



US007507573B2

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

(10) **Patent No.:** US 7,507,573 B2
(b4) **Date of Patent:** Mar. 24, 2009

(54) **MODIFICATION OF PROTEIN GLYCOSYLATION IN METHYLOTROPHIC YEAST**

WO WO 2005/100584 A2 10/2005

(75) Inventors: **Roland Contreras**, Merelbeke (BE); **Nico L. M. Callewaert**, Lichtervelde (BE); **Wouter Vervecken**, Ghent-Ledeberg (BE); **Vladimir Kaigorodov**, Ghent (BE)

(73) Assignees: **VIB, vzw** (BE); **Universiteit Gent** (BE); **Research Corporation Technologies, Inc.**, Tucson, AZ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 191 days.

(21) Appl. No.: **10/713,970**

(22) Filed: **Nov. 14, 2003**

(65) **Prior Publication Data**

US 2005/0106664 A1 May 19, 2005

(51) **Int. Cl.**

C12Q 1/68 (2006.01)
C12N 15/04 (2006.01)
C12P 21/00 (2006.01)
C12P 19/18 (2006.01)
C12P 1/02 (2006.01)

(52) **U.S. Cl.** **435/254.11**; 435/183; 435/193; 435/227; 435/203; 435/254.23; 435/254.6; 435/71.1

(58) **Field of Classification Search** 435/6, 435/193, 254.2, 254.11, 254.23, 320.1; 536/23.2
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,135,854 A	8/1992	MacKay et al.
5,705,616 A	1/1998	Lehle et al.
6,803,225 B2	10/2004	Contreras et al.
7,029,872 B2 *	4/2006	Gerngross
2002/0137134 A1	9/2002	Gerngross

FOREIGN PATENT DOCUMENTS

EP	0 314 096	5/1989
EP	0 548 012 A1	6/1993
EP	0 582 244 A2	2/1994
EP	1 211 310 A1	6/2002
JP	8-336387	12/1996
WO	WO 91/05057	4/1991
WO	WO 92/09694	6/1992
WO	WO 96/21038	7/1996
WO	WO 02/00856 A2	1/2002
WO	WO 02/00879 A2	1/2002
WO	WO 03/056914 A1	7/2003
WO	WO 2004/074499 A2	9/2004

OTHER PUBLICATIONS

Maras, M., et al., "Molecular Cloning and Enzymatic Characterization of a *Trichoderma reesei*, 1, 2— α -D-Mannosidase", *Journal of Biotechnology*, vol. 77, No. 2-3, pp. 255-263 (2000).

Breithauer, R. K., et al., "Glycosylation of *Pichia pastoris*-derived Proteins", *Biotechnol. Appl. Biochem.*, vol. 30, pp. 193-200 (1999).

Kukuruzinska, M. A., et al., "Protein Glycosylation in Yeast", *Ann. Rev. Biochem.*, vol. 56, pp. 915-944 (1987).

Chiba, Y., et al., "Production of Human Compatible High Mannose-Type ($\text{Man}_5\text{GlcNAc}_2$) Sugar Chains in *Saccharomyces cerevisiae*", *The Journal of Biological Chemistry*, vol. 273, No. 41, pp. 26298-26304 (1998).

Maras, M., et al., In Vivo Synthesis of Complex N-Glycans by Expression of Human N-Acetylglucosaminyltransferase I in the Filamentous Fungus *Trichoderma reesei*, *FEBS Letters*, vol. 452, pp. 365-370 (1999).

Nakanishi-Shindo, Y., et al., "Structure of the N-Linked Oligosaccharides That Show the Complete Loss of α -1,6-Polymannose Outer Chain from *och1*, *och1 mnn1*, and *och1 mnn1 alg3* Mutants of *Saccharomyces cerevisiae*", *The Journal of Biological Chemistry*, vol. 268, No. 35, pp. 26338-26345 (1993).

Martinet, W., et al., "Modification of the Protein Glycosylation Pathway in the Methylo trophic Yeast *Pichia pastoris*", *Biotechnology Letters*, vol. 20, No. 12, pp. 1171-1177 (1998).

Maras, M., et al., "In vitro Conversion of the Carbohydrate Moiety of Fungal Glycoproteins to Mammalian-Type Oligosaccharides", *Eur. J. Biochem.*, vol. 249, pp. 701-707 (1997).

Laroy, W., et al., "Cloning of *Trypanosoma cruzi trans-Sialidase* and Expression in *Pichia pastoris*", *Protein Expression and Purification*, vol. 20, pp. 389-393 (2000).

Inoue, T., et al., "Molecular Cloning and Nucleotide Sequence of the 1,2-a-D-Mannosidase Gene, *msdS*, from *Aspergillus saitoi* and Expression of the Gene in Yeast Cells" *Biochimica et Biophysica Acta*, vol. 1253, pp. 141-145 (1995).

Herscovics, A., et al., "Isolation of a Mouse Golgi Mannosidase cDNA, a Member of a Gene Family Conserved from Yeast to Mammals", *The Journal of Biological Chemistry*, vol. 269, No. 13, pp. 9864-9871 (1994).

Lal, A., et al., "Isolation and Expression of Murine and Rabbit cDNAs Encoding an a1,2-Mannosidase Involved in the Processing of Asparagines-linked Oligosaccharides", *The Journal of Biological Chemistry*, vol. 269, No. 13, pp. 9872-9881 (1994).

(Continued)

Primary Examiner—Andrew D Kosar

Assistant Examiner—Kagnew H Gebreyesus

(74) Attorney, Agent, or Firm—Scully, Scott, Murphy & Presser, P.C.

(57) **ABSTRACT**

The present invention relates to methods and genetically engineered methylo trophic yeast strains for producing glycoproteins with mammalian-like glycosylation. The present invention also relates to vectors useful for generating methylo trophic yeast strains capable of producing glycoproteins with mammalian-like glycosylation. Glycoproteins produced from the genetically engineered methylo trophic yeast strains are also provided.

20 Claims, 5 Drawing Sheets