



US009024111B1

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

(10) **Patent No.:** **US 9,024,111 B1**
(45) **Date of Patent:** **May 5, 2015**

(54) **METHODS AND MATERIALS FOR DECONSTRUCTION OF BIOMASS FOR BIOFUELS PRODUCTION**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 369 days.

(21) Appl. No.: **13/436,508**

(22) Filed: **Mar. 30, 2012**

Related U.S. Application Data

(60) Provisional application No. 61/469,860, filed on Mar. 31, 2011.

(51) **Int. Cl.**
C12N 15/82 (2006.01)
C12N 15/63 (2006.01)
C12N 15/09 (2006.01)
A01H 5/04 (2006.01)
C12N 9/42 (2006.01)

(52) **U.S. Cl.**
CPC **C12N 9/2437** (2013.01); **C12N 15/8255** (2013.01); **C12N 15/8247** (2013.01)

(58) **Field of Classification Search**
CPC C12N 15/82; C12N 9/2402; C12N 9/2437; C12N 15/10; C12N 15/52; C12N 15/63; C12N 15/8221
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,391,547 B1 * 5/2002 Jefferson et al. 435/6.13
2008/0233175 A1 * 9/2008 Steer et al. 424/439

OTHER PUBLICATIONS

Genbank Accession No. AJ308623.1, 2005.
Genbank Accession No. AE006641, 2001.
Datta, Supratim, et al., "Ionic Liquid tolerant hyperthermophilic cellulases for biomass pretreatment and hydrolysis", Green Chem., vol. 12, pp. 2338-2345 (2010).

Eckert, K., et al., "Gene cloning, sequencing, and characterization of a family . . .", Appl. Microbiol Biotechnol, vol. 60, pp. 428-436 (2002).

Hadi, M.Z., et al., "Simple and versatile section of *Arabidopsis* transformants"; Plant Cell Rep., vol. 21, pp. 130-135 (2002).

Hadi, M., "Trojan Horse Strategy for Deconstruction of Biomass for Biofuels Production" presentation slides, presentation slides, presented at the European Conference on Synthetic Biology in Sant Feliu Guixols, Spain, Nov. 23-29, 2007.

Hadi, M., et al., "'Trojan Horse' Strategy for Deconstruction of Biomass for Biofuels Production" poster, presented at the Synthetic Biology 4.0 conference in Hong Kong, Oct. 9-12, 2008.

Hadi, M., "Strategy for Deconstruction of Biomass for Biofuels Production" presentation slides, presented to Innovations in BioFuels 2008 conference in Baltimore, MD, May 13-16, 2008.

Huang, Y., et al., "A highly acid-stable and thermostable endo-B-glucanase from the thermoacidophilic archaeon . . ." Biochem J., vol. 385, pp. 581-588 (2005).

Kim, Seungdo, et al., "Global potential bioethanol production from wasted crops and crop residues"; Biomass and Bioenergy, vol. 26, pp. 361-375 (2004).

Simmons, Bruce., "Green Car Congress"; www.greencarcongress.com/2007/06/sandia_national.html, 2007.

Ziegelhoffer, Thomas., et al., "Dramatic effects of truncation and sub-cellular targeting on the accumulation of recombinant microbial cellulase in tobacco"; Molecular Breeding, vol. 8, pp. 147-158 (2001).

* cited by examiner

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(57) **ABSTRACT**

The present invention relates to nucleic acids, peptides, vectors, cells, and plants useful in the production of biofuels. In certain embodiments, the invention relates to nucleic acid sequences and peptides from extremophile organisms, such as SSO1949 and CelA, that are useful for hydrolyzing plant cell wall materials. In further embodiments, the invention relates to modified versions of such sequences that have been optimized for production in one or both of monocot and dicot plants. In other embodiments, the invention provides for targeting peptide production or activity to a certain location within the cell or organism, such as the apoplast. In further embodiments, the invention relates to transformed cells or plants. In additional embodiments, the invention relates to methods of producing biofuel utilizing such nucleic acids, peptides, targeting sequences, vectors, cells, and/or plants.

31 Claims, 13 Drawing Sheets