Claims

1. A method for the production of a sialylated compound in a microorganism, the method comprising:

- culturing a microorganism in a culture medium,

wherein said microorganism comprises at least one nucleic acid encoding a phosphatase converting N-acetylglucosamine-6-phosphate to N-acetylglucosamine, at least one nucleic acid encoding an *N*-acetylmannosamine epimerase converting said N-acetylglucosamine to N-acetylmannosamine; and at least one nucleic acid encoding a sialic acid synthase converting said N-acetylmannosamine to N-acetylneuraminate, and

wherein said microorganism is unable to *i)* convert N-acetylglucosamine-6-P to glucosamine-6-P, *ii)* convert *N*-acetyl-glucosamine to *N*-acetyl-glucosamine-6-P, and *iii)* convert *N*-acetyl-neuraminate to *N*-acetyl-mannosamine; and

- increasing the expression in said microorganism of a nucleic acid encoding said phosphatase, wherein said phosphatase polypeptide comprises:

- a homologue or derivative of any one of SEQ ID NOs: 46, 52, 69, 70, 57, 42, 47, 43 , 50, 59, 65, 54, 58, 60, 45, 67, 44, 48, 51, or 55 having at least ~~80 %, 81 %, 82 %, 83 %, 84 %, 85 %, 86 %, 87 %, 88 %, 89 %,~~ 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, or 99 % overall sequence identity to said polypeptide and <u>said homologue or derivative</u> having phosphatase activity.

2. The method according to claim 1, wherein said phosphatase polypeptide comprises any one of SEQ ID NOs: 46, 52, 69, 70, 57, 42, 47, 43, 50, 59, 65, 54, 58, 60, 45, 67, 44, 48, 51, or 55.

3. Method according to claim 1 or 2, wherein said increased expression is effected by introducing and expressing in a microorganism a nucleic acid encoding any one of said ~~phospatases~~<u>phosphatases</u> as described in claim 1 or 2.

4. Method according to claim 1 or 2, wherein said increased expression is effected by the action of a constitutive promoter.

5. Method according to any one of the preceding claims, wherein said sialylated compound is selected from the group consisting of *N*-acetylneuramic acid, sialylated oligosaccharide, sialylated lipid, sialylated protein, sialylated aglycon.

6. Method according to the claim 5, wherein said sialylated compound is a sialylated oligosaccharide.

7. Method according to claim 6, wherein said sialylated oligosaccharide is sialyllactose.

8. Method according to claim 6, wherein said sialylated oligosaccharide is disialyl lacto-*N*-tetraose.

9. Method according to claim 5, wherein said sialylated compound is *N*-acetylneuraminic acid.

10. Method according to any one of claim 1 to 9 wherein said sialylated compound is a sialylated lacto-*N*-triose, lacto-*N*-tetraose or a lacto-*N*-neotetraose, and wherein said microorganism further comprises the activity of a galactosyltransferase (EC 2.4.1.38).

11. Method according to claim 10 wherein said microorganism is unable to express the genes coding for UDP sugar hydrolase and galactose-1-phosphate uridylyltransferase.

12. Method according to any one of claims 1 to 11, wherein said microorganism produces less than 50%, 40%, 30%, 20%, 10%, 5%, 2% extracellular N-acetylglucosamine than sialylated compound.

13. Method for producing a sialylated oligosaccharide, comprising:

a) culturing a microorganism according to the method of any one of claims 1 to 12, and wherein said microorganism produces internally, activated *N*-acetylneuraminate as donor substrate for a sialyltransferase; and

b) culturing said microorganism in a culture medium comprising an exogenous precursor selected from the group consisting of lactose, *N*-acetyllactosamine, lacto-*N*-biose, galactose, beta-galactoside, and alpha-galactoside such as but not limited to globotriose (Gal-alpha-1,4Gal- beta-1,4Glc)galactose, wherein active uptake into the microorganism of said exogenous precursor occurs and wherein said exogenous precursor is the acceptor substrate for said sialytransferase for producing the sialylated oligosaccharide.

14. Method according to claim 1, wherein any one or more of said *N*-acetylmannosamine epimerase and sialic acid synthase is overexpressed in the microorganism.

15. Method according to claim 1, wherein any one or more of said *N*-acetylmannosamine epimerase and sialic acid synthase is introduced and expressed in the microorganism.

16. Method according to any one of claims 1 to 15, wherein said microorganism is a bacterium, preferably an *Escherichia coli* strain, more preferably an *Escherichia coli* strain which is a K12 strain, even more preferably the *Escherichia coli* K12 strain is *Escherichia coli* MG1655.

17. Method according to any one of claims 1 to 15, wherein said microorganism is a yeast.

18. A microorganism for the production of sialylated compounds wherein said microorganism comprises at least one nucleic acid encoding a phosphatase converting N-acetylglucosamine-6-phosphate to N-acetylglucosamine, at least one nucleic acid encoding an *N*-acetylmannosamine epimerase converting said N-acetylglucosamine to N-acetylmannosamine; and at least one nucleic acid encoding a sialic acid synthase converting said N-acetylmannosamine to N-acetylneuraminate, and

wherein said microorganism is unable to *i)* convert *N*-acetylglucosamine-6-P to glucosamine-6-P, *ii)* convert *N*-acetyl-glucosamine to *N*-acetyl-glucosamine-6-P, and *iii)* convert *N*-acetyl-neuraminate to *N*-acetyl-mannosamine; characterised in that

said microorganism comprises an increased expression of a nucleic acid encoding a phosphatase as defined in claim 1 or 2.

19. Use of a construct comprising:

(i) nucleic acid encoding a phosphatase as defined in claim 1 or 2;

(ii) one or more control sequences capable of driving expression of the nucleic acid sequence of (~~a~~i); and optionally

(iii) a transcription termination sequence;

in a method for producing sialylated compounds.

20. Use of a construct according to claim 19, wherein one of said control sequences is a constitutive promoter.