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(54) Title: TERMITE ENZYMES AND USES THEREOF FOR IN VITRO CONVERSION OF LIGNIN-CONTAINING MATERIALS TO FERMENTABLE PRODUCTS

(57) Abstract: The disclosure provides isolated nucleic acid molecules derived from the gut of the termite R flavipes, recombinant nucleic acid molecules comprising a vector and an isolated heterologous nucleic acid molecule operably inserted therein, whereby, when transformed into an appropriate host cell system, the heterologous nucleic acid sequence is expressed as a polypeptide having an activity similar to that when expressed in the gut of the termite R. flavipes. The recombinant nucleic acid molecules can comprise more than one heterologous nucleic acid molecule such that more than one polypeptide may be expressed by the host system. The expressed polypeptides may be substantially purified, or used in a substantially unpurified form, to be admixed with a lignocellulose source to be converted to a fermentable product such as a sugar or a mixture of sugars. One aspect of the present disclosure, therefore, encompasses methods of converting a lignified plant material to a fermentable product, the method comprising obtaining a series of isolated polypeptides of a termite, wherein the series of polypeptides cooperate to convert a plant lignocellulose to a fermentable product; and incubating the series of polypeptides with a source of lignified plant material, under conditions allowing the polypeptides to cooperatively produce a fermentable product from the lignified plant material.

AMENDED CLAIMS
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CLAIMS

Therefore, the following is claimed:

1. A method of converting a lignified plant material to a fermentable product, the method comprising the steps of:

(a) obtaining a series of isolated polypeptides wherein said polypeptides are encoded by the genome of a termite, wherein the series of polypeptides cooperate to convert a plant lignocellulose to a fermentable product; and

(b) incubating the series of polypeptides with a source of lignified plant material, under conditions allowing the polypeptides to cooperatively produce a fermentable product from the lignified plant material.

2. The method according to claim 1, wherein the isolated polypeptides are derived from the termite *Reticulitermes flavipes*, and not from a symbiont thereof, or a protozoa or a fungus.

3. The method according to claim 1, wherein the isolated polypeptides of the series of isolated polypeptides comprise an endoglucanase, a laccase, an esterase, and a glucosidase.

4. The method according to claim 3, wherein the series of the isolated polypeptides consists of an endoglucanase, an exoglucanase, a laccase, an esterase, and a glucosidase.

5. The method according to claim 1, wherein the isolated polypeptides of the series of isolated polypeptides are recombinant polypeptides, and wherein each polypeptide is expressed from an expression vector of a recombinant expression system, and wherein the recombinant expression system is selected from a eukaryotic cell-based system and a prokaryotic cell-based system.

6. The method according to claim 5, wherein the expression vector is a baculovirus expression vector and the recombinant expression system is a eukaryotic cell-based system.

7. The method according to claim 3, wherein the endoglucanase has an amino acid sequence having about 75% sequence identity with the amino acid sequence SEQ ID NO.: 2.

8. The method according to claim 7, wherein the endoglucanase has the amino acid sequence SEQ ID NO.: 2.

9. The method according to claim 3, wherein the series of isolated polypeptides further comprises an exoglucanase having an amino acid sequence having about 75% sequence identity with the amino acid sequence SEQ ID NO.: 4.

10. The method according to claim 9, wherein the exoglucanase has the amino acid sequence SEQ ID NO.: 4.

11. The method according to claim 3, wherein the laccase has an amino acid sequence having about 75% sequence identity with an amino acid sequence selected from the group consisting of: SEQ ID NOs.: 8, 10, 12, 15, 17, 19, 21, and 23.

12. The method according to claim 11, wherein the laccase has the amino acid sequence selected from the group consisting of: SEQ ID NOs.: 8, 10, 12, 15, 17, 19, 21, and 23.

13. The method according to claim 3, wherein the esterase has an amino acid sequence having about 75% sequence identity with the amino acid sequence selected from the group consisting of: SEQ ID NOs.: 27, 29, 31, and 33.

14. The method according to claim 13, wherein the esterase has the amino acid sequence selected from the group consisting of: SEQ ID NOs.: 27, 29, 31, and 33.

15. The method according to claim 3, wherein the glucosidase has the amino acid sequence SEQ ID NO.: 6.

16. The method according to claim 1, wherein the fermentable product comprises at least one carbohydrate selected from the group consisting of: a glucose, a mannose, a xylose, a galactose, a rhamnose, an arabinose, a glucuronic acid, a mannuronic acid, and a galacturonic acid.

17. The method according to claim 16, wherein the fermentable product comprises glucose.

18. A system for producing a fermentable product from a lignified plant material, wherein the system comprises at least two isolated polypeptides selected from the group consisting of: an endoglucanase, an exoglucanase, a laccase, an esterase, and a glucosidase of the termite *Reticulitermes flavipes* and wherein the at least two isolated polypeptides can cooperate to convert a constituent of the lignified plant material to a fermentable product or a precursor thereof.

19. The system according to claim 18, wherein the nucleotide sequence encoding the endoglucanase hybridizes under high stringency conditions to a nucleotide sequence according to SEQ ID NO.: 1, the nucleotide sequence encoding the exoglucanase hybridizes under high stringency conditions to a nucleotide sequence according to SEQ ID NO.: 3, the nucleic acid molecule encoding the laccase hybridizes under high stringency conditions to a nucleotide sequence selected from the group consisting of: SEQ ID NOS.: 7, 9, 11, 13, 14, 16, 18, 20, and 22, the nucleic acid molecule encoding the esterase hybridizes under high stringency conditions to a nucleotide sequence selected from the group consisting of: SEQ ID NOS.: 26, 28, 30, and 32, and the nucleotide sequence encoding the glucosidase hybridizes under high stringency conditions to SEQ ID NO.: 5.

20. The system according to claim 18, wherein each of the isolated nucleic acid molecules thereof is operably inserted into an expression vector.

21. A recombinant cell comprising an isolated nucleic acid molecule hybridizing under high stringency conditions to a nucleotide sequence encoding a polypeptide selected from the group consisting of: an endoglucanase, an exoglucanase, a laccase, an esterase, and a glucosidase of the termite *Reticulitermes flavipes,* wherein the nucleotide sequence encoding the endoglucanase is according to SEQ ID NO.: 1, the nucleotide sequence encoding the exoglucanase is according to SEQ ID NOS.: 3, the nucleic acid molecule encoding the laccase is selected from the group consisting of: SEQ ID NOS.: 7, 9, 11, 13, 14, 16, 18, 20, and 22, the nucleic acid molecule encoding the esterase is selected from the group consisting of: SEQ ID NOS.: 26, 28, 30, and 32, and the nucleotide sequence encoding the glucosidase is according to SEQ ID NO.: 5.