CLAIMS

1. A method for producing sialylated oligosaccharides, the method comprising the steps of

   a) providing at least one genetically engineered cell which comprises a heterologous sialyltransferase, said heterologous sialyltransferase possessing an α-2,3-sialyltransferase activity and/or an α-2,6-sialyltransferase activity for transferring a sialic acid residue from its nucleotide-activated form as donor substrate to an acceptor molecule, wherein the acceptor molecule is selected from the group consisting of lacto-*N*-triose II and human milk oligosaccharides, wherein the heterologous sialyltransferase is selected from

      i) polypeptides comprising an amino acid sequence of any one of SEQ ID Nos: 34 to 37, 39 to 42, 44, 46 to 48, and 50 to 66; and

      ii) polypeptides comprising or consisting of an amino acid sequence having a similarity of at least 80 % to any one of the amino acid sequences of SEQ ID NOs: 34 to 37, 39 to 42, 44, 46 to 48, and 50 to 66; and

      iii) fragments of polypeptides comprising an amino acid sequence of any one of SEQ I DNOs: 34 to 37, 39 to 42, 44, 46 to 48, and 50 to 66 or any one of the polypeptides of ii);

   b) cultivating the at least one cell in a fermentation broth and under conditions permissive for the production of said sialylated oligosaccharide; and optionally

   c) recovering said sialylated oligosaccharide.

2. The method according to claim 1, wherein the sialylated oligosaccharide is selected from the group consisting of LST-a, LST-b, LST-c and DSLNT.

3. The method according to any one of claims 1 or 2, wherein the fermentation broth contains at least one carbon source, the at least one carbon source is preferably selected from the group consisting of glucose, fructose, sucrose, glycerol, and combinations thereof.

4. The method according to any one of claims 1 to 3, wherein the fermentation broth contains at least one compound selected from the group consisting of *N*-acetylglucosamine, galactose and sialic acid.

5. The method according to any one of claims 1 to 3, wherein the at least one cell is cultivated in the absence of and/or without addition of one or more compounds selected from the group consisting of *N*-acetylglucosamine, galactose and sialic acid.

6. The method according to any one of claims 1 to 5, wherein the at least one cell is cultivated in the presence of at least one selected from the group consisting of lactose, lacto-*N*-triose II, LNT and LN*n*t.

7. A genetically engineered cell for producing sialylated oligosaccharides, wherein the genetically engineered cell comprises a heterologous sialyltransferase, said heterologous sialyltransferase being capable of possessing an α-2,3-sialyltransferase activity and/or an α-2,6-sialyltransferase activity for transferring a sialic acid residue from its nucleotide-activated form as donor substrate to an acceptor molecule,
wherein the heterologous sialyltransferase is selected from
i) polypeptides comprising an amino acid sequence of any one of SEQ ID Nos: 34 to 37, 39 to 42, 44, 46 to 48, and 50 to 66; and
ii) polypeptides comprising or consisting of an amino acid sequence having a similarity of at least 80 % to any one of the amino acid sequences of SEQ ID NOs: 34 to 37, 39 to 42, 44, 46 to 48, and 50 to 66; and
iii) fragments of polypeptides comprising an amino acid sequence of any one of SEQ ID NOs: 34 to 37, 39 to 42, 44, 46 to 48, and 50 to 66 or any one of the polypeptides of ii); and

wherein the acceptor molecule is selected from the group consisting of lacto-*N*-triose II and human milk oligosaccharides.

8. The method according to any one of claims 1 to 6 or the genetically engineered cell according to claim 7, wherein the acceptor molecule is a human milk oligosaccharide selected from the group consisting of trisaccharides, tetrasaccharides and pentasaccharides, preferably selected from the group consisting of 3-fucosyllactose, lacto-*N*-tetraose, lacto-*N*-*neo*tetraose, sialyllacto-*N*-tetraose a, sialyllacto-*N*-tetraose b, lacto-*N*-fucopentaose I and lacto-*N*-fucopentaose II.

~~9. The method according to any one of claims 1 to 6 or 8, or the genetically engineered cell according to claim 7 or 8, wherein the heterologous sialyltransferase, is selected from the group consisting of~~
~~i. polypeptides comprising or consisting of an amino acid sequence as represented by any one of SEQ ID NOs: 34 to 66;~~

~~ii. polypeptides comprising or consisting of an amino acid sequence having a sequence similarity of at least 80 % to any one of the amino acid sequences as represented by any one of SEQ ID NOs: 34 to 66; and~~

~~iii. fragments of any one of the polypeptides of i. and ii.~~

~~10.~~9. The method according to any one of claims 1 to 6 or 8~~ to 9~~, or the genetically engineered cell according to any one of claims 7 to ~~9~~8, wherein

the genetically engineered cell comprises a nucleic acid molecule comprising a nucleotide sequence which encodes the heterologous sialyltransferase, said nucleotide sequence being selected from the group consisting of

i. nucleotide sequences encoding a polypeptide as represented by any one of SEQ ID NOs: 34 to 37, 39 to 42, 44, 46 to 48, and 50 to 66~~34 to 66~~;

~~ii. nucleotide sequences as represented by any one of SEQ ID NOs: 1 to 33;~~

~~iii. nucleotide sequences having at least 80% sequence similarity to one of the nucleotide sequences encoding a polypeptide as represented by any one of SEQ ID NOs: 34 to 66;~~

~~iv. nucleotide sequences having a sequence similarity of at least 80 % to any one of the nucleotide sequences represented by SEQ ID NOs: 1 to 33;~~

ii. nucleotide sequences encoding polypeptides comprising or consisting of an amino acid sequence having a similarity of at least 80 % to any one of the amino acid sequences of SEQ ID NOs: 34 to 37, 39 to 42, 44, 46 to 48, and 50 to 66:

iii. nucleotide sequences encoding fragments of polypeptides comprising an amino acid sequence of any one of SEQ ID NOs: 34 to 37, 39 to 42, 44, 46 to 48, and 50 to 66 or any one of the polypeptides of ii);

~~v.~~iv. nucleotide sequences which are complementary to any one of the nucleotide sequences of i., ii.~~,~~. and iii.~~ and iv.~~; and

~~vi.~~v. fragments of any one of the nucleotide sequences of i., ii., iii.~~,~~. and iv.~~ and v.~~,

wherein said nucleotide sequence is operably linked to at least one nucleic acid expression control sequence effecting transcription and/or translation of said nucleotide sequence encoding the heterologous sialyltransferase in the genetically engineered cell.

~~11~~10. The method according to any one of claims 1 to 6 or 8 to ~~10~~9, or the genetically engineered cell according to any one of claims 7 to ~~10~~9, wherein the genetically engineered cell possesses an increased production of one or more nucleotide-activated sugars selected from the group consisting of CMP-*N*-acetylneuraminic acid, UDP-*N*-acetylglucosamine, UDP-galactose and GDP-fucose as compared to cell before being genetically engineered.

~~12.~~ 11. The method according to any one of claims 1 to 6 or 8 to ~~11~~10, or the genetically engineered cell according to any one of claims 7 to ~~11~~10, wherein the genetically engineered cell possesses an overexpression, as compared to the cell prior to be genetically engineered, of one or more genes encoding for a polypeptide being capable of possessing an enzymatic activity selected from the group consisting of L-glutamine:D-fructose-6-phosphate aminotransferase, *N*-acetylglucosamine-1-phosphate uridyltransferase, glucosamine-1-phosphate acetyl transferase, phosphoglucosamine mutase, glucosamine-6-phosphate-*N*-acetyltransferase, *N*-acetylglucosamine-2-epimerase, UDP-*N*-acetylglucosamine-2-epimerase, sialic acid synthase, phosphoenolpyruvate synthase, CMP-sialic acid synthetase, UDP-galactose-4-epimerase, galactose-1-phosphate uridylyltransferase, phosphoglucomutase, glucose-1-phosphate uridylyltransferase, phosphomannomutase, mannose-1-phosphate guanosyltransferase, GDP-mannose-4,6-dehydratase, GDP-L-fucose synthase and fucosekinase/L-fucose-1-phosphate-guanyltransferase.

~~13.~~12. The method according to any one of claims 1 to 6 or 8 to ~~12~~11, or the genetically engineered cell according to any one of claims 7 to ~~12~~11, wherein the cell lacks or possesses a decreased activity, as compared to the prior to be genetically engineered, of one or more enzymatic activities selected from the group consisting of β-galactosidase, a glucosamine-6-phosphate deaminase, a *N*-acetylglucosamine-6-phosphate deacetylase, a *N*-acetylmannosamine kinase, a *N*-acetylmannosamine-6-phosphate epimerase and a *N*-acetylneuraminic acid aldolase.

~~14.~~13. The method according to any one of claims 1 to 6 or 8 to ~~13~~12, or the genetically engineered cell according to any one of claims 7 to ~~13~~12, wherein one or more of the genes encoding a β-galactosidase, a glucosamine-6-phosphate deaminase, a *N*-acetylglucosamine-6-phosphate deacetylase, a *N*-acetylmannosamine kinase, a *N*-acetylmannosamine-6-phosphate epimerase and a *N*-acetylneuraminic acid aldolase has been deleted or its expression has been inactivated in the genetically engineered cell.

~~15.~~14. The method according to any one of claims 1 to 6 or 8 to ~~14~~13, or the genetically engineered cell according to any one of claims 7 to ~~14~~13, wherein the at least one cell comprises at least one selected from the group consisting of a functional lactose permease, a functional fucose permease and a functional sialic acid transporter (importer), preferably comprises and expresses at least one nucleotide sequence

encoding one selected from the group consisting of a functional lactose permease, a functional fucose permease and a functional sialic acid transporter (importer).

~~16.~~15. The method according to any one of claims 1 to 6 or 8 to ~~15~~14, or the genetically engineered cell according to any one of claims 7 to ~~15~~14, wherein the cell possesses activity of at least one glycosyltransferase selected from the group consisting of a β-1,3-N-acetylglucosaminyltransferase, a β-1,3-galactosyltransferase, a β-1,4-galactosyltransferase, a α-2,3-sialyltransferase and a α-2,6-sialyltransferase.

~~17. A method for producing a sialylated oligosaccharide, the method comprising:~~

~~a) providing a sialyltransferase in a reaction mixture, the sialyltransferase being capable of capable of possessing an α-2,3-sialyltransferase activity and/or an α-2,6-sialyltransferase activity for transferring a sialic acid residue from its nucleotide-activated form as donor substrate to an acceptor molecule, wherein the acceptor molecule is selected from the group consisting of lacto-*N*-triose II and human milk oligosaccharides;~~

~~b) contacting said sialyltransferase with a donor substrate and an acceptor molecule, wherein the acceptor molecule is selected from the group consisting of lacto-*N*-triose II and human milk oligosaccharides.~~

~~18. Use of a sialylated oligosaccharide produced by a method according to any one of claims 1 to 6 and 8 to 17 for manufacturing a nutritional composition, preferably an infant composition.~~

~~19. A nutritional composition comprising at least one sialylated oligosaccharide, wherein said at least one sialylated oligosaccharide has been produced by a method according to any one of claims.~~