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*).

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 one aspect, the present disclosure refers to a method for producing human milk oligosaccharide, which comprises:

(a) culturing the genetically modified microorganism of the disclosure in a medium, wherein the medium further optionally comprises lactose and/or free Mg^{2+}, 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.

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. In one aspect, the present disclosure refers to a medium obtainable by the method disclosed herein or human milk oligosaccharide obtainable by the method disclosed herein. In another aspect, the present disclosure refers to use of the medium or of the human milk oligosaccharide disclosed herein for the preparation of animal feed.

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*.