Claims

1. A method for the production of a desired oligosaccharide by a genetically modified microbial host cell, wherein the desired oligosaccharide is a human milk oligosaccharide comprising a lacto-*N*-triose II (LNT-II; Glc*N*Ac(β1-3)Gal(β1-4)Gluc) as a core trisaccharide, the method comprising the steps of

   - providing a genetically modified microbial host cell that

     - comprises at least one recombinant glycosyltransferase,

     - has the expression or activity of at least one endogenous sugar export protein modified such, that the expression or activity of the sugar export protein is either (i) increased or (ii) decreased or inactivated as compared to a genetically unmodified host cell, so that (i) the export of a precursor of the desired oligosaccharide into the medium is either decreased or abolished, or (ii) the transport of the desired oligosaccharide is increased, respectively, as compared to a genetically unmodified host cell,

   - cultivating the host cell in a medium under conditions permissive for the production of the desired oligosaccharide, whereby the desired oligosaccharide is transported into the medium, and

   - obtaining the desired oligosaccharide from the medium.

2. The method of claim 1, wherein the oligosaccharide is selected from the group consisting of: lacto-*N*-triose II, lacto-*N*-tetraose, lacto-*N*-*neo*tetraose, lacto-*N*-fucopentaose I, lacto-*N*-fucopentaose II, lacto-*N*-fucopentaose III, lacto-*N*-

fucopentaose V, lacto-*N*-difucosylhexose I, lacto-*N*-difucosylhexaose II, lacto-*N*-sialylpentaose LSTa, LSTb, LSTc, disialyllacto-*N*-tetraose, disialyllacto-*N*-*neo*tetraose.

3. The method of any of the preceding claims, wherein the host cell comprises:

    - at least one homologous or heterologous nucleic acid sequence coding for a protein enabling the export of a desired oligosaccharide into the culture medium, wherein said host cell has been modified such, that the expression of the homologous or heterologous nucleic acid sequence is overexpressed; and/or

    - the deletion, disruption, diminishment or inactivation of at least one endogenous nucleic acid sequence coding for an exporter protein that exports precursors of the desired oligosaccharide outside the host cell; and/or

    - the overexpression of at least one homologous or heterologous sequence coding for a protein mediating the import of a precursor of a desired oligosaccharide into said host cell, wherein the precursor is larger than a disaccharide.

4. The method of claim 3, wherein said protein enabling the export of a desired oligosaccharide belongs to the class of secondary active transporters.

5. The method of any of claims 3 or 4, wherein said protein enabling the export of the desired oligosaccharide effects the export of an oligosaccharide a lacto-*N*-triose II (LNT-II; GIc*N*Ac(β1-3)Gal(β1-4)Gluc) as a core trisaccharide.

6. The method of any of claims 3 to 5, wherein said at least one nucleic acid sequence coding for a protein enabling the export of a desired oligosaccharide is an endogenous or a recombinant nucleic acid.

7. The method of any of claims 3 to 6, wherein said nucleic acid sequence coding for the protein enabling the export of a desired oligosaccharide is of bacterial, archeal, plant, yeast or animal origin.

8. The method of any of claims 3 to 6, wherein said at least one nucleic acid sequence coding for a protein enabling the export of a desired oligosaccharide is a gene selected from the group consisting of *yebQ* and *yjhB* from *Escherichia coli*, *proP* from *Mannheimia succiniciproducens* and *setA* from *Cedecea neteri* or functional fragments thereof.

9. The method of any of the preceding claims, wherein said recombinant glycosyltransferase is selected from at least one of the following: a galactosyltransferase, a sialyltransferase, an *N*-acetylglucosaminyltransferase and a fucosyltransferase.

10. The method of any of the preceding claims, wherein said recombinant glycosyltransferase is selected from at least one of the following: β-1,3-*N*-acetylglucosaminyltransferase, β-1,3-galactosyltransferase, β-1,4-galactosyltransferase, β-1,6-galactosyltransferase, α-2,3-sialyltransferase, α-2,6-sialyltansferase, α-1,2-fucosyltransferase, or α-1,3-fucosyltransferase.

11. The method of any of the preceding claims, wherein the host cell comprises (i) a β-1,3-*N*-acetylglucosaminyltransferase, and (ii) a β-1,3-galactosyltransferase or a β-1,4-galactosyltransferase as glycosyltransferases.

12. The method of any of claims 10 or 11, wherein said β-1,3-*N*-acetylglucosaminyltransferase belongs to the class of *IgtA* of *Neisseria meningitides* or *PmnagT* of *Pasteurella multocida*.

13. The method of any of claims 11 or 12, wherein the lacto-*N*-tetraose generating β-1,3-galactosyltransferase is *WbdO*.

14. The method of any of the preceding claims, wherein, where applicable, in the genetically modified host cell the endogenous β-galactosidase gene and the glucosamine-6-phosphate deaminase gene are inactivated or deleted, and wherein

said genetically modified host cell comprises a nucleic acid sequence coding for a functional lactose permease protein.

15. The method of any of the preceding claims, wherein the genetically modified host cell comprises an increased UDP-*N*-acetylglucosamine and UDP-galactose or GDP-fucose or CMP-*N*-acetylneuraminic acid production capability as compared to a genetically unmodified host cell.

16. The method of claim 15, wherein said increased UDP-*N*-acetylglucosamine and UDP-galactose 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.

17. The method according to claim 14 or 16, wherein said genetically modified host cell is cultivated in the presence of glucose, sucrose, glycerol or a combination thereof, but neither by addition or in the presence of *N*-acetylglucosamine or galactose nor in a combination thereof.

18. The method of any of the preceding claims, wherein the desired oligosaccharide is lacto-*N*-triose II, which is produced by total fermentation from a simple carbon source in the host cell by the action of the heterologous expressed glycosyltransferases β-1,4-galactosyltransferase and β-1,3-*N*-acetylglucosaminyltransferase.

19. A genetically modified microbial host cell for the production of a desired oligosaccharide comprising a lacto-*N*-triose II (LNT-II; GIc*N*Ac(β1-3)Gal(β1-4)Gluc) as a core trisaccharide, wherein the host cell

    - comprises at least one recombinant glycosyltransferase, and

- has the expression or activity of at least endogenous sugar transport protein modified such, that the expression or activity of the endogenous sugar transport protein is functionally inactivated for the export of a precursor of the desired oligosaccharide.

20. The genetically modified microbial host cell of claim 19, wherein said at least one recombinant glycosyltransferase is selected from the group consisting of a galactosyltransferase, a sialyltransferase, and an *N*-acetylglucosaminyltransferase.

21. The microbial host cell of claim 19 or 20, comprising (i) a heterologous expressed β-1,3-*N*-acetylglucosaminyltransferase, and (ii) a heterologous expressed β-1,3-galactosyltransferase or a heterologous expressed β-1,4-galactosyltransferase as glycosyltransferases.

22. The microbial host cell of claim 19 or 21, further comprising

- at least one homologous or heterologous nucleic acid sequence coding for a protein enabling the export of the oligosaccharide into a culture medium the host cell is cultivated in, wherein said protein enabling the export of the desired oligosaccharide belongs to the class of secondary active transporters, and wherein said homologous or heterologous nucleic acid sequence coding for a protein enabling the export of a desired oligosaccharide is overexpressed or under control of a promoter sequence enabling the overexpression of said nucleic acid sequence; and/or

- the deletion, disruption, diminishment or inactivation of at least one endogenous nucleic acid sequence coding for an exporter protein that exports precursors of the desired oligosaccharide outside the host cell; and/or,

- the overexpression of at least one homologous or heterologous nucleic acid sequence coding for a protein mediating the import of a precursor of a desired oligosaccharide into said host cell, wherein the precursor is larger than a disaccharide.

23. The microbial host cell of claim 22, wherein said nucleic acid sequence coding for a protein enabling the export of a desired oligosaccharide is of bacterial, archeal, plant, yeast or animal origin.

24. The microbial host cell of claim 22 or 23, wherein said at least one nucleic acid sequence coding for a protein enabling the export of the desired oligosaccharide is an endogenous or a recombinant nucleic acid sequence.

25. The microbial host cell of any of claims 22 to 24, wherein said at least one nucleic acid sequence coding for a protein enabling the export of a desired oligosaccharide is a gene selected from the group consisting of *yebQ* and *yjhB* from *Escherichia coli*, *proP* from *Mannheimia succiniciproducens* and *setA* from *Cedecea neteri* or functional fragments thereof.

26. The microbial host cell of claim 25, wherein the desired oligosaccharide is lacto-*N*-triose II.

27. The microbial host cell of any of claims 23, 25 and 26, wherein the desired oligosaccharide is lacto-*N*-tetraose, the precursor is lacto-*N*-triose II, and wherein the host cell has deleted, disrupted or inactivated at least one nucleic acid sequence coding for an exporter protein that is able to export lacto-*N*-triose II outside the host cell, wherein, preferably, the protein enabling the export of lacto-*N*-tetraose is selected from *YebQ* from *Escherichia coli* BL21(DE3), SpoVB of *Bacillus amyloliquefaciens*, YabM of *Erwinia pyrilfolia*, Bcr of *E. coli* MG1655, YdeA of *E. coli* MG1655, ProP2 of *Haemophilus parainfluenzae*, SetA of *Pectobacterium carotovorum*, FucP of *E. coli* MG1655, MdeA of *Staphylococcus aureus* Bmb9393, ImrA of *Lactococcus lactis*, SetA of *Pseudomonas* sp. MT-1 and SetA of *Beauveria bassiana* D1-5.