

Atty. Dkt. No.: 105153-3301

WHAT IS CLAIMED IS:

1. A method for production of a botulinum toxin, comprising the steps of:
 - (a) providing a working cell bank (WBC) comprising a *Clostridium botulinum* bacterium;
 - (b) adding the working cell bank to a first container containing a vegetable toxin production medium (VTPM), and culturing the *Clostridium botulinum* bacterium in the VTPM under conditions which permit growth of the *Clostridium botulinum* to produce a pre-culture;
 - (c) adding the pre-culture to a second container containing VTPM, and culturing the *Clostridium botulinum* bacterium under conditions that allow production of a botulinum toxin, and;
 - (d) recovering the botulinum toxin;wherein the VTPM is substantially free or free of an animal derived product and comprises at least one plant-derived protein.
2. The method of claim 1, wherein the botulinum toxin is botulinum neurotoxin type A (BoNT/A).
3. The method of claim 1 or 2, wherein the container used in the steps (b) and (c) is a fermentation bag.
4. The method of any one of claims 1-3, wherein the conditions in steps (b) and (c) comprise an anaerobic environment.
5. The method of claim 4, wherein the anaerobic environment has a dissolved oxygen (DO) concentration of < 2%.
6. The method of any one of claims 1-5, wherein the condition in step (b) comprises a temperature of between about 35 °C and about 39 °C, preferably about 37±1°C.
7. The method of any one of claims 1-6, wherein the condition in step (c) comprises a temperature of between about 30 °C and about 37 °C, preferably about 33±1°C.

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8. The method of any one of claims 1-7, wherein the volume ratio of the WCB to the VTPM in step (b) is no greater than about 2.0%, preferably about 0.08%.
9. The method of any one of claims 1-8, wherein the volume ratio of the pre-culture to the VTPM in step (c) is between about 1:2 and about 1:50, preferably about 1:9.
10. The method of any one of claims 1-9, wherein the step (b) is conducted until OD₆₀₀ reaches the range of about 0.1 to about 1.0, preferably about 0.2 to about 0.4.
11. The method of any one of claims 1-10, wherein the step (b) is conducted for between about 10 and about 30 hours, preferably about 19 hours.
12. The method of any one of claims 1-11, wherein the step (c) is conducted for between about 60 and about 80 hours, preferably for about 69±2 hours.
13. The method of any one of claims 1-12, wherein microbiological purity for other microorganisms than *C. botulinum* is tested in the pre-culture after step (b) and before step (c).
14. The method of any one of claims 1-13, wherein microbiological purity for other microorganisms than *C. botulinum* is tested in the culture after step (c) and before step (d).
15. The method of any one of claims 1-14, wherein the plant-derived protein is a wheat peptone, broadbean peptone, potato peptone, pea peptone, rice peptone, or soybean peptone, preferably a wheat peptone.
16. The method of any one of claims 1-15, wherein the wheat peptone concentration in the VTPM is between about 10 g/L and about 30 g/L, preferably about 20 g/L.
17. The method of any one of claims 1-16, wherein the VTPM comprises wheat peptone, yeast extract, D-(+)-Glucose, L-Cysteine hydrochloride monohydrate, and Medical antifoam C emulsion.
18. The method of any one of claims 1-17, wherein the VTPM comprises:
 - between about 5 g/L and about 50 g/L, preferably about 20 g/L, of wheat peptone;
 - between about 5 g/L and about 50 g/L, preferably about 20 g/L, of yeast extract;

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between about 0.05 g/L and about 20 g/L, preferably about 5 g/L, of D-(+)-glucose;
between about 0.05 g/L and about 0.50 g/L, preferably about 0.20 g/L, of L-cysteine hydrochloride monohydrate; and
between about 0.05 g/L and about 0.50 g/L, preferably about 0.24 g/L, of medical antifoam c emulsion.

19. The method of any one of claims 1-18, wherein the pH of the VPTM is between about 6.7 and about 7.2.

20. A composition comprising a *Clostridium botulinum* and a culture medium for producing a botulinum toxin, wherein the medium is free or substantially free of an animal derived product, and comprises at least one plant-derived protein.

21. The composition of claim 20, wherein the plant-derived protein is a wheat peptone.

22. The composition of claim 21, wherein the wheat peptone concentration in the VTPM is between about 5 g/L and about 50 g/L, preferably about 20 g/L.

23. The composition of any one of claims 20-22, wherein the culture medium comprises wheat peptone, yeast extract, D-(+)-Glucose, L-Cysteine hydrochloride monohydrate, and Medical antifoam C emulsion.

24. The composition of any one of claims 20-23, wherein the culture medium comprises:
between about 5 g/L and about 50 g/L, preferably about 20 g/L, of wheat peptone;
between about 5 g/L and about 50 g/L, preferably about 20 g/L, of yeast extract;
between about 1 g/L and about 20 g/L, preferably about 5 g/L, of D-(+)-glucose;
between about 0.05 g/L and about 0.50 g/L, preferably about 0.20 g/L, of L-cysteine hydrochloride monohydrate; and
between about 0.05 g/L and about 0.50 g/L, preferably about 0.24 g/L, of medical antifoam c emulsion.