



US007217541B2

(12) **United States Patent**
Cassels et al.

(10) **Patent No.:** **US 7,217,541 B2**
(45) **Date of Patent:** **May 15, 2007**

(54) **METHOD OF MAKING CS6 ANTIGEN VACCINE FOR TREATING, PREVENTING, OR INHIBITING ENTEROTOXIGENIC *ESCHERICHIA COLI* INFECTIONS**

5,935,838 A 8/1999 Askelöf et al. 435/252.1
6,309,669 B1 10/2001 Setterstrom et al. 424/486
6,902,736 B2 * 6/2005 Alboum et al. 424/242.1
2004/0156829 A1* 8/2004 Wolf et al. 424/93.2

(75) Inventors: **Frederick J. Cassels**, Ellicott City, MD (US); **James F. Wood**, Germantown, MD (US)

FOREIGN PATENT DOCUMENTS
WO WO 02/064162 8/2002

(73) Assignee: **United States of America as represented by the Secretary of the Army**, Washington, DC (US)

OTHER PUBLICATIONS

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 665 days.

Helander et al. J. Clin. Microbiol. 35: 867-872, 1997.*
Jones et al. J. Bacteriol. 146: 841-846, 1981.*
Cassels et al. Toxicon 36 (9): pp. 1231, Sep. 1998.*
Wolf (1997) "Occurrence, Distribution, and Associations of O and H Serogroups, Colonization Factor Antigens, and Toxins of Enterotoxigenic *Escherichia coli*", Clinical Microbiology Reviews, 10(4):569-584.

(21) Appl. No.: **10/370,522**

Cassels, et al. (1998) "Production and microencapsulation of enterotoxigenic *Escherichia coli* colonization factors in human use vaccines" Abstracts of the General Meeting of the Am. Society for Microbiology, Atlanta, GA, Abstract E-92.

(22) Filed: **Feb. 24, 2003**

Katz, et al. (2001) "Clinical evaluation of microencapsulated CS6 vaccine for enterotoxigenic *Escherichia coli* (EPEC) diarrhea in healthy adults" Vaccines for Enteric Diseases Ved, 7-9.

(65) **Prior Publication Data**

US 2004/0005662 A1 Jan. 8, 2004

Katz, et al. (2003) "Oral immunization of adult volunteers with microencapsulated enterotoxigenic *Escherichia coli* (EPEC) CS6 antigen" Vaccine, Butterworth Scientific, Guilford, GB, 21(5-6):341-346.

Related U.S. Application Data

(60) Provisional application No. 60/361,105, filed on Feb. 25, 2002, provisional application No. 60/421,804, filed on Oct. 29, 2002.

Supplementary Partial EP Search Report mailed from EPO May 6, 2005.

* cited by examiner

(51) **Int. Cl.**

A12P 21/04 (2006.01)
C12N 15/09 (2006.01)
C12N 1/20 (2006.01)
C12P 21/06 (2006.01)

Primary Examiner—S. Devi

(74) Attorney, Agent, or Firm—Elizabeth Arwine

(52) **U.S. Cl.** 435/71.1; 435/69.3; 435/69.1; 435/252.22; 435/848

(58) **Field of Classification Search** 435/71.1, 435/69.3, 69.1, 252.33, 848; 424/242.1; 530/350, 825

See application file for complete search history.

(57) **ABSTRACT**

Disclosed herein are methods for making large amounts of highly pure colonization factors. The methods of the present invention differ from prior art methods in that host cells which express the colonization factor of interest are cultured in media comprising more than about 50 µg/l of an antibiotic, the media is centrifuged and then filtered with a 0.2 µm filter tangential flow cartridge and a 300,000 MW cut-off filter, and a divalent cation is added. As disclosed herein the colonization factors made by the method of the present invention may be used in pharmaceutical compositions and methods for treating or preventing enterotoxigenic *Escherichia coli* infections.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,648,081 A * 7/1997 van den Bosch 424/255.1
5,698,416 A 12/1997 Wolf et al. 435/69.1
5,830,479 A * 11/1998 Emery et al. 424/255.1
5,914,114 A 6/1999 Cassels 424/241.1

21 Claims, 5 Drawing Sheets