

Claims

1. Microbial cell naturally synthesizing KDO genetically modified to produce an oligosaccharide characterised in that the KDO biosynthesis of said cell is knocked out or rendered less functional.
- 5 2. A cell according to claim 1 wherein said cell comprises at least one glycosyltransferase with affinity for CMP-KDO, preferably said glycosyltransferase is overexpressed.
3. A cell according to any one of claim 1 to 2, wherein said cell is capable of synthesizing a nucleotide sugar selected from the group: GDP-fucose, GDP-mannose, GDP-rhamnose, CMP-N-acetylneuraminic acid, CMP-N-glycolylneuraminic acid, UDP-glucose, dTDP-
10 UDP-glucose, UDP-galactose, UDP-N-acetylmannosamine, UDP-N-acetylglucosamine, UDP-N-acetylgalactosamine, UDP-glucuronic acid, UDP-xylose, UDP-arabinose, and/or UDP-galacturonic acid
4. The cell according to any one of claims 1 to 3, wherein an ATP-dependent translocator encoding gene is overexpressed and/or an inner membrane protein encoding gene is
15 overexpressed and/or an lauroyl acyltransferase encoding gene is overexpressed and/or an endogenous ATP-dependent translocator encoding gene is modified and/or an endogenous inner membrane protein encoding gene is modified, preferably said inner membrane protein is a transmembrane transporter.
5. The cell according to any one of claims 1 to 4, wherein the cell comprises a KDO-independent
20 lauroyl acyltransferase encoding gene and/or wherein the cell comprises an ATP-binding cassette multidrug transporter encoding gene.
6. Cell according to any one of claim 4 to 5, wherein said overexpression of the ATP-dependent translocator, the inner membrane protein and/or lauroyl acyltransferase is an overexpression of the endogenous gene encoding said protein or obtained by introducing and expressing
25 said protein.
7. Cell according to any one of claim 1 to 6, wherein said modification of said endogenous ATP-dependent translocator encoding gene and/or said endogenous inner membrane protein encoding gene is a point-mutation.
8. Cell according to any one of claim 1 to 7, wherein said ATP-dependent translocator has 80%
30 or more sequence identity to SEQ ID NO: 4 and has ATP-dependent translocator activity; said inner membrane protein has 80% or more sequence identity to SEQ ID NO: 21 and has transmembrane transporter activity and/or said lauroyl acyltransferase has 80% or more sequence identity to SEQ ID NO: 1 and has lauroyl acyltransferase activity.
9. Cell according to any one of claim 5 to 8, wherein said KDO-independent lauroyl
35 acyltransferase encoding gene and/or wherein said ATP-binding cassette multidrug transporter is i) introduced and expressed or ii) overexpressed in said cell.
10. Cell according to any one of claim 1 to 9, wherein the cell comprises expression of the gene encoding the KDO-independent lauroyl acyltransferase of SEQ ID NO: 2 or SEQ ID NO: 3,

- or a protein having at least 80% sequence identity thereto and having KDO-independent lauroyl acyltransferase activity.
11. Cell according to any one of claim 1 to 10, wherein the cell comprises expression of the gene encoding the ATP-binding cassette multidrug transporter of SEQ ID NO: 22, or a protein having at least 80% sequence identity thereto and having transmembrane transporter activity.
12. The cell according to any one of claims 1 to 11, wherein at least one of the genes selected from the group of genes encoding for D-arabinose 5-phosphate isomerase, 3-deoxy-D-*manno*-octulosonate 8-phosphate synthase, 3-deoxy-D-*manno*-octulosonate 8-phosphate phosphatase, and/or 3-deoxy-*manno*-octulosonate cytidyltransferase is rendered less functional or knocked out.
13. Cell according to any one of claim 1 to 12, wherein said cell produces a neutral, sialylated and/or fucosylated oligosaccharide.
14. Cell according to any one of claim 1 to 13, wherein said cell produces a mammalian milk oligosaccharide, preferably a neutral, sialylated and/or fucosylated milk oligosaccharide.
15. Cell according to any one of claim 1 to 14, wherein said glycosyltransferase is a sialyltransferase.
16. Cell according to any one of claim 1 to 15, wherein said cell comprises a deleted or inactivated endogenous beta-galactosidase gene.
17. Cell according to claim 16, wherein said deleted or inactivated beta-galactosidase gene comprises *E. coli lacZ* gene.
18. Cell according to any one of the preceding claims, wherein said microbial cell further comprises a deleted, inactivated, or mutated galactoside-O-acyltransferase (*lacA*) encoding gene.
19. Cell according to any one of the preceding claims, wherein said cell further comprises a nucleic acid sequence encoding at least one of the following additional proteins: an exporter protein or a permease exporting the synthesized oligosaccharide from the microbial cell.
20. Cell according to any one of the preceding claims, wherein said cell is further genetically modified to contain a nucleic acid sequence encoding a glycosidase for the specific degradation of interfering oligosaccharides, such as intermediates, side products or endogenous oligosaccharides generated by bacterial host cell, wherein the expression of said glycosidase is under control of a regulatory sequence.
21. An isolated microbial cell according to any one of claims 1 to 20.
22. Use of a cell according to any one of claims 1 to 21, for the production of sialylated oligosaccharides substantially free of KDO-lactose and/or KDO-oligosaccharide.
23. Method for the fermentative production of an oligosaccharide substantially free of KDO-oligosaccharide, said method comprising: providing a microbial cell according to any one of the claims 1 to 20, cultivating said cells in favourable growing conditions and optionally separating or isolating the oligosaccharide from the culture.

24. Method for producing a sialylated oligosaccharide by fermentation through genetically modified microbial cell naturally synthesizing KDO, the method comprising the steps of:
- a) Obtaining a microbial cell naturally synthesizing KDO and being capable to produce sialylated oligosaccharides and expressing a sialyltransferase with affinity for CMP-KDO, and wherein the KDO-biosynthesis route of said cell is knocked out or rendered less functional;
 - b) Culturing the cell from step a) in favourable growing conditions, thus producing said sialylated oligosaccharide and
 - c) Optionally, separating or isolating the sialylated oligosaccharide from the culture medium.
25. The method of any one of the preceding claims 23 or 24, wherein said culture medium comprises precursor for the production of said oligosaccharide, preferably said culture medium comprises any one or more of lactose, galactose, N-acetylglucosamine N-acetyl-D-lactosamine, Lacto-N-biose, sialic acid, N-acetylneuraminic acid, fucose.
26. Method of any one of claim 23 to 25, wherein said cell produces the precursor internally, preferably said cell produces any one or more of lactose, N-acetyl-D-lactosamine, Lacto-N-biose, sialic acid, N-acetylneuraminic acid, fucose.
27. Method according to any one of claims 23 to 26, or the microbial cell according to any one of claims 1 to 20, wherein said sialylated oligosaccharide is 3'-sialyllactose, 6'-sialyllactose, disialyl lacto-N-tetraose, sialylated lacto-N-triose, sialylated lacto-N-tetraose, sialylated lacto-N-neotetraose, 3-fucosyl-3'-sialyllactose, lacto-N-sialylpentaose LSTa, LSTb, LSTc, LSTd.
28. Method according to any one of claims 23 to 27, or the microbial cell according to any one of claims 1 to 21 or 27, wherein said cell is a Gram-negative microbial cell, preferably said cell is an *Escherichia coli* strain, preferably an *Escherichia coli* strain which is a K12 strain, more preferably the *Escherichia coli* K12 strain is *Escherichia coli* K12 substr. MG1655.
29. The method according to any one of claims 23 to 28, wherein said oligosaccharide is isolated from said culture medium by means of unit operation selected from the group comprising centrifugation, filtration, microfiltration, ultrafiltration, nanofiltration, ion exchange, electrodialysis, chromatography, simulated moving bed chromatography, simulated moving bed ion exchange, evaporation, precipitation, crystallisation, spray drying and any combination thereof.
30. An oligosaccharide produced by the cell according to any one of claims 1 to 21, or 27 and/or according to the method described in any one of claims 23 to 29, wherein said oligosaccharide is added to food formulation, feed formulation, pharmaceutical formulation, cosmetic formulation, or agrochemical formulation.