Claims

1. A method for the purification of lacto-*N*-*neo*tetraose from other carbohydrates, wherein the lacto-*N*-*neo*tetraose is obtained by microbial fermentation, characterized in that the method comprises the steps of:

i. separating the biomass from the fermentation broth;

ii. subjecting the cell-free fermentation broth to ion-exchange treatment for the removal of charged material, wherein the aqueous solution is treated with a cation exchange resin and an anion exchange resin;

iii. subjecting the aqueous solution obtained in step ii. to a membrane filtration step to remove carbohydrates having a molecular weight smaller than that of lacto-*N*-*neo*tetraose, wherein the membrane has a molecular weight cut-off of between about 300 to about 500 Dalton;

iv. subjecting the retentate obtained in step iii. to a membrane filtration step to remove carbohydrates having a molecular weight larger than that of lacto-*N*-*neo*tetraose, wherein the membrane has a molecular weight cut-off of between about 600 to about 800 Dalton; and

v. increasing the concentration of the lacto-*N*-*neo*tetraose in the filtrate of step iv.

~~subjecting an aqueous solution containing lacto-*N*-*neo*tetraose to two membrane filtration steps using different membranes, wherein~~

- ~~one membrane has a molecular weight cut-off of between about 300 to about 500 Dalton, and~~
- ~~the other membrane as a molecular weight cut-off of between about 600 to about 800 Dalton.~~

~~2. The method according to claim 1, wherein the aqueous solution is obtained from a fermentation or enzymatic process for the production of lacto-*N*-*neo*tetraose.~~

~~3. The method according to claim 1 or 2, wherein the aqueous medium is obtained from a fermentation by separating the biomass from the fermentation broth.~~

4.2. The method according to claim 31, wherein separating the biomass from the fermentation broth comprises at least one step of ultrafiltration, preferably two steps of ultrafiltration, more preferably a first ultrafiltration using a membrane having a molecular weight cut-off of about 500 kDa and a second ultrafiltration using a membrane having a molecular weight cut-off of about 150 kDa.

5.3. The method according to any one of claims 1 to 5or 2, wherein the aqueous medium is treated with a cation exchange resin, preferably in H^+ form, and an anion exchange resin, preferably in Cl^- form, more preferably before subjecting the aqueous solution to the membrane filtration steps.

6.4. The method according to any one of claims 1 to 63, wherein the method further comprises a step of dialysis, preferably of electrodialysis.

7.5. A method for the purification of lacto-*N*-*neo*tetraose from other carbohydrates, ,wherein the lacto-*N*-*neo*tetraose is obtained by microbial fermentation, characterized in that the method comprises the steps of

i. separating the biomass from the fermentation broth;

ii. subjecting the cell-free fermentation broth to ion-exchange treatment for the removal of charged material, wherein the aqueous solution is treated with a cation exchange resin and an anion exchange resin;

iii. subjecting the aqueous solution obtained in step ii. to a membrane filtration step to remove carbohydrates having a molecular weight smaller than that of lacto-*N*-*neo*tetraose, wherein the membrane has a molecular weight cut-off of between about 300 to about 500 Dalton;

iv. subjecting the retentate obtained in step iii. to a ~~continuous~~ simulated moving bed chromatography to remove carbohydrates having a molecular weight larger than that of lacto-N-neotetraose; and

v. increasing the concentration of the lacto-*N*-*neo*tetraose in the filtrate of step iv ~~subjecting an aqueous solution containing lacto-*N*-*neo*tetraose to a membrane filtration step using a membrane having a molecular weight~~

~~cut-off of between about 300 to about 500 Dalton, and subjecting the aqueous solution to a continuous chromatography to remove carbohydrates having a molecular weight larger than that of LNnT from the aqueous solution~~.

~~8. The method according to claim 7, wherein the retentate of the membrane filtration step is subjected to the continuous chromatography.~~

~~9. A method for the purification of lacto-*N*-*neo*tetraose from other carbohydrates, characterized in that the method comprises the steps of subjecting an aqueous solution containing lacto-*N*-*neo*tetraose to a membrane filtration step using a membrane having a molecular weight cut-off of between about 600 to about 800 Dalton, and subjecting the aqueous solution to a continuous chromatography to remove carbohydrates having a molecular weight smaller than that of LNnT from the aqueous solution.~~

~~10. The method according to claim 9, wherein the eluate of the continuous chromatography is subjected to the membrane filtration step.~~

~~11. The method according to any one of claims 7 to 10, wherein the aqueous solution is obtained from a fermentation or enzymatic process for the production of lacto-*N*-*neo*tetraose.~~

~~12. The method according to any one of claims 7 to 11, wherein the aqueous medium is obtained from a fermentation by separating the biomass from the fermentation broth.~~

~~13.~~6. The method according to claim ~~12~~5, wherein separating the biomass from the fermentation broth comprises at least one step of ultrafiltration, preferably two steps of ultrafiltration, more preferably a first ultrafiltration using a membrane having a molecular weight cut-off of about 500 kDa and a second

ultrafiltration using a membrane having a molecular weight cut-off of about 150 kDa.

~~14.~~7. The method according to ~~any one of~~ claim~~s 7~~ 5 ~~to 13~~or 6, wherein the aqueous solution is treated with a cation exchange resin ~~preferably~~ in H^+ form, and an anion exchange resin~~, preferably~~ in Cl^- form~~, more preferably before subjecting the aqueous medium to the membrane filtration steps~~.

~~15.~~8. The method according to any one of claims ~~7~~ 5 to ~~14~~7, wherein the method further comprises a step of dialysis, preferably of electrodialysis.

~~16.~~9. The method according to any one of steps 1 to ~~15~~8, wherein the purity of lacto-*N*-*neo*tetraose in the aqueous solution is ≤ 70 %, ≤ 60 %, ≤ 50 %, ≤ 40 %, ≤ 30 %, ≤ 20 %, ≤ 10 %, ≤ 5 % prior to the purification and /or the aqueous solution contains lacto-*N*-*neo*tetraose at a purity of ≥ 80 %, preferably of ≥ 85 % or more preferably ≥ 90 % after the purification.

~~17.~~10. The method according to any one of steps 1 to ~~16~~9, wherein the method comprises the use of at least one glycosidase for hydrolyzing one or more carbohydrates other than LNnT in the fermentation broth.

~~18.~~11. The method according to any one of steps 1 to ~~17~~10, wherein a second microorganism is added to the fermentation broth after fermentative production of LNnT, wherein said second microorganism expresses a β-galactosidase, preferably *E. coli* LacZ, and/or a *N*-acetylhexosaminidase, preferably Bbhl of *B. bifidum*.