Claims

1. Method for the production of sialylated oligosaccharide by a genetically modified cell, comprising the steps of:

- providing a cell capable of producing sialylated oligosaccharide, said cell comprising at least one nucleic acid sequence coding for an enzyme for sialylated oligosaccharide synthesis,
- said cell genetically modified for i) overexpression of an endogenous membrane protein, ii) expression or overexpression of a homologous membrane protein, and/or iii) expression or overexpression of a heterologous membrane protein
- culturing the cell in a medium under conditions permissive for the production of sialylated oligosaccharide
- optionally separating sialylated oligosaccharide from the culture.

2. Method according to claim 1, wherein said cell is genetically modified for the production of sialylated oligosaccharide and wherein said genetically modified cell a) excretes sialylated oligosaccharide at a ratio of the supernatant concentration to whole broth concentration higher than 0,5 and/or b) has an enhanced production of sialylated oligosaccharide compared to a cell with the same genetic makeup but lacking the i) overexpression of the endogenous membrane protein, ii) expression or overexpression of the homologous membrane protein and/or iii) expression or overexpression of the heterologous membrane protein, respectively.

3. Method according to any one of claim 1 or 2, wherein said membrane protein comprises

i) an amino acid sequence encoding a siderophore exporter, preferably a siderophore exporter as part of any one of NOG families COG0477, 0ZVQG, 0ZPI7, 0ZVXV, 0XNN3, COG3182, 0ZW7F, 0XP7I, 0ZVCH, 0XQZX, 0XNQK, 0ZVYD, COG2271, 0XNNX, 0ZZWT, COG2814, 0ZITE, 0ZVC8, 0XT98, 0XNQ6, 0YAQV, 0ZVQA, COG2211, COG3104, 1269U, 0ZW8Z, COG1132, COG1173, COG0842, COG4615, COG0577, COG2274, COG4618, COG4172, COG5265, COG1136, 0XPIZ, COG0444, COG4779, COG4606, COG0601, COG1108, COG3182, COG4214, COG4605, COG2409, COG0841, COG3696, COG0845, COG1033, COG0534, 0Y3TF, COG2244, 0XPYW, COG2223 or bactNOG families 05E8G, 08HFG, 089VA, 07TNI, 05C0R, 07Y9F, 05CSH, 05QRD, 05EDF, 05C6X, 08NGX, 05C2C, 07FU4, 07U9Z, 080SS, 07SFI, 05EYM, 05C57, 08E7F, 07QF7, 05CSP, 07UZE, 07VHC, 08EFJ, 05CT4, 05FCD, 07YDJ, 08MMW, 08TKV, 07XMP, 05BZ1, 05IBP, 05CK8, 05IUH, 05D6C, 08E0J, 08JJ6, 08JJA, 05FDX, 05EGG, 08JN3, 08N1B, 05IDI, 08ITX, 05TVJ, 05DHS, 05CM4, 07RUJ, 05EYF, 07R13, 05BZS, 08IJF, 05UQX, 05C3S, 07U3M, 07R73, 07T1S, 07TJ5, 07XCD, 05DJC, 07RBJ, 05CXP; or

ii) an amino acid sequence encoding an ABC transporter comprising a) a conserved domain GxSGxGKST (SEQ ID NO 94) and b) a conserved domain SGGQxQRxxxxRAxxxxPK (SEQ ID NO 95)

wherein x can be any distinct amino acid; or

iii) an amino acid sequence encoding an MFS transporter comprising a) a conserved domain [AGMS]x[FLMVY]x[DGKNQR]xx[EGST][PRTVY][KR]x[GILMV] (SEQ ID NO 96) and b) a conserved domain [LRST]xxx[AG][AFILV] (SEQ ID NO 97), wherein x can be any distinct amino acid; or

iv) an amino acid sequence encoding a Sugar Efflux Transporter, preferably said membrane protein is an MFS transporter comprising the conserved domain L[FY]AxNR[HN]Y (SEQ ID NO 98), wherein x can be any distinct amino acid.

4. Method according to any one of claims 1 to 3, wherein

i) when said membrane protein is a siderophore exporter, said membrane protein is selected from SEQ ID NOs 9,117, 4, 6, 11, 13, 15, 20, 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, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 99, 100, 101, 102, 103, 104, 105, 106, 107, 109, 110, 111, 112, 113, 114, 115, 116~~, 117~~, 118, 119, 120, 121 or 122 or functional homolog or functional fragment of any one of the above membrane protein or a sequence having at least 80% sequence identity to any one of said SEQ ID NOs 9, 117, 4, 6, 11, 13, 15, 20, 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, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 99, 100, 101, 102, 103, 104, 105, 106, 107, 109, 110, 111, 112, 113, 114, 115, 116~~, 117~~, 118, 119, 120, 121 or 122 and providing i) improved growth speed of the cell and/or ii) improved production and/or efflux of sialylated oligosaccharides;

ii) when said membrane protein is an ABC transporter, said membrane protein is selected from oppF from *Escherichia coli K12 MG1655* with SEQ ID NO 18, lmrA from *Lactococcus lactis subsp. lactis bv. Diacetylactis* with SEQ ID NO 15, Blon_2475 from *B. longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 19 or gsiA from *Escherichia coli K12 MG1655* with SEQ ID NO 63, or functional homolog or functional fragment of any one of the above transporter membrane protein or a sequence having at least 80% sequence identity to any one of said SEQ ID NOs 18, 15, 19 or 63 and providing i) improved growth speed of the cell and/or ii) improved production and/or efflux of sialylated oligosaccharides;

iii) when said membrane protein is an MFS transporter, said membrane protein is selected from SEQ ID Nos 9, 117, 4, 5, 6, 7, 8,~~ 9,~~ 10, 11, 12, 13, 14, 20, 21, 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, 100, 106, 107, 108, 111, 113, 116, ~~117,~~ 118, 119, 121 or 122 and providing improved production and/or efflux of sialylated oligosaccharides or functional homolog or functional fragment of any one of the above transporter membrane protein or a sequence having at least 80% sequence identity to any one of said SEQ ID NOs 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 20, 21, 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, 100, 106, 107, 108, 111, 113, 116, 117, 118, 119, 121 or 122 and providing i) improved growth speed of the cell and/or ii) improved production and/or efflux of sialylated oligosaccharides;

iv) when said membrane protein is a Sugar Efflux Transporter, said membrane protein is selected from SEQ ID NOs 2, 1, 3, 16, 17 or 62, or functional homolog or functional fragment of any one of the above transporter membrane protein or a sequence having at least 80% sequence identity to any one of said SEQ ID NOs 2, 1, 3, 16, 17 or 62 and providing i) improved growth speed of the cell and/or ii) improved production and/or efflux of sialylated oligosaccharides.

5. Method according to any one of the previous claims, the method further comprising at least one of the following steps:

i) Adding to the culture medium a precursor 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 precursor 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 precursor feed;

ii) Adding a precursor 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;

iii) Adding a precursor 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 precursor 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;

iv) Said method resulting in sialylated oligosaccharide 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~~.~~,

preferably, wherein said precursor feed is accomplished by:

~~6. Method according to claim 5, wherein the precursor feed is accomplished by~~ i) adding precursor from the beginning of the cultivating in a concentration of at least 5 mM, preferably in a concentration of

30, 40, 50, 60, 70, 80, 90, 100, 150 mM, more preferably in a concentration > 300 mM~~.~~, and/or

~~7. Method according to any one of claims 5 or 6, wherein said precursor feed is accomplished by~~ ii) adding precursor to the cultivation medium in a concentration, such, that throughout the production phase of the cultivation a precursor concentration of at least 5 mM, preferably 10 mM or 30 mM is obtained~~.~~, preferably

~~8. Method according to any one of claims 5 to 7, wherein the host cells are cultivated for at least about 60, 80, 100, or about 120 hours or in a continuous manner.~~

~~9. Method according to any one of claims 1 to 8, wherein a precursor feed is added to the culture medium and wherein~~ said precursor is chosen from the group comprising lactose, lacto-N-biose (LNB), lacto-N-triose, lacto-N-tetraose (LNT), lacto-N-neotetraose (LNnT), N-acetyl-lactosamine (LacNAc), lacto-N-pentaose (LNP), lacto-N-neopentaose, para lacto-N-pentaose, para lacto-N-neopentaose, lacto-N-novopentaose I, lacto-N-hexaose (LNH), lacto-N-neohexaose (LNnH), para lacto-N-neohexaose (pLNnH), para lacto-N-hexaose (pLNH), lacto-N-heptaose, lacto-N-neoheptaose, para lacto-N-neoheptaose, para lacto-N-heptaose, lacto-N-octaose (LNO), lacto-N-neooctaose, iso lacto-N-octaose, para lacto-N-octaose, iso lacto-N-neooctaose, novo lacto-N-neooctaose, para lacto-N-neooctaose, iso lacto-N-nonaose, novo lacto-N-nonaose, lacto-N-nonaose, lacto-N-decaose, iso lacto-N-decaose, novo lacto-N-decaose, lacto-N-neodecaose, galactosyllactose, a lactose extended with 1, 2, 3, 4, 5, or a multiple of N-acetyllactosamine units and/or 1, 2, 3, 4, 5, or a multiple of lacto-N-biose units, and oligosaccharide containing 1 or multiple N-acetyllactosamine units and/or 1 or multiple lacto-N-biose units or an intermediate into sialylated oligosaccharide, fucosylated and sialylated versions thereof.

~~10.~~ 6. Method according to any one of claims 1 to ~~9~~5, wherein a carbon and energy source, preferably sucrose, glucose, fructose, glycerol, maltose, maltodextrines, trehalose, polyols, starch, succinate, malate, pyruvate, lactate, ethanol, citrate, lactose, is also added, preferably continuously to the culture medium, preferably with the precursor.

~~11.~~ 7. Method according to any one of claims 1 to ~~10~~6, wherein a first phase of exponential cell growth is provided by adding a carbon-based substrate, preferably glucose or sucrose, to the culture medium before the lactose is added to the culture medium in a second phase.

~~12.~~ 8. Method according to any one of claims 1 to ~~11~~7, wherein said sialylated oligosaccharide is 6’-sialyllactose, 3’-sialyllactose, 3-fucosyl-3'-sialyllactose (3'-O-sialyl-3-0-fucosyllactose, FSL), 2’-fucosyl-3’-sialyllactose, 2’-fucosyl-6’-sialyllactose, 3,6-disialyllactose, 6,6’-disialyllactose, sialyllacto-N-tetraose a

(LSTa), fucosyl-LSTa (FLSTa), sialyllacto-N-tetraose b (LSTb), fucosyl-LSTb (FLSTb), sialyllacto-N-neotetraose c (LSTc), fucosyl-LSTc (FLSTc), sialyllacto-N-neotetraose d (LSTd), fucosyl-LSTd (FLSTd), sialyl-lacto-N-hexaose (SLNH), sialyl-lacto-N-neohexaose I (SLNH-I), sialyl-lacto-N-neohexaose II (SLNH-II), disialyl-lacto-N-tetraose (DS-LNT), 6'-O-sialylated-lacto-N-neotetraose, 3'-O-sialylated-lacto-N-tetraose, 6'-sialylN-acetyllactosamine, 3'-sialylN-acetyllactosamine, 3-fucosyl-3'-sialylN-acetyllactosamine (3'-O-sialyl-3-O-fucosyl-N-acetyllactosamine), 3,6-disialylN-acetyllactosamine, 6,6'-disialyl-Nacetyllactosamine, 2'-fucosyl-3'-sialylN-acetyllactosamine, 2'-fucosyl-6'-sialyl-N-acetyllactosamine, 6'-sialyl-LactoNbiose, 3'-sialyl-LactoNbiose, 4-fucosyl-3'-sialyl-LactoNbiose (3'-O-sialyl-4-O-fucosyl-LactoNbiose), 3',6'-disialyl-LactoNbiose, 6,6'-disialyl-LactoNbiose, 2'-fucosyl-3'-sialyl-LactoNbiose, 2'-fucosyl-6'-sialyl-LactoNbiose.

~~13.~~ 9. Method according to any one of claims 1 to ~~12~~8, wherein the method is producing a mixture of sialylated oligosaccharides.

~~14.~~ 10. Method according to any one of claims 1 to ~~13~~9, wherein said genetically modified cell is selected from the group consisting of microorganism, plant, or animal cells, 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 is an insect, fish, bird or non-human mammal, preferably the cell is an *Escherichia coli* cell.

~~15.~~ 11. Host cell genetically modified for the production of sialylated oligosaccharide, wherein the host cell comprises at least one nucleic acid sequence coding for an enzyme for sialylated oligosaccharide synthesis and wherein said cell is genetically modified for i) overexpression of an endogenous membrane protein, ii) expression or overexpression of a homologous membrane protein, and/or iii) expression or overexpression of a heterologous membrane protein, wherein said membrane protein comprises

i) an amino acid sequence encoding a siderophore exporter, preferably a siderophore exporter as part of any one of NOG families COG0477, 0ZVQG, 0ZPI7, 0ZVXV, 0XNN3, COG3182, 0ZW7F, 0XP7I, 0ZVCH, 0XQZX, 0XNQK, 0ZVYD, COG2271, 0XNNX, 0ZZWT, COG2814, 0ZITE, 0ZVC8, 0XT98, 0XNQ6, 0YAQV, 0ZVQA, COG2211, COG3104, 1269U, 0ZW8Z, COG1132, COG1173, COG0842, COG4615, COG0577, COG2274, COG4618, COG4172, COG5265, COG1136, 0XPIZ, COG0444, COG4779, COG4606, COG0601, COG1108, COG3182, COG4214, COG4605, COG2409, COG0841, COG3696, COG0845, COG1033, COG0534, 0Y3TF, COG2244, 0XPYW, COG2223 or bactNOG families 05E8G, 08HFG, 089VA, 07TNI, 05C0R, 07Y9F, 05CSH, 05QRD, 05EDF, 05C6X, 08NGX, 05C2C, 07FU4, 07U9Z, 080SS, 07SFI, 05EYM, 05C57, 08E7F, 07QF7, 05CSP, 07UZE, 07VHC, 08EFJ, 05CT4, 05FCD, 07YDJ, 08MMW, 08TKV, 07XMP, 05BZ1, 05IBP, 05CK8, 05IUH, 05D6C, 08E0J, 08JJ6, 08JJA, 05FDX, 05EGG, 08JN3, 08N1B, 05IDI, 08ITX, 05TVJ, 05DHS, 05CM4, 07RUJ, 05EYF,

07R13, 05BZS, 08IJF, 05UQX, 05C3S, 07U3M, 07R73, 07T1S, 07TJ5, 07XCD, 05DJC, 07RBJ, 05CXP; or

ii) an amino acid sequence encoding an ABC transporter comprising a) a conserved domain GxSGxGKST (SEQ ID NO 94) and b) a conserved domain SGGQxQRxxxxRAxxxxPK (SEQ ID NO 95) wherein x can be any distinct amino acid; or

iii) an amino acid sequence encoding an MFS transporter comprising a) a conserved domain [AGMS]x[FLMVY]x[DGKNQR]xx[EGST][PRTVY][KR]x[GILMV] (SEQ ID NO 96) and b) a conserved domain [LRST]xxx[AG][AFILV] (SEQ ID NO 97), wherein x can be any distinct amino acid; or

iv) an amino acid sequence encoding a Sugar Efflux Transporter, preferably said membrane protein is an MFS transporter comprising the conserved domain L[FY]AxNR[HN]Y (SEQ ID NO 98), wherein x can be any distinct amino acid.

~~16.~~ 12. Cell according to claim ~~15~~11, wherein

i) when said membrane protein is a siderophore exporter, said membrane protein is selected from SEQ ID NOs 9,117, 4, 6, 11, 13, 15, 20, 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, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 99, 100, 101, 102, 103, 104, 105, 106, 107, 109, 110, 111, 112, 113, 114, 115, 116, ~~117,~~ 118, 119, 120, 121 or 122 or functional homolog or functional fragment of any one of the above membrane protein or a sequence having at least 80% sequence identity to any one of said SEQ ID NOs 9,117, 4, 6, 11, 13, 15, 20, 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, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 99, 100, 101, 102, 103, 104, 105, 106, 107, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121 or 122 and providing i) improced growth speed of the cell and/or ii) improved production and/or efflux of sialylated oligosaccharides;

ii) when said membrane protein is an ABC transporter, said membrane protein is selected from oppF from *Escherichia coli K12 MG1655* with SEQ ID NO 18, lmrA from *Lactococcus lactis subsp. lactis bv. Diacetylactis* with SEQ ID NO 15, Blon_2475 from *B. longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 19 or gsiA from *Escherichia coli K12 MG1655* with SEQ ID NO 63, or functional homolog or functional fragment of any one of the above transporter membrane protein or a sequence having at least 80% sequence identity to any one of said SEQ ID NOs 18, 15, 19 or 63 and providing i) improved growth speed of the cell and/or ii) improved production and/or efflux of sialylated oligosaccharides;

iii) when said membrane protein is an MFS transporter, said membrane protein is selected from SEQ ID NOs 9, 117, 4, 5, 6, 7, 8, ~~9,~~ 10, 11, 12, 13, 14, 20, 21, 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, 100, 106, 107, 108, 111, 113, 116, ~~117,~~ 118,

119, 121 or 122 or functional homolog or functional fragment of any one of the above transporter membrane protein or a sequence having at least 80% sequence identity to any one of said SEQ ID NOs 9, 117, 4, 5, 6, 7, 8~~, 9~~, 10, 11, 12, 13, 14, 20, 21, 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, 100, 106, 107, 108, 111, 113, 116,~~117,~~ 118, 119, 121 or 122 and providing i) improved growth speed of the cell and/or ii) improved production and/or efflux of sialylated oligosaccharides;

iv) when said membrane protein is a Sugar Efflux Transporter, said membrane protein is selected from SEQ ID NOs 2, 1, 3, 16, 17 or 62, or functional homolog or functional fragment of any one of the above transporter membrane protein or a sequence having at least 80% sequence identity to any one of said SEQ ID NOs 2, 1, 3, 16, 17 or 62 and providing i) improved growth speed of the cell and/or ii) improved production and/or efflux of sialylated oligosaccharides.

~~17.~~ 13. Cell according to any one of claims ~~15~~11 or ~~16~~12, wherein said cell is selected from the group consisting of microorganism, plant, or animal cells, 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 is an insect, fish, bird or non-human mammal; preferably the cell is an Escherichia coli cell.

~~18.~~ 14. Cell according to any one of claims ~~15~~11 to ~~17~~13, wherein the cell comprises a catabolic pathway for selected mono-, di- or oligosaccharides which is at least partially inactivated, the mono-, di-, or oligosaccharides being involved in and/or required for the synthesis of sialylated oligosaccharide.

~~19.~~ 15. Cell according to any one of claims ~~15~~11 to ~~18~~14, wherein said sialylated oligosaccharide is 6’-sialyllactose, 3’-sialyllactose, 3-fucosyl-3'-sialyllactose (3'-O-sialyl-3-0-fucosyllactose, FSL), 2’-fucosyl-3’-sialyllactose, 2’-fucosyl-6’-sialyllactose, 3,6-disialyllactose, 6,6’-disialyllactose, sialyllacto-N-tetraose a (LSTa), fucosyl-LSTa (FLSTa), sialyllacto-N-tetraose b (LSTb), fucosyl-LSTb (FLSTb), sialyllacto-N-neotetraose c (LSTc), fucosyl-LSTc (FLSTc), sialyllacto-N-neotetraose d (LSTd), fucosyl-LSTd (FLSTd), sialyl-lacto-N-hexaose (SLNH), sialyl-lacto-N-neohexaose I (SLNH-I), sialyl-lacto-N-neohexaose II (SLNH-II), disialyl-lacto-N-tetraose (DS-LNT), 6'-O-sialylated-lacto-N-neotetraose, 3'-O-sialylated-lacto-N-tetraose, 6’-sialylN-acetyllactosamine, 3’-sialylN-acetyllactosamine, 3-fucosyl-3'-sialylN-acetyllactosamine (3'-O-sialyl-3-O-fucosyl-N-acetyllactosamine), 3,6-disialylN-acetyllactosamine, 6,6’-disialyl-Nacetyllactosamine, 2’-fucosyl-3’-sialylN-acetyllactosamine, 2’-fucosyl-6’-sialyl-N-acetyllactosamine, 6’-sialyl-LactoNbiose, 3’-sialyl-LactoNbiose, 4-fucosyl-3'-sialyl-LactoNbiose (3'-O-sialyl-4-O-fucosyl-LactoNbiose), 3’,6’-disialyl-LactoNbiose, 6,6’-disialyl-LactoNbiose, 2’-fucosyl-3’-sialyl-LactoNbiose, 2’-fucosyl-6’-sialyl-LactoNbiose.

~~20.~~ 16. Cell according to any one of claims ~~15~~11 to ~~19~~15, characterized in that it is further transformed to comprise

i) at least one nucleic acid sequence coding for a protein facilitating or promoting the import of substrate required for oligosaccharide synthesis, wherein the protein is selected from the group consisting of lactose transporter, fucose transporter, sialic acid transporter, galactose transporter, mannose transporter, N-acetylglucosamine transporter, N-acetylgalactosamine transporter, ABC-transporter, transporter for a nucleotide- activated sugar and transporter for a nucleobase, nucleoside or nucleotide~~.~~, and/or

~~21. Cell according to any one of claims 15 to 20, characterized in that it is further transformed to comprise~~ ii) at least one nucleic acid sequence coding for a protein selected from the group consisting of nucleotidyltransferase, guanylyltransferase, uridylyltransferase, Fkp, L-fucose kinase, fucose-1-phosphate guanylyltransferase, CMP-sialic acid synthetase, galactose kinase, galactose-1-phosphate uridylyltransferase, glucose kinase, glucose-1-phosphate uridylyltransferase, mannose kinase, mannose-1-phosphate guanylyltransferase, GDP-4-keto-6-deoxy-D-mannose reductase, glucosamine kinase, glucosamine-phosphate acetyltransferase, N-acetyl-glucosamin-phosphate uridylyltransferase, UDP-N-acetylglucosamine 4-epimerase, UDP-N-acetyl-glucosamine 2-epimerase, cytidyltransferase, fructose-6-P-aminotransferase, glucosamine-6-P-aminotransferase, phosphatase, N-acetylglucosamine-2-epimerase, sialic acid synthase, ManNAc kinase, sialic acid synthetase, sialic acid phosphatase.

~~22. Use of a membrane protein selected from the group of membrane proteins as defined in any one of the claims 1 to 14 in the fermentative production of sialylated oligosaccharide.~~

~~23.~~ 17. Use of a cell according to any one of the claims ~~15~~11 to ~~21~~16, in a method for the production of sialylated oligosaccharide~~.~~, preferably said sialylated oligosaccharide is 6’-sialyllactose, 3’-sialyllactose, 3-fucosyl-3'-sialyllactose (3'-O-sialyl-3-0-fucosyllactose, FSL), 2’-fucosyl-3’-sialyllactose, 2’-fucosyl-6’-sialyllactose, 3,6-disialyllactose, 6,6’-disialyllactose, sialyllacto-N-tetraose a (LSTa), fucosyl-LSTa (FLSTa), sialyllacto-N-tetraose b (LSTb), fucosyl-LSTb (FLSTb), sialyllacto-N-neotetraose c (LSTc), fucosyl-LSTc (FLSTc), sialyllacto-N-neotetraose d (LSTd), fucosyl-LSTd (FLSTd), sialyl-lacto-N-hexaose (SLNH), sialyl-lacto-N-neohexaose I (SLNH-I), sialyl-lacto-N-neohexaose II (SLNH-II), disialyl-lacto-N-tetraose (DS-LNT), 6'-O-sialylated-lacto-N-neotetraose, 3'-O-sialylated-lacto-N-tetraose, 6’-sialylN-acetyllactosamine, 3’-sialylN-acetyllactosamine, 3-fucosyl-3'-sialylN-acetyllactosamine (3'-O-sialyl-3-O-fucosyl-N-acetyllactosamine), 3,6-disialylN-acetyllactosamine, 6,6’-disialyl-Nacetyllactosamine, 2’-fucosyl-3’-sialylN-acetyllactosamine, 2’-fucosyl-6’-sialyl-N-acetyllactosamine, 6’-sialyl-LactoNbiose, 3’-sialyl-LactoNbiose, 4-fucosyl-3'-sialyl-LactoNbiose (3'-O-sialyl-4-O-fucosyl-LactoNbiose), 3’,6’-disialyl-LactoNbiose, 6,6’-disialyl-LactoNbiose, 2’-fucosyl-3’-sialyl-LactoNbiose, 2’-fucosyl-6’-sialyl-LactoNbiose.

~~24. Use of a membrane protein according to claim 22 wherein said sialylated oligosaccharide is 6'-sialyllactose, 3'-sialyllactose, 3-fucosyl-3'-sialyllactose (3'-O-sialyl-3-0-fucosyllactose, FSL), 2'-fucosyl-3'-sialyllactose, 2'-fucosyl-6'-sialyllactose, 3,6-disialyllactose, 6,6'-disialyllactose, sialyllacto-N-tetraose a (LSTa), fucosyl-LSTa (FLSTa), sialyllacto-N-tetraose b (LSTb), fucosyl-LSTb (FLSTb), sialyllacto-N-neotetraose c (LSTc), fucosyl-LSTc (FLSTc), sialyllacto-N-neotetraose d (LSTd), fucosyl-LSTd (FLSTd), sialyl-lacto-N-hexaose (SLNH), sialyl-lacto-N-neohexaose I (SLNH-I), sialyl-lacto-N-neohexaose II (SLNH-II), disialyl-lacto-N-tetraose (DS-LNT), 6'-O-sialylated-lacto-N-neotetraose, 3'-O-sialylated-lacto-N-tetraose, 6'-sialylN-acetyllactosamine, 3'-sialylN-acetyllactosamine, 3-fucosyl-3'-sialylN-acetyllactosamine (3'-O-sialyl-3-O-fucosyl-N-acetyllactosamine), 3,6-disialylN-acetyllactosamine, 6,6'-disialyl-Nacetyllactosamine, 2'-fucosyl-3'-sialylN-acetyllactosamine, 2'-fucosyl-6'-sialyl-N-acetyllactosamine, 6'-sialyl-LactoNbiose, 3'-sialyl-LactoNbiose, 4-fucosyl-3'-sialyl-LactoNbiose (3'-O-sialyl-4-O-fucosyl-LactoNbiose), 3',6'-disialyl-LactoNbiose, 6,6'-disialyl-LactoNbiose, 2'-fucosyl-3'-sialyl-LactoNbiose, 2'-fucosyl-6'-sialyl-LactoNbiose.~~

~~25. Use of a cell according to claim 23, wherein said sialylated oligosaccharide is 6'-sialyllactose, 3'-sialyllactose, 3-fucosyl-3'-sialyllactose (3'-O-sialyl-3-0-fucosyllactose, FSL), 2'-fucosyl-3'-sialyllactose, 2'-fucosyl-6'-sialyllactose, 3,6-disialyllactose, 6,6'-disialyllactose, sialyllacto-N-tetraose a (LSTa), fucosyl-LSTa (FLSTa), sialyllacto-N-tetraose b (LSTb), fucosyl-LSTb (FLSTb), sialyllacto-N-neotetraose c (LSTc), fucosyl-LSTc (FLSTc), sialyllacto-N-neotetraose d (LSTd), fucosyl-LSTd (FLSTd), sialyl-lacto-N-hexaose (SLNH), sialyl-lacto-N-neohexaose I (SLNH-I), sialyl-lacto-N-neohexaose II (SLNH-II), disialyl-lacto-N-tetraose (DS-LNT), 6'-O-sialylated-lacto-N-neotetraose, 3'-O-sialylated-lacto-N-tetraose, 6'-sialylN-acetyllactosamine, 3'-sialylN-acetyllactosamine, 3-fucosyl-3'-sialylN-acetyllactosamine (3'-O-sialyl-3-O-fucosyl-N-acetyllactosamine), 3,6-disialylN-acetyllactosamine, 6,6'-disialyl-Nacetyllactosamine, 2'-fucosyl-3'-sialylN-acetyllactosamine, 2'-fucosyl-6'-sialyl-N-acetyllactosamine, 6'-sialyl-LactoNbiose, 3'-sialyl-LactoNbiose, 4-fucosyl-3'-sialyl-LactoNbiose (3'-O-sialyl-4-O-fucosyl-LactoNbiose), 3',6'-disialyl-LactoNbiose, 6,6'-disialyl-LactoNbiose, 2'-fucosyl-3'-sialyl-LactoNbiose, 2'-fucosyl-6'-sialyl-LactoNbiose.~~

~~26.~~ 18. A bacterial cell for the production of sialyllactose, the cell being transformed to comprise at least one nucleic acid sequence coding for a sialyltransferase, characterized in that: the cell in addition is transformed to comprise at least one nucleic acid sequence coding for a membrane protein wherein said membrane protein comprises

i) an amino acid sequence encoding a siderophore exporter, preferably a siderophore exporter as part of any one of NOG families COG0477, 0ZVQG, 0ZPI7, 0ZVXV, 0XNN3, COG3182, 0ZW7F, 0XP7I, 0ZVCH, 0XQZX, 0XNQK, 0ZVYD, COG2271, 0XNNX, 0ZZWT, COG2814, 0ZITE, 0ZVC8, 0XT98, 0XNQ6, 0YAQV, 0ZVQA, COG2211, COG3104, 1269U, 0ZW8Z, COG1132, COG1173, COG0842, COG4615, COG0577, COG2274, COG4618, COG4172, COG5265, COG1136, 0XPIZ, COG0444, COG4779, COG4606, COG0601, COG1108, COG3182, COG4214, COG4605, COG2409, COG0841,

COG3696, COG0845, COG1033, COG0534, 0Y3TF, COG2244, 0XPYW, COG2223 or bactNOG families 05E8G, 08HFG, 089VA, 07TNI, 05C0R, 07Y9F, 05CSH, 05QRD, 05EDF, 05C6X, 08NGX, 05C2C, 07FU4, 07U9Z, 080SS, 07SFI, 05EYM, 05C57, 08E7F, 07QF7, 05CSP, 07UZE, 07VHC, 08EFJ, 05CT4, 05FCD, 07YDJ, 08MMW, 08TKV, 07XMP, 05BZ1, 05IBP, 05CK8, 05IUH, 05D6C, 08E0J, 08JJ6, 08JJA, 05FDX, 05EGG, 08JN3, 08N1B, 05IDI, 08ITX, 05TVJ, 05DHS, 05CM4, 07RUJ, 05EYF, 07R13, 05BZS, 08IJF, 05UQX, 05C3S, 07U3M, 07R73, 07T1S, 07TJ5, 07XCD, 05DJC, 07RBJ, 05CXP, preferably, said membrane protein is selected from SEQ ID NOs 9, 117, 4, 6, 11, 13, 15, 20, 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, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 99, 100, 101, 102, 103, 104, 105, 106, 107, 109, 110, 111, 112, 113, 114, 115, 116, 118, 119, 120, 121 or 122 or functional homolog or functional fragment of any one of the above membrane protein or a sequence having at least 80% sequence identity to any one of said SEQ ID NOs 9, 117, 4, 6, 11, 13, 15, 20, 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, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 99, 100, 101, 102, 103, 104, 105, 106, 107, 109, 110, 111, 112, 113, 114, 115, 116, 118, 119, 120, 121 or 122 and providing i) improved growth speed of the cell and/or ii) improved production and/or efflux of sialylated oligosaccharides; or

ii) an amino acid sequence encoding an ABC transporter comprising a) a conserved domain GxSGxGKST (SEQ ID NO 94) and b) a conserved domain SGGQxQRxxxxRAxxxxPK (SEQ ID NO 95) wherein x can be any distinct amino acid, preferably, said membrane protein is selected from oppF from *Escherichia coli K12 MG1655* with SEQ ID NO 18, lmrA from *Lactococcus lactis subsp. lactis bv. Diacetylactis* with SEQ ID NO 15, Blon_2475 from *B. longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 19 or gsiA from *Escherichia coli K12 MG1655* with SEQ ID NO 63, or functional homolog or functional fragment of any one of the above transporter membrane protein or a sequence having at least 80% sequence identity to any one of said SEQ ID NOs 18, 15, 19 or 63 and providing i) improved growth speed of the cell and/or ii) improved production and/or efflux of sialylated oligosaccharides; or

iii) an amino acid sequence encoding an MFS transporter comprising a) a conserved domain [AGMS]x[FLMVY]x[DGKNQR]xx[EGST][PRTVY][KR]x[GILMV] (SEQ ID NO 96) and b) a conserved domain [LRST]xxx[AG][AFILV] (SEQ ID NO 97), wherein x can be any distinct amino acid, preferably, said membrane protein is selected from SEQ ID NOs 9, 117, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 20, 21, 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, 100, 106, 107, 108, 111, 113, 116, 118, 119, 121 or 122 or functional homolog or functional fragment of any one of the above transporter membrane protein or a sequence having at least 80% sequence identity to any one of said SEQ ID NOs 9, 117, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 20, 21, 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, 100, 106, 107, 108, 111, 113, 116, 118, 119, 121 or 122 and providing i) improved growth speed of the cell and/or ii) improved production and/or efflux of sialylated oligosaccharides; or

iv) an amino acid sequence encoding a Sugar Efflux Transporter, preferably said membrane protein is an MFS transporter comprising the conserved domain L[FY]AxNR[HN]Y (SEQ ID NO: 98), wherein x can be any distinct amino acid. , preferably, said membrane protein is selected from SEQ ID NOs 2, 1, 3, 16, 17 or 62, or functional homolog or functional fragment of any one of the above transporter membrane protein or a sequence having at least 80% sequence identity to any one of said SEQ ID NOs 2, 1, 3, 16, 17 or 62 and providing i) improved growth speed of the cell and/or ii) improved production and/or efflux of sialylated oligosaccharides,

preferably,

27. Bacterial cell according to claim 26, characterized in that the cell is an Escherichia coli cell.

28. Bacterial cell according to any one of claims 26 or 27, wherein

i) when said membrane protein is a siderophore exporter, said membrane protein is selected from SEQ ID NOs 9, 4, 6, 11, 13, 15, 20, 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, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 99, 100, 101, 102, 103, 104, 105, 106, 107, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121 or 122 or functional homolog or functional fragment of any one of the above membrane protein or a sequence having at least 80% sequence identity to any one of said SEQ ID NOs 9, 4, 6, 11, 13, 15, 20, 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, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 99, 100, 101, 102, 103, 104, 105, 106, 107, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121 or 122 and providing improved production and/or efflux of sialylated oligosaccharides;

ii) when said membrane protein is an ABC transporter, said membrane protein is selected from oppF from *Escherichia coli K12 MG1655* with SEQ ID NO 18, lmrA from *Lactococcus lactis subsp. lactis bv. Diacetylactis* with SEQ ID NO 15, Blon_2475 from *B. longum subsp. Infantis* (strain ATCC 15697) with SEQ ID NO 19 or gsiA from *Escherichia coli K12 MG1655* with SEQ ID NO 63, or functional homolog or functional fragment of any one of the above transporter membrane protein or a sequence having at least 80% sequence identity to any one of said SEQ ID NOs 18, 15, 19 or 63 and providing improved production and/or efflux of sialylated oligosaccharides;

iii) when said membrane protein is an MFS transporter, said membrane protein is selected from SEQ ID NOs 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 20, 21, 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, 100, 106, 107, 108, 111, 113, 116, 117, 118, 119, 121 or 122 or functional homolog or functional fragment of any one of the above transporter

~~membrane protein or a sequence having at least 80% sequence identity to any one of said SEQ ID NOs 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 20, 21, 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, 100, 106, 107, 108, 111, 113, 116, 117, 118, 119, 121 or 122 and providing improved production and/or efflux of sialylated oligosaccharides;~~

~~iv) when said membrane protein is a Sugar Efflux Transporter, said membrane protein is selected from SEQ ID NOs 2, 1, 3, 16, 17 or 62, or functional homolog or functional fragment of any one of the above transporter membrane protein or a sequence having at least 80% sequence identity to any one of said SEQ ID NOs 2, 1, 3, 16, 17 or 62 and providing improved production and/or efflux of sialylated oligosaccharides.~~

~~29.~~ 19. Bacterial cell according to ~~any one of claims 26 to 28~~ claim 18, characterized in that it is further transformed to comprise:

i) at least one nucleic acid sequence coding for a protein facilitating or promoting the import of substrate required for oligosaccharide synthesis, wherein the protein is selected from the group consisting of lactose transporter, fucose transporter, sialic acid transporter, galactose transporter, mannose transporter, N-acetylglucosamine transporter, N-acetylgalactosamine transporter, ABC-transporter, transporter for a nucleotide- activated sugar and transporter for a nucleobase, nucleoside or nucleotide~~.~~, and/or

~~30. Bacterial cell according to any one of claims 26 to 29, characterized in that it is further transformed to comprise~~ ii) at least one nucleic acid sequence coding for a protein selected from the group consisting of nucleotidyltransferase, guanylyltransferase, uridylyltransferase, Fkp, L-fucose kinase, fucose-1-phosphate guanylyltransferase, CMP-sialic acid synthetase, galactose kinase, galactose-1-phosphate uridylyltransferase, glucose kinase, glucose-1-phosphate uridylyltransferase, mannose kinase, mannose-1-phosphate guanylyltransferase, GDP-4-keto-6-deoxy-D-mannose reductase, glucosamine kinase, glucosamine-phosphate acetyltransferase, N-acetyl-glucosamin-phosphate uridylyltransferase, UDP-N-acetylglucosamine 4-epimerase, UDP-N-acetyl-glucosamine 2-epimerase, cytidyltransferase, fructose-6-P-aminotransferase, glucosamine-6-P-aminotransferase, phosphatase, N-acetylglucosamine-2-epimerase, sialic acid synthase, ManNAc kinase, sialic acid synthetase, sialic acid phosphatase.

~~31.~~ 20. A method for the production of oligosaccharides, said oligosaccharides being sialyllactose, the method comprising the steps of:

a) providing a bacterial cell according to any one of claims ~~26 to 30~~18 or 19,

b) culturing the cell in a medium under conditions permissive for the production of said oligosaccharides,

c) optionally separating said oligosaccharides from the culture~~.~~,

preferably, said sialyllactose is 3'-sialyllactose and/or 6'-sialyllactose.

~~32. The method according to any one of claims 1 to 14, 22 to 25 or 31, characterized in that culturing is performed using a continuous flow bioreactor.~~

~~33.~~ 21. The method according to any one of claims 1 to ~~14~~10, ~~22~~17 ~~to 25, 31~~ or ~~32~~20, characterized in that the medium comprises substrates required for the synthesis of said oligosaccharides, wherein the substrates are selected from the group consisting of arabinose, threose, erythrose, ribose, ribulose, xylose, glucose, D-2-deoxy-2-amino-glucose, N-acetylglucosamine, glucosamine, fructose, mannose, galactose, N-acetylgalactosamine, galactosamine, sorbose, fucose, N-acetylneuraminic acid, glycoside, non-natural sugar, nucleobase, nucleoside, nucleotide and any possible di- or polymer thereof; lactose, maltose, glycerol, sucrose.

~~34. Method according to any one of claims 1 to 14, 22 to 25, 31 to 33, wherein said sialyllactose is 3'-sialyllactose and/or 6'-sialyllactose.~~