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(54) **MATERIALS AND METHODS RELATING TO THE ATTACHMENT AND DISPLAY OF SUBSTANCES ON CELL SURFACES**

(75) Inventors: **Lothar Steidler; Erik Remaut**, both of Ghent (BE); **Jeremy Mark Wells**, Cambridge (GB)

(73) Assignee: **Vlaams Interuniversitair Instituut voor Biotechnologie (VIB) vzw**, Zwijnaarde (BE)

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- (58) **Field of Search** 424/184.1, 192.1, 424/195.11, 197.11, 200.1; 435/69.3, 320.1, 252.3

(56) **References Cited**

U.S. PATENT DOCUMENTS

- 5,348,867 * 9/1994 Georgiou et al. .
- 5,616,686 4/1997 Fischetti et al. .

FOREIGN PATENT DOCUMENTS

WO 93/18163 9/1993 (WO) .

OTHER PUBLICATIONS

- A. Charbit et al., *J. Bacteriology*, 173 (1): 262–275 (1991).
- A. Charbit et al., *J. Immunology*, 139: 1658–1664 (1987).
- D. Chiswell et al., *TIBTECH*, 10: 80–84 (1992).

- G. Georgiou et al., *TIBTECH*, 11: 6–10 (1993).
- T. Klauser et al., *EMBJ Journal*, 9: 1991–1999 (1990).
- M.G. Kornacker et al., *Mol Micro*, 7: 1101–1109 (1990).
- J. Marmur, *J.Mol.Biol.*, 3: 208–218 (1961).
- WW Navarre et al., *Mol. Micro*, 14: 115–121 (1994).
- RK Saiki et al., *Science*, 230: 1350–1354 (1985).
- O Schneewind et al., *EMBO J*, 12(12): 4803–4811 (1993).
- D. Simon et al., *Biochimie*, 70: 559–566 (1988).
- L. Steidler et al., *Mol.Gen.Genet*, 236: 187–192(1993).
- L. Steidler et al., *J. Bacteriology*, 175(23): 7639–7643 (1993).
- L. Steidler et al., *Appl. Environ. Microbiol*, 55: 984–993 (1989).
- G. Thiry et al., *Appl. Environ. Microbiol*, 55(4): 984–993 (1989).
- NR Waterfield et al., *Gene*, 165: 9–15 (1995).
- JM Wells et al., *Mol. Microbiol.* 8(6): 1155–1162 (1993).
- JM Wells et al., *Environ. Microbiol.* 59(11): 3954–3959 (1993).
- JM Wells et al., *J. Appl. Bact.* 74: 629–6365 (1993).
- Ngugen et al., “Cell–Surface Display of Heterologous Epitopes on *Staphylococcus Xylosus* As A Potential Delivery System For Oral Vaccination”; *Gene*, vol. 128, 1993, Amsterdam, NL. pp. 89–94. See page 91, fig. 1.
- Samuelson, P, et al.; “Cell Surface Display Of Recombinant Proteins On *Staphylococcus Carnosus*”; *Journal of Bacteriology*, vol. 177, No. 6, Mar. 1995, pp. 1470–1476. See abstract; Figures 1,3.
- Oggioni, M.R. et al.; “Immunization of Mice By Oral Colonization with Live Recombinant Commensal Streptococci”; *Vaccine*, 13 (8) pp. 775–779, Jun. 1995, England.
- Steidler, L. et al.; Secretion of Biologically Active Murine Interleukin–2 by *Lactococcus Lactis* Subsp. *Lactis*; *Appl. Environ Microbiol*, Apr. 1995 (4) p1627–9, United States.

* cited by examiner

Primary Examiner—Albert Navarro

(74) *Attorney, Agent, or Firm*—Pennie & Edmonds LLP

(57) **ABSTRACT**

Methods for obtaining surface expression of a desired protein or polypeptide in Gram-positive host organisms are provided. In addition, vectors useful in such methods as well as Gram-positive host organisms transformed with such vectors are disclosed.

24 Claims, 7 Drawing Sheets