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; GlcNAc(β1,3)Gal(β1,4)Gluc) as a core trisaccharide, wherein the desired oligosaccharide is selected from the group consisting of lacto-*N*-tetraose and lacto-*N*-*neo*tetraose, the method comprising the steps of:
   - providing a genetically modified microbial host cell that
     - comprises at least one recombinant glycosyltransferase selected from the group consisting of β-1,3-*N*-acetylglucosaminyltransferase, β-1,3-galactosyltransferase and β-1,4-galactosyltransferse
   - has the expression or activity of at least one endogenous sugar export protein decreased or inactivated, as compared to a genetically unmodified host cell, so that the export of a precursor of the desired oligosaccharide into the medium is either decreased or abolished, wherein said precursor is lacto-N-triose II, or
     comprises at least one homologous or heterologous nucleic acid sequence coding for a protein enabling the export of the 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, wherein said at least one nucleic acid sequence coding for a protein enabling the export of the desired oligosaccharide is a gene selected from the group consisting of *yebQ, ydeA. fucP* and *bcr* from *Escherichia coli*, *spoVB* of *Bacillus amyloliquifaciens*, *yabM* of *Erwinia pyrilfolia*, *proP2* of *Haemophilus parainfluenzae*, *setA* of *Pectobacterium carotovorum*, *Pseudomonas sp*. or *Beauveria bassiana*, *mdeA* of *Staphylococcus aureus* and *imrA* of *Lactococcus lactis*, and functional fragments thereof;
   - 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 said β-1,3-N-acetylglucosaminyltransferase belongs to the class of LgtA of *Neisseria meningitides* or PmnagT of *Pasteurella multocida*.

3. The method of claim 1, wherein the β-1,3-galactosyltransferase is WbdO, a β-1,3-galactosyltransferase derived from *Salomonella enterica* wbdO, or encoded by a gene selected from the group consisting of *wbgO* from *Escherichia coli* and *furA* from *Lutiella nitroferrum.*

4. 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.

5. 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.

6. The method of claim 5, 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.

7. The method of claim 4 or 6, 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.

8. A genetically modified microbial host cell for the production of a desired oligosaccharide wherein the desired oligosaccharide is a human milk oligosaccharide comprising a lacto-N-triose II (LNT-II; GlcNAc(β1,3)Gal(β1,4)Gluc) as a core trisaccharide, wherein the desired oligosaccharide is selected from the group consisting of lacto-*N*-tetraose and lacto-*N*-*neo*tetraose, wherein the host cell
   - comprises at least one recombinant glycosyltransferase selected from the group consisting of β-1,3-*N*-acetylglucosaminyltransferase, β-1,3-galactosyltransferase and β-1,4-galactosyltransferse
   - has the expression or activity of at least one endogenous sugar export protein decreased or inactivated, as compared to a genetically unmodified host cell, so that the export of a precursor of the desired oligosaccharide into the medium is either decreased or abolished, wherein said precursor is lacto-N-triose II, or
     comprises at least one homologous or heterologous nucleic acid sequence coding for a protein enabling the export of the 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, wherein said at least one nucleic acid sequence coding for a protein enabling the export of the desired oligosaccharide is a gene selected from the group consisting of *yebQ, ydeA. fucP* and *bcr* from *Escherichia coli*, *spoVB* of *Bacillus amyloliquifaciens*, *yabM* of *Erwinia pyrilfolia*, *proP2* of *Haemophilus parainfluenzae*, *setA* of *Pectobacterium carotovorum*, *Pseudomonas sp*. or *Beauveria bassiana*, *mdeA* of *Staphylococcus aureus* and *imrA* of *Lactococcus lactis*, and functional fragments thereof.

9. The microbial host cell of claim 8, wherein said β-1,3-N-acetylglucosaminyltransferase belongs to the class of LgtA of *Neisseria meningitides* or PmnagT of *Pasteurella multocida*.

10. The microbial host cell of claim 8 or 9, wherein the β-1,3-galactosyltransferase is WbdO, a β-1,3-galactosyltransferase derived from *Salomonella enterica* wbdO, or encoded by a gene selected from the group consisting of *wbgO* from *Escherichia coli* and *furA* from *Lutiella nitroferrum*.

11. The microbial host cell of any one of claims 8 to 10, 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.

12. The microbial host cell of any one of claims 8 to 11, wherein the microbial 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.

13. The microbial host cell of claim 12, wherein 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.