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Jennewein Biotechnologie GmbH 2827P103WO - GL/GL

Claims

1. Vector, containing a nucleic acid sequence being selected from the group consisting of: a) SEQ ID Nos. 1, 3, or 5 of the attached sequence listing; b) a nucleic acid sequence complementary to SEQ ID Nos. 1, 3, or 5; c) nucleic acid sequences which hybridize under stringent conditions to the nucleic acid sequences defined in a) and b) or their complementary strands, and encoding a polypeptide with alpha-1,3-fucosyltransferase activity, the nucleic acid sequence being operably linked to control sequences recognized by a host cell transformed with the vector.

2. Host cell containing a vector as claimed in claim 1.

3. Host cell according to claim 2, characterized in that the host cell is selected from the group consisting of fungi including yeast, bacteria, insect, animal and plant cells.

4. Host cell according to claims 2 or 3, characterized in that the host cell is an *Escherichia coli* cell.

5. Host cell according to one of claims 2 to 4, characterized in that the nucleic acid encoding the polypeptide with alpha-1,3-fucosyltransferase activity is adapted to the codon usage of the respective cell.

6. Use of a polynucleotide encoding a polypeptide with alpha-1,3-fucosyltransferase activity and comprising a sequence selected from the group consisting of a) SEQ ID Nos. 1, 3, or 5 of the attached sequence listing; b) a nucleic acid sequence complementary to SEQ ID Nos. 1, 3, or 5; c) nucleic acid sequences which hybridize under stringent conditions to the nucleic acid se-

quences defined in a) and b) or their complementary strands, or of the vector as claimed in claim 1, or of a polypeptidewith alpha-1,3-fucosyltransferase activity consisting of an amino acid sequence selected from the group consisting of (a) an amino acid sequence shown in SEQ ID NO. 2, 4, or 6; b) an amino acid sequence of an allelic variant of an amino acid sequence shown in SEQ ID No. 2, 4, or 6, wherein said allelic variant is encoded by a nucleic acid molecule that hybridizes under stringent conditions to the opposite strand of a nucleic acid molecule shown in SEQ ID Nos. 1, 3, or 5; c) an amino acid sequence of an ortholog of an amino acid sequence shown in SEQ ID No. 2, 4, or 6, wherein said ortholog is encoded by a nucleic acid molecule that hybridizes under stringent conditions to the opposite strand of a nucleic acid molecule shown in SEQ ID Nos. 1, 3, or 5; and (d) a fragment of an amino acid sequence shown in SEQ ID No. 2, 4, or 6, wherein said fragment comprises at least 10 contiguous amino acids , for the production of a fucosylated oligosaccharide.

7. Use according to claim 6, characterized in that the production of said fucosylated oligosaccharide is performed by means of a heterologous or homologous expression of the polynucleotide encoding the alpha-1,3-fucosyltransferase.

8. Use according to claim 6 or 7, characterized in that the produced fucosylated oligosaccharide is 3-fucosyllactose.

9. Method for producing fucosylated oligosaccharides, comprising the steps of:

   a. providing a polypeptide with alpha-1,3-fucosyltransferase activity consisting of an amino acid sequence selected from the group consisting of (a) an amino acid sequence shown in SEQ ID NO. 2, 4, or 6; b) an amino acid sequence of an allelic variant of an amino acid sequence shown in SEQ ID No. 2, 4, or 6, wherein said allelic variant is encoded by a nucleic acid molecule that hybridizes under stringent conditions to the

opposite strand of a nucleic acid molecule shown in SEQ ID Nos. 1, 3, or 5; c) an amino acid sequence of an ortholog of an amino acid sequence shown in SEQ ID No. 2, 4, or 6, wherein said ortholog is encoded by a nucleic acid molecule that hybridizes under stringent conditions to the opposite strand of a nucleic acid molecule shown in SEQ ID Nos. 1, 3, or 5; and (d) a fragment of an amino acid sequence shown in SEQ ID No. 2, 4, or 6, wherein said fragment comprises at least 10 contiguous amino acids ,

b. contacting the polypeptide with alpha-1,3-fucosyltransferase activity of step a. with a mixture comprising a donor substrate comprising a fucose residue, and an acceptor substrate comprising a mono- or oligosaccharide under conditions where the polypeptide catalyzes the transfer of a fucose residue from the donor substrate to the acceptor substrate, thereby producing a fucosylated oligosaccharide.

10. Method for producing fucosylated oligosaccharides, comprising the steps of:

a. growing, under suitable nutrient conditions permissive for the production of the fucosylated oligosaccharide, and permissive for the expression of a polypeptide with alpha-1,3-fucosyltransferase activity, a host cell as claimed in claims 2 to 5;

b. providing simultaneously or subsequently to step a. a donor substrate comprising a fucose residue and an acceptor substrate comprising a mono- or oligosaccharide, in order for the alpha-1,3-fucosyltransferase polypeptide catalyzing the transfer of a fucose residue from the donor substrate to the acceptor substrate, thereby producing a fucosylated oligosaccharide; and

c. isolating said fucosylated oligosaccharide from the host cell or the medium of its growth.

11. Method according to claim 9 or 10, characterized in that donor substrate is GDP-fucose.

12. Method according to any of claims 9 to 11, characterized in that the GDP-fucose is provided by an enzyme simultaneously expressed in the host cell or by the metabolism of the host cell.

13. Method according to any of claims 9 to 12, characterized in that the acceptor substrate is selected from the group comprising N- acetylglucosamine, N-acetyllactosamine, galactose, fucose, sialic acid, glucose, lactose or any combination thereof.

14. Use of any of claims 6 or 7, or method of any of claims 9 to 13, characterized in that the produced fucosylated oligosaccharide is a human milk oligosaccharide (HMO).

[0029] Accordingly, the present invention also relates to an isolated polypeptide with alpha-1,3-fucosyltransferase activity consisting of an amino acid sequence selected from the group consisting of :

(a) an amino acid sequence shown in SEQ ID NO: 2, 4 or 6;

b) an amino acid sequence of an allelic variant of an amino acid sequence shown in SEQ ID No. 2, 4 or 6, wherein said allelic variant is encoded by a nucleic acid molecule that hybridizes under stringent conditions to the opposite strand of a nucleic acid molecule shown in SEQ ID Nos. 1, 3 or 5;

c) an amino acid sequence of an ortholog of an amino acid sequence shown in SEQ ID No. 2, 4, or 6, wherein said ortholog is encoded by a nucleic acid molecule that hybridizes under stringent conditions to the opposite strand of a nucleic acid molecule shown in SEQ ID Nos. 1, 3, or 5; and

(d) a fragment of an amino acid sequence shown in SEQ ID No. 2, 4, or 6, wherein said fragment comprises at least 10 contiguous amino acids, and wherein said fragment has an alpha-1,3-fucosyltransferase activity.

[0030] The term "stringent conditions" refers to conditions under which a probe will hybridize to its target subsequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 15 C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Exemplary stringent hybridization conditions can be as following:

Claims

1. Isolated polynucleotide encoding a polypeptide with alpha-1,3-fucosyltransferase activity and comprising a sequence selected from the group consisting of:

a) SEQ ID Nos. 1, 3, or 5 of the attached sequence listing;

b) a nucleic acid sequence complementary to SEQ ID Nos. 1, 3, or 5;

c) nucleic acid sequences which hybridize under stringent conditions to the nucleic acid sequences defined in a) and b) or their complementary strands.

2. The isolated polynucleotide of claim 1 consisting of the SEQ ID Nos. 1, 3, or 5 encoding a polypeptide with alpha-1,3 fucosyltransferase activity.

3. Vector, containing a nucleic acid sequence according to claims 1 or 2 encoding a polypeptide with alpha-1,3-fucosyltransferase activity, the nucleic acid sequence being operably linked to control sequences recognized by a host cell transformed with the vector.

4. An isolated peptide with alpha-1,3-fucosyltransferase activity consisting of an amino acid sequence selected from the group consisting of :

(a) an amino acid sequence shown in SEQ ID NO. 2, 4, or 6;

b) an amino acid sequence of an allelic variant of an amino acid sequence shown in SEQ ID No. 2, 4, or 6, wherein said allelic variant is encoded by a nucleic acid molecule that hybridizes under stringent conditions

to the opposite strand of a nucleic acid molecule shown in SEQ ID Nos. 1, 3, or 5;

c) an amino acid sequence of an ortholog of an amino acid sequence shown in SEQ ID No. 2, 4, or 6, wherein said ortholog is encoded by a nucleic acid molecule that hybridizes under stringent conditions to the opposite strand of a nucleic acid molecule shown in SEQ ID Nos. 1, 3, or 5; and

(d) a fragment of an amino acid sequence shown in SEQ ID No. 2, 4, or 6, wherein said fragment comprises at least 10 contiguous amino acids.

5. Host cell containing a vector as claimed in claim 3.

6. Host cell according to claim 5, characterized in that the host cell is selected from the group consisting of fungi including yeast, bacteria, insect, animal and plant cells.

7. Host cell according to claims 5 or 6, characterized in that the host cell is an *Escherichia coli* cell.

8. Host cell according to one of claims 5 to 7, characterized in that the nucleic acid encoding the polypeptide with alpha-1,3-fucosyltransferase activity is adapted to the codon usage of the respective cell.

9. Use of a polynucleotide as claimed in any of claims 1 or 2, or of the vector as claimed in claim 3, or of the polypeptide as claimed in claim 4, for the production of a fucosylated oligosaccharide, (glyco)protein and/or (glyco)lipid.

10. Use according to claim 9, characterized in that the production of said fucosylated oligosaccharide, (glyco)protein and/or (glyco)lipid is performed by

means of a heterologous or homologous expression of the polynucleotide encoding the alpha-1,3-fucosyltransferase.

11. Use according to claim 9 or 10, characterized in that the fucosylated oligosaccharide is 3-fucosyllactose.

12. Method for producing fucosylated oligosaccharides, (glyco)proteins and (glyco)lipids, comprising the steps of:

a. providing a polypeptide with alpha-1,3-fucosyltransferase activity as claimed in claim 4,

b. contacting the polypeptide with alpha-1,3-fucosyltransferase activity of step a. with a mixture comprising a donor substrate comprising a fucose residue, and an acceptor substrate comprising a mono- or oligosaccharide, (glyco)protein or (glyco)lipid under conditions where the polypeptide catalyzes the transfer of a fucose residue from the donor substrate to the acceptor substrate, thereby producing a fucosylated oligosaccharide, (glyco)protein or (glyco)lipid.

13. Method for producing fucosylated oligosaccharides, (glyco)proteins and (glyco)lipids, comprising the steps of:

a. growing, under suitable nutrient conditions permissive for the production of the fucosylated oligosaccharide, (glyco)protein and/or (glyco)lipid, and permissive for the expression of a polypeptide with alpha-1,3-fucosyltransferase activity, a host cell as claimed in claims 5 to 8;

b. providing simultaneously or subsequently to step a. a donor substrate comprising a fucose residue and an acceptor substrate comprising a

mono- or oligosaccharide, (glyco)protein or (glyco)lipid, in order for the alpha-1,3-fucosyltransferase polypeptide catalyzing the transfer of a fucose residue from the donor substrate to the acceptor substrate, thereby producing a fucosylated oligosaccharide, (glyco)protein or (glyco)lipid; and

c. isolating said fucosylated oligosaccharide, (glyco)protein and/or (glyco)lipid from the host cell or the medium of its growth.

14. Method according to claim 12 or 13, characterized in that donor substrate is GDP-fucose.

15. Method according to any of claims 12 to 14, characterized in that the GDP-fucose is provided by an enzyme simultaneously expressed in the host cell or by the metabolism of the host cell.

16. The fucosylated oligosaccharide, (glyco)protein and/or (glyco)lipid obtained by the use of any of claims 9 to 11 or the by the method of any of claims 12 to 15.

[0029] Accordingly, the present invention also relates to an isolated polypeptide with alpha-1,3-fucosyltransferase activity consisting of an amino acid sequence selected from the group consisting of :

(a) an amino acid sequence shown in SEQ ID NO: 2, 4 or 6; or

b) an amino acid sequence of an allelic variant of an amino acid sequence shown in SEQ ID No. 2, 4 or 6, wherein said allelic variant is encoded by a nucleic acid molecule that hybridizes under stringent conditions to the opposite strand of a nucleic acid molecule shown in SEQ ID Nos. 1, 3 or 5.

[0030] The term "stringent conditions" refers to conditions under which a probe will hybridize to its target subsequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 15 C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Exemplary stringent hybridization conditions can be as following:

Claims

1. Vector, containing a nucleic acid sequence being selected from the group consisting of: a) SEQ ID Nos. 1, 3, or 5 of the attached sequence listing; b) a nucleic acid sequence complementary to SEQ ID Nos. 1, 3, or 5; c) nucleic acid sequences which hybridize under stringent conditions to the nucleic acid sequences defined in a) and b) or their complementary strands, and encoding a polypeptide with alpha-1,3-fucosyltransferase activity, the nucleic acid sequence being operably linked to control sequences recognized by a host cell transformed with the vector.

2. Host cell containing a vector as claimed in claim 1.

3. Host cell according to claim 2, characterized in that the host cell is selected from the group consisting of fungi including yeast, bacteria, insect, animal and plant cells.

4. Host cell according to claims 2 or 3, characterized in that the host cell is an *Escherichia coli* cell.

5. Host cell according to one of claims 2 to 4, characterized in that the nucleic acid encoding the polypeptide with alpha-1,3-fucosyltransferase activity is adapted to the codon usage of the respective cell.

6. Use of a polynucleotide encoding a polypeptide with alpha-1,3-fucosyltransferase activity and comprising a sequence selected from the group consisting of a) SEQ ID Nos. 1, 3, or 5 of the attached sequence listing; b) a nucleic acid sequence complementary to SEQ ID Nos. 1, 3, or 5; c) nucleic acid sequences which hybridize under stringent conditions to the nucleic acid sequences defined in a) and b) or their complementary strands, or of the vector as claimed in claim 1, or of a polypeptide with alpha-1,3-fucosyltransferase activity consisting of an amino acid sequence

selected from the group consisting of (a) an amino acid sequence shown in SEQ ID NO. 2, 4, or 6; or b) an amino acid sequence of an allelic variant of an amino acid sequence shown in SEQ ID No. 2, 4, or 6, wherein said allelic variant is encoded by a nucleic acid molecule that hybridizes under stringent conditions to the opposite strand of a nucleic acid molecule shown in SEQ ID Nos. 1, 3, or 5; for the production of a fucosylated oligosaccharide.

7. Use according to claim 6, characterized in that the production of said fucosylated oligosaccharide is performed by means of a heterologous or homologous expression of the polynucleotide encoding the alpha-1,3-fucosyltransferase.

8. Use according to claim 6 or 7, characterized in that the produced fucosylated oligosaccharide is 3-fucosyllactose.

9. Method for producing fucosylated oligosaccharides, comprising the steps of:

a. providing a polypeptide with alpha-1,3-fucosyltransferase activity consisting of an amino acid sequence selected from the group consisting of (a) an amino acid sequence shown in SEQ ID NO. 2, 4, or 6; b) an amino acid sequence of an allelic variant of an amino acid sequence shown in SEQ ID No. 2, 4, or 6, wherein said allelic variant is encoded by a nucleic acid molecule that hybridizes under stringent conditions to the opposite strand of a nucleic acid molecule shown in SEQ ID Nos. 1, 3, or 5;

b. contacting the polypeptide with alpha-1,3-fucosyltransferase activity of step a. with a mixture comprising a donor substrate comprising a fucose residue, and an acceptor substrate comprising a mono- or oligosaccharide under conditions where the polypeptide catalyzes the

transfer of a fucose residue from the donor substrate to the acceptor substrate, thereby producing a fucosylated oligosaccharide.

10. Method for producing fucosylated oligosaccharides, comprising the steps of:

a. growing, under suitable nutrient conditions permissive for the production of the fucosylated oligosaccharide, and permissive for the expression of a polypeptide with alpha-1,3-fucosyltransferase activity, a host cell as claimed in claims 2 to 5;

b. providing simultaneously or subsequently to step a. a donor substrate comprising a fucose residue and an acceptor substrate comprising a mono- or oligosaccharide, in order for the alpha-1,3-fucosyltransferase polypeptide catalyzing the transfer of a fucose residue from the donor substrate to the acceptor substrate, thereby producing a fucosylated oligosaccharide; and

c. isolating said fucosylated oligosaccharide from the host cell or the medium of its growth.

11. Method according to claim 9 or 10, characterized in that donor substrate is GDP-fucose.

12. Method according to any of claims 9 to 11, characterized in that the GDP-fucose is provided by an enzyme simultaneously expressed in the host cell or by the metabolism of the host cell.

13. Method according to any of claims 9 to 12, characterized in that the acceptor substrate is selected from the group consisting of N- acetylglucosa-

mine, N-acetyllactosamine, galactose, fucose, sialic acid, glucose, lactose or any combination thereof.

14. Use of any of claims 6 or 7, or method of any of claims 9 to 13, characterized in that the produced fucosylated oligosaccharide is a human milk oligosaccharide (HMO).