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 bacterium for the production of 2'-Fucosyllactose, wherein the bacterium is *Escherichia coli* (*E. coli*), and wherein the bacterium comprises: (a) an exogenous gene encoding an α-1,2-fucosyltransferase, wherein the gene encoding the α-1,2-fucosyltransferase is a heterologous gene encoding *futC* from *Helicobacter pylori;* (b) an inducible lysis system, wherein the inducible lysis system is a Mg2+-regulated auto inducible lysis system; and (c) a lysis gene from a bacteriophage and a Mg2+-regulated promoter, wherein expression of the lysis gene is controlled by the Mg2+-regulated promoter.

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

system is an auto-inducible lysis system which is Mg^{2+}-regulated, wherein the cells are lysed under Mg^{2+}-depleted conditions, i.e. when the extracellular Mg^{2+} concentration is below a certain threshold value. Upon lysis of the cells, intracellular HMO may be released into the medium, in which the cells are cultured, leading to an increased HMO concentration in the medium. This may advantageously lead to increased yields of HMO as well as time and cost savings.

The disclosure also provides microorganisms comprising an exogenous gene encoding a lactose permease. The lactose permease is preferably a mutant of LacY, which is less prone to inhibition by intracellular lactose compared to wildtype LacY. This may advantageously lead to an increased yield of HMO.

The present disclosure further provides a method for producing HMOs using the microorganism of the disclosure. In another aspect, the present disclosure refers to a method for producing 2'-Fucosyllactose, which comprises:

(a) culturing the genetically modified bacterium of the disclosure in a medium, wherein the medium comprises lactose and/or free Mg^{2+}, wherein optionally the concentration of the 2'-Fucosyllactose in the medium at the end of the culture is at least 30 g/L, preferably at least 50 g/L.

Also provided is a medium, in which the microorganism of the disclosure has been cultured during the method of producing HMOs, and purified HMOs, obtained by the method for producing HMOs of the disclosure.

Preferred examples:

1. A genetically modified microorganism for the production of human milk oligosaccharide.
2. The genetically modified microorganism according to item 1, wherein the microorganism is a bacterium.
3. The genetically modified microorganism according to item 1 or 2, wherein the microorganism is *Escherichia coli* (*E. coli)*.
4. The genetically modified microorganism according to any one of items 1 to 3, wherein the microorganism comprises an inducible lysis system.
5. The genetically modified microorganism according to item 4, wherein the inducible lysis system is auto-inducible.
6. The genetically modified microorganism according to item 4 or 5, wherein the inducible lysis system is Mg^{2+}-regulated.
7. The genetically modified microorganism according to any one of items 4 to 6, wherein the inducible lysis system is regulated by the exogenous free Mg^{2+} concentration.
8. The genetically modified microorganism according to any one of items 4 to 7, wherein the microorganism comprises a Mg^{2+}-regulated promoter.
9. The genetically modified microorganism according to item 8, wherein the Mg^{2+}-regulated promoter is the Pmgt promoter from the *mgtB* gene of *Salmonella typhimurium* or the PmgtA promoter of *Escherichia coli*.

CLAIMS

1. A genetically modified bacterium for the production of 2'-Fucosyllactose, wherein the bacterium is *Escherichia coli* (*E. coli*), and wherein the bacterium comprises:

(a) an exogenous gene encoding an α-1,2-fucosyltransferase, wherein the gene encoding the α-1,2-fucosyltransferase is a heterologous gene encoding *futC* from *Helicobacter pylori*;

(b) an inducible lysis system, wherein the inducible lysis system is a Mg2+-regulated auto inducible lysis system; and

(c) a lysis gene from a bacteriophage and a Mg2+-regulated promoter, wherein expression of the lysis gene is controlled by the Mg2+-regulated promoter.

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

3. The genetically modified bacterium according to claim 1 or 2, wherein the expression of the lysis gene is controlled by the Mg2+-regulated promoter and by an additional Mg2+-regulated element.

4. The genetically modified bacterium according any one of claims 1 to 3, wherein the bacterium is unable to cleave lactose into glucose and galactose, and/or wherein optionally the lac operon is inactivated, preferably by deletion.

5. The genetically modified bacterium according to any one of claims 1 to 4, wherein the bacterium 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.

6. The genetically modified bacterium according to any one of claims 1 to 5, wherein the gene encoding the α-1,2-fucosyltransferase is driven by a heterologous promoter, wherein optionally the gene encoding the α-1,2-fucosyltransferase is codon-optimized for expression in the bacterium.

7. The genetically modified bacterium according to any one of claims 1 to 6, wherein the bacterium comprises a heterologous gene encoding a chaperone, wherein the chaperone is preferably human Hsp70.

8. The genetically modified bacterium according to any one of claims 1 to 5, wherein the *rcsA* gene, preferably derived from *E coli* is overexpressed; or a gene encoding a Lon protease family protein, preferably *E. coli* Lon protease is inactivated, preferably by deletion; or the *wcaJ* gene is inactivated, preferably by deletion; or the *zwf* gene and the two *pntAB* genes, preferably derived from *E coli*, are overexpressed; or the *gsk* gene, preferably derived from *E coli*, is overexpressed; or the *trxA* gene, preferably derived from *E coli,* is overexpressed; or the *trxB* gene is inactivated, preferably by deletion.

9. A method for producing 2'-Fucosyllactose, which comprises:

(a) culturing the genetically modified bacterium according to any one of claims 1 to 8 in a medium, wherein the medium comprises lactose and/or free Mg2+, wherein optionally the concentration of the 2'-Fucosyllactose in the medium at the end of the culture is at least 30 g/L, preferably at least 50 g/L.

10. The method for producing 2'-Fucosyllactose according to claim 9, which further comprises:

(b) purifying the 2'-Fucosyllactose from the culture medium and/or from the bacterium itself, wherein optionally the 2'-Fucosyllactose is purified from the culture medium after lysis has been induced via the inducible lysis system, or wherein the 2'-Fucosyllactose is purified from the culture medium when the concentration of free Mg2+ in the medium is 10 µM or less or when the lysis can be directly visualized.