



(43) International Publication Date
18 October 2012 (18.10.2012)

(10) International Publication Number
WO 2012/140171 A1

(51) International Patent Classification:

C12P 21/00 (2006.01) C07K 14/34 (2006.01)
C07K 14/195 (2006.01) A61K 39/02 (2006.01)

(21) International Application Number:

PCT/EP2012/056728

(22) International Filing Date:

12 April 2012 (12.04.2012)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/474,815 13 April 2011 (13.04.2011) US
1106225.4 13 April 2011 (13.04.2011) GB

(71) Applicant (for all designated States except US): **GLAXO-SMITHKLINE BIOLOGICALS S.A.** [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **DEHOTTAY, Philippe, Marc, Helene** [BE/BE]; GlaxoSmithKline Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). **GOFFIN, Philippe** [BE/BE]; GlaxoSmithKline Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE).

(74) Agents: **JOHNSTON, Caroline, Louise** et al.; GlaxoSmithKline, Global Patents CN925.1, 980 Great West Road, Brentford Middlesex TW8 9GS (GB).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))
- of inventorship (Rule 4.17(iv))

Published:

- with international search report (Art. 21(3))

(54) Title: FERMENTATION PROCESS

(57) Abstract: The present invention provides a process for periplasmic expression of a bacterial toxoid comprising the steps of: a) growing a culture of a gram negative host cell in a fermentation medium, wherein the host cell is transformed with a polynucleotide, and wherein the polynucleotide encodes the bacterial toxoid and a periplasmic signal sequence; or providing a gram negative host cell wherein the host cell is transformed with a polynucleotide, the polynucleotide encodes the bacterial toxoid and a periplasmic signal sequence and wherein the gram negative host cell comprises the bacterial toxoid expressed in the periplasm; a(i)) inducing expression of the bacterial toxoid; b) maturing the host cell, wherein the maturing step comprises: I) subjecting the host cell to a pH shock; II) incubating the host cell with no feed addition; and/or III) subjecting the host cell to a temperature below -20°C; and c) extracting the bacterial toxoid from the host cell wherein the extraction process comprises osmotic shock.



WO 2012/140171 A1