Another object of the embodiments disclosed herein was to provide a corresponding method enabling LNT and LNnT and their fucosylated derivatives to be biotechnologically produced in an efficient and inexpensive manner.

There is provided 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.

In an aspect, there is provided 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.

In another aspect, there is provided 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.

In another aspect, there is provided the use of a genetically modified microorganism as defined above for *in vivo* synthesis of lacto-N-tetrose or lacto-N-neotetrose or a fucosylated derivative of lacto-N-tetrose or lacto-N-neotetrose.

In another aspect, there is provided 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 as defined above,

(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.

In another aspect, there is provided the genetically modified microorganism as defined above or the use as defined above or the method as defined above, 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*.

A genetically modified microorganism in the present context means a microorganism in which individual genes have been switched off and/or endogenous or exogenous genes have been incorporated (transgenes) in a specific manner using biotechnological methods. A transgene in accordance with the present invention may be a gene imported from a different organism or else a gene which is naturally present in the microorganism concerned that has been integrated by genetic engineering at a different site in the genome and as a result is expressed, for example, under a promoter different from the natural promoter.

Surprisingly, it was found in the course of the present invention that micro-organisms routinely employed in genetic engineering, which transgenically express β1,3-*N*-acetylglucosaminyltransferase and a β1,3-galactosyltransferase or β1,4-galactosyltransferase, can successfully be employed in the synthesis of LNT and LNnT, respectively. It is possible to use here, for example, Leloir

glycosyltransferases (LgtA or LgtB) which firstly react lactose as substrate for glycosylation to give lacto-N-triose II (LNT II) as intermediate and then, in a step dependent on nucleotide-activated sugars, elongate it to give LNT (see fig. 1 and Frey et al. *FASEB J.* **1996**, *10,* 461–70). Examples of transgenes with proven

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