from *Bacillus* predominantly produce β1-4 bonds (resulting in a GOS preparation that predominantly comprises β1-4 bonds, which may also be referred to as "4'-GOS"). Moreover, beta-galactosidase produced by *B. circulans* possesses particularly strong transgalactosylation activity, and thus, GOS prepared by *B. circulans* is sold worldwide.

Since its introduction to the market (1999), approximately more than 100 million of infants have consumed infant formula containing GOS prepared by *B. circulans*. It has been proven to be a safe ingredient, with a GRAS status acknowledged by the FDA.

In the past few years, however, a small number of very rare cases of GOS-related allergy has been reported in South East Asia. Research has shown that certain oligosaccharide structures present in GOS can exert an allergic response in very sensitive subjects [8].

Jyo *et al.* (Occup. Environ. Allergy 3, 12–20 (1992)) determined allergy symptoms after consumption of a lactobacillus beverage containing 6'-GOS produced by fungal beta-galactosidase. Allergy symptoms have also been observed with 4'-GOS produced by bacterial beta-galactosidase. In 2014, Kaneko *et al*. (Biosc. Biotechnol. Biochem. 78, 100-108) observed that GOS produced by treating lactose with a beta-galactosidase derived from *B. circulans* may induce allergic reactions and revealed that the allergies were caused by two tetrasaccharides [Gal β1-4 (Gal β1-4 Gal β1-6) Glc, Gal β1-4 Gal β1-4 Gal β1-3 Glc]. These GOS allergy cases occurred in subjects who already had a history of atopy.

The present inventors aimed at the manufacture of a GOS preparation having reduced allergenicity. In particular, they sought to identify a beta-galactosidase for use in the manufacture of a GOS preparation having a reduced capacity to induce an allergic response in a subject, as compared to a GOS preparation obtained by *Bacillus circulans* beta-galactosidase or by *Aspergillus oryzae* beta-galactosidase.

Summary of the invention

It was surprisingly observed that a GOS preparation produced by a β-galactosidase derived from a microorganism of the genus *Cryptococcus* has an unexpected low allergenicity. More specifically, it was found in an oral challenge test that GOS obtained using a *Cryptococcus terrestris* beta-galactosidase enzyme did not induce allergic symptoms in human subjects suffering from hypersensitivity to GOS prepared by conventional *B. circulans* enzyme.

In one aspect, the present disclosure refers to the use of a beta-galactosidase (EC 3.2.1.23) derived from *Cryptococcus terrestris* (recently renamed *Papiliotrema terrestris*) having an amino acid sequence according to any of SEQ ID NO: 1, 2, 3, or 4, or an amino acid sequence that is at least 95% identical to any of SEQ ID NO: 1, 2, 3, or 4, in

the production of a hypoallergenic galacto-oligosaccharide (GOS) preparation, wherein said GOS preparation has a reduced capacity to induce an (IgE-mediated) allergic response in a subject, as compared to a GOS preparation obtained by transgalactosylation of lactose using a beta-galactosidase derived from *Bacillus circulans* or from *Aspergillus oryzae.* In another aspect, the present disclosure refers to the use of a hypoallergenic GOS preparation obtainable by transgalactosylation of lactose using a beta-galactosidase (EC 3.2.1.23) derived from *Cryptococcus terrestris* (recently renamed *Papiliotrema terrestris*), having an amino acid sequence according to any of SEQ ID NO: 1, 2, 3, or 4, or an amino acid sequence that is at least 95% identical to any of SEQ ID NO: 1, 2, 3, or 4, in the manufacture of a medicament for at least partially preventing an (IgE-mediated) allergic response in a subject known to suffer or having an increased chance to suffer from hypersensitivity to a GOS preparation obtained by transgalactosylation of lactose using a beta-galactosidase derived from B*acillus circulans* or from *Aspergillus oryzae*. In yet another aspect, the present disclosure refers to the use of a nutritional composition in the manufacture of a medicament for at least partially preventing an (IgE-mediated) allergic response in a subject known to suffer or having an increased chance to suffer from hypersensitivity to a GOS preparation obtained by transgalactosylation of lactose using a beta-galactosidase derived from *Bacillus circulans* or from *Aspergillus oryzae*, said nutritional composition comprising (i) a hypoallergenic GOS preparation obtainable by transgalactosylation of lactose using a beta-galactosidase (EC 3.2.1.23) derived from *Cryptococcus terrestris* having an amino acid sequence according to any of SEQ ID NO: 1, 2, 3, or 4, or an amino acid sequence that is at least 95% identical to any of SEQ ID NO: 1, 2, 3, or 4, and (ii) at least one further ingredient selected from the group consisting of a hypoallergenic or non-allergenic protein source, preferably a protein hydrolysate, preferably a non-allergenic protein hydrolysate, free amino acids, probiotics, LC-PUFA's and carbohydrates, such as lactose, saccharose, starch or maltodextrin. In still another aspect, the present disclosure refers to a method for providing a hypoallergenic nutritional composition, comprising (i) contacting a lactose feed with a beta-galactosidase (EC 3.2.1.23) derived from *Cryptococcus terrestris* having an amino acid sequence according to any of SEQ ID NO: 1, 2, 3, or 4, or an amino acid sequence that is at least 95% identical to any of SEQ ID NO: 1, 2, 3, or 4, to provide a hypoallergenic galacto-oligosaccharide (GOS) preparation, and (ii) formulating said hypoallergenic GOS preparation together with at least one further hypoallergenic or non-allergenic ingredient into a hypoallergenic nutritional composition. In yet another aspect, the present disclosure refers to a hypoallergenic nutritional composition obtainable by a method according the present disclosure.

Herewith, the disclosure provides the use of a beta-galactosidase (EC 3.2.1.23) derived from *C. terrestris* in the production of a hypoallergenic galacto-oligosaccharide (GOS)

Claims

1. The use of a beta-galactosidase (EC 3.2.1.23) derived from *Cryptococcus terrestris* (recently renamed *Papiliotrema terrestris*) having an amino acid sequence according to any of SEQ ID NO: 1, 2, 3, or 4, or an amino acid sequence that is at least 95% identical to any of SEQ ID NO: 1, 2, 3, or 4, in the production of a hypoallergenic galacto-oligosaccharide (GOS) preparation, wherein said GOS preparation has a reduced capacity to induce an (IgE-mediated) allergic response in a subject, as compared to a GOS preparation obtained by transgalactosylation of lactose using a beta-galactosidase derived from *Bacillus circulans* or from *Aspergillus oryzae*.

2. The use according to claim 1, wherein the subject is known to suffer or has an increased chance to suffer from hypersensitivity to a GOS preparation obtained by transgalactosylation of lactose using *Bacillus circulans* or *Aspergillus oryzae.*

3. Use of a hypoallergenic GOS preparation obtainable by transgalactosylation of lactose using a beta-galactosidase (EC 3.2.1.23) derived from *Cryptococcus terrestris* (recently renamed *Papiliotrema terrestris*), having an amino acid sequence according to any of SEQ ID NO: 1, 2, 3, or 4, or an amino acid sequence that is at least 95% identical to any of SEQ ID NO: 1, 2, 3, or 4, in the manufacture of a medicament for at least partially preventing an (IgE-mediated) allergic response in a subject known to suffer or having an increased chance to suffer from hypersensitivity to a GOS preparation obtained by transgalactosylation of lactose using a beta-galactosidase derived from *Bacillus circulans* or from *Aspergillus oryzae*.

4. The use according to any one of claims 1 to 3, wherein the beta-galactosidase is derived from *Cryptococcus terrestris* strain MM13-F2171 (Accession Number: NITE BP-02177) or APC-6431 (Accession Number: NITE BP-02178).

5. The use according to any one of claims 1 to 3, wherein the beta-galactosidase comprises an amino acid sequence according to any of SEQ ID NO: 1, 2, 3 or 4, or an amino acid sequence that is at least 97%, preferably at least 98%, and most preferably at least 99% identical to any of SEQ ID NO: 1, 2, 3 or 4.

6. The use according to any of the preceding claims, wherein said hypoallergenic GOS preparation has a decreased score in a Skin Prick Test in the subject and/or in a Basophil Activation Test performed on a blood sample isolated from the subject when compared to a GOS preparation obtained by transgalactosylation of lactose using *Bacillus circulans* or *Aspergillus oryzae* beta-galactosidase.

7. The use according to any of the preceding claims, wherein the subject is of South East Asian origin.

8. The use according to any of the preceding claims, wherein the subject is an adult, an adolescent, or a child, preferably having an age of 18 months or above.

9. Use of a nutritional composition in the manufacture of a medicament for at least partially preventing an (IgE-mediated) allergic response in a subject known to suffer or having an increased chance to suffer from hypersensitivity to a GOS preparation obtained by transgalactosylation of lactose using a beta-galactosidase derived from *Bacillus circulans* or from *Aspergillus oryzae*, said nutritional composition comprising (i) a hypoallergenic GOS preparation obtainable by transgalactosylation of lactose using a beta-galactosidase (EC 3.2.1.23) derived from *Cryptococcus terrestris* having an amino acid sequence according to any of SEQ ID NO: 1, 2, 3, or 4, or an amino acid sequence that is at least 95% identical to any of SEQ ID NO: 1, 2, 3, or 4, and (ii) at least one further ingredient selected from the group consisting of a hypoallergenic or non-allergenic protein source, preferably a protein hydrolysate, preferably a non-allergenic protein hydrolysate, free amino acids, probiotics, LC-PUFA's and carbohydrates, such as lactose, saccharose, starch or maltodextrin.

10. A method for providing a hypoallergenic nutritional composition, comprising (i) contacting a lactose feed with a beta-galactosidase (EC 3.2.1.23) derived from *Cryptococcus terrestris* having an amino acid sequence according to any of SEQ ID NO: 1, 2, 3, or 4, or an amino acid sequence that is at least 95% identical to any of SEQ ID NO: 1, 2, 3, or 4, to provide a hypoallergenic galacto-oligosaccharide (GOS) preparation, and (ii) formulating said hypoallergenic GOS preparation together with at least one further hypoallergenic or non-allergenic ingredient into a hypoallergenic nutritional composition.

11. Method according to claim 10, wherein said at least one further hypoallergenic or non-allergenic ingredient is selected from non-allergenic protein hydrolysates, hydrolysates substantially free of allergenic proteins and hypoallergenic protein sources.

12. The method according to claim 10 or claim 11, wherein the nutritional composition is a composition for pregnant women, a growing up milk, a follow-up formula or an infant formula.

13. The method according to claim 10 or claim 11, wherein the beta-galactosidase (EC 3.2.1.23) derived from *Cryptococcus terrestris* (recently renamed *Papiliotrema terrestris*) is a beta-galactosidase (EC 3.2.1.23) derived from *Cryptococcus terrestris* strain MM13-F2171 (Accession Number: NITE BP-02177) or APC-6431 (Accession Number: NITE BP-02178).

14. The method according to claim 10 or claim 11, wherein the beta-galactosidase (EC 3.2.1.23) derived from *C. terrestris* comprises an amino acid sequence according to any one of SEQ ID NOs: 1 to 4 or an amino acid sequence that is at least 97%, preferably at least 98%, and most preferably at least 99% identical to any one of SEQ ID NOs: 1 to 4.