Summary

According to a first example, provided is a non-sporulating bacterial cell of the genus *Bacillus* for the production of a sialylated oligosaccharide, wherein the *Bacillus* cell possesses a lactose permease, a CMP-NeuNAc biosynthesis pathway and a sialyltransferase.

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 sialylated oligosaccharide.

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

- providing a non-sporulating bacterial cell of the genus *Bacillus*, wherein said *Bacillus* cell possesses a lactose permease, a CMP-NeuNAc biosynthesis pathway and a sialyltransferase;
- cultivating the *Bacillus* cell in a culture medium containing lactose and under conditions that are permissive for the *Bacillus* cell to produce the sialylated oligosaccharide, and
- optionally retrieving the sialylated oligosaccharide from the culture medium and/or the *Bacillus* cell.

According to a fourth example, provided are sialylated oligosaccharides that were produced by a *Bacillus* cell which possesses a lactose permease, a CMP-NeuNAc biosynthesis pathway and a sialyltransferase.

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

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

In one aspect, the present disclosure refers to a non-sporulating *Bacillus* cell for the production of a sialylated oligosaccharide, wherein said *Bacillus* cell has been genetically engineered to possess a lactose permease, a CMP-NeuNAc biosynthesis pathway and a sialyltransferase, 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, 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.

In another aspect, the present disclosure refers to use of a *Bacillus* cell as disclosed herein for the production of a sialylated oligosaccharide.

In another aspect, the present disclosure refers to a method for the production of a sialylated oligosaccharide, the method comprising

- providing a non-sporulating *Bacillus* cell as disclosed herein; - cultivating said *Bacillus* cell in a fermentation broth and under conditions that are permissive for the production of a sialylated oligosaccharide.

CLAIMS

1. A non-sporulating *Bacillus* cell for the production of a sialylated oligosaccharide, wherein said *Bacillus* cell has been genetically engineered to possess a lactose permease, a CMP-NeuNAc biosynthesis pathway and a sialyltransferase, 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, 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.

2. The *Bacillus* cell according to claim 1, wherein the sporulation capability of the *Bacillus* cell 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 CMP-NeuNAc biosynthesis pathway uses GlcN-1P or GlcNAc-6P as intermediate.

5. The *Bacillus* cell according to any one of claims 1 to 4, wherein the CMP-NeuNAc biosynthesis pathway uses a sialic acid salvage pathway.

6. The *Bacillus* cell according to any one of claims 1 to 5, wherein the sialyltransferase is a lactose-accepting sialyltransferase.

7. The *Bacillus* cell according to claim 6, wherein the lactose-accepting sialyltransferase is selected from the group consisting of α-2,3- sialyltransferases, α-2,6-sialyltransferases and α-2,8- sialyltransferases.

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

9. The *Bacillus* cell according to any one of claims 1 to 8, for use in the production of 3'-sialylactose, wherein the CMP-NeuNAc biosynthesis pathway uses GlcN-1P as intermediate, and wherein the sialyltransferase is a α-2,3-sialyltransferase.

10. The *Bacillus* cell according to any one of claims 1 to 9, for use in the production of 6'-sialylactose, wherein the CMP-NeuNAc biosynthesis pathway uses GlcN-1P as intermediate, and wherein the sialyltransferase is a α-2,6-sialyltransferase.

11. Use of a *Bacillus* cell according to any one of claims 1 to 10 for the production of a sialylated oligosaccharide.

12. A method for the production of a sialylated oligosaccharide, the method comprising
    - providing a non-sporulating *Bacillus* cell as defined in any one of claims 1 to 10;
    - cultivating said *Bacillus* cell in a fermentation broth and under conditions that are permissive for the production of a sialylated oligosaccharide.

13. The method for the production of a sialylated oligosaccharide according to claim 12, the method comprising retrieving the sialylated oligosaccharide from the medium and/or the *Bacillus* cell.

14. The method according to claims 12 or 13, wherein the fermentation broth contains lactose.