

Claims

1. A micro-organism genetically modified for the production of at least one glycosylated product characterized in that said micro-organism has a cell wall biosynthesis that is reduced by a deletion, reduced or abolished expression of at least one enzyme within the cell wall biosynthesis pathway,
5 wherein said micro-organism is a bacterium or yeast, and
wherein said cell wall biosynthesis pathway is at least one pathway chosen from:
 - cell wall carbohydrate antigen biosynthesis, preferably O-antigen and/or common-antigen biosynthesis when said micro-organism is a Gram-negative bacterium,
 - 10 - capsular polysaccharide biosynthesis,
 - cell wall protein mannosylation biosynthesis, beta-1,3-glucan biosynthesis, beta-1,6-glucan biosynthesis and/or chitin biosynthesis when said micro-organism is a yeast,
 - mycolic acid and/or arabinogalactan biosynthesis when said micro-organism is a Corynebacterium, Nocardia or Mycobacterium,
 - 15 - teichoic acid biosynthesis when said micro-organism is a Gram-positive bacterium, preferably Bacillus.
2. Micro-organism according to claim 1, wherein said reduced cell wall biosynthesis pathway is combined with the introduction of one or more pathways for the synthesis of one or more nucleotide-activated sugars.
- 20 3. Micro-organism according to any one of claim 1 or 2, wherein said micro-organism is further modified to express one or more glycosyltransferases for production of said glycosylated product.
4. Micro-organism according to any one of claim 1 to 3, wherein said glycosylated product is an oligosaccharide, a glycosylated aglycon, a glycolipid or a glycoprotein.
- 25 5. Micro-organism according to any one of claim 1 to 4, wherein said enzyme within the cell wall biosynthesis pathway is a glycosyltransferase.
6. Micro-organism according to any one of claims 1 to 5, wherein said micro-organism is a bacterium chosen from Escherichia, Bacillus, Lactobacillus, Lactococcus, Corynebacterium.
7. Micro-organism according to any one of claims 1 to 5, wherein said micro-organism is a yeast
30 chosen from Pichia, Hansenula, Komagataella, Saccharomyces.
8. Micro-organism according to any one of claims 1 to 6, wherein the micro-organism is a bacterium with a further cell wall biosynthesis pathway that is reduced by a deletion, reduced or abolished expression of at least one enzyme within said further cell wall biosynthesis pathway chosen from colanic acid biosynthesis, exopolysaccharide biosynthesis and/or
35 lipopolysaccharide biosynthesis.
9. Micro-organism according to any one of claims 1 to 6 and 8, wherein the micro-organism is a Gram-negative bacterium having a reduced cell wall biosynthesis by a reduced O-antigen biosynthesis wherein said reduction in the O-antigen biosynthesis is provided by a deletion,

- reduced or abolished expression of any one or more of the genes present in the O-antigen biosynthesis gene cluster comprising rhamnosyltransferase, putative glycosyltransferase, putative lipopolysaccharide biosynthesis O-acetyl transferase, β -1,6-galactofuranosyltransferase, putative O-antigen polymerase, UDP-galactopyranose mutase, polyisoprenol-linked O-antigen repeat unit flippase, dTDP-4-dehydrorhamnose 3,5-epimerase, dTDP-glucose pyrophosphorylase, dTDP-4-dehydrorhamnose reductase, dTDP-glucose 4,6-dehydratase 1, UTP:glucose-1-phosphate uridylyltransferase.
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10. Micro-organism according to claim 9, wherein said reduction in the O-antigen biosynthesis is provided by a deletion, reduced or abolished expression of any one or more of i) WbbL, WbbK, WbbJ, WbbI, WbbH, glf, rfbX, rfbC, rfbA, rfbD, rfbB, wcaN, preferably as given by SEQ ID NOs: 27 to 38, respectively, or ii) a polypeptide sequence having 80% or more sequence identity to the full-length sequence of any one of the SEQ ID NOs: 27 to 38 and having rhamnosyltransferase activity, glycosyltransferase activity, lipopolysaccharide biosynthesis O-acetyl transferase activity, β -1,6-galactofuranosyltransferase activity, O-antigen polymerase activity, UDP-galactopyranose mutase activity, polyisoprenol-linked O-antigen repeat unit flippase activity, dTDP-4-dehydrorhamnose 3,5-epimerase activity, dTDP-glucose pyrophosphorylase activity, dTDP-4-dehydrorhamnose reductase activity, dTDP-glucose 4,6-dehydratase 1 activity or UTP:glucose-1-phosphate uridylyltransferase activity, respectively.
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11. Micro-organism according to any one of claims 1 to 6 and 8, wherein the micro-organism is a Gram-negative bacterium having a reduced cell wall biosynthesis by a reduced common-antigen biosynthesis wherein said reduction in the common-antigen biosynthesis is provided by a deletion, reduced or abolished expression of any one or more of the genes present in the common-antigen biosynthesis gene cluster comprising UDP-N-acetylglucosamine—undecaprenyl-phosphate N-acetylglucosaminophosphotransferase, enterobacterial common antigen polysaccharide co-polymerase, UDP-N-acetylglucosamine 2-epimerase, UDP-N-acetyl-D-mannosamine dehydrogenase, dTDP-glucose 4,6-dehydratase 2, dTDP-glucose pyrophosphorylase, dTDP-4-amino-4,6-dideoxy-D-galactose acyltransferase, dTDP-4-dehydro-6-deoxy-D-glucose transaminase, lipid III flippase, TDP-N-acetylfucosamine:lipid II N-acetylfucosaminyltransferase, putative enterobacterial common antigen polymerase, UDP-N-acetyl-D-mannosaminuronic acid transferase.
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12. Micro-organism according to claim 11, wherein said reduction in the common-antigen biosynthesis is provided by a deletion, reduced or abolished expression of any one or more of i) rfe, wzzE, wecB, wecC, rffG, rffH, rffC, wecE, wzxE, wecF, wzyE, rffM, preferably as given by SEQ ID NOs: 15 to 26, respectively, or ii) a polypeptide sequence having 80% or more sequence identity to the full-length sequence of any one of the SEQ ID NOs: 15 to 26 and having UDP-N-acetylglucosamine—undecaprenyl-phosphate N-acetylglucosaminophosphotransferase activity, enterobacterial common antigen polysaccharide co-polymerase activity, UDP-N-acetylglucosamine 2-epimerase activity,
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- UDP-N-acetyl-D-mannosamine dehydrogenase activity, dTDP-glucose 4,6-dehydratase 2 activity, dTDP-glucose pyrophosphorylase activity, dTDP-4-amino-4,6-dideoxy-D-galactose acyltransferase activity, dTDP-4-dehydro-6-deoxy-D-glucose transaminase activity, lipid III flippase activity, TDP-N-acetylfucosamine:lipid II N-acetylfucosaminyltransferase activity, enterobacterial common antigen polymerase activity or UDP-N-acetyl-D-mannosaminuronic acid transferase activity, respectively.
13. Micro-organism according to claim 8, wherein said micro-organism is a bacterium having a further reduced cell wall biosynthesis by a reduced colanic acid biosynthesis wherein said reduction in the colanic acid biosynthesis is provided by a deletion, reduced or abolished expression of any one or more of the genes present in the colanic acid biosynthesis gene cluster comprising putative colanic acid biosynthesis protein, putative colanic biosynthesis glycosyl transferase, putative colanic acid biosynthesis pyruvyl transferase, M-antigen undecaprenyl diphosphate flippase, UDP-glucose:undecaprenyl-phosphate glucose-1-phosphate transferase, phosphomannomutase, mannose-1-phosphate guanylyltransferase, colanic acid biosynthesis fucosyltransferase, GDP-mannose mannosyl hydrolase, GDP-L-fucose synthase, GDP-mannose 4,6-dehydratase, colanic acid biosynthesis acetyltransferase, colanic acid biosynthesis fucosyltransferase, putative colanic acid polymerase, colanic acid biosynthesis galactosyltransferase, colanic acid biosynthesis acetyltransferase, colanic acid biosynthesis glucuronosyltransferase, protein-tyrosine kinase, protein-tyrosine phosphatase, outer membrane polysaccharide export protein.
14. Micro-organism according to claim 13, wherein said reduction in the colanic acid biosynthesis is provided by a deletion, reduced or abolished expression of any one or more of i) WcaM, WcaL, WcaK, Wzx, wcaJ, cpsG, cpsB, WcaI, gmm, fcl, gmd, WcaF, WcaE, WcaD, WcaC, WcaB, WcaA, Wzc, wzb, Wza, preferably as given by SEQ ID NOs: 39 to 58, respectively, or ii) a polypeptide sequence having 80% or more sequence identity to the full-length sequence of any one of the SEQ ID NOs: 39 to 58 and having colanic acid biosynthesis protein activity, colanic biosynthesis glycosyl transferase activity, colanic acid biosynthesis pyruvyl transferase activity, M-antigen undecaprenyl diphosphate flippase activity, UDP-glucose:undecaprenyl-phosphate glucose-1-phosphate transferase activity, phosphomannomutase activity, mannose-1-phosphate guanylyltransferase activity, colanic acid biosynthesis fucosyltransferase activity, GDP-mannose mannosyl hydrolase activity, GDP-L-fucose synthase activity, GDP-mannose 4,6-dehydratase activity, colanic acid biosynthesis acetyltransferase activity, colanic acid biosynthesis fucosyltransferase activity, colanic acid polymerase activity, colanic acid biosynthesis galactosyltransferase activity, colanic acid biosynthesis acetyltransferase activity, colanic acid biosynthesis glucuronosyltransferase activity, protein-tyrosine kinase activity, protein-tyrosine phosphatase activity or outer membrane polysaccharide export protein activity, respectively.
15. Micro-organism according to any one of claims 1 to 5 and 7, wherein said micro-organism is

- a yeast having a reduced cell wall biosynthesis by a reduced cell wall protein mannosylation biosynthesis wherein said reduction of the cell wall protein mannosylation biosynthesis is provided by a deletion, reduced or abolished expression of any one or more of Protein-O-mannosyltransferase encoding gene preferably one or more of PMT1, PMT2, PMT3, PMT4, PMT5, PMT6, PMT7, more preferably one or more of PMT1, PMT2, PMT4.
16. Micro-organism according to any one of claims 1 to 6 and 8, wherein said micro-organism is a Corynebacterium, Nocardia or Mycobacterium having a reduced cell wall biosynthesis by a reduced mycolic acid and/or arabinogalactan biosynthesis wherein said reduced mycolic acid and/or arabinogalactan biosynthesis is provided by a reduced expression of any one or more of mycolic acid and/or arabinogalactan biosynthesis genes, preferably by reduced expression of any one or more of accD2, accD3, aftA, aftB or emb.
17. Micro-organism according to any one of claims 1 to 6 and 8, wherein said micro-organism is a Gram-positive bacterium having a reduced cell wall biosynthesis by a reduced teichoic acid biosynthesis wherein said reduced teichoic acid biosynthesis is provided by a reduced expression of any one or more of teichoic acid biosynthesis genes, preferably by reduced expression of any one or more of tagO, tagA, tagB, tagD, tagF, tagG or tagH.
18. Micro-organism according to any one of claims 1 to 17, wherein said glycosylated product is an oligosaccharide with a degree of polymerization higher than 3.
19. Isolated micro-organism according to any one of claims 1 to 18.
20. A method to reduce the viscosity, foaming, and/or airlift of a fermentation process with a micro-organism characterized in that the cell wall biosynthesis of said micro-organism is reduced by deletion, reduced or abolished expression of at least one enzyme within the cell wall biosynthesis pathway, wherein said micro-organism is a bacterium or yeast, and wherein said cell wall biosynthesis pathway is at least one pathway chosen from:
- cell wall carbohydrate antigen biosynthesis, preferably O-antigen and/or common-antigen biosynthesis when said micro-organism is a Gram-negative bacterium,
 - capsular polysaccharide biosynthesis,
 - cell wall protein mannosylation biosynthesis, beta-1,3-glucan biosynthesis, beta-1,6-glucan biosynthesis and/or chitin biosynthesis when said micro-organism is a yeast,
 - mycolic acid and/or arabinogalactan biosynthesis when said micro-organism is a Corynebacterium, Nocardia or Mycobacterium,
 - teichoic acid biosynthesis when said micro-organism is a Gram-positive bacterium, preferably Bacillus.
21. Method according to claim 20, wherein said micro-organism is further modified to produce at least one glycosylated product.
22. Method for the production of glycosylated product by a genetically modified cell, comprising the steps of:

- providing a cell genetically modified for the production of glycosylated product, said cell comprising at least one nucleic acid sequence coding for an enzyme for glycosylated product synthesis,
 - said cell further genetically modified for reduced cell wall biosynthesis by deletion, reduced or abolished expression of at least one enzyme within the cell wall biosynthesis pathway, wherein said cell wall biosynthesis pathway is at least one pathway chosen from cell wall carbohydrate antigen biosynthesis, capsular polysaccharide biosynthesis, cell wall protein mannosylation biosynthesis, beta-1,3-glucan biosynthesis, beta-1,6-glucan biosynthesis, chitin biosynthesis, mycolic acid biosynthesis, arabinogalactan biosynthesis and teichoic acid biosynthesis, preferably wherein said cell wall carbohydrate antigen biosynthesis is O-antigen and/or common-antigen biosynthesis,
 - culturing the cell in a medium under conditions permissive for the production of glycosylated product,
 - optionally separating glycosylated product from the culture.
23. Method according to claim 22, wherein said enzyme for glycosylated product synthesis comprises enzymes involved in nucleotide-activated sugar synthesis and glycosyltransferases.
24. Method according to any one of claim 22 or 23, wherein the genetically modified cell is a micro-organism, preferably bacterium or yeast.
25. Method according to any one of claim 22 to 24, wherein the genetically modified cell is a bacterium, preferably Enterobacteriaceae, more preferably Escherichia.
26. Method according to any one of claim 22 to 24, wherein the genetically modified cell is a yeast, preferably Pichia, Hansenula, Komagataella, Saccharomyces.
27. Method for the production of glycosylated product by a genetically modified Gram-negative bacterial cell, comprising the steps of:
- providing a Gram-negative bacterial cell genetically modified for the production of glycosylated product, said cell comprising at least one nucleic acid sequence coding for an enzyme for glycosylated product synthesis,
 - said cell further genetically modified for reduced cell wall biosynthesis by deletion, reduced or abolished expression of at least one enzyme within the cell wall biosynthesis pathway, said cell wall biosynthesis being cell wall carbohydrate antigen biosynthesis, preferably O-antigen and/or common-antigen biosynthesis
 - culturing the cell in a medium under conditions permissive for the production of glycosylated product,
 - optionally separating glycosylated product from the culture.
28. Method according to claim 27, wherein said enzyme for glycosylated product synthesis comprises enzymes involved in nucleotide-activated sugar synthesis and glycosyltransferases.

29. Method according to any one of claim 27 or 28, wherein said Gram-negative bacterial cell has a further cell wall biosynthesis pathway that is reduced by a deletion, reduced or abolished expression of at least one enzyme within said further cell wall biosynthesis pathway chosen from colanic acid biosynthesis, exopolysaccharide biosynthesis and/or lipopolysaccharide biosynthesis.
30. Method for the production of glycosylated product by a genetically modified yeast cell, comprising the steps of:
- providing a yeast cell genetically modified for the production of glycosylated product, said cell comprising at least one nucleic acid sequence coding for an enzyme for glycosylated product synthesis,
 - said cell further genetically modified for reduced cell wall biosynthesis by deletion, reduced or abolished expression of at least one enzyme within the cell wall biosynthesis pathway, said cell wall biosynthesis being i) cell wall protein mannosylation biosynthesis, ii) beta-1,3-glucan biosynthesis, iii) beta-1,6-glucan biosynthesis, and/or iv) chitin biosynthesis,
 - culturing the cell in a medium under conditions permissive for the production of glycosylated product,
 - optionally separating glycosylated product from the culture.
31. Method according to claim 30, wherein said enzyme for glycosylated product synthesis comprises enzymes involved in nucleotide-activated sugar synthesis and glycosyltransferases.
32. Method for the production of glycosylated product by a genetically modified Corynebacterium, Nocardia or Mycobacterium cell, comprising the steps of:
- providing a Corynebacterium, Nocardia or Mycobacterium cell genetically modified for the production of glycosylated product, said cell comprising at least one nucleic acid sequence coding for an enzyme for glycosylated product synthesis,
 - said cell further genetically modified for reduced cell wall biosynthesis by deletion, reduced or abolished expression of at least one enzyme within the cell wall biosynthesis pathway, said cell wall biosynthesis being i) mycolic acid biosynthesis, and/or ii) arabinogalactan biosynthesis,
 - culturing the cell in a medium under conditions permissive for the production of glycosylated product,
 - optionally separating glycosylated product from the culture.
33. Method according to claim 32, wherein said enzyme for glycosylated product synthesis comprises enzymes involved in nucleotide-activated sugar synthesis and glycosyltransferases.
34. Method for the production of glycosylated product by a genetically modified Bacillus cell, comprising the steps of:

- providing a *Bacillus* cell genetically modified for the production of glycosylated product, said cell comprising at least one nucleic acid sequence coding for an enzyme for glycosylated product synthesis,
 - said cell further genetically modified for reduced cell wall biosynthesis by deletion, reduced or abolished expression of at least one enzyme within the cell wall biosynthesis pathway, said cell wall biosynthesis being teichoic acid biosynthesis,
 - culturing the cell in a medium under conditions permissive for the production of glycosylated product,
 - optionally separating glycosylated product from the culture.
35. Method according to claim 34, wherein said enzyme for glycosylated product synthesis comprises enzymes involved in nucleotide-activated sugar synthesis and glycosyltransferases.
36. A method for the production of glycosylated product, the method comprising the steps of:
- a) providing a cell of a micro-organism according to any one of claims 1 to 19,
 - b) culturing the cell in a medium under conditions permissive for the production of said glycosylated product,
 - c) optionally separating said glycosylated product from the culture.
37. Method according to any one of claims 20 to 36, wherein the cell wall biosynthesis is reduced by deletion, reduced or abolished expression of at least one glycosyltransferase within the cell wall biosynthesis pathway.
38. Method according to any one of claims 20 to 37, wherein said glycosylated product is chosen from saccharide, a glycosylated aglycon, a glycolipid or a glycoprotein.
39. Method according to any one of claims 20 to 38, wherein said glycosylated product is an oligosaccharide, preferably a mammalian milk oligosaccharide.
40. Method according to any one of claims 20 to 39, wherein said glycosylated product is an oligosaccharide, preferably an oligosaccharide with a degree of polymerization higher than 3.
41. Use of a micro-organism according to any one of the claims 1 to 19, in a method for the production of an oligosaccharide, preferably a mammalian milk oligosaccharide.
42. Method according to claim 27, characterized in that the cell is an *Escherichia coli* cell.
43. Method for the production of glycosylated product by a genetically modified cell in a bioreactor, comprising the steps of:
- providing a cell genetically modified for the production of glycosylated product, said cell comprising at least one nucleic acid sequence coding for an enzyme for glycosylated product synthesis,
 - culturing the cell in a medium under conditions permissive for the production of glycosylated product,
- characterized in that the vessel filling of the bioreactor is equal to or higher than 50%.
44. Method according to claim 43, wherein said enzyme for glycosylated product synthesis

comprises enzymes involved in nucleotide-activated sugar synthesis and glycosyltransferases.

45. Method according to any one of claim 43 or 44, wherein said cell is a cell of a micro-organism according to any one of claims 1 to 19.
- 5 46. Method according to any one of claim 43 to 45, wherein said glycosylated product is an oligosaccharide, preferably a mammalian milk oligosaccharide, more preferably chosen from the group of fucosylated oligosaccharide, neutral oligosaccharide or sialylated oligosaccharide, most preferably chosen from 2'-fucosyllactose, 3-fucosyllactose, difucosyllactose, Lacto-N-tetraose, Lacto-N-neotetraose, 3'-sialyllactose, 6'-sialyllactose, 10 lacto-N-fucopentaose II, lacto-N-fucopentaose I, lacto-N-fucopentaose III, lacto-N-fucopentaose V, lacto-N-fucopentaose VI, sialyllacto-N-tetraose d (LSTd), sialyllacto-N-tetraose c (LSTc), sialyllacto-N-tetraose b (LSTb), sialyllacto-N-tetraose a (LSTa).