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 known to suffer or having an increased chance to suffer from hypersensitivity to a GOS preparation obtained by transgalactosylation of lactose using *Bacillus circulans* or *Aspergillus oryzae*, 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 known to suffer or having an increased chance to suffer from hypersensitivity to a GOS preparation obtained by transgalactosylation of lactose using *Bacillus circulans* or *Aspergillus oryzae*, 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*.~~

2~~4~~. The use according to ~~any one of~~ claim~~s~~ 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).

3~~5~~. The use according to ~~any one of~~ claim~~s~~ 1 or 2 ~~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.

~~64~~. 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.

~~75~~. The use according to any of the preceding claims, wherein the subject is of South East Asian origin.

~~86~~. 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.~~

~~107~~. 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~~8. Method according to claim ~~10~~7, 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~~9. The method according to claim ~~10~~ 7 or claim ~~11~~8, wherein the nutritional composition is a composition for pregnant women, a growing up milk, a follow-up formula or an infant formula.

~~13~~10. The method according to claim ~~10~~ 7 or claim ~~11~~8, 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~~11. The method according to claim ~~10~~ 7 or claim ~~11~~8, 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.