Claims

1. A method for producing a fucolactopentaose, the method comprising the steps of:
   a) providing at least one genetically engineered cell that comprises a β-1,3-N-acetylglucosaminyltransferase, a β-1,3-galactosyltransferase, and which has been genetically engineered to express a heterologous fucosyltransferase, wherein said heterologous fucosyltransferase transfers a fucose residue from a donor substrate to an acceptor molecule, said acceptor molecule being lacto-N-tetraose (LNT);
   b) cultivating the at least one genetically engineered cell in the presence of at least one carbon source, and under conditions suitable for the at least one genetically engineered cell to transfer the fucose residue from the donor substrate to the acceptor molecule; and
   c) optionally recovering the fucosylated oligosaccharide.

2. The method according to claim 1, wherein the heterologous fucosyltransferase is selected from the group consisting of polypeptides as represented by any one of SEQ ID NOs: 16 to 30, functional variants of the polypeptides as represented by any one of SEQ ID NOs: 16 to 30, wherein said functional variants have an amino acid sequence which has at least 80 % identity to any one of the amino acid sequences as represented by any one of SEQ ID NOs: 16 to 30, functional fragments of the polypeptides as represented by any one of SEQ ID NOs: 16 to 30, and functional variants of the functional fragments of the polypeptides as represented by any one of SEQ ID NOs: 16 to 30, wherein said functional variants have an amino acid sequence which has at least 80 % identity to any one of the amino acid sequences as represented by any one of SEQ ID NOs: 16 to 30.

3. The method according to claim 1 or 2, wherein the heterologous fucosyltransferase is encoded by a nucleic acid molecule comprising
   i) a nucleotide sequence as represented by any one of SEQ ID NOs: 1 to 15;
   ii) a nucleotide sequence having a sequence identity of at least 80% to one of the nucleotides sequences as represented by any one of SEQ ID NOs: 1 to 15, preferably across the entire length of the sequence;

iii) a nucleotide sequence which encodes a polypeptide having an amino acid sequence as represented by any one of SEQ ID NOs: 16 to 30;

iv) a nucleotide sequence which encodes a polypeptide having an amino acid sequence which has at least 80% identity to any one of the amino acid sequences as represented by any one of SEQ ID NOs: 16 to 30;

v) a nucleotide sequence encoding a functional fragment of any one of the polypeptides according to iii) and iv); or

vi) wherein the nucleic acid molecule hybridizes to a complementary strand of a nucleic acid molecule according to i), ii), iii), iv) or v) under stringent conditions, wherein stringent conditions refers to hybridizing in 4 x SSC at 65 °C and subsequent multiple washings in 0.1 x SSC at 65 °C for – in total - about 1 hour, or to hybridizing at 68 °C in 0.25 M sodium phosphate, pH 7.2, 7 % (w/v) SDS, 1 mM EDTA and 1 % (W/v) BSA for 16 hours and subsequent washing two times with 2 x SSC and 0.1 % (w/v) SDS at 68 °C.

4. A genetically engineered cell for producing a lactofucopentaose, wherein said cell comprises a β-1,3-N-acetylglucosaminyltransferase, a β-1,3-galactosyltransferase, and has been genetically engineered to express a heterologous fucosyltransferase, wherein said heterologous fucosyltransferase transfers a fucose residue from a donor substrate to an acceptor molecule, said acceptor molecule being lacto-N-tetraose.

5. The genetically engineered cell according to claim 4, wherein the heterologous fucosyltransferase is selected from the group consisting of polypeptides as represented by any one of SEQ ID NOs: 16 to 30, functional variants of the polypeptides as represented by any one of SEQ ID NOs: 16 to 30, wherein said functional variants have an amino acid sequence which has at least 80 % identity to any one of the amino acid sequences as represented by any one of SEQ ID NOs: 16 to 30, functional fragments of the polypeptides as represented by any one of SEQ ID NOs: 16 to 30, and functional variants of the functional fragments of the polypeptides as represented by any one of SEQ ID NOs: 16 to 30, wherein said functional variants have an amino acid sequence which has at least 80 % identity to any one of the amino acid sequences as represented by any one of SEQ ID NOs: 16 to 30.

6. The genetically engineered cell according to claim 4 or 5, wherein the heterologous fucosyltransferase is encoded by a nucleic acid molecule comprising

i) a nucleotide sequence as represented by any one of SEQ ID NOs: 1 to 15;

ii) a nucleotide sequence having a sequence identity of at least 80% to one of the nucleotides sequences as represented by any one of SEQ ID NOs: 1 to 15, preferably across the entire length of the sequence;

iii) a nucleotide sequence which encodes a polypeptide having an amino acid sequence as represented by any one of SEQ ID NOs: 16 to 30;

iv) a nucleotide sequence which encodes a polypeptide having an amino acid sequence which has at least 80% identity to any one of the amino acid sequences as represented by any one of SEQ ID NOs: 16 to 30;

v) a nucleotide sequence encoding a functional fragment of any one of the polypeptides according to iii) and iv); or

vi) wherein the nucleic acid molecule hybridizes to a complementary strand of a nucleic acid molecule according to i), ii), iii), iv) or v) under stringent conditions, wherein stringent conditions refers to hybridizing in 4 x SSC at 65 °C and subsequent multiple washings in 0.1 x SSC at 65 °C for – in total - about 1 hour, or to hybridizing at 68 °C in 0.25 M sodium phosphate, pH 7.2, 7 % (w/v) SDS, 1 mM EDTA and 1 % (W/v) BSA for 16 hours and subsequent washing two times with 2 x SSC and 0.1 % (w/v) SDS at 68 °C.

7. The genetically engineered cell according to any one of claims 4 to 6, wherein the cell has been genetically engineered to comprise an increased intracellular GDP-fucose production capability as compared to the cell prior to being genetically engineered, preferably in that the cell has been genetically engineered i) to express a gene encoding a bifunctional fucosekinase/L-fucose-1-phosphate-guanyltransferase catalysing the formation of GDP-fucose from L-fucose, or ii) to overexpress at least one of the genes encoding phosphomannomutase, mannose-1-phosphate guanosyltransferase, GDP-mannose-4,6-dehydratase, and GDP-L-fucose synthase.

8. The genetically engineered cell according to any one of claims 4 to 7, wherein the cell has been genetically engineered to possess an increased import of exogenous L-fucose across its cell membrane, preferably in that the cell has also been genetically engineered to express or overexpress - as compared to the progenitor cell before being genetically engineered - one nucleotide sequence selected from the group consisting of nucleotide sequences encoding the major facilitator transporter FucP from *E. coli*., nucleotide sequences encoding functional variants of the major facilitator transporter FucP from *E. coli*, nucleotide sequences encoding functional fragments of the major facilitator transporter FucP from *E. coli,* and nucleotide sequences encoding functional variants of the functional fragments of the major facilitator transporter FucP from *E. coli.*

9. The genetically engineered cell according to any one of claims 4 to 8, wherein the cell has also been genetically engineered to prevent depletion of the cell's intracellular GDP-fucose pool, preferably in the expression of the cell's gene encoding WcaJ, which catalyses the first step in colonic acid synthesis, is impaired or inactivated, or in that the nucleotide sequence of the gene encoding WcaJ has been altered such that an enzymatically inactive polypeptide is encoded by the altered WcaJ gene.

10. The genetically engineered cell according to any one of claims 4 to 9, wherein the cell has also been genetically engineered in that the genes *fucI* and/or *fucK*, encoding the L-fucose isomerase and the L-fuculose kinase respectively, are deleted, the nucleotide sequence of *fucI* and/or *fucK* is altered to irreversibly inactivate the enzymatic activity of the corresponding polypeptide(s), or in that the expression of *fucI* and/or *fucK* is impaired.

11. The genetically engineered cell according to any one of claims 4 to 10, wherein the cell has also been genetically engineered to possess an increased capability of synthesizing one or more of CMP-*N*-acetylneuraminic acid, UDP-*N*-acetyl-glucosamine and UDP-galactose, as compared to the cell prior to being genetically engineered, wherein preferably said increased UDP-*N*-acetyl-glucosamine, UDP-galactose and more preferably, CMP-*N*-acetylneuraminic acid production capability comprises the overexpression of one or more genes

encoding for proteins comprising the following activities for a: L-glutamine: D-fructose-6-phosphate aminotransferase, *N*-acetyl glucosamine-1-phosphate uridyltransferase/glucosamine-1-phosphate acetyl transferase, phosphoglucosamine mutase, UDP-galactose-4'-epimerase, phosphoglucomutase, glucose-1-phosphate uridylyltransferase, glucosamine-6-phosphate acetyltransferase, *N*-acetylglucosamine 2-epimerase, UDP-*N*-acetylglucosamine-2-epimerase, phosphoenolpyruvate synthetase, sialic acid synthase, sialic acid permease, CMP-sialic acid synthetase.

12. A method for producing a lactofucopentaose, the method comprising the steps of:
    a) providing a fucosyltransferase in a reaction mixture, the fucosyltransferase being capable of transferring a fucose residue from a donor substrate to an acceptor molecule, said acceptor molecule being lacto-N-tetraose; and
    b) contacting said fucosyltransferase with a donor substrate comprising a fucose residue and an acceptor molecule, wherein said acceptor molecule is lacto-N-tetraose.

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