Claims

1. A genetically modified microorganism for *in vivo* synthesis of lacto-N-tetrose, said microorganism comprising

(i) a first transgene coding for β1,3-*N*-acetylglucosaminyltransferase,

and

(ii) a second transgene coding for β1,3-galactosyltransferase,

wherein the expression of the first transgene and the second transgene are controlled by a P_{tac} promoter.

2. A genetically modified microorganism for *in vivo* synthesis of lacto-N-neotetrose, said microorganism comprising

(i) a first transgene coding for β1,3-*N*-acetylglucosaminyltransferase,

and

(ii) a second transgene coding for β1,4-galactosyltransferase,

wherein the expression of the first transgene and the second transgene are controlled by a P_{tac} promoter.

3. The genetically modified microorganism according to claim 1 or 2, said microorganism in addition being genetically modified so as to suppress expression of LacZ and LacA.

4. The genetically modified microorganism according to claim 3, wherein the (i) first transgene has been integrated into the LacZYA locus and the microorganism comprises a further transgene coding for LacY.

5. The genetically modified microorganism according to claim 4, wherein the transgene coding for LacY has been integrated into the FucIK locus.

6. The genetically modified microorganism according to any of claims 1 to 5, said microorganism comprising a further transgene coding for UDP-sugar pyrophosphorylase.

7. The genetically modified microorganism according to any of claims 1 to 6, said microorganism in addition being genetically modified so as to suppress expression of UDP-glucose 4-epimerase.

8. The genetically modified microorganism according to any of claims 1 to 7, wherein one or both, or one, multiple or all, transgenes is/are chromosomally integrated.

9. The genetically modified microorganism according to any of claims 1 to 8, said microorganism comprising a further transgene coding for a

bifunctional enzyme having L-fucokinase activity and L-fucose-1-phosphate guanylyl-transferase activity, and at least one transgene coding for an enzyme capable of alpha1,2-fucosylation, alpha1,3-fucosylation or alpha1,4-fucosylation.

10. The genetically modified microorganism according to claim 9, wherein the transgene coding for said bifunctional enzyme having L-fucokinase activity and L-fucose-1-phosphate guanylyltransferase activity is chromosomally integrated, and the at least one transgene coding for an enzyme capable of alpha1,2-fucosylation, alpha1,3-fucosylation or alpha1,4-fucosylation is expressed on a plasmid vector.

11. The genetically modified microorganism according to claim 9, wherein both the transgene coding for said bifunctional enzyme having L-fucokinase activity and L-fucose-1-phosphate guanylyltransferase activity and the at least one transgene coding for an enzyme capable of alpha1,2-fucosylation, alpha1,3-fucosylation or alpha1,4-fucosylation are chromosomally integrated.

12. The use of a genetically modified microorganism according to any of the preceding claims for *in vivo* synthesis of lacto-N-tetrose or lacto-N-neotetrose or a fucosylated derivative of lacto-N-tetrose or lacto-N-neotetrose.

13. A method of preparing lacto-N-tetrose or lacto-N-neotetrose or a fucosylated derivative of lacto-N-tetrose or lacto-N-neotetrose, comprising the following steps:

(a) providing a genetically modified microorganism according to any of claims 1 to 11,

(b) culturing said genetically modified microorganism under conditions that permit synthesis of lacto-N-tetrose and lacto-N-neotetrose,

(c) optionally adding fucose,

(d) optionally isolating the synthesized lacto-N-tetrose or lacto-N-neotetrose or fucosylated derivative of lacto-N-tetrose or lacto-N-neotetrose.

14. The method according to claim 13, wherein step (b) comprises

- using galactose as carbon source for said microorganism,

or

- using glycerol and galactose as carbon source for said microorganism.

15. The method according to either of claims 13 and 14, wherein step (b) comprises adding one or more carbon source(s), preferably selected from the group consisting of lactose, glucose, glycerol, galactose, and any mixtures thereof, continuously or in batches.

16. The method according to any of claims 13 to 15, said method being carried out by way of a fed batch process with a batch volume in the range from 2 to 30 L, preferably from 3 to 20 L, particularly preferably from 5 to 15 L.

17. The genetically modified microorganism according to any of claims 1 to 11 or the use according to claim 12 or the method according to any of claims 13 to 16, wherein the genetically modified microorganism is selected from the group consisting of bacteria, fungi, and plants, preferably microorganisms of the genera *Corynebacterium, Bevibacterium, Bacillus, Saccharomyces* and *Escherichia*.