

CLAIMS

1. A method for the production of a desired oligosaccharide using a genetically-engineered microbial host cell, the method comprises the steps of:
 - (i) providing a genetically-engineered microbial host cell that is able to
5 produce the desired oligosaccharide, wherein the microbial host cell has been genetically engineered to express at least one heterologous glycosidase which is able to intracellularly degrade metabolic saccharide by-products that are generated during the intracellular biosynthesis of the desired oligosaccharide, and wherein the microbial host cell is able
10 to recycle the degradation products resulting from the enzymatic activity of said glycosidase for the production of the desired oligosaccharide;
 - (ii) cultivating the genetically-engineered microbial host cell under conditions and in a medium permissive for the production of the desired oligosaccharide, thereby producing the desired oligosaccharide; and
15 (iii) optionally, recovering the desired oligosaccharide.
2. A genetically-engineered microbial host cell for producing a desired oligosaccharide, wherein said microbial host cell is
 - a) able to produce the desired oligosaccharide;
 - b) has been genetically engineered to express at least one heterologous
20 glycosidase which is able to intracellularly degrade metabolic saccharide by-products that are generated during the intracellular biosynthesis of the desired oligosaccharide; and
 - c) is able to recycle degradation products resulting from the enzymatic activity of said glycosidase for the production of the desired
25 oligosaccharide.

3. The method according to claim 1 or the genetically-engineered microbial host cell according to claim 2, wherein the heterologous glycosidase is selected from the group consisting of fucosidases, sialidases, hexosaminidases, galactosidases and glucosidases.
- 5 4. The method or the genetically-engineered microbial host cell according to any one of claims 1 to 3, wherein the heterologous glycosidase is selected from the group consisting of α -1,2-fucosidases, α -1,3-fucosidases, α -2,3-sialidases, α -2,6-sialidases, α -2,8-sialidases, β -1,3-galactosidases, β -1,4-galactosidases, β -1,6-galactosidases, β -*N*-acetylhexosaminidases and β -1,3-glucosidases.
- 10 5. The method or the genetically-engineered microbial host cell according to any one of claims 1 to 4, wherein the genetically-engineered microbial host cell has been genetically engineered to express a heterologous glycosyltransferase, preferably as glycosyltransferase selected from the group consisting of fucosyltransferases, sialyltransferases, galactosyltransferases, N-acetyl-
15 glucosaminyltransferases and glucosyltransferases.
6. The method or the genetically-engineered microbial host cell according to any one of claims 1 to 5, wherein the microbial host cell has been genetically engineered to express a heterologous α -1,3-fucosyltransferase and a heterologous α -1,2-fucosidase.
- 20 7. The method or the genetically-engineered microbial host cell according to any one of claims 1 to 5, wherein the microbial host cell has been genetically engineered to express a heterologous α -1,2-fucosyltransferase and a heterologous α -1,3-fucosidase.
- 25 8. The method or the genetically-engineered microbial host cell according to any one of claims 1 to 5, wherein the microbial host cell has been genetically engineered to express a heterologous β -1,3-N-acetylglucosaminyltransferase,

a heterologous α -1,2-fucosyltransferase, a heterologous β -1,3-galactosyltransferase and a heterologous α -1,3-fucosidase.

9. The method or the genetically-engineered microbial host cell according to any one of claims 1 to 5, wherein the microbial host cell has been genetically engineered to express a heterologous α -2,6-sialyltransferase and a heterologous α -2,3-sialidase.
10. The method or the genetically-engineered microbial host cell according to any one of claims 1 to 5, wherein the microbial host cell has been genetically engineered to express a heterologous β -1,3-*N*-acetylglucosaminyltransferase, a heterologous β -1,4-galactosyltransferase and a heterologous β -1,3-galactosidase and/or β -1,3-glucosidase and/or galactan- β -1,3-galactosidase.
11. The method or the genetically-engineered microbial host cell according to any one of claims 1 to 5, wherein the microbial host cell has been genetically engineered to express a heterologous β -1,3-*N*-acetylglucosaminyltransferase, a heterologous β -1,3-galactosyltransferase and a heterologous β -1,3-glucosidase and/or a galactan- β -1,3-galactosidase.
12. The method or the genetically-engineered microbial host cell according to any one of claims 1 to 11, wherein the desired oligosaccharide is a human milk oligosaccharide, preferably a human milk oligosaccharide selected from the group consisting of 2'-fucosyllactose (2'-FL), 3-fucosyllactose (3-FL), 2',3-difucosyllactose, lacto-*N*-triose II, lacto-*N*-tetraose, lacto-*N*-neotetraose, lacto-*N*-fucopentaose I, lacto-*N*-neofucopentaose I, lacto-*N*-fucopentaose II, lacto-*N*-fucopentaose III, lacto-*N*-fucopentaose V, lacto-*N*-neofucopentaose V, lacto-*N*-difucohexaose I, lacto-*N*-difucosylhexaose II, *para*-Lacto-*N*-fucosylhexaose, fucosyl-lacto-*N*-sialylpentaose b, fucosyl-lacto-*N*-sialylpentaose c, fucosyl-lacto-*N*-sialylpentaose c, disialyl-lacto-*N*-fucopentaose, 3-fucosyl-3'-sialyllactose, 3-fucosyl-6'-sialyllactose, lacto-*N*-neodifucohexaose I, 3'-

sialyllactose, 6'-sialyllactose, sialyllacto-*N*-tetraose a (LST-a), sialyllacto-*N*-tetraose b (LST-b), sialyllacto-*N*-tetraose c (LST-c), and disialyllacto-*N*-tetraose.

- 5 13. Use of the genetically-engineered microbial host cell according to any one of claims 2 to 12 for the production of a desired oligosaccharide, preferably an oligosaccharide selected from the group of HMOs.
14. An oligosaccharide, preferably an oligosaccharide selected from the group of HMOs, produced by the method or by the use of a genetically-engineered microbial host cell according to any one of claims 1 to 12 for the manufacture
10 of a nutritional composition.
15. A nutritional composition containing at least one oligosaccharide that has been produced by the method or by the use of a genetically-engineered microbial host cell according to any one of claims 1 to 12, wherein the at least one oligosaccharide is preferably a HMO.

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