donor substrates that are naturally occurring in *B. subtilis* cells, production of a fucosylated oligosaccharide in *Bacillus* requires implementation of a heterologous metabolic pathway in the cell for providing the required donor substrate GDP-fucose.

Chinese patent application CN 109 735 479 A discloses recombinant *Bacillus subtilis* cells for synthesizing 2'-fucosyllactose, wherein the expression of a lactose transport enzyme is enhanced, and which cell expresses a fucose kinase, a phosphate guanine transferase and a fucosyltransferase. The yield of 2'-fucosyllactose in the fermentation medium was reported to be between 0.424 g/L and 1.042 g/L.

It was nonetheless an object of the present invention to provide a bacterial cell of the genus *Bacillus* for the production of fucosylated oligosaccharides.

The object has been achieved by providing a *Bacillus* cell that possesses a lactose permease for import of exogenous lactose into the cell, a *de novo* GDP-fucose biosynthesis pathway for providing GDP-fucose, and a fucosyltransferase for transfer of a fucose moiety from GDP-fucose to lactose. Said *Bacillus* cell can be cultivated in the presence of exogenous lactose to produce a fucosylated oligosaccharide.

Summary

According to a first example, provided is a non-sporulating bacterial cell of the genus *Bacillus* for the production of a fucosylated oligosaccharide, wherein the *Bacillus* cell possesses a lactose permease, a GDP-fucose biosynthesis pathway, and a fucosyltransferase.

In one aspect, the present disclosure refers to a non-sporulating *Bacillus* cell for the production of a fucosylated oligosaccharide, wherein said fucosylated oligosaccharide is a fucosyllactose, wherein said cell has been genetically engineered to possess a lactose permease, a GDP-fucose biosynthesis pathway and a fucosyltransferase.

According to a second example, provided is the use of the non-sporulating bacterial cell of the genus *Bacillus* according to the first example for the production of a fucosylated oligosaccharide.

In another aspect, the present disclosure refers to use of a *Bacillus* cell as disclosed herein for the production of a fucosylated oligosaccharide, wherein said fucosylated oligosaccharide is a fucosyllactose.

According to a third example, provided is a method for the production of a fucosylated oligosaccharide, the method comprising:

- providing a non-sporulating bacterial cell of the genus *Bacillus*, wherein said *Bacillus* cell possesses a lactose permease, a GDP-fucose biosynthetic pathway, and a lactose-accepting fucosyltransferase;
- cultivating the *Bacillus* cell in a culture medium containing lactose and under conditions that are permissive for the *Bacillus* cell to produce the fucosylated oligosaccharide, and
- optionally retrieving the fucosylated oligosaccharide from the culture medium and/or the *Bacillus* cell.

In another aspect, the present disclosure refers to a method for the production of a fucosylated oligosaccharide, wherein said fucosylated oligosaccharide is a fucosyllactose,

- providing a non-sporulating *Bacillus* cell as disclosed herein;
- cultivating said *Bacillus* cell in a medium and under conditions that are permissive for the production of a fucosylated oligosaccharide; and optionally
- retrieving the fucosylated oligosaccharide from the medium and/or the *Bacillus* cell.

According to a fourth example, provided are fucosylated oligosaccharides that were produced by a *Bacillus* cell which possesses a lactose permease, a GDP-fucose biosynthesis pathway and a fucosyltransferase.

According to a fifth example, provided is the use of a fucosylated oligosaccharide that was produced by a *Bacillus* cell which possesses a lactose permease, a GDP-fucose biosynthesis pathway and a fucosyltransferase for the manufacturing of a nutritional composition.

According to a sixth example, provided are nutritional compositions containing at least one fucosylated oligosaccharide that had been produced by a *Bacillus* cell which possesses a lactose permease, a GDP-fucose biosynthesis pathway and a fucosyltransferase.

Detailed description

The present invention concerns *Bacillus* cells for the production of fucosylated oligosaccharides, their use and methods for producing fucosylated oligosaccharides by cultivating said *Bacillus* cells in a culture medium containing lactose, and under conditions that are permissive for the production of said fucosylated oligosaccharide by said *Bacillus* cell.

CLAIMS

1. A non-sporulating *Bacillus* cell for the production of a fucosylated oligosaccharide, wherein said fucosylated oligosaccharide is a fucosyllactose, wherein said cell has been genetically engineered to possess a lactose permease, a GDP-fucose biosynthesis pathway and a fucosyltransferase.

2. The *Bacillus* cell according to claim 1, wherein the sporulation has been impaired by deletion or functional inactivation of one or more of the genes encoding Spo0A, sigma E and sigma F.

3. The *Bacillus* cell according to claim 1 or 2, wherein the lactose permease is *E. coli* LacY or a functional variant thereof.

4. The *Bacillus* cell according to any one of claims 1 to 3, wherein the GDP-fucose biosynthesis pathway is a *de novo* GDP-fucose biosynthesis pathway and/or a salvage pathway.

5. The *Bacillus* cell according to any one of claims 1 to 4, wherein the fucosyltransferase is a lactose-accepting fucosyltransferase, preferably a fucosyltransferase selected from the group of α-1,2-fucosyltransferases and α-1,3-fucosoyltransferases.

6. The *Bacillus* cell according to any one of claims 1 to 5, wherein said *Bacillus* cell lacks any β-galactosidase activity or possesses reduced β-galactosidase activity as compared to a wild-type progenitor *Bacillus* cell of the same species.

7. The *Bacillus* cell according to claim 6, wherein the *Bacillus* cell has been genetically engineered by deletion or functional inactivation of at least one of the genes selected from the group consisting of *yes*Z and *gan*A.

8. The Bacillus cell according to any one of claims 1 to 7, wherein said *Bacillus* cell is a *Bacillus subtilis* cell.

9. Use of a *Bacillus* cell according to any one of claims 1 to 8 for the production of a fucosylated oligosaccharide, wherein said fucosylated oligosaccharide is a fucosyllactose.

10. A method for the production of a fucosylated oligosaccharide, wherein said fucosylated oligosaccharide is a fucosyllactose,
    - providing a non-sporulating *Bacillus* cell as defined in any one of claims 1 to 8;
    - cultivating said *Bacillus* cell in a medium and under conditions that are permissive for the production of a fucosylated oligosaccharide; and optionally
    - retrieving the fucosylated oligosaccharide from the medium and/or the *Bacillus* cell.

11. The method according to claim 10, wherein the medium contains lactose.

12. The method according to claim 10 or 11, wherein the medium contains L-fucose.