Claims

1. A method for the fermentative production of a saccharide comprising at least one *N*-acetylneuraminic acid moiety, the method comprising the steps of
   a) providing at least one genetically engineered microbial cell which comprises
      (i) a sialic acid biosynthesis pathway comprising a glucosamine-6-phosphate *N*-acetyltransferase, an *N*-acetylglucosamine-6-phosphate phosphatase and an *N*-acetylglucosamine 2-epimerase;
      (ii) a cytidine 5'-monophospho-(CMP)-*N*-acetylneuraminic acid synthetase, wherein the genetically engineered microbial cell contains a nucleic acid molecule comprising and expressing a nucleotide sequence encoding the cytidine 5'-monophospho-(CMP)-N-acetylneuraminic acid synthetase, the nucleotide sequence being selected from the group consisting of
      i) nucleotide sequences encoding a polypeptide as represented by SEQ ID NO. 91; and
      ii) a nucleotide sequence as represented by SEQ ID NO. 92; and
      (iii) a heterologous sialyltransferase;
   b) cultivating the at least one genetically-engineered microbial cell in a fermentation broth and under conditions permissive for the production of said saccharide comprising at least one *N*-acetylneuraminic acid moiety; and optionally
   c) recovering said saccharide comprising at least one *N*-acetylneuraminic acid moiety.

2. A genetically-engineered microbial cell for the fermentative production of a saccharide comprising at least one *N*-acetylneuraminic acid moiety, wherein the genetically-engineered microbial cell comprises
   (i) a synthetic sialic acid biosynthesis pathway comprising a glucosamine-6-phosphate *N*-acetyltransferase, an *N*-acetylglucosamine-6-phosphate phosphatase and an *N*-acetylglucosamine 2-epimerase;
   (ii) a cytidine 5'-monophospho-(CMP)-*N*-acetylneuraminic acid synthetase, wherein the genetically engineered microbial cell contains a nucleic acid molecule comprising and expressing a nucleotide sequence encoding the cytidine 5'-monophospho-(CMP)-N-acetylneuraminic acid synthetase, the nucleotide se-

quence being selected from the group consisting of nucleotide sequences encoding a polypeptide as represented by SEQ ID NO. 91

i) nucleotide sequences encoding a polypeptide as represented by SEQ ID NO. 91;

ii) a nucleotide sequence as represented by SEQ ID NO. 92; and

(iii) a heterologous sialyltransferase.

~~3. The method according to claim 1 or the genetically engineered microbial cell according to claim 2, wherein the sialic acid biosynthesis pathway further comprises a) an N-acetylglucosamine-6-phosphate phosphatase and an N-acetylglucosamine 2-epimerasel and/or b) an N-acetylglucosamine-6-phosphate epimerase and an N-acetylmannosamine-6-phosphate phosphatase.~~

~~4. The method according to claim 1 to 3 or the genetically engineered microbial cell according to claim 2 or 3, wherein the genetically engineered microbial cell contains a nucleic acid molecule comprising and expressing a nucleotide sequence selected from the group consisting of~~

~~i) nucleotide sequences encoding a polypeptide as represented by SEQ ID NO. 91;~~

~~ii) a nucleotide sequences as represented by SEQ ID NO. 92;~~

~~iii) nucleotide sequences having at least 80%, 90%, 95%, 96%, 97%, 98%, 99% or more than 99% sequence similarity to the nucleotide sequences encoding a polypeptide as represented by SEQ ID NO. 91;~~

~~iv) nucleotide sequences having at least 80%, 90%, 95%, 96%, 97%, 98%, 99% or more than 99% sequence similarity to the nucleotide sequences encoding a polypeptide as represented by SEQ ID NO. 92;~~

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

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

~~5~~3. The method according to claim 1 ~~to 4~~ or the genetically engineered microbial cell according to ~~any one of claims 2 to 4~~claim 2, wherein the genetically engineered mi-

crobial cell contains a heterologous sialyltransferase that is preferably selected from the group consisting of

I. polypeptides comprising or consisting of an amino acid sequence encoded by a nucleotide sequence of ~~as represented by~~ any one of SEQ ID NOs: 1 to 33;

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 as encoded by a nucleotide sequence of~~represented by~~ any one of SEQ ID NOs: 1 to 33; and

III. fragments of any one of the polypeptides of I. and II.

~~6~~4. The method according to claim 1 or ~~5~~ 3 or the genetically engineered microbial cell according to claim 2 or ~~5~~3, wherein the genetically engineered microbial cell contains a nucleic acid molecule which comprises and expresses a nucleotide sequence encoding the heterologous sialyltransferase possessing α2,3-sialyltransferase activity or α2,6-sialyltransferase activity, the nucleotide sequence being selected from the group consisting of

i. nucleotide sequences ~~encoding a polypeptide~~ as represented by any one of SEQ ID NOs: 1 to 33;

~~ii.~~ nucleotide sequences encoding a polypeptide as represented by any one of SEQ ID NOs: 34 to 66~~;~~

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

~~iv. nucleotide sequences having a sequence similarity of at least 80% to any one of the nucleotide sequences which are complementary to any one of the nucleotide sequences of i., ii., iii. and iv; and~~

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

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

~~7~~5. The method according to any one of claims 1 and 3 to ~~6~~ 4 or the genetically engineered microbial cell according to any one of claims 2 to ~~6~~4, wherein the sialyltransferase transfers a sialic acid residue from a donor substrate to an acceptor

molecule, wherein the acceptor molecule is selected from the group consisting of *N*-acetylglucosamine, galactose, *N*-acetylgalactosamine, lactose, lactulose, *N*-acetyllactosamine, lacto-*N*-biose, lactulose, melibiose, raffinose, lacto-*N*-triose II, 2'-fucosyllactose, 3-fucosyllactose, 3'-sialyllactose, 6'-sialyllactose, 3'-sialyl-*N*-acetyllactosamine, 6'-sialyl-*N*-acetyllactosamine, 3'-galactosyllactose, 6'-galactosyllactose, lacto-*N*-tetraose, lacto-*N*-*neo*tetraose, 2'3-difucosyllactose, 3-fucosyl-3'-sialyllactose, 3-fucosyl-6'-sialyllactose, sialyllacto-*N*-tetraose a, sialyllacto-*N*-tetraose b, sialyllacto-*N*-tetraose c, lacto-*N*-fucopentaose I, lacto-*N*-fucopentaose II, lacto-*N*-fucopentaose III, lacto-*N*-fucopentaose V, lacto-*N*-*neo*fucopentaose I and lacto-*N*-*neo*fucopentaose V.

~~8~~6. The method according to any one of claims 1 and ~~1~~ 3 to ~~7~~5, 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.

~~9~~7. The method according to any one of claims 1 and 3 to ~~8~~6, wherein the at least one genetically engineered microbial cell is cultivated in the absence of and/or without addition of one or more selected from the group consisting of glucosamine, *N*-acetylglucosamine, *N*-acetylmannosamine and *N*-acetylneuraminic acid.

~~10~~8. The method according to any one of claims 1 and 3 to ~~9~~7, wherein the saccharide comprising at least one *N*-acetylneuraminic acid moiety is selected from the group consisting of 3'-sialylgalactose, 6'-sialylgalactose, 3'-sialyl-*N*-acetyllactosamine, 6'-sialyl-*N*-acetyllactosamine, 3'-sialyllactose, 6'-sialyllactose, sialyllacto-*N*-tetraose a, sialyllacto-*N*-tetraose b, sialyllacto-*N*-tetraose c, fucosyl-sialyllacto-*N*-tetraose a, fucosyl-sialyllacto-*N*-tetraose b, fucosyl-sialyllacto-*N*-tetraose c, disialyllacto-*N*-tetraose, fucosyldisialyllacto-*N*-tetraose I, fucosyldisialyllacto-*N*-tetraose II.

~~11~~9. Use of a genetically engineered microbial cell according to any one of claims 2 to ~~7~~5 for the production of a sialylated saccharide in a whole cell fermentation process.

~~12. A sialylated saccharide, wherein said sialyalted saccharide has been produced by the method according to any one of claims 1 to 10 or by the use of the genetically engineered microbial cell according to any one of claims 2 to 7.~~

~~13. Use of the sialylated saccharide according to claim 12 for the manufacture of a nutritional composition.~~

~~14. A nutritional composition containing at least one sialylated saccharide, preferably at least one sialylated oligosaccharide, which has been produced by the method according to any one of claim 1 to 9 or by the genetically engineered microbial cell according to any one of claims 2 to 7.~~

~~15. The nutritional composition according to claim 14, wherein the nutritional composition is selected from the group consisting of medicinal, pharmaceutical, formulations, infant formula and dietary supplements.~~