



US009410157B2

(12) **United States Patent**
Withers, III et al.

(10) **Patent No.:** **US 9,410,157 B2**
(45) **Date of Patent:** **Aug. 9, 2016**

(54) **SYSTEMS AND METHODS FOR THE SECRETION OF RECOMBINANT PROTEINS IN GRAM NEGATIVE BACTERIA**

(75) Inventors: **Sydnor T. Withers, III**, Madison, WI (US); **Miguel A. Dominguez**, Madison, WI (US); **Matthew P. DeLisa**, Ithaca, NY (US); **Charles H. Haitjema**, Ithaca, NY (US)

(73) Assignees: **WISCONSIN ALUMNI RESEARCH FOUNDATION**, Madison, WI (US); **CORNELL UNIVERSITY**, Ithaca, NY (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 81 days.

(21) Appl. No.: **13/192,058**

(22) Filed: **Jul. 27, 2011**

(65) **Prior Publication Data**
US 2012/0225453 A1 Sep. 6, 2012

Related U.S. Application Data

(60) Provisional application No. 61/369,188, filed on Jul. 30, 2010.

(51) **Int. Cl.**
C12N 15/70 (2006.01)
C12P 21/02 (2006.01)

(52) **U.S. Cl.**
CPC **C12N 15/70** (2013.01); **C12P 21/02** (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**
U.S. PATENT DOCUMENTS
7,951,361 B2* 5/2011 Turner et al. 424/93.1

OTHER PUBLICATIONS
Prehna et al., Structure 20, 1154-1166, 2012.*
Ward et al. Nature 341:544-546, 1989.*
Zhang, et al, "Extracellular accumulation of recombinant proteins fused to the carrier protein YebF in *Escherichia coli*," Nat. Biotechnol., Jan. 2006, No. 24, vol. 1, pp. 100-104.

* cited by examiner

Primary Examiner — Nancy T Vogel
(74) *Attorney, Agent, or Firm* — Casimir Jones S.C.

(57) **ABSTRACT**
Disclosed herein are systems and methods for producing recombinant proteins utilizing mutant *E. coli* strains containing expression vectors carrying nucleic acids encoding the proteins, and secretory signal sequences to direct the secretion of the proteins to the culture medium. Host cells transformed with the expression vectors are also provided.

19 Claims, 12 Drawing Sheets