherein the composition is in the form of a dry powder, a dry powder suspended in an oil, or as a solution; and/or wherein the composition further comprises a cryoprotectant; and/or wherein the composition further comprises a stabilizer.

**[0003b]** In another aspect, the present disclosure provides a use of the composition of any one of the preceding claims in the manufacture of a medicament for improving the health of a mammalian gastrointestinal tract, comprising that the composition is to be administered to a mammal in need thereof.

## Composition with bacteria having H5 gene cluster: amount of bacteria

**[0004]** The invention provides a composition comprising a Bifidobacterium longum subsp. infantis comprising a functional H5 gene cluster and at least one oligosaccharide having a Type I or Type II core, where the H5 cluster comprises at least B1oh_2175, Blon_2l76, and Blon_2l77, more typically where the functional H5 cluster comprises Blon_2l7l,

B1oh_2173, B1oh_2174, B1oh_2175, B1oh_2176, Blon_2l77, and galT. An organism with a functional H5 cluster will transport and consume LNB oligosaccharides. In one preferred mode if the invention, the Bifidobacterium longum subsp. infantis is Bifidobacterium longum subsp. infantis EVC001 deposited under ATCC Accession No. PTA-125180. The

Bifidobacterium may be present in the composition of this invention at a concentration of from 1 million cfu/g to 500 billion cfu/g, and/or the Bifidobacterium may be present at a concentration of from 5 Billion cfu/g to 100 Billion cfu/g, and/or the Bifidobacterium may be present at a concentration of from 10 Billion cfu/g to 50 Billion cfu/g. Alternatively, the Bifidobacterium may be present in the composition at a concentration of from 50 million to 4 or 5 billion cfu/g.

## Activated bacteria - functionality

**[0005]** Preferably, the Bifidobacterium longum subsp. infantis in the compositions of this invention is activated. In the compositions of this invention, the Bifidobacterium preferably contains a LNB transport system capable of internalizing one or more oligosaccharides before the oligosaccharide is hydrolyzed and is capable of hydrolyzing the internalized

oligosaccharide, where the oligosaccharide has the structure of an oligosaccharide found in a mammalian milk, and the mammalian milk may be human, bovine, pig, rabbit, goat, sheep, camel milk, or mixtures thereof. Such Bifidobacterium will have a higher binding affinity to mammalian mucosal cells than Bifidobacteria of the same species cultivated in the absence of complex oligosaccharides.

## Claims

1. A composition comprising a *Bifidobacterium longum* subsp. *infantis* comprising a functional H5 gene cluster, wherein the *Bifidobacterium longum* subsp. *infantis* is *Bifidobacterium longum* subsp. *infantis* EVC001 deposited under ATCC Accession No. PTA-125180;

wherein the composition is in the form of a dry powder, a dry powder suspended in an oil, or as a solution; and/or wherein the composition further comprises a cryoprotectant; and/or wherein the composition further comprises a stabilizer.

2. The composition of claim 1, wherein the H5 cluster comprises Blon_2175, Blon_2176, and Blon_2177; or

wherein the functional H5 cluster comprises Blon_2171, Blon_2173, Blon_2174, Blon_2175, Blon_2176, Blon_2177, and galT; or

wherein the *Bifidobacterium* comprises upregulated genes selected from the group consisting of Blon_0042, Blon_R0015, Blon_R0017, Blon_R0021, Blon_R0022, and combinations thereof; or

wherein the *Bifidobacterium* comprises downregulated genes selected from the group consisting of Blon_0518, Blon_0785, Blon_2167, Blon_2168 and combinations thereof; or

wherein the Bifidobacterium comprises an upregulated Blon_0042 gene; or

wherein the Bifidobacterium comprises a downregulated Blon_2168 gene; or

wherein the *Bifidobacterium* comprises upregulated genes selected from the group consisting of Blon_0879, Blon_0880, Blon_0881, Blon_0882, Blon_2177, Blon_2334, Blon_2335, Blon_2336, Blon_2337, Blon_2338, Blon_2339, Blon_2343, Blon_2344, Blon_2346, Blon_2347, and Blon_2331; or

wherein the *Bifidobacterium* expresses a gene coding for a sialidase or a fucosidase; or

wherein the *Bifidobacterium* expresses a gene coding for a sialic acid or a fucose transporter.

3. The composition of any one of claims 1-2, wherein the *Bifidobacterium longum* subsp. *infantis* is activated, or

wherein the activator is from the group consisting of N-acetyl-glucosamine/galactosamine (NAG), Dimeric N-acetylglucosamine, Fucose, Sialic Acid, Lacto-N-biose (LNB), Galacto-N-biose and Fuc α1,2Galβ; or

wherein the composition further comprises an activator selected from the group consisting of any one of N-acetyl-glucosamine/galactosamine (NAG), Dimeric N-acetylglucosamine, Fucose, Sialic Acid, Lacto-N-biose, Galacto-N-biose and Fuc α1,2Galβ; or

wherein the *Bifidobacterium* contains a LNB transport system capable of internalizing one or more oligosaccharide having a Type I or Type II core before the oligosaccharide is hydrolyzed and is capable of hydrolyzing the internalized oligosaccharide; or

wherein the oligosaccharide can be obtained from mammalian milk selected from human, bovine, pig, rabbit, goat, sheep, camel milk, or mixtures thereof; or

wherein the *Bifidobacterium* has been cultured in the presence of at least one mammalian milk oligosaccharide; or

wherein the *Bifidobacterium* has a higher binding affinity to mammalian mucosal cells than *Bifidobacteria* of the same species cultivated in the absence of complex oligosaccharides.

4. The composition of any one of claims 1-3, wherein the *Bifidobacterium* is present at a concentration of from 1 Million cfu/g to 500 Billion cfu/g; or

wherein the *Bifidobacterium* is present at a concentration of from 5 Billion cfu/g to 100 Billion cfu/g; or

wherein the *Bifidobacterium* is present at a concentration of from 10 Billion cfu/g to 50 Billion cfu/g or from 50 million cfu/g to 5 billion cfu/g.

5. The composition of any one of claims 1-4, further comprising an isolated complex oligosaccharide;

wherein the complex oligosaccharide is a mammalian milk oligosaccharide (MMO); or

wherein the complex oligosaccharide is isolated from a mammalian milk source; or

wherein the mammalian milk source is human, bovine, pigs, rabbits, goats, sheep, or camel; or

wherein the mammalian milk oligosaccharide (MMO) comprises oligosaccharide molecules found in human milk oligosaccharides (HMO), bovine milk oligosaccharides (BMO),

bovine colostrum oligosaccharides (BCO), goat milk oligosaccharides (GMO), or a combination thereof; or

wherein the bovine source is from bovine milk, bovine colostrum, bovine colostrum concentrate, or mixtures thereof; or

wherein the bovine colostrum oligosaccharide comprises any of Hex(4); Hex(4) HexNAc(2); or Hex(3) HexNAc(1) NeuAc(1) at levels greater than 1%; or

wherein the complex oligosaccharide is from whey permeate; or

wherein the mammalian milk oligosaccharide (MMO) comprises lacto-N-biose, lacto-N-triose, N-acetyllactosamime, lacto-N-neotriose, lacto-N-tetraose, lacto-N-neotetraose, fucosyllactose, lacto-N- fucopentose, lactodifucotetraose, sialyllactose, disialyllactone-N-tetraose, 2'- fucosyllactose, 3'-sialyllactoseamin, 3'-fucosyllactose, 3'-sialyl-3-fucosyllactose, 3'- sialyllactose, 6'-sialyllactosamine, 6'-sialyllactose, difucosyllactose, lacto-N- fucosylpentose I, lacto-N-fucosylpentose II, lacto-N-fucosylpentose III, lacto-N- fucosylpentose V, sialyllacto-N-tetraose, their derivatives, or combinations thereof.

6. The composition of claim 5, wherein the complex oligosaccharide comprises at least one of (3Hex,4HexNac,1Fuc), (1gal,1glcnac,1neuac), or (1Glu,1Gal,1NeuAc (3' or 6')); or

wherein the complex oligosaccharide is less than 50% fucosylated; or

wherein the complex oligosaccharide comprise one of the following ratios of constituents: 1) a ratio of Hex(2) NeuAc(1) : hex(2) hexnac(1) less than 5.0; 2) a ratio of hex(2) hexnac(1) : hex (3) hexnac(1) of greater than 1.0; 3) a ratio of hex(2) hexnac(1) : hex (3) hexnac(2) of greater than 2.0; 4) a ratio of hex(3) : hex (3) hexnac(1) neuac(1) of less than 100; or 5) a ratio of hex(2) hexnac(1) : hex (4) NeuAc(2) NeuGc(1) of greater than 10.

7. The composition of claim 5 or 6, wherein the complex oligosaccharide is at least 20% of the weight of the composition; or

wherein the complex oligosaccharide is at least 50% of the weight of the composition; or

wherein the complex oligosaccharide is at least 80% of the weight of the composition.

8. The composition of any one of claims 5 to 7, wherein fucosyllactose and/or sialyllactose comprises 1-5% of the total oligosaccharides; or

wherein fucosyllactose and/or sialyllactose comprises 5-20% of the total oligosaccharides; or

wherein fucosyllactose and/or sialyllactose comprises 20-50% of the total oligosaccharides; or

wherein the mass ratio of the complex oligosaccharide to the fucosyllactose and/or sialyllactose is from 20: 1 to 1: 10; or

wherein the complex oligosaccharide comprises a plurality of oligosaccharides with 3 to 10 residues (DP3-10 oligosaccharides).

9. The composition of any one of claims 5-8, wherein the mammalian milk oligosaccharide (MMO) comprises lacto-N-biose; or

wherein the mammalian milk oligosaccharide (MMO) comprises lacto-N-triose

10. The composition of any one of claims 5-9, wherein the complex oligosaccharide comprises a plant-derived oligosaccharide;

optionally wherein the plant oligosaccharide is from carrots, peas, broccoli, onions, tomatoes, peppers, rice, wheat, oats, bran, oranges, cocoa, olives, apples, grapes, sugar beets, cabbage, com, or a mixture thereof; or

optionally wherein the plant oligosaccharide is from orange peels, cocoa hulls, olive pomace, tomato skins, grape pomace, com silage, or a mixture thereof; or

optionally wherein the plant-derived oligosaccharides are between 2 and 10 sugar residues (DP2-DP10), between 3 and 10 sugar residues (DP3-DP10), between 5 and 10 sugar resides (DP5-DP10), or up to DP20.

11. The composition of any one of claims 5-10, wherein the composition provides a total dietary intake of oligosaccharide in an amount of 0.001-100 grams per day; or

wherein the oligosaccharide is in an amount of 1-20 grams, 3-20 grams, or 5-10 grams; or

wherein the oligosaccharide is in an amount of 10, 15, 20, 25, 30, 35, 40, 45, or 50 grams; or

wherein at least one of the oligosaccharides has a Type I core; or

wherein at least one of the oligosaccharides has a Type II core.

12. The composition of any one of claims 1-11, wherein the composition further comprises galactooligosaccharide (GOS); or

wherein the composition further comprises a protein source rich in threonine, N-acetyl-threonine, gamma-glutamylthreonine, or a combination thereof; or

wherein the composition further comprises a secondary metabolite;

optionally wherein the secondary metabolite comprises a short chain fatty acid; or

wherein the secondary metabolite comprises acetate, lactate, or combinations thereof.

13. The composition of any one of claims 1-12, wherein the composition is in the form of a dry powder, a dry powder suspended in an oil, or as a solution;

wherein the dry powder is spray dried or freeze-dried; or

wherein the composition is freeze-dried in the presence of a suitable cryoprotectant.

14. The composition of any one of claims 1-13, wherein the composition further comprises a cryoprotectant;

optionally wherein the cryoprotectant is glucose, lactose, raffinose, sucrose, trehalose, adonitol, glycerol, mannitol, methanol, polyethylene glycol, propylene glycol, ribitol, alginate, bovine serum albumin, carnitine, citrate, cysteine, dextran, dimethyl sulfoxide, sodium glutamate, glycine betaine, glycogen, hypotaurine, peptone, polyvinyl pyrrolidone, or taurine, mammalian milk oligosaccharides, chitin, chitosan, other polysaccharides, or a combination thereof.

15. The composition of any of one of claims 1-14, further comprising a stabilizer;

optionally wherein the stabilizer is a flow agent; or

optionally wherein the stabilizer is a milk protein.

16. The composition of any one of claims 1-15, wherein the composition is a powder with a water activity level of less than 0.35, less than 0.30, less than 0.25, less than 0.2, less than or less than 0.1; and wherein the composition is:

an anhydrous composition; or

suspended in an oil; or

in the form of a dry powder suspended in an oil.

17. The composition of claim 16, wherein the oil is a medium chain triglyceride.

wherein the composition is suspended in syrup having oligosaccharide at least 57% where water activity is low enough to keep *Bifidobacterium* dormant.

18. The composition of any one of claims 1-17, wherein the composition is in the form of a packet, sachet, orally disintegrating tablet, foodstuff, capsule, lozenge, effervescent tablet, suppository, enema, capsule, dry powder, dry powder suspended in an oil, chewable composition, syrup, or gel; or

wherein the capsule or tablet has an enteric coating; or

wherein the enteric coating comprises one or more of fatty acids, waxes, shellac, plastics, plant fibers, methyl acrylate-methacrylic acid copolymers, cellulose acetate succinate, hydroxy propyl methyl cellulose phthalate, hydroxy propyl methyl cellulose acetate succinate, polyvinyl acetate phthalate (PVAP), methyl methacrylate-methacrylic acid copolymers, cellulose acetate trimellitate, sodium alginate, and zein; or

wherein the composition is a pharmaceutical composition, dietary supplement, nutritional product, food product, probiotic, and/or prebiotic; or

wherein the composition is formulated as a unit dose medicament.

19. Use of the composition of any one of the preceding claims in the manufacture of a medicament for improving the health of a mammalian gastrointestinal tract, comprising that the composition is to be administered to a mammal in need thereof.

20. The use of claim 19, wherein the use is for increasing levels of said administered *Bifidobacterium* in the feces of that mammal to greater than 10% of the total microbiome found in that feces; or

wherein a complex oligosaccharide is to be administered prior to the administration of *Bifidobacterium*; or

wherein a complex oligosaccharide is to be administered contemporaneously with the administration of *Bifidobacterium*; or

wherein a complex oligosaccharide is to be administered after the administration of *Bifidobacterium*; or

wherein the complex oligosaccharide is to be provided in a daily dose of from 1 to 20 grams; or

wherein the complex oligosaccharide is to be provided in a daily dose of from 1 to 10 grams; or

wherein the *Bifidobacterium* is to be provided in a daily dose of from 1 million to 100 billion colony forming units (CFUs); or

wherein the *Bifidobacterium* is to be provided in a daily dose of from 5 to 50 billion CFUs; or

wherein the subject in need thereof is to be administered a dose once daily or in multiple, optionally two, three, four, five, six, sub doses to be administered at appropriate intervals throughout the day; or

wherein the mammal is a human, a cow, a pig, a rabbit, a goat, a sheep, a cat, a dog, a horse, or a camel; or

wherein the mammal is a human infant; or

wherein the infant was delivered via cesarean section; or

wherein the infant was delivered vaginally; or

wherein the infant is being fed with infant formula which contains no appreciable quantity of mammalian milk oligosaccharides; or

wherein the infant is from birth to about 36 months post-conception; or

wherein the infant is from birth to weaning age; or

wherein the infant is nursed by a mother who is FUC-2 deficient as measured by a genetic test or the absence of complex fucosylated oligosaccharides in her milk; or

wherein the human is a pregnant woman; or

wherein the pregnant woman is in at least the third trimester of pregnancy.