Claims

1. A method for the production of α-1,2-fucosyllactose, the method comprising the steps of:

a) providing a polypeptide with α-1,2-fucosyltransferase activity and with the ability to use lactose as acceptor substrate wherein said polypeptide

i) comprises an amino acid sequence encoding a conserved domain G-Y-[F/Y]-Q-[N/S] (SEQ ID NO: 72) and an amino acid sequence encoding a conserved domain (X, no K/V)XXXX[I/L]H[I/L]R[R/L]GD[F/Y](X, no C/M) (SEQ ID NO: 74) wherein X can be any distinct amino acid excluding a lysine and a valine residue from the first position of said domain and excluding a cysteine and a methionine residue from the last position of said domain, and/or

ii) is selected from the group consisting of:

i) any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 09, 10, 11, 12, 13, 14, 15, 16, 17, 19, 21, 22, 25, 26, 29, 33, 34, 35, 36, 37, 38, 39, 40, 41, 43, 44, 45, 46, 47, 48, 50, 51, 52, 53, 54, 55, 56, 57, 58, 61, 62, 63, 66, 67 or 76~~ of the attached sequence listing~~, ~~or~~

ii) an amino acid sequence having 80% or more sequence identity to the full-length amino acid sequence of any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78 or 79~~ of the attached sequence listing~~, ~~or~~

iii) an amino acid sequence of an allelic variant of an amino acid sequence shown in any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78 or 79, ~~or~~

iv) an amino acid sequence of an ortholog of an amino acid sequence shown in any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78 or 79, and

v) a functional fragment of an amino acid sequence shown in any one of SEQ ID NOs 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78 or 79,

preferably said polypeptide belongs to the glycosyltransferase 11 (GT11) family,

b) contacting the polypeptide with α-1,2-fucosyltransferase activity of step a) with a mixture comprising GDP-fucose as donor substrate, and lactose as acceptor substrate, under conditions

where the polypeptide catalyses the transfer of a fucose residue from the donor substrate to the acceptor substrate, thereby producing α-1,2-fucosyllactose,

c) optionally separating said α-1,2-fucosyllactose.

2. Method according to claim 1, wherein said polypeptide is selected from the group consisting of:

i) any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 70, 75, 76, 77, 78 or 79,

ii) an amino acid sequence having 80% or more sequence identity to the full-length amino acid sequence of any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 70, 75, 76, 77, 78 or 79,

iii) an amino acid sequence of an allelic variant of an amino acid sequence shown in any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 70, 75, 76, 77, 78 or 79,

iv) an amino acid sequence of an ortholog of an amino acid sequence shown in any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 70, 75, 76, 77, 78 or 79, and

v) a functional fragment of an amino acid sequence shown in any one of SEQ ID NOs 01, 02, 03, 04, 05, 06, 07, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 70, 75, 76, 77, 78 or 79,

preferably said polypeptide belongs to the glycosyltransferase 11 (GT11) family.

3. Method according to claim 1, wherein said polypeptide

(i) comprises an amino acid sequence encoding a conserved domain G-Y-[F/Y]-Q-[N/S] (SEQ ID NO: 72) and an amino acid sequence encoding a conserved domain (X, no K/V)XXXX[I/L]H[I/L]R[R/L]GD[F/Y](X, no C/M) (SEQ ID NO: 74) wherein X can be any distinct amino acid excluding a lysine and a valine residue from the first position of said domain and excluding a cysteine and a methionine residue from the last position of said domain, and

ii) is selected from the group consisting of:

(i) any one of SEQ ID NO 08, 32, 64, 68 and 69, or

ii) an amino acid sequence having 80% or more sequence identity to the full-length amino acid sequence of any one of SEQ ID NO 08, 32, 64, 68 and 69,

iii) an amino acid sequence of an allelic variant of an amino acid sequence shown in any one of SEQ ID NO 08, 32, 64, 68 and 69,

iv) an amino acid sequence of an ortholog of an amino acid sequence shown in any one of SEQ ID NO 08, 32, 64, 68 and 69, and

v) a functional fragment of an amino acid sequence shown in any one of SEQ ID NOs 08, 32, 64, 68 and 69,

preferably said polypeptide belongs to the glycosyltransferase 11 (GT11) family.

~~2.~~4. Method according to any one of claims 1 to 3 for the production of α-1,2-fucosyllactose resulting in a diFL concentration to 2'fucosyllactose concentration ratio of less than 1:5, preferably a α-1,2-fucosyllactose purity of 80% or more.

~~3.~~5. Method according to any one of claims 1 to 4 ~~1 or 2~~, wherein said polypeptide is provided in a cell free system.

~~4.~~6. Method according to any one of claims 1 to 4~~or 2~~, wherein said polypeptide is produced by a cell comprising a polynucleotide encoding said polypeptide.

~~5.~~7. Method according to any one of claim 1, 2, 3, 4 or 6~~4~~, wherein said GDP-fucose and/or lactose is provided by a cell producing said GDP-fucose and/or lactose.

~~6. A method according to any one of claims 1, 2, 4 or 5, the method comprising the steps of:~~

~~i) providing a cell genetically modified for the production of α-1,2-fucosyllactose, said cell comprising at least one nucleic acid sequence coding for an enzyme for α-1,2-fucosyllactose synthesis,~~

~~said cell comprising the expression of said polypeptide with α-1,2-fucosyltransferase activity and with the ability to use lactose as acceptor substrate,~~

~~ii) cultivating the cell in a medium under conditions permissive for the production of α-1,2-fucosyllactose,~~

~~iii) optionally separating the α-1,2-fucosyllactose from the cultivation.~~

~~7.~~8. Method according to claim ~~4~~6 or 7, the method comprising the steps of:

a) providing a ~~host~~ cell expressing said polypeptide with α-1,2-fucosyltransferase activity and with the ability to use lactose as acceptor substrate,

b) growing, under suitable nutrient conditions permissive for the production of the α-1,2-fucosyllactose, and permissive for the expression of said polypeptide with α-1,2-fucosyltransferase activity, said ~~host~~ cell;

c) providing simultaneously or subsequently to step b) a donor substrate GDP-fucose and the acceptor substrate lactose, in order for the α-1,2-fucosyltransferase polypeptide to catalyse the transfer of a fucose residue from GDP-fucose to lactose, thereby producing α-1,2-fucosyllactose;

d) optionally separating said α-1,2-fucosyllactose from the ~~host~~ cell or the medium of its growth.

~~8.~~9. M~~A m~~ethod according to ~~any one of~~ claim ~~8~~6 ~~or 7~~, wherein the ~~host~~ cell is transformed or transfected

to express an exogenous polypeptide with alpha-1,2-fucosyltransferase activity and with the ability to use lactose as an acceptor substrate.

9.10. Method according to any one of claims 6 to 9 ~~4 to 8~~ characterized in that the GDP-fucose and/or lactose is provided by an enzyme simultaneously expressed in the ~~host~~ cell or by the metabolism of the host cell.

~~10. The method of any one of claims 1 to 9, further comprising purification of α-1,2-fucosyllactose.~~

~~11. Method for the production of 2'-fucosyllactose according to any one of the preceding claims, the method further comprising at least one of the following steps:~~

~~i) adding to the culture medium a lactose feed comprising at least 50, more preferably at least 75, more preferably at least 100, more preferably at least 120, more preferably at least 150 gram of lactose per litre of initial reactor volume wherein the total reactor volume ranges from 250 mL (millilitre) to 10.000 m^3 (cubic meter), preferably in a continuous manner, and preferably so that the final volume of the culture medium is not more than three-fold, preferably not more than two-fold, more preferably less than 2-fold of the volume of the culture medium before the addition of said lactose feed;~~

~~ii) adding to the culture medium a GDP-fucose feed at a concentration enabling the host cell to synthesize 2'-fucosyllactose with a diFL concentration to 2'fucosyllactose concentration ratio of less than 1:5, preferably in a continuous manner and preferably so that the final volume of the culture medium is not more than three-fold, preferably not more than two-fold, more preferably less than 2-fold of the volume of the culture medium before the addition of said GDP-fucose feed;~~

~~iii) adding to the culture medium a carbon-based substrate feed at a concentration enabling the host cell to synthesize GDP-fucose at an optimal concentration for 2'fucosyllactose synthesis with a diFL concentration to 2'fucosyllactose concentration ratio of less than 1:5, preferably in a continuous manner;~~

~~iv) adding to the culture medium a carbon-based substrate feed at a concentration enabling the host cell to internally synthesize lactose at an optimal concentration for 2'fucosyllactose synthesis;~~

~~v) adding a lactose feed, a GDP-fucose feed and/or a carbon-based substrate feed in a continuous manner to the culture medium over the course of 1 day, 2 days, 3 days, 4 days, 5 days by means of at least one feeding solution;~~

~~vi) adding a lactose feed in a continuous manner to the culture medium over the course of 1 day, 2 days, 3 days, 4 days, 5 days by means of a feeding solution and wherein the concentration of said lactose feeding solution is 50 g/L, preferably 75 g/L, more preferably 100 g/L, more preferably 125 g/L, more preferably 150 g/L, more preferably 175 g/L, more preferably 200 g/L, more preferably 225 g/L, more preferably 250 g/L, more preferably 275 g/L, more preferably 300 g/L,~~

~~more preferably 325 g/L, more preferably 350 g/L, more preferably 375 g/L, more preferably, 400 g/L, more preferably 450 g/L, more preferably 500 g/L, even more preferably, 550 g/L, most preferably 600 g/L; and wherein preferably the pH of said solution is set between 3 and 7 and wherein preferably the temperature of said feed solution is kept between 20°C and 80°C;~~

~~vii) said method resulting in a 2' fucosyllactose concentration of at least 50 g/L, preferably at least 75 g/L, more preferably at least 90 g/L, more preferably at least 100 g/L, more preferably at least 125 g/L, more preferably at least 150 g/L, more preferably at least 175 g/L, more preferably at least 200 g/L in the final volume of said culture medium.~~

~~12.~~11. A ~~H~~host cell genetically modified for the production of alpha-1,2-fucosyllactose, wherein the host cell comprises at least one nucleic acid sequence coding for an enzyme for α-1,2-fucosyllactose synthesis; said cell comprising the expression of a polypeptide with α-1,2-fucosyltransferase activity and with the ability to use lactose as acceptor substrate wherein said polypeptide

i) comprises an amino acid sequence encoding a conserved domain G-Y-[F/Y]-Q-[N/S] (SEQ ID NO: 72) and an amino acid sequence encoding a conserved domain (X, no K/V)XXXX[I/L]H[I/L]R[R/L]GD[F/Y](X, no C/M) (SEQ ID NO: 74) wherein X can be any distinct amino acid excluding a lysine and a valine residue from the first position of said domain and excluding a cysteine and a methionine residue from the last position of said domain; and/or,

ii) is selected from the group consisting of:

i) any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 09, 10, 11, 12, 13, 14, 15, 16, 17, 19, 21, 22, 25, 26, 29, 33, 34, 35, 36, 37, 38, 39, 40, 41, 43, 44, 45, 46, 47, 48, 50, 51, 52, 53, 54, 55, 56, 57, 58, 61, 62, 63, 66, 67 or 76~~ of the attached sequence listing~~, ~~or~~

ii) an amino acid sequence having 80% or more sequence identity to the full-length amino acid sequence of any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78 or 79~~ of the attached sequence listing~~, ~~or~~

iii) an amino acid sequence of an allelic variant of an amino acid sequence shown in any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78 or 79, ~~or~~

iv) an amino acid sequence of an ortholog of an amino acid sequence shown in any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78 or 79, and

v) a functional fragment of an amino acid sequence shown in any one of SEQ ID NOs 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78 or 79,

preferably said polypeptide belongs to the glycosyl transferase 11 (GT11) family.

12. A host cell according to claim 11, wherein said polypeptide is selected from the group consisting of:

i) any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 70, 75, 76, 77, 78 or 79,

ii) an amino acid sequence having 80% or more sequence identity to the full-length amino acid sequence of any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 70, 75, 76, 77, 78 or 79,

iii) an amino acid sequence of an allelic variant of an amino acid sequence shown in any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 70, 75, 76, 77, 78 or 79,

iv) an amino acid sequence of an ortholog of an amino acid sequence shown in any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 70, 75, 76, 77, 78 or 79, and

v) a functional fragment of an amino acid sequence shown in any one of SEQ ID NOs 01, 02, 03, 04, 05, 06, 07, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 70, 75, 76, 77, 78 or 79,

preferably said polypeptide belongs to the glycosyltransferase 11 (GT11) family.

13. A host cell according to claim 11, wherein said polypeptide

(i) comprises an amino acid sequence encoding a conserved domain G-Y-[F/Y]-Q-[N/S] (SEQ ID NO: 72) and an amino acid sequence encoding a conserved domain (X, no K/V)XXXX[I/L]H[I/L]R[R/L]GD[F/Y](X, no C/M) (SEQ ID NO: 74) wherein X can be any distinct amino acid excluding a lysine and a valine residue from the first position of said domain and excluding a cysteine and a methionine residue from the last position of said domain, and

ii) is selected from the group consisting of:

(i) any one of SEQ ID NO 08, 32, 64, 68 and 69,

ii) an amino acid sequence having 80% or more sequence identity to the full-length amino acid sequence of any one of SEQ ID NO 08, 32, 64, 68 and 69,

iii) an amino acid sequence of an allelic variant of an amino acid sequence shown in any one of SEQ ID NO 08, 32, 64, 68 and 69,

iv) an amino acid sequence of an ortholog of an amino acid sequence shown in any one of SEQ ID NO 08, 32, 64, 68 and 69, and

v) a functional fragment of an amino acid sequence shown in any one of SEQ ID NOs 08, 32, 64, 68 and 69,

preferably said polypeptide belongs to the glycosyltransferase 11 (GT11) family.

~~13.~~14. A host cell~~Cell~~ according to any one of claims 11 to 13~~claim 12~~, the host cell comprising

i) a sequence comprising a polynucleotide encoding said polypeptide with lactose binding α-1,2-fucosyltransferase activity wherein said sequence is foreign to the host cell and is integrated in the genome of the host cell, or

ii) containing a vector comprising a polynucleotide encoding said polypeptide, wherein the polynucleotide being operably linked to control sequences recognized by a host cell transformed with the vector.

15. A host cell according to any one of claims 11 to 14, wherein said cell heterologously expresses said polypeptide with α-1,2-fucosyltransferase activity.

~~14.~~16. A host c~~C~~ell according to any one of claims 11 to 15~~12 or 13~~, wherein said cell is selected from the group consisting of a microorganism, plant and~~, or~~ animal cell~~s~~, preferably said microorganism is a bacterium, fungus or a yeast, preferably said plant is a rice, cotton, rapeseed, soy, maize or corn plant, preferably said animal cell is an insect, fish, bird or non-human mammal; more preferably the host cell is a microorganism; even more preferably said host cell is an Escherichia coli cell.

~~15. Host cell according to any one of claim 12 to 14, characterized in that the host cell is a cell of a bacterium, preferably of an *Escherichia coli* strain, more preferably of an *Escherichia coli* strain which is a K12 strain, even more preferably the *Escherichia coli* K12 strain is *Escherichia coli* MG1655.~~

~~16. Host cell according to any one of claim 12 to 14, characterized in that the host cell is a yeast cell.~~

17. A ~~H~~host cell according to any one of claims 11 to 16~~12 to 16~~, characterized in that the polynucleotide encoding the polypeptide with lactose binding alpha-1,2-fucosyltransferase activity is adapted to the codon usage of the respective host cell.

18. Method for the production of α-1,2-fucosyllactose, comprising the steps of:

a) providing a cell according to any one of claims 11 to 17~~12 to 17~~,

b) cultivating the cell in a medium under conditions permissive for the production of α-1,2-fucosyllactose,

c) optionally, separating said α-1,2-fucosyllactose from the cultivation.

~~19. Use of a host cell according to any one of claim 12 to 17 for the production of α-1,2-fucosyllactose.~~

~~20. A microorganism heterologously expressing a lactose binding alpha-1,2-fucosyltransferase polypeptide wherein said polypeptide~~

~~i) comprises an amino acid sequence encoding a conserved domain G-Y-[F/Y]-Q-[N/S] (SEQ ID NO: 72) and an amino acid sequence encoding a conserved domain (X, no K/V)XXXX[I/L]H[I/L]R[R/L]GD[F/Y](X, no C/M) (SEQ ID NO: 74) wherein X can be any distinct amino acid excluding a lysine and a valine residue from the first position of said domain and excluding a cysteine and a methionine residue from the last position of said domain, and/or~~

~~ii) is selected from the group consisting of:~~

~~i) any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 09, 10, 11, 12, 13, 14, 15, 16, 17, 19, 21, 22, 25, 26, 29, 33, 34, 35, 36, 37, 38, 39, 40, 41, 43, 44, 45, 46, 47, 48, 50, 51, 52, 53, 54, 55, 56, 57, 58, 61, 62, 63, 66, 67 or 76 of the attached sequence listing, or~~

~~ii) an amino acid sequence having 80% or more sequence identity to the full-length amino acid sequence of any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78 or 79 of the attached sequence listing, or~~

~~iii) an amino acid sequence of an allelic variant of an amino acid sequence shown in any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78 or 79, or~~

~~iv) an amino acid sequence of an ortholog of an amino acid sequence shown in any one of SEQ ID NO 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78 or 79, and~~

~~v) a functional fragment of an amino acid sequence shown in any one of SEQ ID NOs 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78 or 79,~~

~~preferably said polypeptide belongs to the glycosyl transferase 11 (GT11) family.~~

~~21. Use of a microorganism according to claim 20 for the production of alpha-1,2-fucosyllactose.~~

~~22.~~19. ~~The~~ M~~m~~ethod of any one of claims 1 to 10~~1, 18, 19~~ or 18~~21~~ further comprising a step of separating said alpha-1,2-fucosyllactose from the ~~host~~ cell or the medium of its growth, preferably wherein said separation comprises at least one of the following steps: clarification, ultrafiltration, nanofiltration, reverse osmosis, microfiltration, activated charcoal or carbon treatment, tangential flow high-

performance filtration, tangential flow ultrafiltration, affinity chromatography, ion exchange chromatography, hydrophobic interaction chromatography and/or gel filtration, ligand exchange chromatography.

~~23. The method of any one of claims 1 to 11, 18, 19, 21 or 22, wherein said separation comprises at least one of the following steps: clarification, ultrafiltration, nanofiltration, reverse osmosis, microfiltration, activated charcoal or carbon treatment, tangential flow high-performance filtration, tangential flow ultrafiltration, affinity chromatography, ion exchange chromatography, hydrophobic interaction chromatography and/or gel filtration, ligand exchange chromatography.~~

~~24.~~20. ~~The~~ ~~M~~~~m~~ethod of any one of claims 1 to 10~~11~~, 18 or 19~~18, 19, 21 to 23~~, further comprising purification of alpha-1,2-fucosyllactose, preferably wherein said purification of said alpha-1,2-fucosyllactose comprises at least one of the following steps: use of activated charcoal or carbon, use of charcoal, nanofiltration, ultrafiltration or ion exchange, use of alcohols, use of aqueous alcohol mixtures, crystallization, evaporation, precipitation, drying, spray drying or lyophilization.

~~25. The method of claim 24, wherein said purification of said alpha-1,2-fucosyllactose comprises at least one of the following steps: use of activated charcoal or carbon, use of charcoal, nanofiltration, ultrafiltration or ion exchange, use of alcohols, use of aqueous alcohol mixtures, crystallization, evaporation, precipitation, drying, spray drying or lyophilization.~~

~~26. The method of any one of claim 1 to 11, 21 to 25, wherein the polypeptide is produced in a fungal, yeast, bacterial, insect, animal or plant expression system.~~

~~27. The method of claim 26, wherein the host cell is a cell of a bacterium, preferably of an *Escherichia coli* strain, more preferably of an *Escherichia coli* strain which is a K12 strain, even more preferably the *Escherichia coli* K12 strain is *Escherichia coli* MG1655.~~

~~28. The method of claim 26, wherein the host cell is a yeast cell.~~

21. Method according to any one of claims 1 to 10, 18 to 20, the method further comprising at least one of the following steps:

i) adding to the culture medium a lactose feed comprising at least 50, more preferably at least 75, more preferably at least 100, more preferably at least 120, more preferably at least 150 gram of lactose per litre of initial reactor volume wherein the total reactor volume ranges from 250 mL (millilitre) to 10.000 m^3 (cubic meter), preferably in a continuous manner, and preferably so that the final volume of the culture medium is not more than three-fold, preferably not more than two-fold, more preferably less than 2-fold of the volume of the culture medium before the addition of said lactose feed;

ii) adding to the culture medium a GDP-fucose feed at a concentration enabling the host cell to synthesize 2'-fucosyllactose with a diFL concentration to 2'fucosyllactose concentration ratio of less than 1:5, preferably in a continuous manner and preferably so that the final volume of the culture medium is not more than three-fold, preferably not more than two-fold, more preferably

less than 2-fold of the volume of the culture medium before the addition of said GDP-fucose feed;

iii) adding to the culture medium a carbon-based substrate feed at a concentration enabling the host cell to synthesize GDP-fucose at an optimal concentration for 2'fucosyllactose synthesis with a diFL concentration to 2'fucosyllactose concentration ratio of less than 1:5, preferably in a continuous manner;

iv) adding to the culture medium a carbon-based substrate feed at a concentration enabling the host cell to internally synthesize lactose at an optimal concentration for 2'fucosyllactose synthesis;

v) adding a lactose feed, a GDP-fucose feed and/or a carbon-based substrate feed in a continuous manner to the culture medium over the course of 1 day, 2 days, 3 days, 4 days, 5 days by means of at least one feeding solution;

vi) adding a lactose feed in a continuous manner to the culture medium over the course of 1 day, 2 days, 3 days, 4 days, 5 days by means of a feeding solution and wherein the concentration of said lactose feeding solution is 50 g/L, preferably 75 g/L, more preferably 100 g/L, more preferably 125 g/L, more preferably 150 g/L, more preferably 175 g/L, more preferably 200 g/L, more preferably 225 g/L, more preferably 250 g/L, more preferably 275 g/L, more preferably 300 g/L, more preferably 325 g/L, more preferably 350 g/L, more preferably 375 g/L, more preferably, 400 g/L, more preferably 450 g/L, more preferably 500 g/L, even more preferably, 550 g/L, most preferably 600 g/L; and wherein preferably the pH of said solution is set between 3 and 7 and wherein preferably the temperature of said feed solution is kept between 20°C and 80°C;

vii) said method resulting in a 2'-fucosyllactose concentration of at least 50 g/L, preferably at least 75 g/L, more preferably at least 90 g/L, more preferably at least 100 g/L, more preferably at least 125 g/L, more preferably at least 150 g/L, more preferably at least 175 g/L, more preferably at least 200 g/L in the final volume of said culture medium.

~~29.~~22. The method of any one of claims 1 to 10~~11~~, 18 to 21~~18, 19, 21 to 28~~, wherein the lactose concentration in the culture medium ranges from 50 to 150 g/L and/or wherein the final concentration of 2'-fucosyllactose ranges between 70 g/L to 200 g/L.

~~30. The method of any one of claims 1 to 11, 18, 19, 21 to 29 wherein the final concentration of 2'-fucosyllactose ranges between 70 g/L to 200 g/L.~~

23. Use of a host cell according to any one of claims 11 to 17~~any one of claim 12 to 17~~ for the production of α-1,2-fucosyllactose.

~~31.~~24. Use of a polypeptide as described in the method of any one of claim 1 to~~or~~ 10 for the production of α-1,2-fucosyllactose.

~~32. A method for the production of α-1,2-fucosyllactose, the method comprising the steps of:~~

~~a) providing a polypeptide with α-1,2-fucosyltransferase activity and with the ability to use lactose~~

as acceptor substrate

b) contacting the polypeptide with α-1,2-fucosyltransferase activity of step a) with a mixture comprising GDP-fucose as donor substrate, and lactose as acceptor substrate, under conditions where the polypeptide catalyses the transfer of a fucose residue from the donor substrate to the acceptor substrate,

thereby producing α 1,2 fucosyllactose,

c) wherein said catalysis results in a diFL concentration to 2'fucosyllactose concentration ratio of less than 1:5

d) optionally separating said α 1,2 fucosyllactose.

33. A method for the production of α-1,2-fucosyllactose, the method comprising the steps of:

a) providing a polypeptide with α-1,2-fucosyltransferase activity and with the ability to use lactose as acceptor substrate

b) contacting the polypeptide with α 1,2 fucosyltransferase activity of step a) with a mixture comprising GDP fucose as donor substrate, and lactose as acceptor substrate, under conditions where the polypeptide catalyses the transfer of a fucose residue from the donor substrate to the acceptor substrate, thereby producing α 1,2 fucosyllactose,

c) wherein said catalysis results in a 2'fucosyllactose purity of 80% or more.

d) optionally separating said α-1,2-fucosyllactose.

34. Method for the production of 2'-fucosyllactose comprising at least one of the following steps:

i) adding to the culture medium a lactose feed comprising at least 50, more preferably at least 75, more preferably at least 100, more preferably at least 120, more preferably at least 150 gram of lactose per litre of initial reactor volume wherein the total reactor volume ranges from 250 mL (millilitre) to 10.000 m^3 (cubic meter), preferably in a continuous manner, and preferably so that the final volume of the culture medium is not more than three fold, preferably not more than two fold, more preferably less than 2 fold of the volume of the culture medium before the addition of said lactose feed;

ii) adding to the culture medium a GDP-fucose feed at a concentration enabling the host cell to synthesize 2' fucosyllactose with a diFL concentration to 2'fucosyllactose concentration ratio of less than 1:5, preferably in a continuous manner and preferably so that the final volume of the culture medium is not more than three-fold, preferably not more than two-fold, more preferably less than 2-fold of the volume of the culture medium before the addition of said GDP-fucose feed;

iii) adding to the culture medium a carbon based substrate feed at a concentration enabling the host cell to synthesize GDP-fucose at an optimal concentration for 2'fucosyllactose synthesis with a diFL concentration to 2'fucosyllactose concentration ratio of less than 1:5, preferably in a continuous manner;

iv) adding to the culture medium a carbon-based substrate feed at a concentration enabling the host cell to internally synthesize lactose at an optimal concentration for 2'fucosyllactose synthesis;

v) adding a lactose feed, a GDP-fucose feed and/or a carbon-based substrate feed in a continuous manner to the culture medium over the course of 1 day, 2 days, 3 days, 4 days, 5 days by means of at least one feeding solution;

vi) adding a lactose feed in a continuous manner to the culture medium over the course of 1 day, 2 days, 3 days, 4 days, 5 days by means of a feeding solution and wherein the concentration of said lactose feeding solution is 50 g/L, preferably 75 g/L, more preferably 100 g/L, more preferably 125 g/L, more preferably 150 g/L, more preferably 175 g/L, more preferably 200 g/L, more preferably 225 g/L, more preferably 250 g/L, more preferably 275 g/L, more preferably 300 g/L, more preferably 325 g/L, more preferably 350 g/L, more preferably 375 g/L, more preferably, 400 g/L, more preferably 450 g/L, more preferably 500 g/L, even more preferably, 550 g/L, most preferably 600 g/L; and wherein preferably the pH of said solution is set between 3 and 7 and wherein preferably the temperature of said feed solution is kept between 20°C and 80°C;

vii) said method resulting in a 2' fucosyllactose concentration of at least 50 g/L, preferably at least 75 g/L, more preferably at least 90 g/L, more preferably at least 100 g/L, more preferably at least 125 g/L, more preferably at least 150 g/L, more preferably at least 175 g/L, more preferably at least 200 g/L in the final volume of said culture medium and preferably a diFL concentration to 2'FL concentration ratio lower than 1:5, more preferably 1:20, even more preferably 1:40.

35. Method for the production of 2'-fucosyllactose comprising at least one of the following steps:

i) adding to the culture medium a lactose feed comprising at least 50, more preferably at least 75, more preferably at least 100, more preferably at least 120, more preferably at least 150 gram of lactose per litre of initial reactor volume wherein the total reactor volume ranges from 250 mL (millilitre) to 10.000 m^3 (cubic meter), preferably in a continuous manner, and preferably so that the final volume of the culture medium is not more than three-fold, preferably not more than two-fold, more preferably less than 2-fold of the volume of the culture medium before the addition of said lactose feed;

ii) adding to the culture medium a GDP-fucose feed at a concentration enabling the host cell to synthesize 2'-fucosyllactose with a purity of at least 80%, preferably in a continuous manner and preferably so that the final volume of the culture medium is not more than three-fold, preferably not more than two-fold, more preferably less than 2-fold of the volume of the culture medium before the addition of said GDP-fucose feed;

iii) adding to the culture medium a carbon-based substrate feed at a concentration enabling the host cell to synthesize GDP-fucose at an optimal concentration for 2'fucosyllactose synthesis with a purity of at least 80%, preferably in a continuous manner;

iv) adding to the culture medium a carbon-based substrate feed at a concentration enabling the host cell to internally synthesize lactose at an optimal concentration for 2'fucosyllactose synthesis;

v) adding a lactose feed, a GDP-fucose feed and/or a carbon-based substrate feed in a continuous manner to the culture medium over the course of 1 day, 2 days, 3 days, 4 days, 5 days by means of at least one feeding solution;

vi) adding a lactose feed in a continuous manner to the culture medium over the course of 1 day, 2 days, 3 days, 4 days, 5 days by means of a feeding solution and wherein the concentration of said lactose feeding solution is 50 g/L, preferably 75 g/L, more preferably 100 g/L, more preferably 125 g/L, more preferably 150 g/L, more preferably 175 g/L, more preferably 200 g/L, more preferably 225 g/L, more preferably 250 g/L, more preferably 275 g/L, more preferably 300 g/L, more preferably 325 g/L, more preferably 350 g/L, more preferably 375 g/L, more preferably, 400 g/L, more preferably 450 g/L, more preferably 500 g/L, even more preferably, 550 g/L, most preferably 600 g/L; and wherein preferably the pH of said solution is set between 3 and 7 and wherein preferably the temperature of said feed solution is kept between 20°C and 80°C;

vii) said method resulting in a 2' fucosyllactose concentration of at least 50 g/L, preferably at least 75 g/L, more preferably at least 90 g/L, more preferably at least 100 g/L, more preferably at least 125 g/L, more preferably at least 150 g/L, more preferably at least 175 g/L, more preferably at least 200 g/L in the final volume of said culture medium and preferably a 2'FL purity of 80% or more.