Claims

1. Method for the production of fucosylated oligosaccharides using a genetically modified prokaryotic host cell, the method comprising the steps of:

- providing a prokaryotic host cell, which has been genetically modified, such, that at least (i) the activity of a fructose-6-phosphate-converting enzyme, which in the unmodified host cell has a regular level, is lowered or abolished; (ii) at least one gene encoding an enzyme necessary for the *de novo* synthesis of GDP-fucose is overexpressed in the host cell; (iii) an exogenous gene, encoding an alpha-1,2-fucosyltransferase and/or alpha-1,3-fucosyltransferase, is expressed in the host cell;

- cultivating and/or growing said genetically modified host cell in a cultivation medium from a carbon and/or energy source that is selected from at least one of the following: glycerol, succinate, malate, pyruvate, lactate, ethanol, citrate; and

- providing lactose to the cultivation medium;

thereby producing the fucosylated oligosaccharide obtainable from the medium the host cell is cultivated in.

2. The method of claim 1, wherein the fucosylated oligosaccharide is selected from the group consisting of 2'-fucosyllactose, 3-fucosyllactose or difucosyllactose.

3. The method of claim 1 or 2, wherein the host cell is selected from the group consisting of bacterial host cells, preferably selected from an *Escherichia coli* strain, a *Lactobacillus* species or a *Corynebacterium glutamicum* strain.

4. The method of any of the preceding claims, wherein the fructose-6-phosphate pool in the cell is increased by lowering or abolishing the activity of a fructose-6-phosphate converting enzyme that is selected from the group of phosphofructokinase, glucose-6-

phosphate isomerase, fructose-6-phosphate aldolase, a transketolase, or a transaldolase, and/or by increasing the activity of a fructose-1,6-bisphosphate phosphatase.

5. The method of any of the preceding claims, wherein the genes encoding enzymes necessary for the de novo synthesis of GDP-fucose are a phosphomannomutase encoding gene, preferably *manB*, a mannose-1-phosphate guanosyltransferase encoding gene, preferably *manC*, a GDP-mannose-4,6-dehydratase encoding gene, preferably *gmd*, and a GDP-L-fucose synthase encoding gene, preferably *wcaG*.

6. The method of any of the preceding claims, wherein the gene encoding at least one fucosyltransferase exhibits an alpha-1,2-fucosyltransferase and/or alpha-1,3 fucosyltransferase activity.

7. The method of claim 6, wherein the gene encoding an alpha-1,2-fucosyltransferase is selected from the group consisting of *wbgL* from *E. coli* O126 or *fucT2* from *Helicobacter pylori*.

8. The method of claim 6, wherein the gene encoding an alpha-1,3-fucosyltransferase is selected from the group consisting of alpha-1,3-fucosyltransferase genes of the species *Akkermansia muciniphila*, *Bacteroides fragilis*,*Helicobacter pylori*, or *Helicobacter hepaticus*.

9. The method of any of the preceding claims, wherein the host cell is further genetically modified to express a gene encoding a protein which enables or facilitates the export of the desired fucosylated oligosaccharide into the culture medium.

10. The method of any of the preceding claims, wherein an endogenous or exogenous permease for the import of lactose is overexpressed.

11. The method of any of the preceding claims, wherein the genes the host cell is modified in or with are endogenous or exogenous genes.

12. The method of any of the preceding claims, wherein at least one of the genes the host cell is modified in or with is overexpressed upon endogenous or exogenous induction or in a constitutive manner.

13. The method of claim 9, wherein the gene encoding a protein which enables or facilitates the export of the desired fucosylated oligosaccharide is a sugar efflux transporter, preferably selected from yberc0001_9420 and SetA.

14. The method of any of claims 4 to 13, wherein the fructose-1,6-bisphosphate phosphatase is encoded by a gene which is a functional active variant of the fructose-1,6-bisphosphate phosphatase (*fbpase*) from *Pisum sativum*.

15. The method of any of claims 10 to 13, wherein the lactose permease is *E. coli* LacY.

16. The method of any of the preceding claims, wherein the providing of lactose is accomplished by adding lactose from the beginning of the cultivating in a concentration of at least 5mM, preferably in a con-centration of 30, 40, 50, 60, 70, 80, 90, 100, 150 mM, more preferably in a concentration > 300 mM.

17. The method of any of the preceding claims wherein providing of lactose is accomplished by adding lactose to the cultivation medium in a concentration, such, that throughout the production phase of the cultivation a lactose concentration of at least 5 mM, preferably 10 mM or 30 mM is obtained.

18. The method of any of the preceding claims, wherein the host cells are cultivated for at least about 60, 80, 100, or about 120 hours or in a continuous manner.

19. The method of any of the preceding claims, wherein the exogenous genes are integrated into the genome of the host strain.

20. A prokaryotic cell for the production of a fucosylated oligosaccharide, characterized in that the cell is genetically modified, such, that at least (i) the activity of a fructose-6-phosphate-converting enzyme, which in the unmodified host cell has a regular level, is lowered or abolished, (ii) at least one gene encoding an enzyme necessary for the *de novo* synthesis of GDP-fucose is overexpressed, (iii) an exogenous gene, encoding an alpha-1,2-fucosyltransferase and/or an alpha-1,3-fucosyltranferase is expressed in the cell.

21. The prokaryotic cell of claim 20, wherein the cell is selected from an *Escherichia coli* strain, a *Lactobacillus* strain or a *Corynebacterium* strain.

22. The prokaryotic cell of claim 20 or 21, wherein the intracellular pool of fructose-6-phosphate is increased by (i) lowering or abolishing the activity of a fructose-6-phosphate converting enzyme that is selected from the group of phosphofructokinase, glucose-6-phosphate isomerase, fructose-6-phosphate aldolase, a transketolase, or a transaldolase, or (ii) increasing the fructose-1,6-bisphosphate phosphatase activity.

23. The prokaryotic cell of any of claims 19 to 22, wherein the genes encoding enzymes necessary for the *de novo* synthesis of GDP-fucose are overexpressed.

24. The prokaryotic cell of any of claims 19 to 23, wherein the exogenous genes encoding alpha-1,2-fucosylltransferases and/or alpha-1,3-fucosyltransferases are selected from *wbgL* from *E. coli* O126 or *fucT2* from *Helicobacter pylori*, referring to alpha-1,2-fucosylltransferases, and genes of the species *Akkermansia muciniphila*, *Bacteroides fragilis*,*Helicobacter pylori*, or *H. hepaticus,* referring to alpha-1,3-fucosyltransferases.

25. Use of a prokaryotic cell of any of claims 19 to 24 for the production of a fucosylated oligosaccharide, preferably of 2'-fucosyllactose, 3-fucosyllactose or difucosyllactose.