On the other hand, HMO production in genetically modified microorganisms holds promise to provide stable and safe means of production at low cost. US 7,521,212 describes a method for producing oligosaccharides by a genetically modified *E. coli* cell from the precursor lactose, involving the inactivation of the *lacZ* gene. EP 2 675 899, EP 2 877 574, EP 2 440 661 and EP 3 191 499 further describe different fucosyltransferases for producing 2-FL or 3-FL in genetically modified microorganisms. Huang *et al*., 2017 describe the biosynthesis of 2-FL or 3-FL in genetically modified *Escherichia coli* (*E. coli*) and multiple modular optimization strategies thereto (Huang *et al*., Metab Eng. 2017; (41):23-38). EP 2 440 661 describes *E. coli* expressing sugar exporters to increase export of 2-FL into the medium.

2-FL consists of lactose and fucose (Pereira & McDonald, Sci Rep. 2012; 84(1):637-655). The biosynthesis pathway of 2-(3-)-FL in *Escherichia coli* is shown schematically in Fig.1. *E. coli* is able to incorporate the precursors glucose and lactose into the cells via specific transporters. Glucose is absorbed via the phosphotransferase system and converted into glucose-6-phosphate. The latter serves as a source of carbon and energy to drive growth and as a precursor for GDP-L-fucose, an intermediate of the colanic acid biosynthesis. The *de novo* biosynthetic pathway of GDP-L-fucose is derived from the glycolytic intermediate fructose 6-phosphate and is based on the enzymes ManA (mannose 6-phosphate isomerase), ManB (phosphomanno-mutase), the GTP-dependent ManC (mannose 1-phosphate guanylyltransferase), Gmd (GDP-D-mannose 4,6-dehydratase) and the NADPH-dependent WcaG (GDP-L-fucose synthase), all derived from *E. coli* (FIG.2). The second precursor, lactose, is absorbed into the cells via the lactose permease LacY, a lactose/H^+-symporter. The key enzyme for 2-FL production is the α-1,2-fucosyltransferase (FutC) derived from *Helicobacter pylori*, which transfers the fucose portion of the donor GDP-L-fucose to the acceptor lactose, producing 2-FL (FIG.2). By replacing the *futC* gene with the *futA* gene from *H. pylori*, which encodes an α-1,3-fucosyltransferase, the biosynthesis pathway can be used for the production of 3'-Fucosyllactose.

Despite recent advances, there is still a need for improved methods of biotechnological production of HMOs, and particularly 2-FL and/or 3-FL, in genetically modified microorganisms. Relying on the known 2-FL biosynthetic pathway in *E. coli*, the invention significantly improves the synthesis of HMOs and their release into the medium.

SUMMARY

The present disclosure provides microorganisms for the improved production of HMOs and HMO production methods using the same.

In one aspect, the present disclosure refers to a genetically modified microorganism for the production of human milk oligosaccharide, wherein the microorganism is optionally a bacterium, and preferably *Escherichia coli* (*E. coli*), wherein the microorganism comprises an inducible lysis system, wherein the inducible lysis system is optionally auto inducible.

In particular the present disclosure provides microorganisms comprising an inducible lysis system, which allows for the easy extraction of intracellular and extracellular HMOs at the same time without the need of additional processing steps to obtain the intracellular fraction. A preferred example of the inducible lysis

CLAIMS

1. A genetically modified microorganism for the production of human milk oligosaccharide, wherein the microorganism is optionally a bacterium, and preferably *Escherichia coli* (*E. coli*), wherein the microorganism comprises an inducible lysis system, wherein the inducible lysis system is optionally auto inducible.

2. The genetically modified microorganism according to claim 1, wherein the inducible lysis system is Mg2+-regulated, and/or wherein the inducible lysis system is optionally regulated by the exogenous free Mg2+ concentration, and/or wherein the microorganism optionally comprises a Mg2+-regulated promoter, and/or wherein the microorganism further optionally comprises an additional Mg2+-regulated element, preferably the 5'UTR of the mgtA gene of *Escherichia coli*.

3. The genetically modified microorganism according to claims 1 or 2, wherein the microorganism comprises a lysis genes, wherein the lysis gene is optionally a lysis gene from a bacteriophage.

4. The genetically modified microorganism according to any one of claims 1 to 3, wherein the microorganism comprises a Mg2+-regulated promoter and lysis genes, wherein expression of the lysis gene(s) is controlled by the Mg2+-regulated promoter, and optionally by an additional Mg2+- regulated element.

5. The genetically modified microorganism according any one of claims 1 to 6, wherein the microorganism is unable to cleave lactose into glucose and galactose, and/or wherein optionally the Iac operon is inactivated, preferably by deletion.

6. The genetically modified microorganism according to any one of claims 1 to 5, wherein the microorganism comprises an exogenous gene encoding a lactose permease, wherein the lactose permease is preferably the *E. coli* LacY protein, wherein the lactose permease optionally has A198V and/or S209I mutations.

7. The genetically modified microorganism according to any one of claims 1 to 6, wherein the human milk oligosaccharide is 2'-Fucosyllactose and/or 3'-Fucosyllactose.

8. The genetically modified microorganism according to any one of claims 1 to 7, wherein the so microorganism comprises an exogenous gene encoding a fucosyltransferase, wherein the fucosyltransferase is preferably an a-1,2-fucosyltransferase and/or an a-1,3-fucosyltransferase, wherein the gene encoding a fucosyltransferase is optionally a heterologous gene and/or wherein the gene encoding a fucosyltransferase is optionally driven by a heterologous promoter, wherein optionally the gene encoding a fucosyltransferase is codon-optimized for expression in the microorganism.

9. The genetically modified microorganism according to any one of claims 1 to 8, wherein the microorganism comprises a heterologous gene encoding a chaperone, wherein the chaperone is preferably human Hsp70.

10. The genetically modified microorganism according to any one of claims 1 to 7, wherein optionally the rcsA gene, preferably derived from *E coli*, is overexpressed; and/or optionally a gene encoding a Lon protease family protein, preferably *E. coli* Lon protease, is inactivated, preferably by deletion; and/or optionally the wcaJ gene is inactivated, preferably by deletion; and/or optionally the zwf gene and the two pntAB genes, preferably derived from *E coli*, are overexpressed; and /or optionally the gsk gene, preferably derived from *E coli*, is overexpressed; and/or optionally the trxA gene, preferably derived from *E coli*, is overexpressed; and/or optionally the trxB gene is inactivated, preferably by deletion.

11. A method for producing human milk oligosaccharide, which comprises:

(a) culturing the genetically modified microorganism according to any one of claims 1 to 10 in a medium, wherein the medium further optionally comprises lactose and/or free Mg2+, wherein optionally the concentration of the human milk oligosaccharide in the medium at the end of the culture is at least 30 g/L, preferably at least 50 g/L, wherein preferably the human milk oligosaccharide is 2'-Fucosyllactose and/or 3'-Fucosyllactose.

12. The method for producing human milk oligosaccharide according to claim 11, which further comprises: (b) purifying the human milk oligosaccharide from the culture medium and/or from the microorganism itself, wherein optionally the human milk oligosaccharide is purified from the culture medium after lysis has been induced via the inducible lysis system, and/or wherein optionally the human milk oligosaccharide is purified from the culture medium

when the concentration of free Mg^{2+} in the medium is 10 µM or less and/or when the lysis can be directly visualized.

13. Medium obtainable by the method according to claim 11 or human milk oligosaccharide obtainable by the method according to claim 12.

14. Use of the medium or of the human milk oligosaccharide according to claim 13 for the preparation of animal feed.