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(54) **FUNGAL ENDOGLUCANASES, THEIR PRODUCTION AND USE**

(56) **References Cited**

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U.S. PATENT DOCUMENTS
5,475,101 A 12/1995 Ward et al.
6,187,732 B1 * 2/2001 Fowler et al. 510/226

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FOREIGN PATENT DOCUMENTS

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CN 101171333 A 4/2008
WO 95/02043 A1 1/1995
WO 95/33386 A1 12/1995
WO 97/08325 A2 3/1997
WO 00/14206 A2 3/2000
WO 02/12462 A2 2/2002
WO 02/12465 2/2002
WO 2007/118935 A1 10/2007

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OTHER PUBLICATIONS

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Broun et al., Catalytic plasticity of fatty acid modification enzymes underlying chemical diversity of plant lipids. *Science*, 1998, vol. 282: 1315-1317.*

(86) PCT No.: **PCT/FI2009/051042**

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Chica et al., Semi-rational approaches to engineering enzyme activity: combining the benefits of directed evolution and rational design. *Curr. Opin. Biotechnol.*, 2005, vol. 16: 378-384.*

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Devos et al., Practical limits of function prediction. *Proteins: Structure, Function, and Genetics*. 2000, vol. 41: 98-107.*

(65) **Prior Publication Data**

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Seffernick et al., Melamine deaminase and Atrazine chlorohydrolase: 98 percent identical but functionally different. *J. Bacteriol.*, 2001, vol. 183 (8): 2405-2410.*

(30) **Foreign Application Priority Data**

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Sen et al., Developments in directed evolution for improving enzyme functions. *Appl. Biochem. Biotechnol.*, 2007, vol. 143: 212-223.*

(51) **Int. Cl.**

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C11D 3/37 (2006.01)

Whisstock et al., Prediction of protein function from protein sequence. *Q. Rev. Biophys.*, 2003, vol. 36 (3): 307-340.*

Witkowski et al., Conversion of b-ketoacyl synthase to a Malonyl Decarboxylase by replacement of the active cysteine with glutamine. *Biochemistry*, 1999, vol. 38: 11643-11650.*

(52) **U.S. Cl.**

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English Translation of Office Action issued for corresponding Chinese Patent Application No. 200980153611.7. This document was provided by the Applicant's foreign associate, Jul. 31, 2012.

International Search Report Relating to corresponding PCT/FI2009/051042, Nov. 8, 2012.

Altschul, et al., "Basic Local Alignment Search Tool," *J. Mol. Biol.* (1990) 215, 403-410.

Bailey, et al., "Induction, Isolation and Testing of Stable *Trichoderma reesei* Mutants With Improved Production of Solubilizing Cellulase," *Enzyme Microb. Technol.*, 1981, vol. 3, April.

Bendtsen, et al., "Improved Prediction of Signal Peptides: SignalP 3.0," *J. Mol. Biol.* (2004) 340, 783-795.

(Continued)

(58) **Field of Classification Search**

USPC 435/209, 262, 263, 69.1, 91.1, 320.1, 435/252.3, 254.11; 426/48, 53, 635; 536/23.1, 23.2; 510/114, 276, 300; 530/350

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(57) **ABSTRACT**

Novel fungal endoglucanases Ce15 and Ce112 are disclosed. The endoglucanases are conveniently produced by recombinant technology, and means for their production are described. The endoglucanases are used for treating cellulosic material, especially in textile industry, e.g. in biofinishing or biostoning. They may also be used in detergents, in animal feed and/or in pulp and paper industry, or in hydrolysis of lignocellulosic material for, e.g. bioethanol production.

See application file for complete search history.

23 Claims, 7 Drawing Sheets