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(54) Title: METHOD FOR DEGRADING OR CONVERTING CELLULOSIC MATERIAL

(57) Abstract: Provided are methods for degrading or converting a cellulosic material, comprising: treating the cellulosic material with an enzyme composition in the presence of a polypeptide having catalase activity; and enzyme composition used for degrading or converting a cellulosic material comprising one or more (e.g., several) enzymes having cellulolytic and/or hemicellulolytic activity and a polypeptide having catalase activity.



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METHODS FOR DEGRADING OR CONVERTING CELLULOSIC MATERIAL

Reference to a Sequence Listing

5 This application contains a Sequence Listing in computer readable form. The computer readable form is incorporated herein by reference.

Background of the Invention

Field of the Invention

10 The present invention relates to methods for degrading or converting cellulosic material and enzyme composition for degrading or converting cellulosic material.

Description of the Related Art

15 Catalases [hydrogen peroxide: hydrogen peroxide oxidoreductases (EC 1.11.1.6)] are enzymes which catalyze the conversion of hydrogen peroxide (H_2O_2) to oxygen (O_2) and water (H_2O). These ubiquitous enzymes have been purified from a variety of animal tissues, plants and microorganisms (Chance and Maehly, 1955, *Methods Enzymol.* 2: 764-791).

20 Catalase preparations are used commercially for diagnostic enzyme kits, for the enzymatic production of sodium gluconate from glucose, for the neutralization of H_2O_2 waste, for removal of H_2O_2 from textile fabrics, and for the removal of H_2O_2 and/or generation of O_2 in foods and beverages.

25 Cellulose is a polymer of simple sugars covalently linked by beta-1,4-bonds. Many microorganisms produce enzymes that hydrolyze beta-linked glucans. These enzymes include endoglucanases, cellobiohydrolases, and beta-glucosidases. Endoglucanases digest the cellulose polymer at random locations, opening it to attack by cellobiohydrolases. Cellobiohydrolases sequentially release molecules of cellobiose from the ends of the cellulose polymer. Cellobiose is a water-soluble beta-1,4-linked dimer of glucose. Beta-glucosidases hydrolyze cellobiose to glucose.

30 The conversion of lignocellulosic material has the advantages of the ready availability of large amounts of feedstock and the desirability of avoiding burning or land filling the materials. Wood, agricultural residues, herbaceous crops, and municipal solid wastes have been considered as feedstocks. These materials primarily consist of cellulose, hemicellulose, and lignin. Once the lignocellulose is converted to simple sugars, *e.g.*, glucose, the simple sugars can further be converted to many useful substances, *e.g.*, fuel, potable ethanol, fermentation products and/or chemicals (*e.g.*, acids, alcohols, ketones, gases, and the like).

35 It would be advantageous in the art to improve methods for degrading or converting a cellulosic material.

Summary of the Invention

The present invention relates to methods for degrading or converting a cellulosic material, comprising: treating the cellulosic material with an enzyme composition in the presence of a polypeptide having catalase activity.

The present invention also relates to methods for producing a fermentation product,

(a) saccharifying a cellulosic material with an enzyme composition in the presence of a polypeptide having catalase activity;

(b) fermenting the saccharified cellulosic material with one or more (*e.g.*, several) fermenting microorganisms to produce the fermentation product; and

(c) recovering the fermentation product from the fermentation.

The present invention further relates to methods of fermenting a cellulosic material, comprising: fermenting the cellulosic material with one or more (*e.g.*, several) fermenting microorganisms, wherein the cellulosic material is hydrolyzed with an enzyme composition in the presence of a polypeptide having catalase activity.

The present invention even further relates to an enzyme composition for degrading or converting a cellulosic material comprising enzymes having cellulolytic activity and/or xylan degrading activity and a polypeptide having catalase activity; and the uses thereof.

Brief Description of the Figures

Figure 1 shows the genomic DNA sequence (SEQ ID NO: 3) and the amino acid sequence (SEQ ID NO: 4) of a *Talaromyces stipitatus* catalase gene.

Figure 2 shows the genomic DNA sequence (SEQ ID NO: 5) and the amino acid sequence (SEQ ID NO: 6) of a *Humicola insolens* catalase gene.

Figure 3 shows the genomic DNA sequence (SEQ ID NO: 7) and the amino acid sequence (SEQ ID NO: 8) of a *Penicillium emersonii* catalase gene.

Definitions

Catalase activity: The term "catalase activity" is defined herein as a hydrogen-peroxide:hydrogen-peroxide oxidoreductase activity (EC 1.11.1.6) that catalyzes the conversion of 2 H₂O₂ to O₂ + 2 H₂O. For purposes of the present invention, catalase activity is determined

according to U.S. Patent No. <http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahhtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=5646025.PN.&OS=PN/5646025&RS=PN/5646025> - h0#h0<http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahhtml%2FPTO%2Fsrchnum.htm>

&r=1&f=G&l=50&s1=5646025.PN.&OS=PN/5646025&RS=PN/5646025 - h2#h25,646,025. One unit of catalase activity equals the amount of enzyme that catalyzes the oxidation of 1 μ mole of hydrogen peroxide under the assay conditions.

In one aspect, the catalases used in the present invention have at least 20%, *e.g.*, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% of the catalase activity of the mature polypeptide of SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6, or the mature polypeptide of SEQ ID NO: 8.

Acetylxytan esterase: The term "acetylxytan esterase" means a carboxylesterase (EC 3.1.1.72) that catalyzes the hydrolysis of acetyl groups from polymeric xylan, acetylated xylose, acetylated glucose, alpha-naphthyl acetate, and *p*-nitrophenyl acetate. For purposes of the present invention, acetylxytan esterase activity is determined using 0.5 mM *p*-nitrophenylacetate as substrate in 50 mM sodium acetate pH 5.0 containing 0.01% TWEEN™ 20 (polyoxyethylene sorbitan monolaurate). One unit of acetylxytan esterase is defined as the amount of enzyme capable of releasing 1 μ mole of *p*-nitrophenolate anion per minute at pH 5, 25°C.

Alpha-L-arabinofuranosidase: The term "alpha-L-arabinofuranosidase" means an alpha-L-arabinofuranoside arabinofuranohydrolase (EC 3.2.1.55) that catalyzes the hydrolysis of terminal non-reducing alpha-L-arabinofuranoside residues in alpha-L-arabinosides. The enzyme acts on alpha-L-arabinofuranosides, alpha-L-arabinans containing (1,3)- and/or (1,5)-linkages, arabinoxylans, and arabinogalactans. Alpha-L-arabinofuranosidase is also known as arabinosidase, alpha-arabinosidase, alpha-L-arabinosidase, alpha-arabinofuranosidase, polysaccharide alpha-L-arabinofuranosidase, alpha-L-arabinofuranoside hydrolase, L-arabinosidase, or alpha-L-arabinanase. For purposes of the present invention, alpha-L-arabinofuranosidase activity is determined using 5 mg of medium viscosity wheat arabinoxylan (Megazyme International Ireland, Ltd., Bray, Co. Wicklow, Ireland) per ml of 100 mM sodium acetate pH 5 in a total volume of 200 μ l for 30 minutes at 40°C followed by arabinose analysis by AMINEX® HPX-87H column chromatography (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Alpha-glucuronidase: The term "alpha-glucuronidase" means an alpha-D-glucosiduronate glucuronohydrolase (EC 3.2.1.139) that catalyzes the hydrolysis of an alpha-D-glucuronoside to D-glucuronate and an alcohol. For purposes of the present invention, alpha-glucuronidase activity is determined according to de Vries, 1998, *J. Bacteriol.* 180: 243-249. One unit of alpha-glucuronidase equals the amount of enzyme capable of releasing 1 μ mole of glucuronic or 4-O-methylglucuronic acid per minute at pH 5, 40°C.

Beta-glucosidase: The term "beta-glucosidase" means a beta-D-glucoside glucohydrolase (E.C. 3.2.1.21) that catalyzes the hydrolysis of terminal non-reducing beta-D-glucose residues with

the release of beta-D-glucose. For purposes of the present invention, beta-glucosidase activity is determined using *p*-nitrophenyl-beta-D-glucopyranoside as substrate according to the procedure of Venturi *et al.*, 2002, Extracellular beta-D-glucosidase from *Chaetomium thermophilum* var. *coprophilum*: production, purification and some biochemical properties, *J. Basic Microbiol.* 42: 55-66. One unit of beta-glucosidase is defined as 1.0 μ mole of *p*-nitrophenolate anion produced per minute at 25°C, pH 4.8 from 1 mM *p*-nitrophenyl-beta-D-glucopyranoside as substrate in 50 mM sodium citrate containing 0.01% TWEEN® 20.

Beta-xylosidase: The term "beta-xylosidase" means a beta-D-xyloside xylohydrolase (E.C. 3.2.1.37) that catalyzes the exo-hydrolysis of short beta (1→4)-xylooligosaccharides to remove successive D-xylose residues from non-reducing termini. For purposes of the present invention, one unit of beta-xylosidase is defined as 1.0 μ mole of *p*-nitrophenolate anion produced per minute at 40°C, pH 5 from 1 mM *p*-nitrophenyl-beta-D-xyloside as substrate in 100 mM sodium citrate containing 0.01% TWEEN® 20.

cDNA: The term "cDNA" means a DNA molecule that can be prepared by reverse transcription from a mature, spliced, mRNA molecule obtained from a eukaryotic or prokaryotic cell. cDNA lacks intron sequences that may be present in the corresponding genomic DNA. The initial, primary RNA transcript is a precursor to mRNA that is processed through a series of steps, including splicing, before appearing as mature spliced mRNA.

Cellobiohydrolase: The term "cellobiohydrolase" means a 1,4-beta-D-glucan cellobiohydrolase (E.C. 3.2.1.91 and E.C. 3.2.1.176) that catalyzes the hydrolysis of 1,4-beta-D-glucosidic linkages in cellulose, celooligosaccharides, or any beta-1,4-linked glucose containing polymer, releasing cellobiose from the reducing or non-reducing ends of the chain (Teeri, 1997, Crystalline cellulose degradation: New insight into the function of cellobiohydrolases, *Trends in Biotechnology* 15: 160-167; Teeri *et al.*, 1998, *Trichoderma reesei* cellobiohydrolases: why so efficient on crystalline cellulose?, *Biochem. Soc. Trans.* 26: 173-178). Cellobiohydrolase activity is determined according to the procedures described by Lever *et al.*, 1972, *Anal. Biochem.* 47: 273-279; van Tilbeurgh *et al.*, 1982, *FEBS Letters*, 149: 152-156; van Tilbeurgh and Claeysens, 1985, *FEBS Letters*, 187: 283-288; and Tomme *et al.*, 1988, *Eur. J. Biochem.* 170: 575-581. In the present invention, the Tomme *et al.* method can be used to determine cellobiohydrolase activity.

Cellulosic material: The term "cellulosic material" means any material containing cellulose. The predominant polysaccharide in the primary cell wall of biomass is cellulose, the second most abundant is hemicellulose, and the third is pectin. The secondary cell wall, produced after the cell has stopped growing, also contains polysaccharides and is strengthened by polymeric lignin covalently cross-linked to hemicellulose. Cellulose is a homopolymer of anhydrocellobiose and thus a linear beta-(1-4)-D-glucan, while hemicelluloses include a variety of compounds, such as xylans, xyloglucans, arabinoxylans, and mannans in complex branched structures with a spectrum

of substituents. Although generally polymorphous, cellulose is found in plant tissue primarily as an insoluble crystalline matrix of parallel glucan chains. Hemicelluloses usually hydrogen bond to cellulose, as well as to other hemicelluloses, which help stabilize the cell wall matrix.

Cellulose is generally found, for example, in the stems, leaves, hulls, husks, and cobs of plants or leaves, branches, and wood of trees. The cellulosic material can be, but is not limited to, agricultural residue, herbaceous material (including energy crops), municipal solid waste, pulp and paper mill residue, waste paper, and wood (including forestry residue) (see, for example, Wiseloge *et al.*, 1995, in Handbook on Bioethanol (Charles E. Wyman, editor), pp.105-118, Taylor & Francis, Washington D.C.; Wyman, 1994, *Bioresource Technology* 50: 3-16; Lynd, 1990, *Applied Biochemistry and Biotechnology* 24/25: 695-719; Mosier *et al.*, 1999, Recent Progress in Bioconversion of Lignocellulosics, in *Advances in Biochemical Engineering/Biotechnology*, T. Scheper, managing editor, Volume 65, pp.23-40, Springer-Verlag, New York). It is understood herein that the cellulose may be in the form of lignocellulose, a plant cell wall material containing lignin, cellulose, and hemicellulose in a mixed matrix. In a preferred aspect, the cellulosic material is any biomass material. In another preferred aspect, the cellulosic material is lignocellulose, which comprises cellulose, hemicelluloses, and lignin.

In one aspect, the cellulosic material is agricultural residue. In another aspect, the cellulosic material is herbaceous material (including energy crops). In another aspect, the cellulosic material is municipal solid waste. In another aspect, the cellulosic material is pulp and paper mill residue. In another aspect, the cellulosic material is waste paper. In another aspect, the cellulosic material is wood (including forestry residue).

In another aspect, the cellulosic material is arundo. In another aspect, the cellulosic material is bagasse. In another aspect, the cellulosic material is bamboo. In another aspect, the cellulosic material is corn cob. In another aspect, the cellulosic material is corn fiber. In another aspect, the cellulosic material is corn stover. In another aspect, the cellulosic material is miscanthus. In another aspect, the cellulosic material is orange peel. In another aspect, the cellulosic material is rice straw. In another aspect, the cellulosic material is switchgrass. In another aspect, the cellulosic material is wheat straw.

In another aspect, the cellulosic material is aspen. In another aspect, the cellulosic material is eucalyptus. In another aspect, the cellulosic material is fir. In another aspect, the cellulosic material is pine. In another aspect, the cellulosic material is poplar. In another aspect, the cellulosic material is spruce. In another aspect, the cellulosic material is willow.

In another aspect, the cellulosic material is algal cellulose. In another aspect, the cellulosic material is bacterial cellulose. In another aspect, the cellulosic material is cotton linter. In another aspect, the cellulosic material is filter paper. In another aspect, the cellulosic material is microcrystalline cellulose. In another aspect, the cellulosic material is phosphoric-acid treated

cellulose.

In another aspect, the cellulosic material is an aquatic biomass. As used herein the term "aquatic biomass" means biomass produced in an aquatic environment by a photosynthesis process. The aquatic biomass can be algae, emergent plants, floating-leaf plants, or submerged plants.

The cellulosic material may be used as is or may be subjected to pretreatment, using conventional methods known in the art, as described herein. In a preferred aspect, the cellulosic material is pretreated.

Cellulolytic enzyme or cellulase: The term "cellulolytic enzyme" or "cellulase" means one or more (*e.g.*, several) enzymes that hydrolyze a cellulosic material. Such enzymes include endoglucanase(s), cellobiohydrolase(s), beta-glucosidase(s), or combinations thereof. The two basic approaches for measuring cellulolytic activity include: (1) measuring the total cellulolytic activity, and (2) measuring the individual cellulolytic activities (endoglucanases, cellobiohydrolases, and beta-glucosidases) as reviewed in Zhang *et al.*, Outlook for cellulase improvement: Screening and selection strategies, 2006, *Biotechnology Advances* 24: 452-481. Total cellulolytic activity is usually measured using insoluble substrates, including Whatman №1 filter paper, microcrystalline cellulose, bacterial cellulose, algal cellulose, cotton, pretreated lignocellulose, *etc.* The most common total cellulolytic activity assay is the filter paper assay using Whatman №1 filter paper as the substrate. The assay was established by the International Union of Pure and Applied Chemistry (IUPAC) (Ghose, 1987, Measurement of cellulase activities, *Pure Appl. Chem.* 59: 257-68).

For purposes of the present invention, cellulolytic enzyme activity is determined by measuring the increase in hydrolysis of a cellulosic material by cellulolytic enzyme(s) under the following conditions: 1-50 mg of cellulolytic enzyme protein/g of cellulose in PCS (or other pretreated cellulosic material) for 3-7 days at a suitable temperature, *e.g.*, 50°C, 55°C, or 60°C, compared to a control hydrolysis without addition of cellulolytic enzyme protein. Typical conditions are 1 ml reactions, washed or unwashed PCS, 5% insoluble solids, 50 mM sodium acetate pH 5, 1 mM MnSO₄, 50°C, 55°C, or 60°C, 72 hours, sugar analysis by AMINEX® HPX-87H column (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Coding sequence: The term "coding sequence" means a polynucleotide, which directly specifies the amino acid sequence of a polypeptide. The boundaries of the coding sequence are generally determined by an open reading frame, which begins with a start codon such as ATG, GTG, or TTG and ends with a stop codon such as TAA, TAG, or TGA. The coding sequence may be a genomic DNA, cDNA, synthetic DNA, or a combination thereof.

Control sequences: The term "control sequences" means nucleic acid sequences necessary for expression of a polynucleotide encoding a mature polypeptide of the present

invention. Each control sequence may be native (*i.e.*, from the same gene) or foreign (*i.e.*, from a different gene) to the polynucleotide encoding the polypeptide or native or foreign to each other. Such control sequences include, but are not limited to, a leader, polyadenylation sequence, propeptide sequence, promoter, signal peptide sequence, and transcription terminator. At a minimum, the control sequences include a promoter, and transcriptional and translational stop signals. The control sequences may be provided with linkers for the purpose of introducing specific restriction sites facilitating ligation of the control sequences with the coding region of the polynucleotide encoding a polypeptide.

Endoglucanase: The term “endoglucanase” means an endo-1,4-(1,3;1,4)-beta-D-glucan 4-glucanohydrolase (E.C. 3.2.1.4) that catalyzes endohydrolysis of 1,4-beta-D-glycosidic linkages in cellulose, cellulose derivatives (such as carboxymethyl cellulose and hydroxyethyl cellulose), lichenin, beta-1,4 bonds in mixed beta-1,3 glucans such as cereal beta-D-glucans or xyloglucans, and other plant material containing cellulosic components. Endoglucanase activity can be determined by measuring reduction in substrate viscosity or increase in reducing ends determined by a reducing sugar assay (Zhang *et al.*, 2006, *Biotechnology Advances* 24: 452-481). For purposes of the present invention, endoglucanase activity is determined using carboxymethyl cellulose (CMC) as substrate according to the procedure of Ghose, 1987, *Pure and Appl. Chem.* 59: 257-268, at pH 5, 40°C.

Expression: The term “expression” includes any step involved in the production of a polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

Expression vector: The term “expression vector” means a linear or circular DNA molecule that comprises a polynucleotide encoding a polypeptide and is operably linked to control sequences that provide for its expression.

Family 61 glycoside hydrolase: The term “Family 61 glycoside hydrolase” or “Family GH61” or “GH61” means a polypeptide falling into the glycoside hydrolase Family 61 according to Henrissat B., 1991, A classification of glycosyl hydrolases based on amino-acid sequence similarities, *Biochem. J.* 280: 309-316, and Henrissat B., and Bairoch A., 1996, Updating the sequence-based classification of glycosyl hydrolases, *Biochem. J.* 316: 695-696. The enzymes in this family were originally classified as a glycoside hydrolase family based on measurement of very weak endo-1,4-beta-D-glucanase activity in one family member. The structure and mode of action of these enzymes are non-canonical and they cannot be considered as bona fide glycosidases. However, they are kept in the CAZy classification on the basis of their capacity to enhance the breakdown of lignocellulose when used in conjunction with a cellulase or a mixture of cellulases.

Feruloyl esterase: The term “feruloyl esterase” means a 4-hydroxy-3-methoxycinnamoyl-sugar hydrolase (EC 3.1.1.73) that catalyzes the hydrolysis of 4-hydroxy-3-methoxycinnamoyl

(feruloyl) groups from esterified sugar, which is usually arabinose in "natural" substrates, to produce ferulate (4-hydroxy-3-methoxycinnamate). Feruloyl esterase is also known as ferulic acid esterase, hydroxycinnamoyl esterase, FAE-III, cinnamoyl ester hydrolase, FAEA, cinnAE, FAE-I, or FAE-II. For purposes of the present invention, feruloyl esterase activity is determined using 0.5 mM *p*-nitrophenylferulate as substrate in 50 mM sodium acetate pH 5.0. One unit of feruloyl esterase equals the amount of enzyme capable of releasing 1 μ mole of *p*-nitrophenolate anion per minute at pH 5, 25°C.

Fragment: The term "fragment" means a polypeptide having one or more (*e.g.*, several) amino acids absent from the amino and/or carboxyl terminus of a mature polypeptide main; wherein the fragment has catalase activity. In one aspect, a fragment contains at least 632 amino acid residues, *e.g.*, at least 670 amino acid residues or at least 708 amino acid residues of SEQ ID NO: 2. In another aspect, a fragment contains at least 622 amino acid residues, *e.g.*, at least 659 amino acid residues or at least 696 amino acid residues of SEQ ID NO: 4. In another aspect, a fragment contains at least 652 amino acid residues, *e.g.*, at least 689 amino acid residues or at least 727 amino acid residues of SEQ ID NO: 6. In another aspect, a fragment contains at least 614 amino acid residues, *e.g.*, at least 650 amino acid residues or at least 686 amino acid residues of SEQ ID NO: 8.

Hemicellulolytic enzyme or hemicellulase: The term "hemicellulolytic enzyme" or "hemicellulase" means one or more (*e.g.*, several) enzymes that hydrolyze a hemicellulosic material. See, for example, Shallom, D. and Shoham, Y. Microbial hemicellulases. *Current Opinion In Microbiology*, 2003, 6(3): 219-228). Hemicellulases are key components in the degradation of plant biomass. Examples of hemicellulases include, but are not limited to, an acetylmannan esterase, an acetylxytan esterase, an arabinanase, an arabinofuranosidase, a coumaric acid esterase, a feruloyl esterase, a galactosidase, a glucuronidase, a glucuronoyl esterase, a mannanase, a mannosidase, a xylanase, and a xylosidase. The substrates of these enzymes, the hemicelluloses, are a heterogeneous group of branched and linear polysaccharides that are bound via hydrogen bonds to the cellulose microfibrils in the plant cell wall, crosslinking them into a robust network. Hemicelluloses are also covalently attached to lignin, forming together with cellulose a highly complex structure. The variable structure and organization of hemicelluloses require the concerted action of many enzymes for its complete degradation. The catalytic modules of hemicellulases are either glycoside hydrolases (GHs) that hydrolyze glycosidic bonds, or carbohydrate esterases (CEs), which hydrolyze ester linkages of acetate or ferulic acid side groups. These catalytic modules, based on homology of their primary sequence, can be assigned into GH and CE families. Some families, with an overall similar fold, can be further grouped into clans, marked alphabetically (*e.g.*, GH-A). A most informative and updated classification of these and other carbohydrate active enzymes is available in the Carbohydrate-Active Enzymes (CAZy)

database. Hemicellulolytic enzyme activities can be measured according to Ghose and Bisaria, 1987, *Pure & Appl. Chem.* 59: 1739-1752, at a suitable temperature, *e.g.*, 50°C, 55°C, or 60°C, and pH, *e.g.*, 5.0 or 5.5.

High stringency conditions: The term "high stringency conditions" means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 50% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 65°C.

Host cell: The term "host cell" means any cell type that is susceptible to transformation, transfection, transduction, or the like with a nucleic acid construct or expression vector comprising a catalase of the present invention. The term "host cell" encompasses any progeny of a parent cell that is not identical to the parent cell due to mutations that occur during replication.

Low stringency conditions: The term "low stringency conditions" means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 25% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 50°C.

Mature polypeptide: The term "mature polypeptide" means a polypeptide in its final form following translation and any post-translational modifications, such as N-terminal processing, C-terminal truncation, glycosylation, phosphorylation, etc. In one embodiment, the mature polypeptide is amino acids 1 to 746 of SEQ ID NO: 2. In another embodiment, the mature polypeptide is amino acids 20 to 733 of SEQ ID NO: 4 based on the SignalP program that predicts amino acids 1 to 19 of SEQ ID NO: 4 are a signal peptide. In another embodiment, the mature polypeptide is amino acids 20 to 765 of SEQ ID NO: 6 based on the SignalP program that predicts amino acids 1 to 19 of SEQ ID NO: 6 are a signal peptide. In another embodiment, the mature polypeptide is amino acids 20 to 741 of SEQ ID NO: 8 based on the SignalP program that predicts amino acids 1 to 19 of SEQ ID NO: 8 are a signal peptide. It is known in the art that a host cell may produce a mixture of two or more different mature polypeptides (*i.e.*, with a different C-terminal and/or N-terminal amino acid) expressed by the same polynucleotide.

Mature polypeptide coding sequence: The term "mature polypeptide coding sequence" means a polynucleotide that encodes a mature polypeptide having catalase activity. In one embodiment, the mature polypeptide coding sequence is nucleotides 1 to 2351 of SEQ ID NO: 1 or the cDNA sequence thereof. In another embodiment, the mature polypeptide coding sequence is nucleotides 58 to 2418 of SEQ ID NO: 3 or the cDNA sequence thereof based on the SignalP program that predicts nucleotides 1 to 57 of SEQ ID NO: 3 encode a signal peptide. In another embodiment, the mature polypeptide coding sequence is nucleotides 58 to 3040 of SEQ ID NO: 5

or the cDNA sequence thereof based on the SignalP program that predicts nucleotides 1 to 57 of SEQ ID NO: 5 encode a signal peptide. In another embodiment, the mature polypeptide coding sequence is nucleotides 58 to 2476 of SEQ ID NO: 7 or the cDNA sequence thereof based on the SignalP program that predicts nucleotides 1 to 57 of SEQ ID NO: 7 encode a signal peptide.

5 Medium stringency conditions: The term "medium stringency conditions" means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 35% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 55°C.

10 Medium-high stringency conditions: The term "medium-high stringency conditions" means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and either 35% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 60°C.

15 Nucleic acid construct: The term "nucleic acid construct" means a nucleic acid molecule, either single- or double-stranded, which is isolated from a naturally occurring gene or is modified to contain segments of nucleic acids in a manner that would not otherwise exist in nature or which is synthetic, which comprises one or more control sequences.

Operably linked: The term "operably linked" means a configuration in which a control
20 sequence is placed at an appropriate position relative to the coding sequence of a polynucleotide such that the control sequence directs expression of the coding sequence.

Polypeptide having cellulolytic enhancing activity: The term "polypeptide having cellulolytic enhancing activity" means a GH61 polypeptide that catalyzes the enhancement of the hydrolysis of a cellulosic material by enzyme having cellulolytic activity. For purposes of the
25 present invention, cellulolytic enhancing activity is determined by measuring the increase in reducing sugars or the increase of the total of cellobiose and glucose from the hydrolysis of a cellulosic material by cellulolytic enzyme under the following conditions: 1-50 mg of total protein/g of cellulose in PCS, wherein total protein is comprised of 50-99.5% w/w cellulolytic enzyme protein and 0.5-50% w/w protein of a GH61 polypeptide having cellulolytic enhancing activity for 1-7 days
30 at a suitable temperature, e.g., 50°C, 55°C, or 60°C, and pH, e.g., 5.0 or 5.5, compared to a control hydrolysis with equal total protein loading without cellulolytic enhancing activity (1-50 mg of cellulolytic protein/g of cellulose in PCS). In a preferred aspect, a mixture of CELLUCLAST® 1.5L (Novozymes A/S, Bagsværd, Denmark) in the presence of 2-3% of total protein weight *Aspergillus oryzae* beta-glucosidase (recombinantly produced in *Aspergillus oryzae* according to WO
35 02/095014) or 2-3% of total protein weight *Aspergillus fumigatus* beta-glucosidase (recombinantly

produced in *Aspergillus oryzae* as described in WO 2002/095014) of cellulase protein loading is used as the source of the cellulolytic activity.

The GH61 polypeptides having cellulolytic enhancing activity enhance the hydrolysis of a cellulosic material catalyzed by enzyme having cellulolytic activity by reducing the amount of cellulolytic enzyme required to reach the same degree of hydrolysis preferably at least 1.01-fold, *e.g.*, at least 1.05-fold, at least 1.10-fold, at least 1.25-fold, at least 1.5-fold, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 10-fold, or at least 20-fold.

Pretreated corn stover: The term "PCS" or "Pretreated Corn Stover" means a cellulosic material derived from corn stover by treatment with heat and dilute sulfuric acid, alkaline pretreatment, or neutral pretreatment.

Sequence identity: The relatedness between two amino acid sequences or between two nucleotide sequences is described by the parameter "sequence identity".

For purposes of the present invention, the sequence identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16: 276-277), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labeled "longest identity" (obtained using the *-nobrief* option) is used as the percent identity and is calculated as follows:

$$(\text{Identical Residues} \times 100) / (\text{Length of Alignment} - \text{Total Number of Gaps in Alignment})$$

For purposes of the present invention, the sequence identity between two deoxyribonucleotide sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *supra*) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *supra*), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EDNAFULL (EMBOSS version of NCBI NUC4.4) substitution matrix. The output of Needle labeled "longest identity" (obtained using the *-nobrief* option) is used as the percent identity and is calculated as follows:

$$(\text{Identical Deoxyribonucleotides} \times 100) / (\text{Length of Alignment} - \text{Total Number of Gaps in Alignment})$$

Variant: The term "variant" means a polypeptide having catalase activity comprising an alteration, *i.e.*, a substitution, insertion, and/or deletion, at one or more (*e.g.*, several) positions. A substitution means replacement of the amino acid occupying a position with a different amino acid; a deletion means removal of the amino acid occupying a position; and an insertion means adding an amino acid adjacent to and immediately following the amino acid occupying a position.

Very high stringency conditions: The term “very high stringency conditions” means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 50% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 70°C.

Very low stringency conditions: The term “very low stringency conditions” means for probes of at least 100 nucleotides in length, prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and 25% formamide, following standard Southern blotting procedures for 12 to 24 hours. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 45°C.

Xylan-containing material: The term “xylan-containing material” means any material comprising a plant cell wall polysaccharide containing a backbone of beta-(1-4)-linked xylose residues. Xylans of terrestrial plants are heteropolymers possessing a beta-(1-4)-D-xylopyranose backbone, which is branched by short carbohydrate chains. They comprise D-glucuronic acid or its 4-O-methyl ether, L-arabinose, and/or various oligosaccharides, composed of D-xylose, L-arabinose, D- or L-galactose, and D-glucose. Xylan-type polysaccharides can be divided into homoxylans and heteroxylans, which include glucuronoxylans, (arabino)glucuronoxylans, (glucurono)arabinoxylans, arabinoxylans, and complex heteroxylans. See, for example, Ebringerova *et al.*, 2005, *Adv. Polym. Sci.* 186: 1–67.

In the processes of the present invention, any material containing xylan may be used. In a preferred aspect, the xylan-containing material is lignocellulose.

Xylan degrading activity or xylanolytic activity: The term “xylan degrading activity” or “xylanolytic activity” means a biological activity that hydrolyzes xylan-containing material. The two basic approaches for measuring xylanolytic activity include: (1) measuring the total xylanolytic activity, and (2) measuring the individual xylanolytic activities (*e.g.*, endoxylanases, beta-xylosidases, arabinofuranosidases, alpha-glucuronidases, acetylxylan esterases, feruloyl esterases, and alpha-glucuronoyl esterases). Recent progress in assays of xylanolytic enzymes was summarized in several publications including Biely and Puchard, Recent progress in the assays of xylanolytic enzymes, 2006, *Journal of the Science of Food and Agriculture* 86(11): 1636-1647; Spanikova and Biely, 2006, Glucuronoyl esterase - Novel carbohydrate esterase produced by *Schizophyllum commune*, *FEBS Letters* 580(19): 4597-4601; Herrmann, Vrsanska, Jurickova, Hirsch, Biely, and Kubicek, 1997, The beta-D-xylosidase of *Trichoderma reesei* is a multifunctional beta-D-xylan xylohydrolase, *Biochemical Journal* 321: 375-381.

Total xylan degrading activity can be measured by determining the reducing sugars formed from various types of xylan, including, for example, oat spelt, beechwood, and larchwood xylans, or by photometric determination of dyed xylan fragments released from various covalently dyed

xylans. The most common total xylanolytic activity assay is based on production of reducing sugars from polymeric 4-O-methyl glucuronoxylan as described in Bailey, Biely, Poutanen, 1992, Interlaboratory testing of methods for assay of xylanase activity, *Journal of Biotechnology* 23(3): 257-270. Xylanase activity can also be determined with 0.2% AZCL-arabinoxylan as substrate in 0.01% TRITON® X-100 (4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol) and 200 mM sodium phosphate buffer pH 6 at 37°C. One unit of xylanase activity is defined as 1.0 μ mole of azurine produced per minute at 37°C, pH 6 from 0.2% AZCL-arabinoxylan as substrate in 200 mM sodium phosphate pH 6 buffer.

For purposes of the present invention, xylan degrading activity is determined by measuring the increase in hydrolysis of birchwood xylan (Sigma Chemical Co., Inc., St. Louis, MO, USA) by xylan-degrading enzyme(s) under the following typical conditions: 1 ml reactions, 5 mg/ml substrate (total solids), 5 mg of xylanolytic protein/g of substrate, 50 mM sodium acetate pH 5, 50°C, 24 hours, sugar analysis using *p*-hydroxybenzoic acid hydrazide (PHBAH) assay as described by Lever, 1972, A new reaction for colorimetric determination of carbohydrates, *Anal. Biochem* 47: 273-279.

Xylanase: The term "xylanase" means a 1,4-beta-D-xylan-xylohydrolase (E.C. 3.2.1.8) that catalyzes the endohydrolysis of 1,4-beta-D-xylosidic linkages in xylans. For purposes of the present invention, xylanase activity is determined with 0.2% AZCL-arabinoxylan as substrate in 0.01% TRITON® X-100 and 200 mM sodium phosphate buffer pH 6 at 37°C. One unit of xylanase activity is defined as 1.0 μ mole of azurine produced per minute at 37°C, pH 6 from 0.2% AZCL-arabinoxylan as substrate in 200 mM sodium phosphate pH 6 buffer.

Detailed Description of the Invention

Methods of Processing Cellulosic Material

The present invention relates to methods for degrading or converting a cellulosic material, comprising: treating the cellulosic material with an enzyme composition in the presence of a polypeptide having catalase activity. In one aspect, the method further comprises recovering the degraded or converted cellulosic material.

The present invention also relates to methods for producing a fermentation product, comprising:

(a) saccharifying a cellulosic material with an enzyme composition in the presence of a polypeptide having catalase activity;

(b) fermenting the saccharified cellulosic material with one or more (*e.g.*, several) fermenting microorganisms to produce the fermentation product; and

(c) recovering the fermentation product from the fermentation.

The present invention further relates to methods of fermenting a cellulosic material, comprising: fermenting the cellulosic material with one or more (*e.g.*, several) fermenting microorganisms, wherein the cellulosic material is hydrolyzed with an enzyme composition in the presence of a polypeptide having catalase activity. In one aspect, the fermenting of the cellulosic material produces a fermentation product. In another aspect, the method further comprises recovering the fermentation product from the fermentation.

In the methods described above, the presence of the polypeptide having catalase activity increases the hydrolysis of the cellulosic material compared to the absence of the polypeptide having catalase activity.

The methods of the present invention can be used to saccharify the cellulosic material to fermentable sugars and to convert the fermentable sugars to many useful fermentation products, *e.g.*, fuel, potable ethanol, and/or platform chemicals (*e.g.*, acids, alcohols, ketones, gases, and the like). The production of a desired fermentation product from the cellulosic material typically involves pretreatment, enzymatic hydrolysis (saccharification), and fermentation.

The processing of the cellulosic material according to the present invention can be accomplished using processes conventional in the art. Moreover, the methods of the present invention can be implemented using any conventional biomass processing apparatus configured to operate in accordance with the invention.

Hydrolysis (saccharification) and fermentation, separate or simultaneous, include, but are not limited to, separate hydrolysis and fermentation (SHF); simultaneous saccharification and fermentation (SSF); simultaneous saccharification and co-fermentation (SSCF); hybrid hydrolysis and fermentation (HHF); separate hydrolysis and co-fermentation (SHCF); hybrid hydrolysis and co-fermentation (HHCF); and direct microbial conversion (DMC), also sometimes called consolidated bioprocessing (CBP). SHF uses separate process steps to first enzymatically hydrolyze the cellulosic material to fermentable sugars, *e.g.*, glucose, cellobiose, cellotriose, and pentose monomers, and then ferment the fermentable sugars to ethanol. In SSF, the enzymatic hydrolysis of the cellulosic material and the fermentation of sugars to ethanol are combined in one step (Philippidis, G. P., 1996, Cellulose bioconversion technology, in *Handbook on Bioethanol: Production and Utilization*, Wyman, C. E., ed., Taylor & Francis, Washington, DC, 179-212). SSCF involves the co-fermentation of multiple sugars (Sheehan, J., and Himmel, M., 1999, Enzymes, energy and the environment: A strategic perspective on the U.S. Department of Energy's research and development activities for bioethanol, *Biotechnol. Prog.* 15: 817-827). HHF involves a separate hydrolysis step, and in addition a simultaneous saccharification and hydrolysis step, which can be carried out in the same reactor. The steps in an HHF process can be carried out at different temperatures, *i.e.*, high temperature enzymatic saccharification followed by SSF at a lower temperature that the fermentation strain can tolerate. DMC combines all three processes (enzyme

production, hydrolysis, and fermentation) in one or more (*e.g.*, several) steps where the same organism is used to produce the enzymes for conversion of the cellulosic material to fermentable sugars and to convert the fermentable sugars into a final product (Lynd, L. R., Weimer, P. J., van Zyl, W. H., and Pretorius, I. S., 2002, Microbial cellulose utilization: Fundamentals and biotechnology, *Microbiol. Mol. Biol. Reviews* 66: 506-577). It is understood herein that any method known in the art comprising pretreatment, enzymatic hydrolysis (saccharification), fermentation, or a combination thereof can be used in the practicing the methods of the present invention.

A conventional apparatus can include a fed-batch stirred reactor, a batch stirred reactor, a continuous flow stirred reactor with ultrafiltration, and/or a continuous plug-flow column reactor (Fernanda de Castilhos Corazza, Flávio Faria de Moraes, Gisella Maria Zanin and Ivo Neitzel, 2003, Optimal control in fed-batch reactor for the cellobiose hydrolysis, *Acta Scientiarum. Technology* 25: 33-38; Gusakov, A. V., and Sinitsyn, A. P., 1985, Kinetics of the enzymatic hydrolysis of cellulose: 1. A mathematical model for a batch reactor process, *Enz. Microb. Technol.* 7: 346-352), an attrition reactor (Ryu, S. K., and Lee, J. M., 1983, Bioconversion of waste cellulose by using an attrition bioreactor, *Biotechnol. Bioeng.* 25: 53-65), or a reactor with intensive stirring induced by an electromagnetic field (Gusakov, A. V., Sinitsyn, A. P., Davydkin, I. Y., Davydkin, V. Y., Protas, O. V., 1996, Enhancement of enzymatic cellulose hydrolysis using a novel type of bioreactor with intensive stirring induced by electromagnetic field, *Appl. Biochem. Biotechnol.* 56: 141-153). Additional reactor types include: fluidized bed, upflow blanket, immobilized, and extruder type reactors for hydrolysis and/or fermentation.

Pretreatment. In practicing the methods of the present invention, any pretreatment process known in the art can be used to disrupt plant cell wall components of cellulosic material (Chandra *et al.*, 2007, Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics? *Adv. Biochem. Engin./Biotechnol.* 108: 67-93; Galbe and Zacchi, 2007, Pretreatment of lignocellulosic materials for efficient bioethanol production, *Adv. Biochem. Engin. / Biotechnol.* 108: 41-65; Hendriks and Zeeman, 2009, Pretreatments to enhance the digestibility of lignocellulosic biomass, *Bioresource Technol.* 100: 10-18; Mosier *et al.*, 2005, Features of promising technologies for pretreatment of lignocellulosic biomass, *Bioresource Technol.* 96: 673-686; Taherzadeh and Karimi, 2008, Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review, *Int. J. of Mol. Sci.* 9: 1621-1651; Yang and Wyman, 2008, Pretreatment: the key to unlocking low-cost cellulosic ethanol, *Biofuels Bioproducts and Biorefining-Biofpr.* 2: 26-40).

The cellulosic material can also be subjected to particle size reduction, pre-soaking, wetting, washing, and/or conditioning prior to pretreatment using methods known in the art.

Conventional pretreatments include, but are not limited to, steam pretreatment (with or without explosion), dilute acid pretreatment, hot water pretreatment, alkaline pretreatment, lime pretreatment, wet oxidation, wet explosion, ammonia fiber explosion, organosolv pretreatment, and

biological pretreatment. Additional pretreatments include ammonia percolation, ultrasound, electroporation, microwave, supercritical CO₂, supercritical H₂O, ozone, ionic liquid, and gamma irradiation pretreatments.

The cellulosic material can be pretreated before hydrolysis and/or fermentation. Pretreatment is preferably performed prior to the hydrolysis. Alternatively, the pretreatment can be carried out simultaneously with enzyme hydrolysis to release fermentable sugars, such as glucose, xylose, and/or cellobiose. In most cases the pretreatment step itself results in some conversion of biomass to fermentable sugars (even in absence of enzymes).

Steam Pretreatment. In steam pretreatment, cellulosic material is heated to disrupt the plant cell wall components, including lignin, hemicellulose, and cellulose to make the cellulose and other fractions, *e.g.*, hemicellulose, accessible to enzymes. Cellulosic material is passed to or through a reaction vessel where steam is injected to increase the temperature to the required temperature and pressure and is retained therein for the desired reaction time. Steam pretreatment is preferably done at 140-230°C, more preferably 160-200°C, and most preferably 170-190°C, where the optimal temperature range depends on any addition of a chemical catalyst. Residence time for the steam pretreatment is preferably 1-30 minutes, more preferably 1-15 minutes, even more preferably 3-12 minutes, and most preferably 4-10 minutes, where the optimal residence time depends on temperature range and any addition of a chemical catalyst. Steam pretreatment allows for relatively high solids loadings, so that cellulosic material is generally only moist during the pretreatment. The steam pretreatment is often combined with an explosive discharge of the material after the pretreatment, which is known as steam explosion, that is, rapid flashing to atmospheric pressure and turbulent flow of the material to increase the accessible surface area by fragmentation (Duff and Murray, 1996, *Bioresource Technology* 855: 1-33; Galbe and Zacchi, 2002, *Appl. Microbiol. Biotechnol.* 59: 618-628; U.S. Patent Application No. 20020164730). During steam pretreatment, hemicellulose acetyl groups are cleaved and the resulting acid autocatalyzes partial hydrolysis of the hemicellulose to monosaccharides and oligosaccharides. Lignin is removed to only a limited extent.

A catalyst such as H₂SO₄ or SO₂ (typically 0.3 to 3% w/w) is often added prior to steam pretreatment, which decreases the time and temperature, increases the recovery, and improves enzymatic hydrolysis (Ballesteros *et al.*, 2006, *Appl. Biochem. Biotechnol.* 129-132: 496-508; Varga *et al.*, 2004, *Appl. Biochem. Biotechnol.* 113-116: 509-523; Sassner *et al.*, 2006, *Enzyme Microb. Technol.* 39: 756-762).

Chemical Pretreatment: The term "chemical treatment" refers to any chemical pretreatment that promotes the separation and/or release of cellulose, hemicellulose, and/or lignin. Examples of suitable chemical pretreatment processes include, for example, dilute acid pretreatment, lime pretreatment, wet oxidation, ammonia fiber/freeze explosion (AFEX), ammonia percolation (APR),

ionic liquid, and organosolv pretreatments.

In dilute acid pretreatment, cellulosic material is mixed with dilute acid, typically H₂SO₄, and water to form a slurry, heated by steam to the desired temperature, and after a residence time flashed to atmospheric pressure. The dilute acid pretreatment can be performed with a number of reactor designs, e.g., plug-flow reactors, counter-current reactors, or continuous counter-current shrinking bed reactors (Duff and Murray, 1996, *supra*; Schell *et al.*, 2004, *Bioresource Technol.* 91: 179-188; Lee *et al.*, 1999, *Adv. Biochem. Eng. Biotechnol.* 65: 93-115).

Several methods of pretreatment under alkaline conditions can also be used. These alkaline pretreatments include, but are not limited to, lime pretreatment, wet oxidation, ammonia percolation (APR), and ammonia fiber/freeze explosion (AFEX).

Lime pretreatment is performed with calcium carbonate, sodium hydroxide, or ammonia at low temperatures of 85-150°C and residence times from 1 hour to several days (Wyman *et al.*, 2005, *Bioresource Technol.* 96: 1959-1966; Mosier *et al.*, 2005, *Bioresource Technol.* 96: 673-686). WO 2006/110891, WO 2006/110899, WO 2006/110900, and WO 2006/110901 disclose pretreatment methods using ammonia.

Wet oxidation is a thermal pretreatment performed typically at 180-200°C for 5-15 minutes with addition of an oxidative agent such as hydrogen peroxide or over-pressure of oxygen (Schmidt and Thomsen, 1998, *Bioresource Technol.* 64: 139-151; Palonen *et al.*, 2004, *Appl. Biochem. Biotechnol.* 117: 1-17; Varga *et al.*, 2004, *Biotechnol. Bioeng.* 88: 567-574; Martin *et al.*, 2006, *J. Chem. Technol. Biotechnol.* 81: 1669-1677). The pretreatment is performed at preferably 1-40% dry matter, more preferably 2-30% dry matter, and most preferably 5-20% dry matter, and often the initial pH is increased by the addition of alkali such as sodium carbonate.

A modification of the wet oxidation pretreatment method, known as wet explosion (combination of wet oxidation and steam explosion), can handle dry matter up to 30%. In wet explosion, the oxidizing agent is introduced during pretreatment after a certain residence time. The pretreatment is then ended by flashing to atmospheric pressure (WO 2006/032282).

Ammonia fiber explosion (AFEX) involves treating cellulosic material with liquid or gaseous ammonia at moderate temperatures such as 90-100°C and high pressure such as 17-20 bar for 5-10 minutes, where the dry matter content can be as high as 60% (Gollapalli *et al.*, 2002, *Appl. Biochem. Biotechnol.* 98: 23-35; Chundawat *et al.*, 2007, *Biotechnol. Bioeng.* 96: 219-231; Alizadeh *et al.*, 2005, *Appl. Biochem. Biotechnol.* 121: 1133-1141; Teymouri *et al.*, 2005, *Bioresource Technol.* 96: 2014-2018). AFEX pretreatment results in the depolymerization of cellulose and partial hydrolysis of hemicellulose. Lignin-carbohydrate complexes are cleaved.

Organosolv pretreatment delignifies cellulosic material by extraction using aqueous ethanol (40-60% ethanol) at 160-200°C for 30-60 minutes (Pan *et al.*, 2005, *Biotechnol. Bioeng.* 90: 473-481; Pan *et al.*, 2006, *Biotechnol. Bioeng.* 94: 851-861; Kurabi *et al.*, 2005, *Appl. Biochem. Biotechnol.* 121:

219-230). Sulphuric acid is usually added as a catalyst. In organosolv pretreatment, the majority of hemicellulose is removed.

Other examples of suitable pretreatment methods are described by Schell *et al.*, 2003, *Appl. Biochem. and Biotechnol.* Vol. 105-108, p. 69-85, and Mosier *et al.*, 2005, *Bioresource Technology* 96: 673-686, and U.S. Published Application 2002/0164730.

In one aspect, the chemical pretreatment is preferably carried out as an acid treatment, and more preferably as a continuous dilute and/or mild acid treatment. The acid is typically sulfuric acid, but other acids can also be used, such as acetic acid, citric acid, nitric acid, phosphoric acid, tartaric acid, succinic acid, hydrogen chloride, or mixtures thereof. Mild acid treatment is conducted in the pH range of preferably 1-5, more preferably 1-4, and most preferably 1-3. In one aspect, the acid concentration is in the range from preferably 0.01 to 20 wt % acid, more preferably 0.05 to 10 wt % acid, even more preferably 0.1 to 5 wt % acid, and most preferably 0.2 to 2.0 wt % acid. The acid is contacted with cellulosic material and held at a temperature in the range of preferably 160-220°C, and more preferably 165-195°C, for periods ranging from seconds to minutes to, *e.g.*, 1 second to 60 minutes.

In another aspect, pretreatment is carried out as an ammonia fiber explosion step (AFEX pretreatment step).

In another aspect, pretreatment takes place in an aqueous slurry. In preferred aspects, cellulosic material is present during pretreatment in amounts preferably between 10-80 wt %, more preferably between 20-70 wt %, and most preferably between 30-60 wt %, such as around 50 wt %. The pretreated cellulosic material can be unwashed or washed using any method known in the art, *e.g.*, washed with water.

Mechanical Pretreatment: The term "mechanical pretreatment" refers to various types of grinding or milling (*e.g.*, dry milling, wet milling, or vibratory ball milling).

Physical Pretreatment: The term "physical pretreatment" refers to any pretreatment that promotes the separation and/or release of cellulose, hemicellulose, and/or lignin from cellulosic material. For example, physical pretreatment can involve irradiation (*e.g.*, microwave irradiation), steaming/steam explosion, hydrothermolysis, and combinations thereof.

Physical pretreatment can involve high pressure and/or high temperature (steam explosion). In one aspect, high pressure means pressure in the range of preferably about 300 to about 600 psi, more preferably about 350 to about 550 psi, and most preferably about 400 to about 500 psi, such as around 450 psi. In another aspect, high temperature means temperatures in the range of about 100 to about 300°C, preferably about 140 to about 235°C. In a preferred aspect, mechanical pretreatment is performed in a batch-process, steam gun hydrolyzer system that uses high pressure and high temperature as defined above, *e.g.*, a Sunds Hydrolyzer available from Sunds Defibrator AB, Sweden.

Combined Physical and Chemical Pretreatment: Cellulosic material can be pretreated both

physically and chemically. For instance, the pretreatment step can involve dilute or mild acid treatment and high temperature and/or pressure treatment. The physical and chemical pretreatments can be carried out sequentially or simultaneously, as desired. A mechanical pretreatment can also be included.

5 Accordingly, in a preferred aspect, cellulosic material is subjected to mechanical, chemical, or physical pretreatment, or any combination thereof, to promote the separation and/or release of cellulose, hemicellulose, and/or lignin.

Biological Pretreatment: The term "biological pretreatment" refers to any biological pretreatment that promotes the separation and/or release of cellulose, hemicellulose, and/or lignin from cellulosic material. Biological pretreatment techniques can involve applying lignin-solubilizing microorganisms (see, for example, Hsu, T.-A., 1996, Pretreatment of biomass, in *Handbook on Bioethanol: Production and Utilization*, Wyman, C. E., ed., Taylor & Francis, Washington, DC, 179-212; Ghosh and Singh, 1993, Physicochemical and biological treatments for enzymatic/microbial conversion of cellulosic biomass, *Adv. Appl. Microbiol.* 39: 295-333; McMillan, J. D., 1994, Pretreating lignocellulosic biomass: a review, in *Enzymatic Conversion of Biomass for Fuels Production*, Himmel, M. E., Baker, J. O., and Overend, R. P., eds., ACS Symposium Series 566, American Chemical Society, Washington, DC, chapter 15; Gong, C. S., Cao, N. J., Du, J., and Tsao, G. T., 1999, Ethanol production from renewable resources, in *Advances in Biochemical Engineering/Biotechnology*, Scheper, T., ed., Springer-Verlag Berlin Heidelberg, Germany, 65: 207-241; Olsson and Hahn-Hagerdal, 1996, Fermentation of lignocellulosic hydrolysates for ethanol production, *Enz. Microb. Tech.* 18: 312-331; and Vallander and Eriksson, 1990, Production of ethanol from lignocellulosic materials: State of the art, *Adv. Biochem. Eng./Biotechnol.* 42: 63-95).

Saccharification. In the hydrolysis step, also known as saccharification, the cellulosic material, e.g., pretreated, is hydrolyzed to break down cellulose and alternatively also hemicellulose to sugars, such as glucose, cellobiose, xylose, xylulose, arabinose, mannose, galactose, and/or soluble oligosaccharides. The sugars, and/or soluble oligosaccharides can further be further used to produce an alcohol (e.g., arabinitol, n-butanol, isobutanol, ethanol, glycerol, methanol, ethylene glycol, 1,3-propanediol [propylene glycol], butanediol, glycerin, sorbitol, and xylitol); an alkane (e.g., pentane, hexane, heptane, octane, nonane, decane, undecane, and dodecane), a cycloalkane (e.g., cyclopentane, cyclohexane, cycloheptane, and cyclooctane), an alkene (e.g. pentene, hexene, heptene, and octene); an amino acid (e.g., aspartic acid, glutamic acid, glycine, lysine, serine, and threonine); a gas (e.g., methane, hydrogen (H₂), carbon dioxide (CO₂), and carbon monoxide (CO)); isoprene; a ketone (e.g., acetone); an organic acid (e.g., acetic acid, acetic acid, adipic acid, ascorbic acid, citric acid, 2,5-diketo-D-gluconic acid, formic acid, fumaric acid, glucaric acid, gluconic acid, glucuronic acid, glutaric acid, 3-hydroxypropionic acid, itaconic acid,

lactic acid, malic acid, malonic acid, oxalic acid, oxaloacetic acid, propionic acid, succinic acid, and xylonic acid); and polyketide.

The hydrolysis is performed enzymatically by an enzyme composition in the presence of a polypeptide having catalase activity of the present invention. The enzymes of the compositions and the polypeptide having catalase activity can also be added simultaneously or sequentially.

Enzymatic hydrolysis is preferably carried out in a suitable aqueous environment under conditions that can be readily determined by one skilled in the art. In one aspect, hydrolysis is performed under conditions suitable for the activity of the enzyme(s), *i.e.*, optimal for the enzyme(s). The hydrolysis can be carried out as a fed batch or continuous process where the cellulosic material is fed gradually to, for example, an enzyme containing hydrolysis solution.

The saccharification is generally performed in stirred-tank reactors or fermentors under controlled pH, temperature, and mixing conditions. Suitable process time, temperature and pH conditions can readily be determined by one skilled in the art. For example, the saccharification can last up to 200 hours, but is typically performed for preferably about 12 to about 120 hours, *e.g.*, about 16 to about 72 hours or about 24 to about 48 hours. The temperature is in the range of preferably about 25°C to about 70°C, *e.g.*, about 30°C to about 65°C, about 40°C to about 60°C, or about 50°C to about 55°C. The pH is in the range of preferably about 3 to about 8, *e.g.*, about 3.5 to about 7, about 4 to about 6, or about 5.0 to about 5.5. The dry solids content is in the range of preferably about 5 to about 50 wt %, *e.g.*, about 10 to about 40 wt % or about 20 to about 30 wt %.

Enzyme Composition

The enzyme compositions can comprise any protein that is useful in degrading or converting cellulosic material. The compositions may comprise one enzyme as the major enzymatic component, *e.g.*, a mono-component composition, or multiple enzymes. The compositions may be prepared in accordance with methods known in the art and may be in the form of a liquid or a dry composition. The compositions may be stabilized in accordance with methods known in the art.

In one aspect, an enzyme composition for degrading or converting a cellulosic material comprises one or more (*e.g.*, several) enzymes having cellulolytic and/or hemicellulolytic activity and a polypeptide having catalase activity.

In an embodiment, the enzyme composition comprises or further comprises one or more (*e.g.*, several) proteins selected from the group consisting of a cellulase, a GH61 polypeptide having cellulolytic enhancing activity, a hemicellulase, an esterase, an expansin, a laccase, a ligninolytic enzyme, a pectinase, a peroxidase, a protease, and a swollenin. In another aspect, the cellulase is preferably one or more (*e.g.*, several) enzymes selected from the group consisting of an endoglucanase, a cellobiohydrolase, and a beta-glucosidase. In another aspect, the

hemicellulase is preferably one or more (*e.g.*, several) enzymes selected from the group consisting of an acetylmannan esterase, an acetylxylan esterase, an arabinanase, an arabinofuranosidase, a coumaric acid esterase, a feruloyl esterase, a galactosidase, a glucuronidase, a glucuronoyl esterase, a mannanase, a mannosidase, a xylanase, and a xylosidase.

5 In another embodiment, the enzyme composition comprises one or more (*e.g.*, several) cellulolytic enzymes. In another aspect, the enzyme composition comprises or further comprises one or more (*e.g.*, several) hemicellulolytic enzymes. In another aspect, the enzyme composition comprises one or more (*e.g.*, several) cellulolytic enzymes and one or more (*e.g.*, several) hemicellulolytic enzymes. In another aspect, the enzyme composition comprises one or more (*e.g.*,
10 several) enzymes selected from the group of cellulolytic enzymes and hemicellulolytic enzymes. In another aspect, the enzyme composition comprises an endoglucanase. In another aspect, the enzyme composition comprises a cellobiohydrolase. In another aspect, the enzyme composition comprises a beta-glucosidase. In another aspect, the enzyme composition comprises a polypeptide having cellulolytic enhancing activity. In another aspect, the enzyme composition
15 comprises an endoglucanase and a polypeptide having cellulolytic enhancing activity. In another aspect, the enzyme composition comprises a cellobiohydrolase and a polypeptide having cellulolytic enhancing activity. In another aspect, the enzyme composition comprises a beta-glucosidase and a polypeptide having cellulolytic enhancing activity. In another aspect, the enzyme composition comprises an endoglucanase and a cellobiohydrolase. In another aspect, the enzyme
20 composition comprises an endoglucanase and a beta-glucosidase. In another aspect, the enzyme composition comprises a cellobiohydrolase and a beta-glucosidase. In another aspect, the enzyme composition comprises an endoglucanase, a cellobiohydrolase, and a polypeptide having cellulolytic enhancing activity. In another aspect, the enzyme composition comprises an endoglucanase, a beta-glucosidase, and a polypeptide having cellulolytic enhancing activity. In
25 another aspect, the enzyme composition comprises a cellobiohydrolase, a beta-glucosidase, and a polypeptide having cellulolytic enhancing activity. In another aspect, the enzyme composition comprises an endoglucanase, a cellobiohydrolase, and a beta-glucosidase. In another aspect, the enzyme composition comprises an endoglucanase, a cellobiohydrolase, a beta-glucosidase, and a polypeptide having cellulolytic enhancing activity.

30 In another embodiment, the enzyme composition comprises an acetylmannan esterase. In another aspect, the enzyme composition comprises an acetylxylan esterase. In another aspect, the enzyme composition comprises an arabinanase (*e.g.*, alpha-L-arabinanase). In another aspect, the enzyme composition comprises an arabinofuranosidase (*e.g.*, alpha-L-arabinofuranosidase). In another aspect, the enzyme composition comprises a coumaric acid esterase. In another aspect,
35 the enzyme composition comprises a feruloyl esterase. In another aspect, the enzyme composition comprises a galactosidase (*e.g.*, alpha-galactosidase and/or beta-galactosidase). In another

aspect, the enzyme composition comprises a glucuronidase (*e.g.*, alpha-D-glucuronidase). In another aspect, the enzyme composition comprises a glucuronoyl esterase. In another aspect, the enzyme composition comprises a mannanase. In another aspect, the enzyme composition comprises a mannosidase (*e.g.*, beta-mannosidase). In another aspect, the enzyme composition comprises a xylanase. In a preferred aspect, the xylanase is a Family 10 xylanase. In another aspect, the enzyme composition comprises a xylosidase (*e.g.*, beta-xylosidase).

In another embodiment, the enzyme composition comprises an esterase. In another aspect, the enzyme composition comprises an expansin. In another aspect, the enzyme composition comprises a laccase. In another aspect, the enzyme composition comprises a ligninolytic enzyme. In a preferred aspect, the ligninolytic enzyme is a manganese peroxidase. In another preferred aspect, the ligninolytic enzyme is a lignin peroxidase. In another preferred aspect, the ligninolytic enzyme is a H₂O₂-producing enzyme. In another aspect, the enzyme composition comprises a pectinase. In another aspect, the enzyme composition comprises a peroxidase. In another aspect, the enzyme composition comprises a protease. In another aspect, the enzyme composition comprises a swollenin.

In the methods of the present invention, the enzyme(s) can be added prior to or during saccharification, saccharification and fermentation, or fermentation. The enzymes having cellulolytic and/or hemicellulolytic activity and a polypeptide having catalase activity can be added simultaneously or sequentially.

One or more (*e.g.*, several) components of the enzyme composition may be wild-type proteins, recombinant proteins, or a combination of wild-type proteins and recombinant proteins. For example, one or more (*e.g.*, several) components may be native proteins of a cell, which is used as a host cell to express recombinantly one or more (*e.g.*, several) other components of the enzyme composition. One or more (*e.g.*, several) components of the enzyme composition may be produced as monocomponents, which are then combined to form the enzyme composition. The enzyme composition may be a combination of multicomponent and monocomponent protein preparations.

The enzymes used in the methods of the present invention may be in any form suitable for use, such as, for example, a fermentation broth formulation or a cell composition, a cell lysate with or without cellular debris, a semi-purified or purified enzyme preparation, or a host cell as a source of the enzymes. The enzyme composition may be a dry powder or granulate, a non-dusting granulate, a liquid, a stabilized liquid, or a stabilized protected enzyme. Liquid enzyme preparations may, for instance, be stabilized by adding stabilizers such as a sugar, a sugar alcohol or another polyol, and/or lactic acid or another organic acid according to established processes.

The optimum amounts of the enzymes and polypeptide(s) having catalase activity depend on several factors including, but not limited to, the mixture of component cellulolytic enzymes, the

cellulosic material, the concentration of cellulosic material, the pretreatment(s) of the cellulosic material, temperature, time, pH, and inclusion of fermenting organism (*e.g.*, yeast for Simultaneous Saccharification and Fermentation).

5 In a preferred aspect, an effective amount of cellulolytic or hemicellulolytic enzyme to the cellulosic material is about 0.5 to about 50 mg, preferably about 0.5 to about 40 mg, more preferably about 0.5 to about 25 mg, more preferably about 0.75 to about 20 mg, more preferably about 0.75 to about 15 mg, even more preferably about 0.5 to about 10 mg, and most preferably about 1.0 to about 10 mg per g of the cellulosic material.

10 In another preferred aspect, an effective amount of polypeptide(s) having catalase activity to the cellulosic material is about 0.001 to about 100.0 mg, preferably about 0.01 to about 50 mg, more preferably about 0.01 to about 40 mg, more preferably about 0.01 to about 30 mg, more preferably about 0.01 to about 20 mg, more preferably about 0.01 to about 10 mg, more preferably about 0.025 to about 8 mg, more preferably about 0.05 to about 6 mg, more preferably about 0.075 to about 5 mg, more preferably about 0.1 to about 4 mg, even more preferably about 0.15 to about 3 mg, and most preferably about 0.25 to about 1.0 mg per g of the cellulosic material.

15 In another preferred aspect, an effective amount of polypeptide(s) having catalase activity to cellulolytic or hemicellulolytic enzyme is about 0.005 to about 1.0 g, preferably about 0.01 to about 1.0 g, more preferably about 0.15 to about 0.75 g, more preferably about 0.15 to about 0.5 g, more preferably about 0.1 to about 0.5 g, even more preferably about 0.1 to about 0.5 g, and most preferably about 0.05 to about 0.2 g per g of cellulolytic or hemicellulolytic enzyme.

20 In another aspect, an effective amount of a GH61 polypeptide having cellulolytic enhancing activity to cellulosic material is about 0.01 to about 50.0 mg, preferably about 0.01 to about 40 mg, more preferably about 0.01 to about 30 mg, more preferably about 0.01 to about 20 mg, more preferably about 0.01 to about 10 mg, more preferably about 0.01 to about 5 mg, more preferably about 0.025 to about 1.5 mg, more preferably about 0.05 to about 1.25 mg, more preferably about 0.075 to about 1.25 mg, more preferably about 0.1 to about 1.25 mg, even more preferably about 0.15 to about 1.25 mg, and most preferably about 0.25 to about 1.0 mg per g of cellulosic material.

25 In another aspect, an effective amount of a GH61 polypeptide having cellulolytic enhancing activity to cellulolytic enzyme protein is about 0.005 to about 1.0 g, preferably about 0.01 to about 1.0 g, more preferably about 0.15 to about 0.75 g, more preferably about 0.15 to about 0.5 g, more preferably about 0.1 to about 0.5 g, even more preferably about 0.1 to about 0.5 g, and most preferably about 0.05 to about 0.2 g per g of cellulolytic enzyme protein.

30 The polypeptides having cellulolytic enzyme activity or hemicellulolytic enzyme activity as well as other proteins/polypeptides useful in the degradation of the cellulosic material, *e.g.*, polypeptides having cellulolytic enhancing activity (collectively hereinafter "polypeptides having enzyme activity") can be derived or obtained from any suitable origin, including, bacterial, fungal,

yeast, plant, or mammalian origin. The term "obtained" also means herein that the enzyme may have been produced recombinantly in a host organism employing methods described herein, wherein the recombinantly produced enzyme is either native or foreign to the host organism or has a modified amino acid sequence, *e.g.*, having one or more (*e.g.*, several) amino acids that are deleted, inserted and/or substituted, *i.e.*, a recombinantly produced enzyme that is a mutant and/or a fragment of a native amino acid sequence or an enzyme produced by nucleic acid shuffling processes known in the art. Encompassed within the meaning of a native enzyme are natural variants and within the meaning of a foreign enzyme are variants obtained recombinantly, such as by site-directed mutagenesis or shuffling.

A polypeptide having enzyme activity may be a bacterial polypeptide. For example, the polypeptide may be a gram positive bacterial polypeptide such as a *Bacillus*, *Streptococcus*, *Streptomyces*, *Staphylococcus*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Clostridium*, *Geobacillus*, *Caldicellulosiruptor*, *Acidothermus*, *Thermobifidia*, or *Oceanobacillus* polypeptide having enzyme activity, or a Gram negative bacterial polypeptide such as an *E. coli*, *Pseudomonas*, *Salmonella*, *Campylobacter*, *Helicobacter*, *Flavobacterium*, *Fusobacterium*, *Ilyobacter*, *Neisseria*, or *Ureaplasma* polypeptide having enzyme activity.

In one aspect, the polypeptide is a *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus firmus*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus stearothermophilus*, *Bacillus subtilis*, or *Bacillus thuringiensis* polypeptide having enzyme activity.

In another aspect, the polypeptide is a *Streptococcus equisimilis*, *Streptococcus pyogenes*, *Streptococcus uberis*, or *Streptococcus equi* subsp. *Zooepidemicus* polypeptide having enzyme activity.

In another aspect, the polypeptide is a *Streptomyces achromogenes*, *Streptomyces avermitilis*, *Streptomyces coelicolor*, *Streptomyces griseus*, or *Streptomyces lividans* polypeptide having enzyme activity.

The polypeptide having enzyme activity may also be a fungal polypeptide, and more preferably a yeast polypeptide such as a *Candida*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, *Schizosaccharomyces*, or *Yarrowia* polypeptide having enzyme activity; or more preferably a filamentous fungal polypeptide such as an *Acremonium*, *Agaricus*, *Alternaria*, *Aspergillus*, *Aureobasidium*, *Botryosphaeria*, *Ceriporiopsis*, *Chaetomidium*, *Chrysosporium*, *Claviceps*, *Cochliobolus*, *Coprinopsis*, *Coptotermes*, *Corynascus*, *Cryphonectria*, *Cryptococcus*, *Diplodia*, *Exidia*, *Filibasidium*, *Fusarium*, *Gibberella*, *Holomastigotoides*, *Humicola*, *Irpex*, *Lentinula*, *Leptosphaeria*, *Magnaporthe*, *Melanocarpus*, *Meripilus*, *Mucor*, *Myceliophthora*, *Neocallimastix*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Phanerochaete*, *Piromyces*, *Poitrasia*, *Pseudoplectania*, *Pseudotrichonympha*, *Rhizomucor*, *Schizophyllum*, *Scytalidium*, *Talaromyces*, *Thermoascus*,

Thielavia, *Tolypocladium*, *Trichoderma*, *Trichophaea*, *Verticillium*, *Volvariella*, or *Xylaria* polypeptide having enzyme activity.

In one aspect, the polypeptide is a *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae*, *Saccharomyces diastaticus*, *Saccharomyces douglasii*, *Saccharomyces kluyveri*,
5 *Saccharomyces norbensis*, or *Saccharomyces oviformis* polypeptide having enzyme activity.

In one aspect, the polypeptide is an *Acremonium cellulolyticus*, *Aspergillus aculeatus*, *Aspergillus awamori*, *Aspergillus fumigatus*, *Aspergillus foetidus*, *Aspergillus japonicus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Chrysosporium keratinophilum*, *Chrysosporium lucknowense*, *Chrysosporium tropicum*, *Chrysosporium merdarium*, *Chrysosporium inops*,
10 *Chrysosporium pannicola*, *Chrysosporium queenslandicum*, *Chrysosporium zonatum*, *Fusarium bactridioides*, *Fusarium cerealis*, *Fusarium crookwellense*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium graminum*, *Fusarium heterosporum*, *Fusarium negundi*, *Fusarium oxysporum*, *Fusarium reticulatum*, *Fusarium roseum*, *Fusarium sambucinum*, *Fusarium sarcochroum*, *Fusarium sporotrichioides*, *Fusarium sulphureum*, *Fusarium torulosum*, *Fusarium trichothecioides*, *Fusarium venenatum*, *Humicola grisea*, *Humicola insolens*, *Humicola lanuginosa*,
15 *Irpex lacteus*, *Mucor miehei*, *Myceliophthora thermophila*, *Neurospora crassa*, *Penicillium funiculosum*, *Penicillium purpurogenum*, *Phanerochaete chrysosporium*, *Thielavia achromatica*, *Thielavia albomyces*, *Thielavia albopilosa*, *Thielavia australeinsis*, *Thielavia fimeti*, *Thielavia microspora*, *Thielavia ovispora*, *Thielavia peruviana*, *Thielavia spededonium*, *Thielavia setosa*,
20 *Thielavia subthermophila*, *Thielavia terrestris*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, *Trichoderma viride*, or *Trichophaea saccata* polypeptide having enzyme activity.

Chemically modified or protein engineered mutants of polypeptides having enzyme activity may also be used.

25 One or more (e.g., several) components of the enzyme composition may be a recombinant component, i.e., produced by cloning of a DNA sequence encoding the single component and subsequent cell transformed with the DNA sequence and expressed in a host (see, for example, WO 91/17243 and WO 91/17244). The host is preferably a heterologous host (enzyme is foreign to host), but the host may under certain conditions also be a homologous host (enzyme is native to host). Monocomponent cellulolytic proteins may also be prepared by purifying such a protein from
30 a fermentation broth.

In one aspect, the one or more (e.g., several) cellulolytic enzymes comprise a commercial cellulolytic enzyme preparation. Examples of commercial cellulolytic enzyme preparations suitable for use in the present invention include, for example, CELLIC® CTec Ctec3 (Novozymes A/S),
35 CELLIC® CTec CTec2 (Novozymes A/S), CELLIC® CTec (Novozymes A/S), CELLUCLAST™ (Novozymes A/S), NOVOZYME™ 188 (Novozymes A/S), CELLUZYME™ (Novozymes A/S),

CEREFLO™ (Novozymes A/S), and ULTRAFLO™ (Novozymes A/S), ACCELERASE™ (Genencor Int.), LAMINEX™ (Genencor Int.), SPEZYME™ CP (Genencor Int.), FILTRASE® NL (DSM); METHAPLUS® S/L 100 (DSM), ROHAMENT™ 7069 W (Röhm GmbH), FIBREZYME® LDI (Dyadic International, Inc.), FIBREZYME® LBR (Dyadic International, Inc.), or VISCOSTAR® 150L (Dyadic International, Inc.). The cellulase enzymes are added in amounts effective from about 0.001 to about 5.0 wt % of solids, e.g., about 0.025 to about 4.0 wt % of solids or about 0.005 to about 2.0 wt % of solids.

Examples of bacterial endoglucanases that can be used in the method of the present invention, include, but are not limited to, an *Acidothermus cellulolyticus* endoglucanase (WO 91/05039; WO 93/15186; U.S. Patent No. 5,275,944; WO 96/02551; U.S. Patent No. 5,536,655, WO 00/70031, WO 05/093050); *Thermobifida fusca* endoglucanase III (WO 05/093050); and *Thermobifida fusca* endoglucanase V (WO 05/093050).

Examples of fungal endoglucanases that can be used in the present invention, include, but are not limited to, a *Trichoderma reesei* endoglucanase I (Penttila *et al.*, 1986, *Gene* 45: 253-263; *Trichoderma reesei* Cel7B endoglucanase I (GENBANK™ accession no. M15665); *Trichoderma reesei* endoglucanase II (Saloheimo, *et al.*, 1988, *Gene* 63:11-22; *Trichoderma reesei* Cel5A endoglucanase II (GENBANK™ accession no. M19373); *Trichoderma reesei* endoglucanase III (Okada *et al.*, 1988, *Appl. Environ. Microbiol.* 64: 555-563; GENBANK™ accession no. AB003694); *Trichoderma reesei* endoglucanase V (Saloheimo *et al.*, 1994, *Molecular Microbiology* 13: 219-228; GENBANK™ accession no. Z33381); *Aspergillus aculeatus* endoglucanase (Ooi *et al.*, 1990, *Nucleic Acids Research* 18: 5884); *Aspergillus kawachii* endoglucanase (Sakamoto *et al.*, 1995, *Current Genetics* 27: 435-439); *Erwinia carotovora* endoglucanase (Saarilahti *et al.*, 1990, *Gene* 90: 9-14); *Fusarium oxysporum* endoglucanase (GENBANK™ accession no. L29381); *Humicola grisea* var. *thermoidea* endoglucanase (GENBANK™ accession no. AB003107); *Melanocarpus albomyces* endoglucanase (GENBANK™ accession no. MAL515703); *Neurospora crassa* endoglucanase (GENBANK™ accession no. XM_324477); *Humicola insolens* endoglucanase V; *Myceliophthora thermophila* CBS 117.65 endoglucanase; basidiomycete CBS 495.95 endoglucanase; basidiomycete CBS 494.95 endoglucanase; *Thielavia terrestris* NRRL 8126 CEL6B endoglucanase; *Thielavia terrestris* NRRL 8126 CEL6C endoglucanase; *Thielavia terrestris* NRRL 8126 CEL7C endoglucanase; *Thielavia terrestris* NRRL 8126 CEL7E endoglucanase; *Thielavia terrestris* NRRL 8126 CEL7F endoglucanase; *Cladorrhinum foecundissimum* ATCC 62373 CEL7A endoglucanase; and *Trichoderma reesei* strain No. VTT-D-80133 endoglucanase (GENBANK™ accession no. M15665).

Examples of cellobiohydrolases useful in the present invention include, but are not limited to, *Trichoderma reesei* cellobiohydrolase I; *Trichoderma reesei* cellobiohydrolase II; *Humicola*

insolens cellobiohydrolase I; *Myceliophthora thermophila* cellobiohydrolase II; *Thielavia terrestris* cellobiohydrolase II (CEL6A); *Chaetomium thermophilum* cellobiohydrolase I; and *Chaetomium thermophilum* cellobiohydrolase II.

Examples of beta-glucosidases useful in the present invention include, but are not limited to, *Aspergillus oryzae* beta-glucosidase; *Aspergillus fumigatus* beta-glucosidase; *Penicillium brasilianum* IBT 20888 beta-glucosidase; *Aspergillus niger* beta-glucosidase; and *Aspergillus aculeatus* beta-glucosidase.

The *Aspergillus oryzae* beta-glucosidase can be obtained according to WO 2002/095014. The *Aspergillus fumigatus* beta-glucosidase can be obtained according to WO 2005/047499. The *Penicillium brasilianum* beta-glucosidase can be obtained according to WO 2007/019442. The *Aspergillus niger* beta-glucosidase can be obtained according to Dan *et al.*, 2000, *J. Biol. Chem.* 275: 4973-4980. The *Aspergillus aculeatus* beta-glucosidase can be obtained according to Kawaguchi *et al.*, 1996, *Gene* 173: 287-288.

The beta-glucosidase may be a fusion protein. In one aspect, the beta-glucosidase is the *Aspergillus oryzae* beta-glucosidase variant BG fusion protein or the *Aspergillus oryzae* beta-glucosidase fusion protein obtained according to WO 2008/057637.

Other useful endoglucanases, cellobiohydrolases, and beta-glucosidases are disclosed in numerous Glycosyl Hydrolase families using the classification according to Henrissat B., 1991, A classification of glycosyl hydrolases based on amino-acid sequence similarities, *Biochem. J.* 280: 309-316, and Henrissat B., and Bairoch A., 1996, Updating the sequence-based classification of glycosyl hydrolases, *Biochem. J.* 316: 695-696.

Other cellulolytic enzymes that may be used in the present invention are described in EP 495,257, EP 531,315, EP 531,372, WO 89/09259, WO 94/07998, WO 95/24471, WO 96/11262, WO 96/29397, WO 96/034108, WO 97/14804, WO 98/08940, WO 98/012307, WO 98/13465, WO 98/015619, WO 98/015633, WO 98/028411, WO 99/06574, WO 99/10481, WO 99/025846, WO 99/025847, WO 99/031255, WO 2000/009707, WO 2002/050245, WO 2002/0076792, WO 2002/101078, WO 2003/027306, WO 2003/052054, WO 2003/052055, WO 2003/052056, WO 2003/052057, WO 2003/052118, WO 2004/016760, WO 2004/043980, WO 2004/048592, WO 2005/001065, WO 2005/028636, WO 2005/093050, WO 2005/093073, WO 2006/074005, WO 2006/117432, WO 2007/071818, WO 2007/071820, WO 2008/008070, WO 2008/008793, U.S. Patent No. 4,435,307, U.S. Patent No. 5,457,046, U.S. Patent No. 5,648,263, U.S. Patent No. 5,686,593, U.S. Patent No. 5,691,178, U.S. Patent No. 5,763,254, and U.S. Patent No. 5,776,757.

In the methods of the present invention, any GH61 polypeptide having cellulolytic enhancing activity can be used.

In a first aspect, the GH61 polypeptide having cellulolytic enhancing activity comprises the following motifs:

[ILMV]-P-X(4,5)-G-X-Y-[ILMV]-X-R-X-[EQ]-X(4)-[HNQ] and [FW]-[TF]-K-[AIV],

wherein X is any amino acid, X(4,5) is any amino acid at 4 or 5 contiguous positions, and X(4) is any amino acid at 4 contiguous positions.

The polypeptide comprising the above-noted motifs may further comprise:

5 H-X(1,2)-G-P-X(3)-[YW]-[AILMV],
[EQ]-X-Y-X(2)-C-X-[EHQN]-[FILV]-X-[ILV], or
H-X(1,2)-G-P-X(3)-[YW]-[AILMV] and [EQ]-X-Y-X(2)-C-X-[EHQN]-[FILV]-X-[ILV],

wherein X is any amino acid, X(1,2) is any amino acid at 1 position or 2 contiguous positions, X(3) is any amino acid at 3 contiguous positions, and X(2) is any amino acid at 2
10 contiguous positions. In the above motifs, the accepted IUPAC single letter amino acid abbreviation is employed.

In a preferred aspect, the GH61 polypeptide having cellulolytic enhancing activity further comprises H-X(1,2)-G-P-X(3)-[YW]-[AILMV]. In another preferred aspect, the GH61 polypeptide having cellulolytic enhancing activity further comprises [EQ]-X-Y-X(2)-C-X-[EHQN]-[FILV]-X-[ILV].
15 In another preferred aspect, the GH61 polypeptide having cellulolytic enhancing activity further comprises H-X(1,2)-G-P-X(3)-[YW]-[AILMV] and [EQ]-X-Y-X(2)-C-X-[EHQN]-[FILV]-X-[ILV].

In a second aspect, the GH61 polypeptide having cellulolytic enhancing activity comprises the following motif:

[ILMV]-P-x(4,5)-G-x-Y-[ILMV]-x-R-x-[EQ]-x(3)-A-[HNQ],

20 wherein x is any amino acid, x(4,5) is any amino acid at 4 or 5 contiguous positions, and x(3) is any amino acid at 3 contiguous positions. In the above motif, the accepted IUPAC single letter amino acid abbreviation is employed.

Examples of GH61 polypeptides having cellulolytic enhancing activity useful in the methods of the present invention include, but are not limited to, polypeptides having cellulolytic enhancing activity from *Thielavia terrestris* (WO 2005/074647, WO/2008/148131 and WO 2011/035027); polypeptides having cellulolytic enhancing activity from *Thermoascus aurantiacus* (WO 2005/074656 and WO 2010/065830); polypeptides having cellulolytic enhancing activity from *Trichoderma reesei* (WO 2007/089290); and polypeptides having cellulolytic enhancing activity from *Myceliophthora thermophila* (WO 2009/085935; WO 2009/085859; WO 2009/085864; and
30 WO 2009/085868); polypeptides having cellulolytic enhancing activity from *Aspergillus fumigatus* (WO 2010/138754); and polypeptides having cellulolytic enhancing activity from *Penicillium pinophilum* (WO 2011/005867), *Thermoascus* sp. (WO 2011/039319), *Penicillium* sp. (WO 2011/041397), and *Thermoascus crustaceus* (WO 2011/041504).

In one aspect, the GH61 polypeptide having cellulolytic enhancing activity is used in the presence of a soluble activating divalent metal cation according to WO 2008/151043, e.g.,
35 manganese sulfate.

In one aspect, the GH61 polypeptide having cellulolytic enhancing activity is used in the presence of a dioxy compound, a bicyclic compound, a heterocyclic compound, a nitrogen-containing compound, a quinone compound, a sulfur-containing compound, or a liquor obtained from a pretreated cellulosic material such as pretreated corn stover (PCS).

5 The dioxy compound may include any suitable compound containing two or more oxygen atoms. In some aspects, the dioxy compounds contain a substituted aryl moiety as described herein. The dioxy compounds may comprise one or more (*e.g.*, several) hydroxyl and/or hydroxyl derivatives, but also include substituted aryl moieties lacking hydroxyl and hydroxyl derivatives. Non-limiting examples of dioxy compounds include pyrocatechol or catechol; caffeic acid; 3,4-
10 dihydroxybenzoic acid; 4-*tert*-butyl-5-methoxy-1,2-benzenediol; pyrogallol; gallic acid; methyl-3,4,5-trihydroxybenzoate; 2,3,4-trihydroxybenzophenone; 2,6-dimethoxyphenol; sinapinic acid; 3,5-dihydroxybenzoic acid; 4-chloro-1,2-benzenediol; 4-nitro-1,2-benzenediol; tannic acid; ethyl gallate; methyl glycolate; dihydroxyfumaric acid; 2-butyne-1,4-diol; (croconic acid; 1,3-propanediol; tartaric
15 acid; 2,4-pentanediol; 3-ethoxy-1,2-propanediol; 2,4,4'-trihydroxybenzophenone; *cis*-2-butene-1,4-diol; 3,4-dihydroxy-3-cyclobutene-1,2-dione; dihydroxyacetone; acrolein acetal; methyl-4-hydroxybenzoate; 4-hydroxybenzoic acid; and methyl-3,5-dimethoxy-4-hydroxybenzoate; or a salt or solvate thereof.

 The bicyclic compound may include any suitable substituted fused ring system as described herein. The compounds may comprise one or more (*e.g.*, several) additional rings, and are not
20 limited to a specific number of rings unless otherwise stated. In one aspect, the bicyclic compound is a flavonoid. In another aspect, the bicyclic compound is an optionally substituted isoflavonoid. In another aspect, the bicyclic compound is an optionally substituted flavylum ion, such as an optionally substituted anthocyanidin or optionally substituted anthocyanin, or derivative thereof. Non-limiting examples of bicyclic compounds include epicatechin; quercetin; myricetin; taxifolin;
25 kaempferol; morin; acacetin; naringenin; isorhamnetin; apigenin; cyanidin; cyanin; kuromanin; keracyanin; or a salt or solvate thereof.

 The heterocyclic compound may be any suitable compound, such as an optionally substituted aromatic or non-aromatic ring comprising a heteroatom, as described herein. In one aspect, the heterocyclic is a compound comprising an optionally substituted heterocycloalkyl
30 moiety or an optionally substituted heteroaryl moiety. In another aspect, the optionally substituted heterocycloalkyl moiety or optionally substituted heteroaryl moiety is an optionally substituted 5-membered heterocycloalkyl or an optionally substituted 5-membered heteroaryl moiety. In another aspect, the optionally substituted heterocycloalkyl or optionally substituted heteroaryl moiety is an optionally substituted moiety selected from pyrazolyl, furanyl, imidazolyl, isoxazolyl, oxadiazolyl,
35 oxazolyl, pyrrolyl, pyridyl, pyrimidyl, pyridazinyl, thiazolyl, triazolyl, thienyl, dihydrothieno-pyrazolyl, thianaphthenyl, carbazolyl, benzimidazolyl, benzothienyl, benzofuranyl, indolyl, quinoliny,

benzotriazolyl, benzothiazolyl, benzooxazolyl, benzimidazolyl, isoquinolinyl, isoindolyl, acridinyl, benzoisazolyl, dimethylhydantoin, pyrazinyl, tetrahydrofuranyl, pyrrolinyl, pyrrolidinyl, morpholinyl, indolyl, diazepinyl, azepinyl, thiepinyl, piperidinyl, and oxepinyl. In another aspect, the optionally substituted heterocycloalkyl moiety or optionally substituted heteroaryl moiety is an optionally substituted furanyl. Non-limiting examples of heterocyclic compounds include (1,2-dihydroxyethyl)-3,4-dihydroxyfuran-2(5H)-one; 4-hydroxy-5-methyl-3-furanone; 5-hydroxy-2(5H)-furanone; [1,2-dihydroxyethyl]furan-2,3,4(5H)-trione; α -hydroxy- γ -butyrolactone; ribonic γ -lactone; aldohexuronic acid γ -lactone; gluconic acid δ -lactone; 4-hydroxycoumarin; dihydrobenzofuran; 5-(hydroxymethyl)furfural; furoin; 2(5H)-furanone; 5,6-dihydro-2H-pyran-2-one; and 5,6-dihydro-4-hydroxy-6-methyl-2H-pyran-2-one; or a salt or solvate thereof.

The nitrogen-containing compound may be any suitable compound with one or more nitrogen atoms. In one aspect, the nitrogen-containing compound comprises an amine, imine, hydroxylamine, or nitroxide moiety. Non-limiting examples of nitrogen-containing compounds include acetone oxime; violuric acid; pyridine-2-aldoxime; 2-aminophenol; 1,2-benzenediamine; 2,2,6,6-tetramethyl-1-piperidinyloxy; 5,6,7,8-tetrahydrobiopterin; 6,7-dimethyl-5,6,7,8-tetrahydropterine; and maleamic acid; or a salt or solvate thereof.

The quinone compound may be any suitable compound comprising a quinone moiety as described herein. Non-limiting examples of quinone compounds include 1,4-benzoquinone; 1,4-naphthoquinone; 2-hydroxy-1,4-naphthoquinone; 2,3-dimethoxy-5-methyl-1,4-benzoquinone or coenzyme Q₀; 2,3,5,6-tetramethyl-1,4-benzoquinone or duroquinone; 1,4-dihydroxyanthraquinone; 3-hydroxy-1-methyl-5,6-indolinedione or adrenochrome; 4-tert-butyl-5-methoxy-1,2-benzoquinone; pyrroloquinoline quinone; or a salt or solvate thereof.

The sulfur-containing compound may be any suitable compound comprising one or more sulfur atoms. In one aspect, the sulfur-containing comprises a moiety selected from thionyl, thioether, sulfinyl, sulfonyl, sulfamide, sulfonamide, sulfonic acid, and sulfonic ester. Non-limiting examples of sulfur-containing compounds include ethanethiol; 2-propanethiol; 2-propene-1-thiol; 2-mercaptoethanesulfonic acid; benzenethiol; benzene-1,2-dithiol; cysteine; methionine; glutathione; cystine; or a salt or solvate thereof.

In one aspect, an effective amount of such a compound described above to cellulosic material as a molar ratio to glucosyl units of cellulose is about 10^{-6} to about 10, *e.g.*, about 10^{-6} to about 7.5, about 10^{-6} to about 5, about 10^{-6} to about 2.5, about 10^{-6} to about 1, about 10^{-5} to about 1, about 10^{-5} to about 10^{-1} , about 10^{-4} to about 10^{-1} , about 10^{-3} to about 10^{-1} , and about 10^{-3} to about 10^{-2} . In another aspect, an effective amount of such a compound described above is about 0.1 μ M to about 1 M, *e.g.*, about 0.5 μ M to about 0.75 M, about 0.75 μ M to about 0.5 M, about 1 μ M to about 0.25 M, about 1 μ M to about 0.1 M, about 5 μ M to about 50 mM, about 10 μ M to about 25 mM, about 50 μ M to about 25 mM, about 10 μ M to about 10 mM, about 5 μ M to about 5 mM,

and about 0.1 mM to about 1 mM.

The term "liquor" means the solution phase, either aqueous, organic, or a combination thereof, arising from treatment of a lignocellulose and/or hemicellulose material in a slurry, or monosaccharides thereof, *e.g.*, xylose, arabinose, mannose, *etc.*, under conditions as described herein, and the soluble contents thereof. A liquor for cellulolytic enhancement of a GH61 polypeptide can be produced by treating a lignocellulose or hemicellulose material (or feedstock) by applying heat and/or pressure, optionally in the presence of a catalyst, *e.g.*, acid, optionally in the presence of an organic solvent, and optionally in combination with physical disruption of the material, and then separating the solution from the residual solids. Such conditions determine the degree of cellulolytic enhancement obtainable through the combination of liquor and a GH61 polypeptide during hydrolysis of a cellulosic substrate by a cellulase preparation. The liquor can be separated from the treated material using methods standard in the art, such as filtration, sedimentation, or centrifugation.

In one aspect, an effective amount of the liquor to cellulose is about 10^{-6} to about 10 g per g of cellulose, *e.g.*, about 10^{-6} to about 7.5 g, about 10^{-6} to about 5, about 10^{-6} to about 2.5 g, about 10^{-6} to about 1 g, about 10^{-5} to about 1 g, about 10^{-5} to about 10^{-1} g, about 10^{-4} to about 10^{-1} g, about 10^{-3} to about 10^{-1} g, and about 10^{-3} to about 10^{-2} g per g of cellulose.

In one embodiment, the one or more (*e.g.*, several) hemicellulolytic enzymes comprise a commercial hemicellulolytic enzyme preparation. Examples of commercial hemicellulolytic enzyme preparations suitable for use in the present invention include, for example, SHEARZYME™ (Novozymes A/S), CELLIC® HTec (Novozymes A/S), CELLIC® HTec2 (Novozymes A/S), VISCOZYME® (Novozymes A/S), ULTRAFLO® (Novozymes A/S), PULPZYME® HC (Novozymes A/S), MULTIFECT® Xylanase (Genencor), ACCELLERASE® XY (Genencor), ACCELLERASE® XC (Genencor), ECOPULP® TX-200A (AB Enzymes), HSP 6000 Xylanase (DSM), DEPOL™ 333P (Biocatalysts Limit, Wales, UK), DEPOL™ 740L (Biocatalysts Limit, Wales, UK), and DEPOL™ 762P (Biocatalysts Limit, Wales, UK).

Examples of xylanases useful in the methods of the present invention include, but are not limited to, *Aspergillus aculeatus* xylanase (GeneSeqP: AAR63790; WO 94/21785); *Aspergillus fumigatus* xylanases (WO 2006/078256); *Penicillium pinophilum* (WO 2011/041405); *Penicillium* sp. (WO 2010/126772); *Thielavia terrestris* NRRL 8126 (WO 2009/079210); and *Trichophaea saccata* GH10 (WO 2011/057083).

Examples of beta-xylosidases useful in the methods of the present invention include, but are not limited to, *Trichoderma reesei* beta-xylosidase (UniProtKB/TrEMBL accession number Q92458); *Talaromyces emersonii* (SwissProt accession number Q8X212); and *Neurospora crassa* (SwissProt accession number Q7SOW4).

Examples of acetylxylan esterases useful in the methods of the present invention include,

but are not limited to, acetylxyylan esterases from *Aspergillus aculeatus* (WO 2010/108918); *Chaetomium globosum* (Uniprot accession number Q2GWX4); *Chaetomium gracile* (GeneSeqP accession number AAB82124); *Humicola insolens* DSM 1800 (WO 2009/073709); *Hypocrea jecorina* (WO 2005/001036); *Myceliophthora thermophila* (WO 2010/014880); *Neurospora crassa* (UniProt accession number q7s259); *Phaeosphaeria nodorum* (Uniprot accession number Q0UHH1); and *Thielavia terrestris* NRRL 8126 (WO 2009/042846).

Examples of ferulic acid esterases useful in the methods of the present invention include, but are not limited to, *Humicola insolens* DSM 1800 feruloyl esterase (WO 2009/076122), *Neurospora crassa* feruloyl esterase (UniProt accession number Q9HGR3), and *Neosartorya fischeri* feruloyl esterase (UniProt Accession number A1D9T4).

Examples of arabinofuranosidases useful in the methods of the present invention include, but are not limited to, arabinofuranosidases from *Aspergillus niger* (GeneSeqP accession number AAR94170); *Humicola insolens* DSM 1800 (WO 2006/114094 and WO 2009/073383); and *M. giganteus* (WO 2006/114094).

Examples of alpha-glucuronidases useful in the methods of the present invention include, but are not limited to, alpha-glucuronidases from *Aspergillus clavatus* (UniProt accession number alcc12); *Aspergillus fumigatus* (SwissProt accession number Q4WW45); *Aspergillus niger* (Uniprot accession number Q96WX9); *Aspergillus terreus* (SwissProt accession number Q0CJP9); *Humicola insolens* (WO 2010/014706); *Penicillium aurantiogriseum* (WO 2009/068565); *Talaromyces emersonii* (UniProt accession number Q8X211); and *Trichoderma reesei* (Uniprot accession number Q99024).

The polypeptides having enzyme activity used in the methods of the present invention may be produced by fermentation of the above-noted microbial strains on a nutrient medium containing suitable carbon and nitrogen sources and inorganic salts, using procedures known in the art (see, e.g., Bennett, J.W. and LaSure, L. (eds.), *More Gene Manipulations in Fungi*, Academic Press, CA, 1991). Suitable media are available from commercial suppliers or may be prepared according to published compositions (e.g., in catalogues of the American Type Culture Collection). Temperature ranges and other conditions suitable for growth and enzyme production are known in the art (see, e.g., Bailey, J.E., and Ollis, D.F., *Biochemical Engineering Fundamentals*, McGraw-Hill Book Company, NY, 1986).

The fermentation can be any method of cultivation of a cell resulting in the expression or isolation of an enzyme or protein. Fermentation may, therefore, be understood as comprising shake flask cultivation, or small- or large-scale fermentation (including continuous, batch, fed-batch, or solid state fermentations) in laboratory or industrial fermentors performed in a suitable medium and under conditions allowing the enzyme to be expressed or isolated. The resulting enzymes produced by the methods described above may be recovered from the fermentation

medium and purified by conventional procedures.

The compositions may be a fermentation broth formulation or a cell composition, as described herein. In some embodiments, the composition is a cell-killed whole broth containing organic acid(s), killed cells and/or cell debris, and culture medium.

5 In one aspect, the present invention relates to a whole broth formulation or cell culture composition comprising one or more (e.g., several) enzymes having cellulolytic and/or hemicellulolytic activity and a polypeptide having catalase activity.

The term "fermentation broth" as used herein refers to a preparation produced by cellular fermentation that undergoes no or minimal recovery and/or purification. For example, fermentation
10 broths are produced when microbial cultures are grown to saturation, incubated under carbon-limiting conditions to allow protein synthesis (e.g., expression of enzymes by host cells) and secretion into cell culture medium. The fermentation broth can contain unfractionated or fractionated contents of the fermentation materials derived at the end of the fermentation. Typically, the fermentation broth is unfractionated and comprises the spent culture medium and cell debris
15 present after the microbial cells (e.g., filamentous fungal cells) are removed, e.g., by centrifugation. In some embodiments, the fermentation broth contains spent cell culture medium, extracellular enzymes, and viable and/or nonviable microbial cells.

In an embodiment, the fermentation broth formulation and cell compositions comprise a first organic acid component comprising at least one 1-5 carbon organic acid and/or a salt thereof and
20 a second organic acid component comprising at least one 6 or more carbon organic acid and/or a salt thereof. In a specific embodiment, the first organic acid component is acetic acid, formic acid, propionic acid, a salt thereof, or a mixture of two or more of the foregoing and the second organic acid component is benzoic acid, cyclohexanecarboxylic acid, 4-methylvaleric acid, phenylacetic acid, a salt thereof, or a mixture of two or more of the foregoing.

25 In one aspect, the composition contains an organic acid(s), and optionally further contains killed cells and/or cell debris. In one embodiment, the killed cells and/or cell debris are removed from a cell-killed whole broth to provide a composition that is free of these components.

The fermentation broth formulations or cell compositions may further comprise a preservative and/or anti-microbial (e.g., bacteriostatic) agent, including, but not limited to, sorbitol,
30 sodium chloride, potassium sorbate, and others known in the art.

The cell-killed whole broth or composition may contain the unfractionated contents of the fermentation materials derived at the end of the fermentation. Typically, the cell-killed whole broth or composition contains the spent culture medium and cell debris present after the microbial cells (e.g., filamentous fungal cells) are grown to saturation, incubated under carbon-limiting conditions
35 to allow protein synthesis (e.g., expression of cellulase and/or glucosidase enzyme(s)). In some embodiments, the cell-killed whole broth or composition contains the spent cell culture medium,

extracellular enzymes, and killed filamentous fungal cells. In some embodiments, the microbial cells present in the cell-killed whole broth or composition can be permeabilized and/or lysed using methods known in the art.

A whole broth or cell composition as described herein is typically a liquid, but may contain insoluble components, such as killed cells, cell debris, culture media components, and/or insoluble enzyme(s). In some embodiments, insoluble components may be removed to provide a clarified liquid composition.

The whole broth formulations and cell compositions may be produced by a method described in WO 90/15861 or WO 2010/096673.

In one aspect, the present invention relates to use of the enzyme composition of the present invention in degrading or converting a cellulosic material.

Fermentation. The fermentable sugars obtained from the hydrolyzed cellulosic material can be fermented by one or more (*e.g.*, several) fermenting microorganisms capable of fermenting the sugars directly or indirectly into a desired fermentation product. "Fermentation" or "fermentation process" refers to any fermentation process or any process comprising a fermentation step. Fermentation processes also include fermentation processes used in the consumable alcohol industry (*e.g.*, beer and wine), dairy industry (*e.g.*, fermented dairy products), leather industry, and tobacco industry. The fermentation conditions depend on the desired fermentation product and fermenting organism and can easily be determined by one skilled in the art.

In the fermentation step, sugars, released from the cellulosic material as a result of the pretreatment and enzymatic hydrolysis steps, are fermented to a product, *e.g.*, ethanol, by a fermenting organism, such as yeast. Hydrolysis (saccharification) and fermentation can be separate or simultaneous, as described herein.

Any suitable hydrolyzed cellulosic material can be used in the fermentation step in practicing the present invention. The material is generally selected based on the desired fermentation product, *i.e.*, the substance to be obtained from the fermentation, and the process employed, as is well known in the art.

The term "fermentation medium" is understood herein to refer to a medium before the fermenting microorganism(s) is(are) added, such as, a medium resulting from a saccharification process, as well as a medium used in a simultaneous saccharification and fermentation process (SSF).

"Fermenting microorganism" refers to any microorganism, including bacterial and fungal organisms, suitable for use in a desired fermentation process to produce a fermentation product. The fermenting organism can be hexose and/or pentose fermenting organisms, or a combination thereof. Both hexose and pentose fermenting organisms are well known in the art. Suitable fermenting microorganisms are able to ferment, *i.e.*, convert, sugars, such as glucose, xylose,

xylulose, arabinose, maltose, mannose, galactose, and/or oligosaccharides, directly or indirectly into the desired fermentation product. Examples of bacterial and fungal fermenting organisms producing ethanol are described by Lin *et al.*, 2006, *Appl. Microbiol. Biotechnol.* 69: 627-642.

Examples of fermenting microorganisms that can ferment hexose sugars include bacterial
5 and fungal organisms, such as yeast. Preferred yeast includes strains of *Candida*, *Kluyveromyces*, and *Saccharomyces*, e.g., *Candida sonorensis*, *Kluyveromyces marxianus*, and *Saccharomyces cerevisiae*.

Examples of fermenting organisms that can ferment pentose sugars in their native state include bacterial and fungal organisms, such as some yeast. Preferred xylose fermenting yeast
10 include strains of *Candida*, preferably *C. sheatae* or *C. sonorensis*; and strains of *Pichia*, preferably *P. stipitis*, such as *P. stipitis* CBS 5773. Preferred pentose fermenting yeast include strains of *Pachysolen*, preferably *P. tannophilus*. Organisms not capable of fermenting pentose sugars, such as xylose and arabinose, may be genetically modified to do so by methods known in the art.

Examples of bacteria that can efficiently ferment hexose and pentose to ethanol include, for
15 example, *Bacillus coagulans*, *Clostridium acetobutylicum*, *Clostridium thermocellum*, *Clostridium phytofermentans*, *Geobacillus* sp., *Thermoanaerobacter saccharolyticum*, and *Zymomonas mobilis* (Philippidis, 1996, *supra*).

Other fermenting organisms include strains of *Bacillus*, such as *Bacillus coagulans*; *Candida*, such as *C. sonorensis*, *C. methanosorbosa*, *C. diddensiae*, *C. parapsilosis*, *C. naedodendra*, *C. blankii*, *C. entomophila*, *C. brassicae*, *C. pseudotropicalis*, *C. boidinii*, *C. utilis*, and *C. scephatae*;
20 *Clostridium*, such as *C. acetobutylicum*, *C. thermocellum*, and *C. phytofermentans*; *E. coli*, especially *E. coli* strains that have been genetically modified to improve the yield of ethanol; *Geobacillus* sp.; *Hansenula*, such as *Hansenula anomala*; *Klebsiella*, such as *K. oxytoca*; *Kluyveromyces*, such as *K. marxianus*, *K. lactis*, *K. thermotolerans*, and *K. fragilis*;
25 *Schizosaccharomyces*, such as *S. pombe*; *Thermoanaerobacter*, such as *Thermoanaerobacter saccharolyticum*; and *Zymomonas*, such as *Zymomonas mobilis*.

In a preferred aspect, the yeast is a *Bretannomyces*. In a more preferred aspect, the yeast is *Bretannomyces clausenii*. In another preferred aspect, the yeast is a *Candida*. In another more preferred aspect, the yeast is *Candida sonorensis*. In another more preferred aspect, the yeast is
30 *Candida boidinii*. In another more preferred aspect, the yeast is *Candida blankii*. In another more preferred aspect, the yeast is *Candida brassicae*. In another more preferred aspect, the yeast is *Candida diddensii*. In another more preferred aspect, the yeast is *Candida entomophillia*. In another more preferred aspect, the yeast is *Candida pseudotropicalis*. In another more preferred aspect, the yeast is *Candida scephatae*. In another more preferred aspect, the yeast is *Candida utilis*. In
35 another preferred aspect, the yeast is a *Clavispora*. In another more preferred aspect, the yeast is *Clavispora lusitaniae*. In another more preferred aspect, the yeast is *Clavispora opuntiae*. In

another preferred aspect, the yeast is a *Kluyveromyces*. In another more preferred aspect, the yeast is *Kluyveromyces fragilis*. In another more preferred aspect, the yeast is *Kluyveromyces marxianus*. In another more preferred aspect, the yeast is *Kluyveromyces thermotolerans*. In another preferred aspect, the yeast is a *Pachysolen*. In another more preferred aspect, the yeast is

5 *Pachysolen tannophilus*. In another preferred aspect, the yeast is a *Pichia*. In another more preferred aspect, the yeast is a *Pichia stipitis*. In another preferred aspect, the yeast is a *Saccharomyces* spp. In another more preferred aspect, the yeast is *Saccharomyces cerevisiae*. In another more preferred aspect, the yeast is *Saccharomyces distaticus*. In another more preferred aspect, the yeast is *Saccharomyces uvarum*.

10 In a preferred aspect, the bacterium is a *Bacillus*. In a more preferred aspect, the bacterium is *Bacillus coagulans*. In another preferred aspect, the bacterium is a *Clostridium*. In another more preferred aspect, the bacterium is *Clostridium acetobutylicum*. In another more preferred aspect, the bacterium is *Clostridium phytofermentans*. In another more preferred aspect, the bacterium is *Clostridium thermocellum*. In another more preferred aspect, the bacterium is *Geobacillus* sp. In

15 another more preferred aspect, the bacterium is a *Thermoanaerobacter*. In another more preferred aspect, the bacterium is *Thermoanaerobacter saccharolyticum*. In another preferred aspect, the bacterium is a *Zymomonas*. In another more preferred aspect, the bacterium is *Zymomonas mobilis*.

Commercially available yeast suitable for ethanol production include, e.g., BIOFERM™ AFT and XR (NABC - North American Bioproducts Corporation, GA, USA), ETHANOL RED™ yeast

20 (Fermentis/Lesaffre, USA), FALI™ (Fleischmann's Yeast, USA), FERMIOL™ (DSM Specialties), GERT STRAND™ (Gert Strand AB, Sweden), and SUPERSTART™ and THERMOSACC™ fresh yeast (Ethanol Technology, WI, USA).

In a preferred aspect, the fermenting microorganism has been genetically modified to

25 provide the ability to ferment pentose sugars, such as xylose utilizing, arabinose utilizing, and xylose and arabinose co-utilizing microorganisms.

The cloning of heterologous genes into various fermenting microorganisms has led to the construction of organisms capable of converting hexoses and pentoses to ethanol (co-fermentation) (Chen and Ho, 1993, Cloning and improving the expression of *Pichia stipitis* xylose

30 reductase gene in *Saccharomyces cerevisiae*, *Appl. Biochem. Biotechnol.* 39-40: 135-147; Ho *et al.*, 1998, Genetically engineered *Saccharomyces* yeast capable of effectively cofermenting glucose and xylose, *Appl. Environ. Microbiol.* 64: 1852-1859; Kotter and Ciriacy, 1993, Xylose fermentation by *Saccharomyces cerevisiae*, *Appl. Microbiol. Biotechnol.* 38: 776-783; Walfridsson *et al.*, 1995, Xylose-metabolizing *Saccharomyces cerevisiae* strains overexpressing the TKL1 and

35 TAL1 genes encoding the pentose phosphate pathway enzymes transketolase and transaldolase,

Appl. Environ. Microbiol. 61: 4184-4190; Kuyper *et al.*, 2004, Minimal metabolic engineering of *Saccharomyces cerevisiae* for efficient anaerobic xylose fermentation: a proof of principle, *FEMS Yeast Research* 4: 655-664; Beall *et al.*, 1991, Parametric studies of ethanol production from xylose and other sugars by recombinant *Escherichia coli*, *Biotech. Bioeng.* 38: 296-303; Ingram *et al.*, 1998, Metabolic engineering of bacteria for ethanol production, *Biotechnol. Bioeng.* 58: 204-214; Zhang *et al.*, 1995, Metabolic engineering of a pentose metabolism pathway in ethanologenic *Zymomonas mobilis*, *Science* 267: 240-243; Deanda *et al.*, 1996, Development of an arabinose-fermenting *Zymomonas mobilis* strain by metabolic pathway engineering, *Appl. Environ. Microbiol.* 62: 4465-4470; WO 2003/062430, xylose isomerase).

In a preferred aspect, the genetically modified fermenting microorganism is *Candida sonorensis*. In another preferred aspect, the genetically modified fermenting microorganism is *Escherichia coli*. In another preferred aspect, the genetically modified fermenting microorganism is *Klebsiella oxytoca*. In another preferred aspect, the genetically modified fermenting microorganism is *Kluyveromyces marxianus*. In another preferred aspect, the genetically modified fermenting microorganism is *Saccharomyces cerevisiae*. In another preferred aspect, the genetically modified fermenting microorganism is *Zymomonas mobilis*.

It is well known in the art that the organisms described above can also be used to produce other substances, as described herein.

The fermenting microorganism is typically added to the degraded cellulosic material or hydrolysate and the fermentation is performed for about 8 to about 96 hours, *e.g.*, about 24 to about 60 hours. The temperature is typically between about 26°C to about 60°C, *e.g.*, about 32°C or 50°C, and about pH 3 to about pH 8, *e.g.*, pH 4-5, 6, or 7.

In one aspect, the yeast and/or another microorganism are applied to the degraded cellulosic material and the fermentation is performed for about 12 to about 96 hours, such as typically 24-60 hours. In another aspect, the temperature is preferably between about 20°C to about 60°C, *e.g.*, about 25°C to about 50°C, about 32°C to about 50°C, or about 32°C to about 50°C, and the pH is generally from about pH 3 to about pH 7, *e.g.*, about pH 4 to about pH 7. However, some fermenting organisms, *e.g.*, bacteria, have higher fermentation temperature optima. Yeast or another microorganism is preferably applied in amounts of approximately 10^5 to 10^{12} , preferably from approximately 10^7 to 10^{10} , especially approximately 2×10^8 viable cell count per ml of fermentation broth. Further guidance in respect of using yeast for fermentation can be found in, *e.g.*, "The Alcohol Textbook" (Editors K. Jacques, T.P. Lyons and D.R. Kelsall, Nottingham University Press, United Kingdom 1999), which is hereby incorporated by reference.

For ethanol production, following the fermentation the fermented slurry is distilled to extract the ethanol. The ethanol obtained according to the methods of the invention can be used as, *e.g.*, fuel ethanol, drinking ethanol, *i.e.*, potable neutral spirits, or industrial ethanol.

A fermentation stimulator can be used in combination with any of the processes described herein to further improve the fermentation process, and in particular, the performance of the fermenting microorganism, such as, rate enhancement and ethanol yield. A "fermentation stimulator" refers to stimulators for growth of the fermenting microorganisms, in particular, yeast.

5 Preferred fermentation stimulators for growth include vitamins and minerals. Examples of vitamins include multivitamins, biotin, pantothenate, nicotinic acid, meso-inositol, thiamine, pyridoxine, para-aminobenzoic acid, folic acid, riboflavin, and Vitamins A, B, C, D, and E. See, for example, Alfenore *et al.*, Improving ethanol production and viability of *Saccharomyces cerevisiae* by a vitamin feeding strategy during fed-batch process, Springer-Verlag (2002), which is hereby
10 incorporated by reference. Examples of minerals include minerals and mineral salts that can supply nutrients comprising P, K, Mg, S, Ca, Fe, Zn, Mn, and Cu.

Fermentation products: A fermentation product can be any substance derived from the fermentation. The fermentation product can be, without limitation, an alcohol (*e.g.*, arabinitol, n-butanol, isobutanol, ethanol, glycerol, methanol, ethylene glycol, 1,3-propanediol [propylene
15 glycol], butanediol, glycerin, sorbitol, and xylitol); an alkane (*e.g.*, pentane, hexane, heptane, octane, nonane, decane, undecane, and dodecane), a cycloalkane (*e.g.*, cyclopentane, cyclohexane, cycloheptane, and cyclooctane), an alkene (*e.g.* pentene, hexene, heptene, and octene); an amino acid (*e.g.*, aspartic acid, glutamic acid, glycine, lysine, serine, and threonine); a gas (*e.g.*, methane, hydrogen (H₂), carbon dioxide (CO₂), and carbon monoxide (CO)); isoprene; a
20 ketone (*e.g.*, acetone); an organic acid (*e.g.*, acetic acid, acetonetic acid, adipic acid, ascorbic acid, citric acid, 2,5-diketo-D-gluconic acid, formic acid, fumaric acid, glucaric acid, gluconic acid, glucuronic acid, glutaric acid, 3-hydroxypropionic acid, itaconic acid, lactic acid, malic acid, malonic acid, oxalic acid, oxaloacetic acid, propionic acid, succinic acid, and xylonic acid); and polyketide. The fermentation product can also be protein as a high value product.

25 In a preferred aspect, the fermentation product is an alcohol. It will be understood that the term "alcohol" encompasses a substance that contains one or more hydroxyl moieties. In a more preferred aspect, the alcohol is n-butanol. In another more preferred aspect, the alcohol is isobutanol. In another more preferred aspect, the alcohol is ethanol. In another more preferred aspect, the alcohol is methanol. In another more preferred aspect, the alcohol is arabinitol. In
30 another more preferred aspect, the alcohol is butanediol. In another more preferred aspect, the alcohol is ethylene glycol. In another more preferred aspect, the alcohol is glycerin. In another more preferred aspect, the alcohol is glycerol. In another more preferred aspect, the alcohol is 1,3-propanediol. In another more preferred aspect, the alcohol is sorbitol. In another more preferred aspect, the alcohol is xylitol. See, for example, Gong, C. S., Cao, N. J., Du, J., and Tsao, G. T.,
35 1999, Ethanol production from renewable resources, in *Advances in Biochemical Engineering/Biotechnology*, Scheper, T., ed., Springer-Verlag Berlin Heidelberg, Germany, 65:

207-241; Silveira, M. M., and Jonas, R., 2002, The biotechnological production of sorbitol, *Appl. Microbiol. Biotechnol.* 59: 400-408; Nigam, P., and Singh, D., 1995, Processes for fermentative production of xylitol – a sugar substitute, *Process Biochemistry* 30 (2): 117-124; Ezeji, T. C., Qureshi, N. and Blaschek, H. P., 2003, Production of acetone, butanol and ethanol by *Clostridium beijerinckii* BA101 and *in situ* recovery by gas stripping, *World Journal of Microbiology and Biotechnology* 19 (6): 595-603.

In another preferred aspect, the fermentation product is an alkane. The alkane can be an unbranched or a branched alkane. In another more preferred aspect, the alkane is pentane. In another more preferred aspect, the alkane is hexane. In another more preferred aspect, the alkane is heptane. In another more preferred aspect, the alkane is octane. In another more preferred aspect, the alkane is nonane. In another more preferred aspect, the alkane is decane. In another more preferred aspect, the alkane is undecane. In another more preferred aspect, the alkane is dodecane.

In another preferred aspect, the fermentation product is a cycloalkane. In another more preferred aspect, the cycloalkane is cyclopentane. In another more preferred aspect, the cycloalkane is cyclohexane. In another more preferred aspect, the cycloalkane is cycloheptane. In another more preferred aspect, the cycloalkane is cyclooctane.

In another preferred aspect, the fermentation product is an alkene. The alkene can be an unbranched or a branched alkene. In another more preferred aspect, the alkene is pentene. In another more preferred aspect, the alkene is hexene. In another more preferred aspect, the alkene is heptene. In another more preferred aspect, the alkene is octene.

In another preferred aspect, the fermentation product is an amino acid. In another more preferred aspect, the organic acid is aspartic acid. In another more preferred aspect, the amino acid is glutamic acid. In another more preferred aspect, the amino acid is glycine. In another more preferred aspect, the amino acid is lysine. In another more preferred aspect, the amino acid is serine. In another more preferred aspect, the amino acid is threonine. See, for example, Richard, A., and Margaritis, A., 2004, Empirical modeling of batch fermentation kinetics for poly(glutamic acid) production and other microbial biopolymers, *Biotechnology and Bioengineering* 87 (4): 501-515.

In another preferred aspect, the fermentation product is a gas. In another more preferred aspect, the gas is methane. In another more preferred aspect, the gas is H₂. In another more preferred aspect, the gas is CO₂. In another more preferred aspect, the gas is CO. See, for example, Kataoka, N., A. Miya, and K. Kiriya, 1997, Studies on hydrogen production by continuous culture system of hydrogen-producing anaerobic bacteria, *Water Science and Technology* 36 (6-7): 41-47; and Gunaseelan V.N. in *Biomass and Bioenergy*, Vol. 13 (1-2), pp. 83-114, 1997, Anaerobic digestion of biomass for methane production: A review.

In another preferred aspect, the fermentation product is isoprene.

In another preferred aspect, the fermentation product is a ketone. It will be understood that the term "ketone" encompasses a substance that contains one or more ketone moieties. In another more preferred aspect, the ketone is acetone. See, for example, Qureshi and Blaschek, 2003,
5 *supra*.

In another preferred aspect, the fermentation product is an organic acid. In another more preferred aspect, the organic acid is acetic acid. In another more preferred aspect, the organic acid is acetonetic acid. In another more preferred aspect, the organic acid is adipic acid. In another more preferred aspect, the organic acid is ascorbic acid. In another more preferred aspect, the organic
10 acid is citric acid. In another more preferred aspect, the organic acid is 2,5-diketo-D-gluconic acid. In another more preferred aspect, the organic acid is formic acid. In another more preferred aspect, the organic acid is fumaric acid. In another more preferred aspect, the organic acid is glucaric acid. In another more preferred aspect, the organic acid is gluconic acid. In another more preferred aspect, the organic acid is glucuronic acid. In another more preferred aspect, the organic acid is glutaric acid. In another preferred aspect, the organic acid is 3-hydroxypropionic acid. In another
15 more preferred aspect, the organic acid is itaconic acid. In another more preferred aspect, the organic acid is lactic acid. In another more preferred aspect, the organic acid is malic acid. In another more preferred aspect, the organic acid is malonic acid. In another more preferred aspect, the organic acid is oxalic acid. In another more preferred aspect, the organic acid is propionic acid. In another more preferred aspect, the organic acid is succinic acid. In another more preferred aspect, the organic acid is xylonic acid. See, for example, Chen, R., and Lee, Y. Y., 1997, Membrane-mediated extractive fermentation for lactic acid production from cellulosic biomass, *Appl. Biochem. Biotechnol.* 63-65: 435-448.

In another preferred aspect, the fermentation product is polyketide.

25 Recovery. The fermentation product(s) can be optionally recovered from the fermentation medium using any method known in the art including, but not limited to, chromatography, electrophoretic procedures, differential solubility, distillation, or extraction. For example, alcohol is separated from the fermented cellulosic material and purified by conventional methods of distillation. Ethanol with a purity of up to about 96 vol.% can be obtained, which can be used as, for
30 example, fuel ethanol, drinking ethanol, *i.e.*, potable neutral spirits, or industrial ethanol.

Polypeptides Having Catalase Activity

In the methods of the present invention, the polypeptide having catalase activity can be any polypeptide having catalase activity. The polypeptide having catalase activity may be present as an
35 enzyme in the enzyme composition and/or as one or more protein components added to the composition. In a preferred aspect, the polypeptide having catalase activity is foreign to one or

more components of the cellulolytic enzyme composition.

The polypeptide having catalase activity may be obtained from microorganisms of any genus. In one aspect, the polypeptide obtained from a given source is secreted extracellularly.

The polypeptide having catalase activity may be a bacterial polypeptide. For example, the
5 polypeptide may be a gram positive bacterial polypeptide such as a *Bacillus*, *Streptococcus*,
Streptomyces, *Staphylococcus*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Clostridium*,
Geobacillus, or *Oceanobacillus* polypeptide having catalase activity, or a Gram negative bacterial
polypeptide such as an *E. coli*, *Pseudomonas*, *Salmonella*, *Campylobacter*, *Helicobacter*,
Flavobacterium, *Fusobacterium*, *Ilyobacter*, *Neisseria*, or *Ureaplasma* polypeptide having catalase
10 activity.

In one aspect, the polypeptide having catalase activity is a *Bacillus alkalophilus*, *Bacillus*
amyloliquefaciens, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus*
firmus, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*,
Bacillus stearothermophilus, *Bacillus subtilis*, or *Bacillus thuringiensis* polypeptide having catalase
15 activity.

In another aspect, the polypeptide having catalase activity is a *Streptococcus equisimilis*,
Streptococcus pyogenes, *Streptococcus uberis*, or *Streptococcus equi* subsp. *Zooepidemicus*
polypeptide having catalase activity.

In another aspect, the polypeptide having catalase activity is a *Streptomyces*
20 *achromogenes*, *Streptomyces avermitilis*, *Streptomyces coelicolor*, *Streptomyces griseus*, or
Streptomyces lividans polypeptide having catalase activity.

The polypeptide having catalase activity may also be a fungal polypeptide, and more
preferably a yeast polypeptide such as a *Candida*, *Kluyveromyces*, *Pichia*, *Saccharomyces*,
Schizosaccharomyces, or *Yarrowia* polypeptide having catalase activity; or more preferably a
25 filamentous fungal polypeptide such as an *Acremonium*, *Agaricus*, *Alternaria*, *Aspergillus*,
Aureobasidium, *Botryosphaeria*, *Ceriporiopsis*, *Chaetomidium*, *Chrysosporium*, *Claviceps*,
Cochliobolus, *Coprinopsis*, *Coptotermes*, *Corynascus*, *Cryphonectria*, *Cryptococcus*, *Diplodia*,
Exidia, *Filibasidium*, *Fusarium*, *Gibberella*, *Holomastigotoides*, *Humicola*, *Irpex*, *Lentinula*,
Leptosphaeria, *Magnaporthe*, *Melanocarpus*, *Meripilus*, *Mucor*, *Myceliophthora*, *Neocallimastix*,
30 *Neurospora*, *Paecilomyces*, *Penicillium*, *Phanerochaete*, *Piromyces*, *Poitrasia*, *Pseudoplectania*,
Pseudotrichonympha, *Rhizomucor*, *Schizophyllum*, *Scytalidium*, *Talaromyces*, *Thermoascus*,
Thielavia, *Tolypocladium*, *Trichoderma*, *Trichophaea*, *Verticillium*, *Volvariella*, or *Xylaria*
polypeptide having catalase activity.

In another aspect, the polypeptide is a *Saccharomyces carlsbergensis*, *Saccharomyces*
35 *cerevisiae*, *Saccharomyces diastaticus*, *Saccharomyces douglasii*, *Saccharomyces kluyveri*,
Saccharomyces norbensis, or *Saccharomyces oviformis* polypeptide having catalase activity.

In another aspect, the polypeptide is an *Acremonium cellulolyticus*, *Aspergillus aculeatus*, *Aspergillus awamori*, *Aspergillus fumigatus*, *Aspergillus foetidus*, *Aspergillus japonicus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Chrysosporium keratinophilum*, *Chrysosporium lucknowense*, *Chrysosporium tropicum*, *Chrysosporium merdarium*, *Chrysosporium inops*,
5 *Chrysosporium pannicola*, *Chrysosporium queenslandicum*, *Chrysosporium zonatum*, *Fusarium bactridioides*, *Fusarium cerealis*, *Fusarium crookwellense*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium graminum*, *Fusarium heterosporum*, *Fusarium negundi*, *Fusarium oxysporum*, *Fusarium reticulatum*, *Fusarium roseum*, *Fusarium sambucinum*, *Fusarium sarcochroum*, *Fusarium sporotrichioides*, *Fusarium sulphureum*, *Fusarium torulosum*, *Fusarium*
10 *trichothecioides*, *Fusarium venenatum*, *Humicola grisea*, *Humicola insolens*, *Humicola lanuginosa*, *Irpex lacteus*, *Mucor miehei*, *Myceliophthora thermophila*, *Neurospora crassa*, *Penicillium emersonii*, *Penicillium funiculosum*, *Penicillium purpurogenum*, *Phanerochaete chrysosporium*, *Talaromyces stipitatus*, *Thermoascus aurantiacus*, *Thielavia achromatica*, *Thielavia albomyces*, *Thielavia albopilosa*, *Thielavia australeinsis*, *Thielavia fimeti*, *Thielavia microspora*, *Thielavia*
15 *ovispora*, *Thielavia peruviana*, *Thielavia spededonium*, *Thielavia setosa*, *Thielavia subthermophila*, *Thielavia terrestris*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, or *Trichoderma viride* polypeptide having catalase activity.

In a preferable embodiment, the polypeptide having catalase activity is a catalase from *Thermoascus*, *Talaromyces*, *Humicola*, or *Penicillium*. In a more preferable embodiment, the
20 polypeptide having catalase activity is a catalase from *Thermoascus aurantiacus*, *Talaromyces stipitatus*, *Humicola insolens*, or *Penicillium emersonii*.

Non-limiting examples of suitable catalases and coding sequence thereof are listed below.

SEQ ID NOs: 1 and 2: a polynucleotide and a polypeptide of a catalase from *Thermoascus aurantiacus*, as described in JP 2004261137A.

25 SEQ ID NOs: 3 and 4: a polynucleotide and a polypeptide of a catalase from *Talaromyces stipitatus*, which can be prepared as shown in Examples 9-13.

SEQ ID NOs: 5 and 6: a polynucleotide and a polypeptide of a catalase from *Humicola insolens*, which can be prepared as shown in Examples 14-20.

30 SEQ ID NOs: 7 and 8: a polynucleotide and a polypeptide of a catalase from *Penicillium emersonii*, which can be prepared as shown in Examples 21-27.

SEQ ID NOs: 9 and 10: a polynucleotide and a polypeptide of a catalase from *Thermus Brockianus*, as described in WO 2005/044994.

SEQ ID NOs: 11 and 12: a polynucleotide and a polypeptide of a catalase from *Saccharomyces pastorianus*, as described in WO 2007/105350.

35 SEQ ID NOs: 13 and 14: a polynucleotide and a polypeptide of a catalase from *Saccharomyces pastorianus* as described in WO 2007/105350.

SEQ ID NOs: 15 and 16: a polynucleotide and a polypeptide of a catalase from *Penicillium pinophilum*, as described in WO 2009/104622.

SEQ ID NOs: 17 and 18: a polynucleotide and a polypeptide of a catalase from *Humicola grisea*, as described in WO 2009/104622.

5 SEQ ID NOs: 19 and 20: a polynucleotide and a polypeptide of a catalase from *Thielavia terrestris*, as described in WO 2010/074972.

SEQ ID NOs: 21 and 22: a polynucleotide and a polypeptide of a catalase from *Bacillus thermoglucosidasius*, as described in JP 11243961A.

10 SEQ ID NOs: 23 and 24: a polynucleotide and a polypeptide of a catalase from *Aspergillus oryzae*, as described in JP 2002223772A.

SEQ ID NOs: 25 and 26: a polynucleotide and a polypeptide of a catalase from *Thermoascus aurantiacus*, as described in JP 2007143405A.

SEQ ID NOs: 27 and 28: a polynucleotide and a polypeptide of a catalase from *Bacillus thermoglucosidasius*, as described in US 6,022,721.

15 SEQ ID NOs: 29 and 30: a polynucleotide and a polypeptide of a catalase from *Bacillus thermoglucosidasius*, as described in US 6,022,721.

SEQ ID NOs: 31 and 32: a polynucleotide and a polypeptide of a catalase from *Alcaligenes aquamarinus*, as described in WO 98/00526.

20 SEQ ID NOs: 33 and 34: a polynucleotide and a polypeptide of a catalase from *Microscilla furvescens*, as described in WO 98/00526.

SEQ ID NOs: 35 and 36: a polynucleotide and a polypeptide of a catalase from *Aspergillus niger*, as described in US 5,360,901.

SEQ ID NO 37: a polypeptide of *Humicola grisea* thermotolerant catalase (GENESEQP: AXQ55105, disclosed in WO2009104622).

25 In an embodiment, the catalases used in the present invention have a sequence identity to the mature polypeptide of the mature polypeptide of SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6, the mature polypeptide of SEQ ID NO: 8, the mature polypeptide of SEQ ID NO: 10, the mature polypeptide of SEQ ID NO: 12, the mature polypeptide of SEQ ID NO: 14, the mature polypeptide of SEQ ID NO: 16, the mature polypeptide of SEQ ID NO: 18, the mature polypeptide of SEQ ID NO: 20, the mature polypeptide of SEQ ID NO: 22, the mature polypeptide of SEQ ID NO: 24, the mature polypeptide of SEQ ID NO: 26, the mature polypeptide of SEQ ID NO: 28, the mature polypeptide of SEQ ID NO: 30, the mature polypeptide of SEQ ID NO: 32, the mature polypeptide of SEQ ID NO: 34, the mature polypeptide of SEQ ID NO: 36, the mature polypeptide of SEQ ID NO: 37, of at least 60%, e.g., at least 65%, at
30 of SEQ ID NO: 18, the mature polypeptide of SEQ ID NO: 20, the mature polypeptide of SEQ ID NO: 22, the mature polypeptide of SEQ ID NO: 24, the mature polypeptide of SEQ ID NO: 26, the mature polypeptide of SEQ ID NO: 28, the mature polypeptide of SEQ ID NO: 30, the mature polypeptide of SEQ ID NO: 32, the mature polypeptide of SEQ ID NO: 34, the mature polypeptide of SEQ ID NO: 36, the mature polypeptide of SEQ ID NO: 37, of at least 60%, e.g., at least 65%, at
35 least 70%, at least 75%, at least 78%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at

least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%, which have catalase activity.

In one embodiment, the mature polypeptide is amino acids 1 to 746 of SEQ ID NO: 2. In another aspect, the mature polypeptide is amino acids 20 to 733 of SEQ ID NO: 4 based on the
5 SignalP program that predicts amino acids 1 to 19 of SEQ ID NO: 4 are a signal peptide. In another aspect, the mature polypeptide is amino acids 20 to 765 of SEQ ID NO: 6 based on the SignalP program that predicts amino acids 1 to 19 of SEQ ID NO: 6 are a signal peptide. In another aspect, the mature polypeptide is amino acids 20 to 741 of SEQ ID NO: 8 based on the SignalP program that predicts amino acids 1 to 19 of SEQ ID NO: 8 are a signal peptide. It is
10 known in the art that a host cell may produce a mixture of two or more different mature polypeptides (*i.e.*, with a different C-terminal and/or N-terminal amino acid) expressed by the same polynucleotide.

In another embodiment, the catalases used in the present invention are encoded by a polynucleotide that hybridizes under low stringency conditions, medium stringency conditions,
15 medium-high stringency conditions, high stringency conditions, or very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 1, the mature polypeptide coding sequence of SEQ ID NO: 3, the mature polypeptide coding sequence of SEQ ID NO: 5, or the mature polypeptide coding sequence of SEQ ID NO: 7, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii) (Sambrook *et al.*, 1989, *Molecular Cloning, A Laboratory Manual*,
20 2d edition, Cold Spring Harbor, New York).

For purposes of the present invention, hybridization indicates that the polynucleotide hybridizes to a labeled nucleic acid probe corresponding to (i) SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 7; (ii) the mature polypeptide coding sequence of SEQ ID NO: 1, the mature polypeptide coding sequence of SEQ ID NO: 3, the mature polypeptide coding sequence of
25 SEQ ID NO: 5, or the mature polypeptide coding sequence of SEQ ID NO: 7; (iii) the cDNA sequence thereof; (iv) the full-length complement thereof; or (v) a subsequence thereof; under very low to very high stringency conditions. Molecules to which the nucleic acid probe hybridizes under these conditions can be detected using, for example, X-ray film or any other detection means known in the art.

In an embodiment, the catalases used in the present invention are encoded by a polynucleotide having at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 78%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence
35 identity to the mature polypeptide coding sequence of SEQ ID NO: 1, the mature polypeptide

coding sequence of SEQ ID NO: 3, the mature polypeptide coding sequence of SEQ ID NO: 5, or the mature polypeptide coding sequence of SEQ ID NO: 7; or the cDNA sequence thereof.

In one embodiment, the mature polypeptide coding sequence is nucleotides 1 to 2351 of SEQ ID NO: 1 or the cDNA sequence thereof. In another embodiment, the mature polypeptide coding sequence is nucleotides 58 to 2418 of SEQ ID NO: 3 or the cDNA sequence thereof based on the SignalP program that predicts nucleotides 1 to 57 of SEQ ID NO: 3 encode a signal peptide. In another embodiment, the mature polypeptide coding sequence is nucleotides 58 to 3040 of SEQ ID NO: 5 or the cDNA sequence thereof based on the SignalP program that predicts nucleotides 1 to 57 of SEQ ID NO: 5 encode a signal peptide. In another embodiment, the mature polypeptide coding sequence is nucleotides 58 to 2476 of SEQ ID NO: 7 or the cDNA sequence thereof based on the SignalP program that predicts nucleotides 1 to 57 of SEQ ID NO: 7 encode a signal peptide.

In another embodiment, the catalases used in the present invention relates to variants of the mature polypeptide of SEQ ID NO: 2, variants of the mature polypeptide of SEQ ID NO: 4, variants of the mature polypeptide of SEQ ID NO: 6, or variants of the mature polypeptide of SEQ ID NO: 8, comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the mature polypeptide of SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6, or the mature polypeptide of SEQ ID NO: 8 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. The amino acid changes may be of a minor nature, that is conservative amino acid substitutions or insertions that do not significantly affect the folding and/or activity of the protein; small deletions, typically of 1-30 amino acids; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue; a small linker peptide of up to 20-25 residues; or a small extension that facilitates purification by changing net charge or another function, such as a poly-histidine tract, an antigenic epitope or a binding domain.

Examples of conservative substitutions are within the groups of basic amino acids (arginine, lysine and histidine), acidic amino acids (glutamic acid and aspartic acid), polar amino acids (glutamine and asparagine), hydrophobic amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tryptophan and tyrosine), and small amino acids (glycine, alanine, serine, threonine and methionine). Amino acid substitutions that do not generally alter specific activity are known in the art and are described, for example, by H. Neurath and R.L. Hill, 1979, *In, The Proteins*, Academic Press, New York. Common substitutions are Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, and Asp/Gly.

Alternatively, the amino acid changes are of such a nature that the physico-chemical properties of the polypeptides are altered. For example, amino acid changes may improve the thermal stability of the polypeptide, alter the substrate specificity, change the pH optimum, and the

like.

Essential amino acids in a polypeptide can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, 1989, *Science* 244: 1081-1085). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for catalase activity to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al.*, 1996, *J. Biol. Chem.* 271: 4699-4708. The active site of the enzyme or other biological interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction, or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos *et al.*, 1992, *Science* 255: 306-312; Smith *et al.*, 1992, *J. Mol. Biol.* 224: 899-904; Wlodaver *et al.*, 1992, *FEBS Lett.* 309: 59-64. The identity of essential amino acids can also be inferred from an alignment with a related polypeptide.

Single or multiple amino acid substitutions, deletions, and/or insertions can be made and tested using known methods of mutagenesis, recombination, and/or shuffling, followed by a relevant screening procedure, such as those disclosed by Reidhaar-Olson and Sauer, 1988, *Science* 241: 53-57; Bowie and Sauer, 1989, *Proc. Natl. Acad. Sci. USA* 86: 2152-2156; WO 95/17413; or WO 95/22625. Other methods that can be used include error-prone PCR, phage display (e.g., Lowman *et al.*, 1991, *Biochemistry* 30: 10832-10837; U.S. Patent No. 5,223,409; WO 92/06204), and region-directed mutagenesis (Derbyshire *et al.*, 1986, *Gene* 46: 145; Ner *et al.*, 1988, *DNA* 7: 127).

Mutagenesis/shuffling methods can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides expressed by host cells (Ness *et al.*, 1999, *Nature Biotechnology* 17: 893-896). Mutagenized DNA molecules that encode active polypeptides can be recovered from the host cells and rapidly sequenced using standard methods in the art. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide.

The polypeptide may be a hybrid polypeptide in which a region of one polypeptide is fused at the N-terminus or the C-terminus of a region of another polypeptide.

The polypeptide may be a fusion polypeptide or cleavable fusion polypeptide in which another polypeptide is fused at the N-terminus or the C-terminus of the polypeptide of the present invention. A fusion polypeptide is produced by fusing a polynucleotide encoding another polypeptide to a polynucleotide of the present invention. Techniques for producing fusion polypeptides are known in the art, and include ligating the coding sequences encoding the polypeptides so that they are in frame and that expression of the fusion polypeptide is under control of the same promoter(s) and terminator. Fusion polypeptides may also be constructed

using intein technology in which fusion polypeptides are created post-translationally (Cooper *et al.*, 1993, *EMBO J.* 12: 2575-2583; Dawson *et al.*, 1994, *Science* 266: 776-779).

A fusion polypeptide can further comprise a cleavage site between the two polypeptides. Upon secretion of the fusion protein, the site is cleaved releasing the two polypeptides. Examples of cleavage sites include, but are not limited to, the sites disclosed in Martin *et al.*, 2003, *J. Ind. Microbiol. Biotechnol.* 3: 568-576; Svetina *et al.*, 2000, *J. Biotechnol.* 76: 245-251; Rasmussen-Wilson *et al.*, 1997, *Appl. Environ. Microbiol.* 63: 3488-3493; Ward *et al.*, 1995, *Biotechnology* 13: 498-503; and Contreras *et al.*, 1991, *Biotechnology* 9: 378-381; Eaton *et al.*, 1986, *Biochemistry* 25: 505-512; Collins-Racie *et al.*, 1995, *Biotechnology* 13: 982-987; Carter *et al.*, 1989, *Proteins: Structure, Function, and Genetics* 6: 240-248; and Stevens, 2003, *Drug Discovery World* 4: 35-48.

Examples of commercial catalase preparations suitable for use in the present invention include, for example, Terminox Ultra 50L/200L (Novozymes A/S), Catzyme 25L (Novozymes A/S), GC118 (Danisco A/S), Oxygone T100/T400 (Danisco A/S), ASC Super 200L (Mitsubishi Chemicals, Japan), and Reyonet 200L (Nagase, Japan).

Nucleic Acid Constructs

An isolated polynucleotide encoding a polypeptide, *e.g.*, a cellulolytic enzyme, a polypeptide having catalase activity, a polypeptide having cellulolytic enhancing activity, etc., may be manipulated in a variety of ways to provide for expression of the polypeptide by constructing a nucleic acid construct comprising an isolated polynucleotide encoding the polypeptide operably linked to one or more (*e.g.*, several) control sequences that direct the expression of the coding sequence in a suitable host cell under conditions compatible with the control sequences. A polynucleotide may be manipulated in a variety of ways to provide for expression of the polypeptide. Manipulation of the *polynucleotide* prior to its insertion into a vector may be desirable or necessary depending on the expression vector. The techniques for modifying polynucleotide sequences utilizing recombinant DNA methods are well known in the art.

The control sequence may be a promoter, a polynucleotide that is recognized by a host cell for expression of a polynucleotide encoding a polypeptide. The promoter contains transcriptional control sequences that mediate the expression of the polypeptide. The promoter may be any polynucleotide that shows transcriptional activity in the host cell including mutant, truncated, and hybrid promoters, and may be obtained from genes encoding extracellular or intracellular polypeptides either homologous or heterologous to the host cell.

Examples of suitable promoters for directing transcription of the nucleic acid constructs of the present invention in a bacterial host cell are the promoters obtained from the *Bacillus amyloliquefaciens* alpha-amylase gene (*amyQ*), *Bacillus licheniformis* alpha-amylase gene (*amyL*), *Bacillus licheniformis* penicillinase gene (*penP*), *Bacillus stearothermophilus* maltogenic amylase

gene (*amyM*), *Bacillus subtilis* levansucrase gene (*sacB*), *Bacillus subtilis* *xylA* and *xylB* genes, *Bacillus thuringiensis* *cryIIIA* gene (Agaisse and Lereclus, 1994, *Molecular Microbiology* 13: 97-107), *E. coli* *lac* operon, *E. coli* *trc* promoter (Egon *et al.*, 1988, *Gene* 69: 301-315), *Streptomyces coelicolor* agarase gene (*dagA*), and prokaryotic beta-lactamase gene (Villa-Kamaroff *et al.*, 1978, *Proc. Natl. Acad. Sci. USA* 75: 3727-3731), as well as the *tac* promoter (DeBoer *et al.*, 1983, *Proc. Natl. Acad. Sci. USA* 80: 21-25). Further promoters are described in "Useful proteins from recombinant bacteria" in Gilbert *et al.*, 1980, *Scientific American* 242: 74-94; and in Sambrook *et al.*, 1989, *supra*. Examples of tandem promoters are disclosed in WO 99/43835.

Examples of suitable promoters for directing transcription of the nucleic acid constructs of the present invention in a filamentous fungal host cell are promoters obtained from the genes for *Aspergillus nidulans* acetamidase, *Aspergillus niger* neutral alpha-amylase, *Aspergillus niger* acid stable alpha-amylase, *Aspergillus niger* or *Aspergillus awamori* glucoamylase (*glaA*), *Aspergillus oryzae* TAKA amylase, *Aspergillus oryzae* alkaline protease, *Aspergillus oryzae* triose phosphate isomerase, *Fusarium oxysporum* trypsin-like protease (WO 96/00787), *Fusarium venenatum* amyloglucosidase (WO 00/56900), *Fusarium venenatum* Daria (WO 00/56900), *Fusarium venenatum* Quinn (WO 00/56900), *Rhizomucor miehei* lipase, *Rhizomucor miehei* aspartic proteinase, *Trichoderma reesei* beta-glucosidase, *Trichoderma reesei* cellobiohydrolase I, *Trichoderma reesei* cellobiohydrolase II, *Trichoderma reesei* endoglucanase I, *Trichoderma reesei* endoglucanase II, *Trichoderma reesei* endoglucanase III, *Trichoderma reesei* endoglucanase IV, *Trichoderma reesei* endoglucanase V, *Trichoderma reesei* xylanase I, *Trichoderma reesei* xylanase II, *Trichoderma reesei* beta-xylosidase, as well as the NA2-tpi promoter (a modified promoter from an *Aspergillus* neutral alpha-amylase gene in which the untranslated leader has been replaced by an untranslated leader from an *Aspergillus* triose phosphate isomerase gene; non-limiting examples include modified promoters from an *Aspergillus niger* neutral alpha-amylase gene in which the untranslated leader has been replaced by an untranslated leader from an *Aspergillus nidulans* or *Aspergillus oryzae* triose phosphate isomerase gene); and mutant, truncated, and hybrid promoters thereof.

In a yeast host, useful promoters are obtained from the genes for *Saccharomyces cerevisiae* enolase (ENO-1), *Saccharomyces cerevisiae* galactokinase (GAL1), *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (ADH1, ADH2/GAP), *Saccharomyces cerevisiae* triose phosphate isomerase (TPI), *Saccharomyces cerevisiae* metallothionein (CUP1), and *Saccharomyces cerevisiae* 3-phosphoglycerate kinase. Other useful promoters for yeast host cells are described by Romanos *et al.*, 1992, *Yeast* 8: 423-488.

The control sequence may also be a transcription terminator, which is recognized by a host cell to terminate transcription. The terminator is operably linked to the 3'-terminus of the

polynucleotide encoding the polypeptide. Any terminator that is functional in the host cell may be used in the present invention.

Preferred terminators for bacterial host cells are obtained from the genes for *Bacillus clausii* alkaline protease (*aprH*), *Bacillus licheniformis* alpha-amylase (*amyL*), and *Escherichia coli* ribosomal RNA (*rrnB*).

Preferred terminators for filamentous fungal host cells are obtained from the genes for *Aspergillus nidulans* anthranilate synthase, *Aspergillus niger* glucoamylase, *Aspergillus niger* alpha-glucosidase, *Aspergillus oryzae* TAKA amylase, and *Fusarium oxysporum* trypsin-like protease.

Preferred terminators for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* enolase, *Saccharomyces cerevisiae* cytochrome C (CYC1), and *Saccharomyces cerevisiae* glyceraldehyde-3-phosphate dehydrogenase. Other useful terminators for yeast host cells are described by Romanos *et al.*, 1992, *supra*.

The control sequence may also be an mRNA stabilizer region downstream of a promoter and upstream of the coding sequence of a gene which increases expression of the gene.

Examples of suitable mRNA stabilizer regions are obtained from a *Bacillus thuringiensis cryIIIA* gene (WO 94/25612) and a *Bacillus subtilis* SP82 gene (Hue *et al.*, 1995, *Journal of Bacteriology* 177: 3465-3471).

The control sequence may also be a leader, a nontranslated region of an mRNA that is important for translation by the host cell. The leader is operably linked to the 5'-terminus of the polynucleotide encoding the polypeptide. Any leader that is functional in the host cell may be used.

Preferred leaders for filamentous fungal host cells are obtained from the genes for *Aspergillus oryzae* TAKA amylase and *Aspergillus nidulans* triose phosphate isomerase.

Suitable leaders for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* enolase (ENO-1), *Saccharomyces cerevisiae* 3-phosphoglycerate kinase, *Saccharomyces cerevisiae* alpha-factor, and *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (ADH2/GAP).

The control sequence may also be a polyadenylation sequence, a sequence operably linked to the 3'-terminus of the polynucleotide and, when transcribed, is recognized by the host cell as a signal to add polyadenosine residues to transcribed mRNA. Any polyadenylation sequence that is functional in the host cell may be used.

Preferred polyadenylation sequences for filamentous fungal host cells are obtained from the genes for *Aspergillus nidulans* anthranilate synthase, *Aspergillus niger* glucoamylase, *Aspergillus niger* alpha-glucosidase, *Aspergillus oryzae* TAKA amylase, and *Fusarium oxysporum* trypsin-like protease.

Useful polyadenylation sequences for yeast host cells are described by Guo and Sherman,

1995, *Mol. Cellular Biol.* 15: 5983-5990.

The control sequence may also be a signal peptide coding region that encodes a signal peptide linked to the N-terminus of a polypeptide and directs the polypeptide into the cell's secretory pathway. The 5'-end of the coding sequence of the polynucleotide may inherently contain a signal peptide coding sequence naturally linked in translation reading frame with the segment of the coding sequence that encodes the polypeptide. Alternatively, the 5'-end of the coding sequence may contain a signal peptide coding sequence that is foreign to the coding sequence. A foreign signal peptide coding sequence may be required where the coding sequence does not naturally contain a signal peptide coding sequence. Alternatively, a foreign signal peptide coding sequence may simply replace the natural signal peptide coding sequence in order to enhance secretion of the polypeptide. However, any signal peptide coding sequence that directs the expressed polypeptide into the secretory pathway of a host cell may be used.

Effective signal peptide coding sequences for bacterial host cells are the signal peptide coding sequences obtained from the genes for *Bacillus* NCIB 11837 maltogenic amylase, *Bacillus licheniformis* subtilisin, *Bacillus licheniformis* beta-lactamase, *Bacillus stearothermophilus* alpha-amylase, *Bacillus stearothermophilus* neutral proteases (*nprT*, *nprS*, *nprM*), and *Bacillus subtilis* *prsA*. Further signal peptides are described by Simonen and Palva, 1993, *Microbiological Reviews* 57: 109-137.

Effective signal peptide coding sequences for filamentous fungal host cells are the signal peptide coding sequences obtained from the genes for *Aspergillus niger* neutral amylase, *Aspergillus niger* glucoamylase, *Aspergillus oryzae* TAKA amylase, *Humicola insolens* cellulase, *Humicola insolens* endoglucanase V, *Humicola lanuginosa* lipase, and *Rhizomucor miehei* aspartic proteinase.

Useful signal peptides for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* alpha-factor and *Saccharomyces cerevisiae* invertase. Other useful signal peptide coding sequences are described by Romanos *et al.*, 1992, *supra*.

The control sequence may also be a propeptide coding sequence that encodes a propeptide positioned at the N-terminus of a polypeptide. The resultant polypeptide is known as a proenzyme or propolypeptide (or a zymogen in some cases). A propolypeptide is generally inactive and can be converted to an active polypeptide by catalytic or autocatalytic cleavage of the propeptide from the propolypeptide. The propeptide coding sequence may be obtained from the genes for *Bacillus subtilis* alkaline protease (*aprE*), *Bacillus subtilis* neutral protease (*nprT*), *Myceliophthora thermophila* laccase (WO 95/33836), *Rhizomucor miehei* aspartic proteinase, and *Saccharomyces cerevisiae* alpha-factor.

Where both signal peptide and propeptide sequences are present, the propeptide sequence is positioned next to the N-terminus of a polypeptide and the signal peptide sequence is

positioned next to the N-terminus of the propeptide sequence.

It may also be desirable to add regulatory sequences that regulate expression of the polypeptide relative to the growth of the host cell. Examples of regulatory systems are those that cause expression of the gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Regulatory systems in prokaryotic systems include the *lac*, *tac*, and *trp* operator systems. In yeast, the ADH2 system or GAL1 system may be used. In filamentous fungi, the *Aspergillus niger* glucoamylase promoter, *Aspergillus oryzae* TAKA alpha-amylase promoter, and *Aspergillus oryzae* glucoamylase promoter may be used. Other examples of regulatory sequences are those that allow for gene amplification. In eukaryotic systems, these regulatory sequences include the dihydrofolate reductase gene that is amplified in the presence of methotrexate, and the metallothionein genes that are amplified with heavy metals. In these cases, the polynucleotide encoding the polypeptide would be operably linked with the regulatory sequence.

Expression Vectors

The various nucleotide and control sequences described above may be joined together to produce a recombinant expression vector that may include one or more (*e.g.*, several) convenient restriction sites to allow for insertion or substitution of a polynucleotide encoding a polypeptide, *e.g.*, a cellulolytic enzyme, a polypeptide having catalase activity, a polypeptide having cellulolytic enhancing activity, etc., at such sites. Alternatively, the polynucleotide may be expressed by inserting the polynucleotide or a nucleic acid construct comprising the polynucleotide into an appropriate vector for expression. In creating the expression vector, the coding sequence is located in the vector so that the coding sequence is operably linked with the appropriate control sequences for expression.

The recombinant expression vector may be any vector (*e.g.*, a plasmid or virus) that can be conveniently subjected to recombinant DNA procedures and can bring about expression of the polynucleotide. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vector may be a linear or closed circular plasmid.

The vector may be an autonomously replicating vector, *i.e.*, a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.*, a plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one that, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. Furthermore, a single vector or plasmid or

two or more vectors or plasmids that together contain the total DNA to be introduced into the genome of the host cell, or a transposon, may be used.

The vector preferably contains one or more (*e.g.*, several) selectable markers that permit easy selection of transformed, transfected, transduced, or the like cells. A selectable marker is a gene the product of which provides for biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs, and the like.

Examples of bacterial selectable markers are *Bacillus licheniformis* or *Bacillus subtilis* *dal* genes, or markers that confer antibiotic resistance such as ampicillin, chloramphenicol, kanamycin, neomycin, spectinomycin, or tetracycline resistance. Suitable markers for yeast host cells include, but are not limited to, ADE2, HIS3, LEU2, LYS2, MET3, TRP1, and URA3. Selectable markers for use in a filamentous fungal host cell include, but are not limited to, *amdS* (acetamidase), *argB* (ornithine carbamoyltransferase), *bar* (phosphinothricin acetyltransferase), *hph* (hygromycin phosphotransferase), *niaD* (nitrate reductase), *pyrG* (orotidine-5'-phosphate decarboxylase), *sC* (sulfate adenylyltransferase), and *trpC* (anthranilate synthase), as well as equivalents thereof. Preferred for use in an *Aspergillus* cell are *Aspergillus nidulans* or *Aspergillus oryzae* *amdS* and *pyrG* genes and a *Streptomyces hygroscopicus* *bar* gene.

The vector preferably contains an element(s) that permits integration of the vector into the host cell's genome or autonomous replication of the vector in the cell independent of the genome.

For integration into the host cell genome, the vector may rely on the polynucleotide encoding the polypeptide or any other element of the vector for integration into the genome by homologous or non-homologous recombination. Alternatively, the vector may contain additional polynucleotides for directing integration by homologous recombination into the genome of the host cell at a precise location(s) in the chromosome(s). To increase the likelihood of integration at a precise location, the integrational elements should contain a sufficient number of nucleic acids, such as 100 to 10,000 base pairs, 400 to 10,000 base pairs, and 800 to 10,000 base pairs, which have a high degree of sequence identity to the corresponding target sequence to enhance the probability of homologous recombination. The integrational elements may be any sequence that is homologous with the target sequence in the genome of the host cell. Furthermore, the integrational elements may be non-encoding or encoding polynucleotides. On the other hand, the vector may be integrated into the genome of the host cell by non-homologous recombination.

For autonomous replication, the vector may further comprise an origin of replication enabling the vector to replicate autonomously in the host cell in question. The origin of replication may be any plasmid replicator mediating autonomous replication that functions in a cell. The term "origin of replication" or "plasmid replicator" means a polynucleotide that enables a plasmid or vector to replicate *in vivo*.

Examples of bacterial origins of replication are the origins of replication of plasmids pBR322,

pUC19, pACYC177, and pACYC184 permitting replication in *E. coli*, and pUB110, pE194, pTA1060, and pAM β 1 permitting replication in *Bacillus*.

Examples of origins of replication for use in a yeast host cell are the 2 micron origin of replication, ARS1, ARS4, the combination of ARS1 and CEN3, and the combination of ARS4 and CEN6.

Examples of origins of replication useful in a filamentous fungal cell are AMA1 and ANS1 (Gems *et al.*, 1991, *Gene* 98: 61-67; Cullen *et al.*, 1987, *Nucleic Acids Res.* 15: 9163-9175; WO 00/24883). Isolation of the AMA1 gene and construction of plasmids or vectors comprising the gene can be accomplished according to the methods disclosed in WO 00/24883.

More than one copy of a polynucleotide may be inserted into a host cell to increase production of a polypeptide. An increase in the copy number of the polynucleotide can be obtained by integrating at least one additional copy of the sequence into the host cell genome or by including an amplifiable selectable marker gene with the polynucleotide where cells containing amplified copies of the selectable marker gene, and thereby additional copies of the polynucleotide, can be selected for by cultivating the cells in the presence of the appropriate selectable agent.

The procedures used to ligate the elements described above to construct the recombinant expression vectors are well known to one skilled in the art (see, *e.g.*, Sambrook *et al.*, 1989, *supra*).

Host Cells

Recombinant host cells comprising a polynucleotide encoding a polypeptide, *e.g.*, a cellulolytic enzyme, a polypeptide having catalase activity, a polypeptide having cellulolytic enhancing activity, etc., can be advantageously used in the recombinant production of the polypeptide. A construct or vector comprising a polynucleotide is introduced into a host cell so that the construct or vector is maintained as a chromosomal integrant or as a self-replicating extra-chromosomal vector as described earlier. The term "host cell" encompasses any progeny of a parent cell that is not identical to the parent cell due to mutations that occur during replication. The choice of a host cell will to a large extent depend upon the gene encoding the polypeptide and its source.

The host cell may be any cell useful in the recombinant production of a polypeptide, *e.g.*, a prokaryote or a eukaryote.

The prokaryotic host cell may be any Gram-positive or Gram-negative bacterium. Gram-positive bacteria include, but not limited to, *Bacillus*, *Clostridium*, *Enterococcus*, *Geobacillus*, *Lactobacillus*, *Lactococcus*, *Oceanobacillus*, *Staphylococcus*, *Streptococcus*, and *Streptomyces*. Gram-negative bacteria include, but not limited to, *Campylobacter*, *E. coli*, *Flavobacterium*, *Fusobacterium*, *Helicobacter*, *Ilyobacter*, *Neisseria*, *Pseudomonas*, *Salmonella*, and *Ureaplasma*.

The bacterial host cell may be any *Bacillus* cell including, but not limited to, *Bacillus*

alkalophilus, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus firmus*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus stearothermophilus*, *Bacillus subtilis*, and *Bacillus thuringiensis* cells.

5 The bacterial host cell may also be any *Streptococcus* cell including, but not limited to, *Streptococcus equisimilis*, *Streptococcus pyogenes*, *Streptococcus uberis*, and *Streptococcus equi* subsp. *Zooepidemicus* cells.

 The bacterial host cell may also be any *Streptomyces* cell including, but not limited to, *Streptomyces achromogenes*, *Streptomyces avermitilis*, *Streptomyces coelicolor*, *Streptomyces*
10 *griseus*, and *Streptomyces lividans* cells.

 The introduction of DNA into a *Bacillus* cell may be effected by protoplast transformation (see, e.g., Chang and Cohen, 1979, *Mol. Gen. Genet.* 168: 111-115), using competent cells transformation (see, e.g., Young and Spizizen, 1961, *J. Bacteriol.* 81: 823-829, or Dubnau and Davidoff-Abelson, 1971, *J. Mol. Biol.* 56: 209-221), electroporation (see, e.g., Shigekawa and
15 Dower, 1988, *Biotechniques* 6: 742-751), or conjugation (see, e.g., Koehler and Thorne, 1987, *J. Bacteriol.* 169: 5271-5278). The introduction of DNA into an *E. coli* cell may be effected by protoplast transformation (see, e.g., Hanahan, 1983, *J. Mol. Biol.* 166: 557-580) or electroporation (see, e.g., Dower *et al.*, 1988, *Nucleic Acids Res.* 16: 6127-6145). The introduction of DNA into a *Streptomyces* cell may be effected by protoplast transformation and electroporation (see, e.g.,
20 Gong *et al.*, 2004, *Folia Microbiol. (Praha)* 49: 399-405), conjugation (see, e.g., Mazodier *et al.*, 1989, *J. Bacteriol.* 171: 3583-3585), or transduction (see, e.g., Burke *et al.*, 2001, *Proc. Natl. Acad. Sci. USA* 98: 6289-6294). The introduction of DNA into a *Pseudomonas* cell may be effected by electroporation (see, e.g., Choi *et al.*, 2006, *J. Microbiol. Methods* 64: 391-397) or conjugation (see, e.g., Pinedo and Smets, 2005, *Appl. Environ. Microbiol.* 71: 51-57). The introduction of DNA into a
25 *Streptococcus* cell may be effected by natural competence (see, e.g., Perry and Kuramitsu, 1981, *Infect. Immun.* 32: 1295-1297), protoplast transformation (see, e.g., Catt and Jollick, 1991, *Microbios* 68: 189-207), electroporation (see, e.g., Buckley *et al.*, 1999, *Appl. Environ. Microbiol.* 65: 3800-3804) or conjugation (see, e.g., Clewell, 1981, *Microbiol. Rev.* 45: 409-436). However, any method known in the art for introducing DNA into a host cell can be used.

30 The host cell may also be a eukaryote, such as a mammalian, insect, plant, or fungal cell. "Fungi" as used herein includes the phyla Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota as well as the Oomycota and all mitosporic fungi (as defined by Hawksworth *et al.*, *In, Ainsworth and Bisby's Dictionary of The Fungi*, 8th edition, 1995, CAB International, University Press, Cambridge, UK).

35 The fungal host cell may be a yeast cell. "Yeast" as used herein includes ascosporogenous yeast (Endomycetales), basidiosporogenous yeast, and yeast belonging to the Fungi Imperfecti

(Blastomycetes). Since the classification of yeast may change in the future, for the purposes of this invention, yeast shall be defined as described in *Biology and Activities of Yeast* (Skinner, F.A., Passmore, S.M., and Davenport, R.R., eds, *Soc. App. Bacteriol. Symposium Series No. 9*, 1980).

The yeast host cell may be a *Candida*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Saccharomyces*,
 5 *Schizosaccharomyces*, or *Yarrowia* cell such as a *Kluyveromyces lactis*, *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae*, *Saccharomyces diastaticus*, *Saccharomyces douglasii*, *Saccharomyces kluyveri*, *Saccharomyces norbensis*, *Saccharomyces oviformis*, or *Yarrowia lipolytica* cell.

The fungal host cell may be a filamentous fungal cell. "Filamentous fungi" include all
 10 filamentous forms of the subdivision Eumycota and Oomycota (as defined by Hawksworth *et al.*, 1995, *supra*). The filamentous fungi are generally characterized by a mycelial wall composed of chitin, cellulose, glucan, chitosan, mannan, and other complex polysaccharides. Vegetative growth is by hyphal elongation and carbon catabolism is obligately aerobic. In contrast, vegetative growth by yeasts such as *Saccharomyces cerevisiae* is by budding of a unicellular thallus and carbon
 15 catabolism may be fermentative.

The filamentous fungal host cell may be an *Acremonium*, *Aspergillus*, *Aureobasidium*,
Bjerkandera, *Ceriporiopsis*, *Chrysosporium*, *Coprinus*, *Coriolus*, *Cryptococcus*, *Filibasidium*,
Fusarium, *Humicola*, *Magnaporthe*, *Mucor*, *Myceliophthora*, *Neocallimastix*, *Neurospora*,
Paecilomyces, *Penicillium*, *Phanerochaete*, *Phlebia*, *Piromyces*, *Pleurotus*, *Schizophyllum*,
 20 *Talaromyces*, *Thermoascus*, *Thielavia*, *Tolypocladium*, *Trametes*, or *Trichoderma* cell.

For example, the filamentous fungal host cell may be an *Aspergillus awamori*, *Aspergillus foetidus*, *Aspergillus fumigatus*, *Aspergillus japonicus*, *Aspergillus nidulans*, *Aspergillus niger*,
Aspergillus oryzae, *Bjerkandera adusta*, *Ceriporiopsis aneirina*, *Ceriporiopsis caregiea*,
Ceriporiopsis gilvescens, *Ceriporiopsis pannocinta*, *Ceriporiopsis rivulosa*, *Ceriporiopsis subrufa*,
 25 *Ceriporiopsis subvermispora*, *Chrysosporium inops*, *Chrysosporium keratinophilum*,
Chrysosporium lucknowense, *Chrysosporium merdarium*, *Chrysosporium pannicola*,
Chrysosporium queenslandicum, *Chrysosporium tropicum*, *Chrysosporium zonatum*, *Coprinus cinereus*,
Coriolus hirsutus, *Fusarium bactridioides*, *Fusarium cerealis*, *Fusarium crookwellense*,
Fusarium culmorum, *Fusarium graminearum*, *Fusarium graminum*, *Fusarium heterosporum*,
 30 *Fusarium negundi*, *Fusarium oxysporum*, *Fusarium reticulatum*, *Fusarium roseum*, *Fusarium sambucinum*,
Fusarium sarcochroum, *Fusarium sporotrichioides*, *Fusarium sulphureum*, *Fusarium torulosum*,
Fusarium trichothecioides, *Fusarium venenatum*, *Humicola insolens*, *Humicola lanuginosa*,
Mucor miehei, *Myceliophthora thermophila*, *Neurospora crassa*, *Penicillium purpurogenum*,
Phanerochaete chrysosporium, *Phlebia radiata*, *Pleurotus eryngii*, *Thielavia terrestris*,
 35 *Trametes villosa*, *Trametes versicolor*, *Trichoderma harzianum*, *Trichoderma koningii*,
Trichoderma longibrachiatum, *Trichoderma reesei*, or *Trichoderma viride* cell.

Fungal cells may be transformed by a process involving protoplast formation, transformation of the protoplasts, and regeneration of the cell wall in a manner known *per se*. Suitable procedures for transformation of *Aspergillus* and *Trichoderma* host cells are described in EP 238023, Yelton *et al.*, 1984, *Proc. Natl. Acad. Sci. USA* 81: 1470-1474, and Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422. Suitable methods for transforming *Fusarium* species are described by Malardier *et al.*, 1989, *Gene* 78: 147-156, and WO 96/00787. Yeast may be transformed using the procedures described by Becker and Guarente, In Abelson, J.N. and Simon, M.I., editors, *Guide to Yeast Genetics and Molecular Biology, Methods in Enzymology*, Volume 194, pp 182-187, Academic Press, Inc., New York; Ito *et al.*, 1983, *J. Bacteriol.* 153: 163; and Hinnen *et al.*, 1978, *Proc. Natl. Acad. Sci. USA* 75: 1920.

Methods of Production

Methods for producing a polypeptide, *e.g.*, a cellulolytic enzyme, a polypeptide having catalase activity, a polypeptide having cellulolytic enhancing activity, etc., comprise (a) cultivating a cell, which in its wild-type form produces the polypeptide, under conditions conducive for production of the polypeptide; and (b) recovering the polypeptide. . In a preferred aspect, the cell is an *Aspergillus*, *Thermoascus*, *Talaromyces*, *Trichoderma*, *Humicola*, or *Penicillium* cell. In a more preferred aspect, the cell is an *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus fumigatus*, *Thermoascus aurantiacus*, *Talaromyces stipitatus*, *Trichoderma reesei*, *Humicola insolens*, or *Penicillium emersonii* cell.

Alternatively, methods for producing a polypeptide, *e.g.*, a cellulolytic enzyme, a polypeptide having catalase activity, a polypeptide having cellulolytic enhancing activity, etc., comprise (a) cultivating a recombinant host cell under conditions conducive for production of the polypeptide; and (b) recovering the polypeptide.

The host cells are cultivated in a nutrient medium suitable for production of the polypeptide using methods known in the art. For example, the cell may be cultivated by shake flask cultivation, or small-scale or large-scale fermentation (including continuous, batch, fed-batch, or solid state fermentations) in laboratory or industrial fermentors in a suitable medium and under conditions allowing the polypeptide to be expressed and/or isolated. The cultivation takes place in a suitable nutrient medium comprising carbon and nitrogen sources and inorganic salts, using procedures known in the art. Suitable media are available from commercial suppliers or may be prepared according to published compositions (*e.g.*, in catalogues of the American Type Culture Collection). If the polypeptide is secreted into the nutrient medium, the polypeptide can be recovered directly from the medium. If the polypeptide is not secreted, it can be recovered from cell lysates.

The polypeptide may be detected using methods known in the art that are specific for the polypeptides. These detection methods include, but are not limited to, use of specific antibodies,

formation of an enzyme product, or disappearance of an enzyme substrate. For example, an enzyme assay may be used to determine the activity of the polypeptide. The polypeptides having cellulolytic enhancing activity are detected using the methods described herein.

5 The polypeptide may be recovered using methods known in the art. For example, the polypeptide may be recovered from the nutrient medium by conventional procedures including, but not limited to, collection, centrifugation, filtration, extraction, spray-drying, evaporation, or precipitation. In one aspect, the whole fermentation broth is recovered.

10 The polypeptide may be purified by a variety of procedures known in the art including, but not limited to, chromatography (*e.g.*, ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (*e.g.*, preparative isoelectric focusing), differential solubility (*e.g.*, ammonium sulfate precipitation), SDS-PAGE, or extraction (see, *e.g.*, *Protein Purification*, Janson and Ryden, editors, VCH Publishers, New York, 1989) to obtain substantially pure polypeptides.

15 In an alternative aspect, the polypeptide is not recovered, but rather a host cell expressing a polypeptide is used as a source of the polypeptide.

The present invention is further described by the following examples that should not be construed as limiting the scope of the invention.

Examples

20 Strain

The fungal strain NN70 was obtained from Centraalbureau voor Schimmelcultures named as CBS 375.48. The strain NN70 was identified as *Talaromyces stipitatus*, based on both morphological characteristics and ITS rDNA sequence.

25 The fungal strain NN38 was isolated from a soil sample collected from China by the dilution plate method with PDA medium at 45 °C. It was then purified by transferring a single conidium onto a YG agar plate. The strain NN38 was identified as *Humicola insolens*, based on both morphological characteristics and ITS rDNA sequence.

30 The fungal strain NN051602 was isolated from a compost sample collected from China by the dilution plate method with PDA medium at 45 °C. It was then purified by transferring a single conidium onto a YG agar plate. The strain NN051602 was identified as *Penicillium emersonii*, based on both morphological characteristics and ITS rDNA sequence.

Media

35 PDA medium was composed of 39 grams of potato dextrose agar and deionized water to 1 liter.

YG agar plate was composed of 5.0 g of yeast extract, 10.0 g of glucose, 20.0 g of agar,

and deionized water to 1 liter.

YPM medium contained 1% yeast extract, 2% of peptone, and 2% of maltose in deionized water.

YPG medium contained 0.4% of yeast extract, 0.1% of KH_2PO_4 , 0.05% of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$,
5 1.5% glucose in deionized water.

Minimal medium plates were composed of 342 g of sucrose, 20 ml of salt solution, 20 g of agar, and deionized water to 1 liter. The salt solution was composed of 2.6% KCl, 2.6% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 7.6% KH_2PO_4 , 2 ppm $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, 20 ppm $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 40 ppm $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 40 ppm $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$, 40 ppm $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, and 400 ppm $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$.

Example 1: Boosting effect of *Thermoascus aurantiacus* catalase or *Talaromyces stipitatus* catalase on hydrolysis of pretreated corn stover (PCS)

Catalase from *Thermoascus aurantiacus* (as shown in SEQ ID NO: 2) was expressed by *Aspergillus niger* and purified as described in J.P. Publication 2004261137A. Catalase from
15 *Talaromyces stipitatus* (as shown in SEQ ID NO: 4) was cloned, expressed and purified as Examples 9-13. Corn stover was pretreated at the U.S. Department of Energy National Renewable Energy Laboratory (NREL) using dilute sulfuric acid at conditions of 190°C, 1 minute residence time, 0.05 g acid/g dry biomass, and at a 30% total solid concentration in a pretreatment reactor.

PCS was hydrolyzed at an initial total solid (TS) of 10% and total weight of hydrolysis
20 system of 20 g. *Trichoderma reesei* cellulase composition (CELLIC® CTec2 available from Novozymes A/S, Bagsvaerd, Denmark) was added into the PCS for enzymatic hydrolysis with a ratio of the *Trichoderma reesei* cellulase composition to cellulose of 0.5% (w/w), namely 5 mg/g cellulose based on protein amount. *Thermoascus aurantiacus* catalase or *Talaromyces stipitatus* catalase at dosages shown in Table 1 below was added into the hydrolysis system. The hydrolysis
25 system without the addition of catalase was used as a control. The flasks were incubated at 50°C for 72 hours, with shaking at 130 rpm. Unless specified otherwise, the total hydrolysis time was 72 hours. After hydrolysis was completed, the sugar was analyzed by High Performance Liquid Chromatography (HPLC).

For HPLC measurement, the collected samples were filtered using 0.22 μm syringe filters
30 (Millipore, Bedford, MA, USA) and the filtrates were analyzed for sugar content as described below. The sugar concentrations of samples diluted in 0.005 M H_2SO_4 were measured using a 7.8×300 mm AMINEX® HPX-87H column (Bio-Rad Laboratories, Inc., Hercules, CA, USA) by elution with 0.005 M H_2SO_4 at 65°C at a flow rate of 0.7 ml per minute, and quantification by integration of the glucose signal from refractive index detection (CHEMSTATION®, AGILENT® 1100 HPLC, Agilent
35 Technologies, Santa Clara, CA, USA) calibrated by pure sugar samples. The resultant glucose was used to calculate the percentage of glucose yield from glucans for each reaction. Measured

sugar concentrations were adjusted for the appropriate dilution factor. The net concentrations of enzymatically-produced sugars were determined by adjusting the measured sugar concentrations for corresponding background sugar concentrations in unwashed biomass at zero time point. All HPLC data processing was performed using MICROSOFT EXCEL™ software (Microsoft, Richland, WA, USA).

The degree of glucose conversion to glucose was calculated according to the publication by Zhu, Y., *et al.* 2010, *Bioresource Technology*. 102(3): 2897-2903.

The results as shown in Table 1 demonstrated that PCS conversion to glucose can be improved significantly by adding small amounts of catalase.

Table 1. Effect of catalase from *T. aurantiacus* or catalase from *T. stipitatus* on the glucose conversion of PCS

Dosage of catalase ($\mu\text{g/g}$ cellulose)	0 (control)	<i>T. aurantiacus</i> catalase			<i>T. stipitatus</i> catalase		
		50	250	500	50	250	500
Glucose conversion (%)	49.7 $\pm$ 0.4	54.9 $\pm$ 0.1	61.7 $\pm$ 1.8	63 $\pm$ 0.7	56.7 $\pm$ 0.4	60.6 $\pm$ 1.9	60.4 $\pm$ 2.5

Example 2: Boosting effect of *Talaromyces stipitatus* catalase on mono-components of cellulases

Avicel® PH-101 (Fluka 11365, Sigma-Aldrich (Shanghai), Shanghai, China), a kind of microcrystalline cellulose, was hydrolyzed at a final concentration of 5 g/l and total volume of the hydrolysis system of 0.5 ml. The pH was adjusted and kept at 5.0 by 50 mM sodium acetate. In addition, ascorbic acid was present with a final concentration of 5 mM or absent in the hydrolysis system. Manganese (II) sulfate was present with a final concentration of 1 mM in the hydrolysis system.

Cellobiohydrolase (CBH) I from *Aspergillus fumigatus* (WO 2011/057140), cellobiohydrolase (CBH) II from *Aspergillus fumigatus* (WO 2011/057140), endoglucanase (EG) I from *Trichoderma reesei* (WO 2011/057140) and beta-glucanase (BG) from *Aspergillus oryzae* (WO 02/095014) were cloned, expressed and purified. These mono-components were applied to the hydrolysis of Avicel® individually. 10 mg mono-component of cellulase/g Avicel® and 5 mg catalase/g Avicel® were used. The tubes were incubated at 50°C for 72 hours, with shaking at 600 rpm. All experiments were performed in triplicates.

HPLC analysis of the extent of hydrolysis was performed according to the procedure described in Example 1.

The extent of cellulose conversion was calculated based on the mass ratio of solubilized glucosyl units to the initial mass of insoluble cellulose. Only glucose and cellobiose were measured for soluble sugars, as cellodextrins longer than cellobiose were present in negligible concentrations (due to enzymatic hydrolysis). The extent of total cellulose conversion was calculated using the

following equation:

$$\% \text{ conversion} = \frac{[\text{cellobiose}] (\text{mg/ml})/1.053 + ([\text{glucose}] (\text{mg/ml})/1.111)}{[\text{Cellulose}] (\text{mg/ml})} \times 100$$

(Equation 1)

The 1.111 and 1.053 factors for glucose and cellobiose, respectively, take into account the increase in mass when the glucosyl units in cellulose (average molecular mass of 162 daltons) are converted to glucose (molecular mass of 180 daltons) or cellobiose glucosyl units (average molecular mass of 171 daltons).

Table 2. Effect of catalase from *T. stipitatus* on glucose conversion of Avicel.

	Mono-component without Ascorbic acid					Mono-component with Ascorbic acid				
	Control	BG	CBH I	CBH II	EG	Control	BG	CBH I	CBH II	EG
Control (%)	0.0±0.0	0.0±0.0	13.7±0.2	9.0±0.3	2.5±0.0	0.0±0.0	0.0±0.0	11.4±0.1	8.0±0.3	1.9±0.1
Catalase (%)	0.0%±0.0	0.0±0.0	13.4±0.2	9.1±0.1	2.4±0.1	0.0±0.0	2.4±0.3	15.8±1.3	12.7±0.3	4.8±0.0

As shown in Table 2, in the presence of ascorbic acid, the hydrolysis of every mono-component of cellulase can be boosted by *T. stipitatus* catalase.

Example 3: Boosting effect of *Humicola insolens* catalase on hydrolysis of PCS

Preparation of PCS and set-up of hydrolysis system were the same as example 1. Catalase from *Humicola insolens* was cloned, expressed and purified as shown in Examples 14-20.

PCS was hydrolyzed at an initial TS of 10% and total weight of hydrolysis system of 20 g. *Trichoderma reesei* cellulase composition (CELLIC® CTec2 available from Novozymes A/S, Bagsvaerd, Denmark) was utilized for enzymatic hydrolysis. Five percent by weight Ctec2 was replaced by *H. insolens* catalase based on protein amount and the total enzyme dose was 4mg/g cellulose. The hydrolysis system with 4mg *Trichoderma reesei* cellulase composition /g cellulose but without catalase was used as a control. The flasks were incubated at 50°C for 72 hours, with shaking at 130 rpm. The total hydrolysis time was 72 hours.

The calculation of glucose conversion was the same as example 1 and the boosting effect was shown in table 3.

Table 3. Effect of catalase from *Humicola insolens* on glucose conversion of PCS.

	Control	<i>H. insolens</i> Catalase
Glucose conversion (%)	50.4±1.1	58.4±0.9

Example 4: Boosting effect of *Humicola insolens* catalase on hydrolysis of PCS

Preparation of PCS and set-up of hydrolysis system were the same as example 1. Catalase from *Humicola insolens* was cloned, expressed and purified and purified as shown in Examples 14-

20.

PCS was hydrolyzed at an initial TS of 10% and total weight of hydrolysis system of 20 g. *Trichoderma reesei* cellulase composition (CELLIC® CTec3 available from Novozymes A/S, Bagsvaerd, Denmark) was utilized for enzymatic hydrolysis. Five percent by weight Ctec3 was replaced by *H. insolens* catalase based on protein amount and the total enzyme dose was 4mg/g cellulose. The hydrolysis system with 4mg *Trichoderma reesei* cellulase composition/g cellulose but without catalase was used as a control. The flasks were incubated at 50°C for 72 hours, with shaking at 130 rpm. The total hydrolysis time was 72 hours.

The calculation of glucose conversion was the same as example 1 and the boosting effect was shown in table 4.

Table 4. Effect of catalase from *Humicola insolens* on glucose conversion of PCS.

	Control	<i>H. insolens</i> Catalase
Glucose conversion (%)	70.9±1.4	80.1±1.2

Example 5: Synergetic effect of *Thermoascus aurantiacus* catalase and *Thermoascus aurantiacus* GH61A on hydrolysis of PCS

PCS was prepared according to the procedure as described in Example 1, and hydrolyzed at initial TS of 10% and total weight of hydrolysis system of 20 g. The pH was adjusted to 5.0 using 10 M sodium hydroxide. *Trichoderma reesei* cellulase composition (CELLUCLAST® in the presence of 10% of total protein weight *Aspergillus fumigatus* beta-glucosidase (WO 2005/047499), available from Novozymes A/S, Bagsvaerd, Denmark) was added into PCS for enzymatic hydrolysis with a ratio of the *Trichoderma reesei* cellulase composition to cellulose of 0.8% (w/w). Catalase from *Thermoascus aurantiacus*, GH61A polypeptide from *Thermoascus aurantiacus* (WO 2005/074656), or the combination thereof were added into the hydrolysis system, respectively. The dosage of catalase, GH61A polypeptide, or the combination thereof was calculated based on the weight of cellulose. The hydrolysis system with *Trichoderma reesei* cellulase composition but without catalase and GH61 was used as a control. The flasks were incubated at 50°C for 72 hours, with shaking at 130 rpm. All experiments were performed in triplicates. HPLC analysis of the extent of hydrolysis was performed according to the procedure described in Example 1. The conversion of PCS to glucose after 72-hour hydrolysis was shown in Table 5 below.

Table 5. Synergetic effect of *Thermoascus aurantiacus* catalase and *Thermoascus aurantiacus* GH61A on hydrolysis of PCS

	Control	Catalase alone (µg/g cellulose)		GH61A alone (µg/g cellulose)		Catalase+GH61A (µg/g cellulose)	
		80	160	80	160	80+80	160+160
Glucose conversion (%)	66.9±1.1	75.9±0.0	82.8±0.4	70.6±0.2	71.8±0.2	88.7±3.0	93.2±0.8

As shown in Table 5, catalase or GH61A polypeptide alone boosted the hydrolysis of PCS. It was surprisingly found that, when catalase and GH61A polypeptide were used simultaneously, the hydrolysis was improved significantly. The results indicated that catalase and GH61A polypeptide have a significant synergistic effect on the hydrolysis of PCS.

5

Example 6: Boosting effect of *Penicillium emersonii* catalase on hydrolysis of PCS

Preparation of PCS and set-up of hydrolysis system were the same as example 1. Catalase from *P. emersonii* was cloned, expressed and purified as shown in Examples 21-27.

PCS was hydrolyzed at an initial TS of 10% and total weight of hydrolysis system of 20 g.

10 *Trichoderma reesei* cellulase composition (CELLIC® CTec2 available from Novozymes A/S, Bagsvaerd, Denmark) was added into PCS for enzymatic hydrolysis. Five percent by weight of Ctec2 was replaced with *P. emersonii* catalase based on protein amount and the total enzyme dose was 4 mg/g cellulose. The hydrolysis system with *Trichoderma reesei* cellulase composition but without catalase was used as a control. The flasks were incubated at 50°C for 72 hours, with
15 shaking at 130 rpm.

The calculation of glucose conversion was the same as example 1 and the boosting effect was shown in table 6.

Table 6. Effect of catalase from *P. emersonii* on glucose conversion of PCS.

	Control	<i>P. emersonii</i> Catalase
Glucose conversion (%)	48.6±0.7	54.3±0.8

20 Example 7: Boosting effect of *Thermoascus aurantiacus* catalase on hydrolysis of PCS in relatively high TS

Preparation of PCS and set-up of hydrolysis system were the same as example 1. PCS was hydrolyzed at an initial TS of 20% and total weight of hydrolysis system of 20 g. *Trichoderma reesei* cellulase composition (CELLIC® CTec2 available from Novozymes A/S, Bagsvaerd,
25 Denmark) was added into PCS for enzymatic hydrolysis. Five percent by weight of Ctec2 was replaced by *T. aurantiacus* catalase based on protein amount and the total enzyme dose was 7 mg/g cellulose. The hydrolysis system with *Trichoderma reesei* cellulase composition but without catalase was used as a control. The flasks were incubated at 50°C for 72 hours, with shaking at 130 rpm. The calculation of glucose conversion was the same as example 1 and the boosting
30 effect of catalases was shown in table 7.

Table 7. Effect of catalase from *Thermoascus aurantiacus* on glucose conversion of PCS.

	Control	5% <i>T. aurantiacus</i> catalase replacement
Glucose conversion (%)	58.6±1.4	64.8±0.8

Example 8: Boosting effect of *Thermoascus aurantiacus* catalase on hydrolysis of PCS in relatively high TS

Preparation of PCS and set-up of hydrolysis system were the same as example 1. PCS was hydrolyzed at an initial TS of 20% and total weight of hydrolysis system of 20 g. *Trichoderma reesei* cellulase composition (CELLIC® CTec3 available from Novozymes A/S, Bagsvaerd, Denmark) was utilized for enzymatic hydrolysis. Five percent by weight of Ctec3 was replaced by *T. aurantiacus* catalase based on protein amount and the total enzyme dose was 6 mg/g cellulose. The hydrolysis system with *Trichoderma reesei* cellulase composition but without catalase was used as a control. The flasks were incubated at 50°C for 72 hours, with shaking at 130 rpm.

The calculation of glucose conversion was the same as example 1 and the boosting effect of catalases was shown in table 8.

Table 8. Effect of catalase from *Thermoascus aurantiacus* on glucose conversion of PCS.

	Control	5% <i>T. aurantiacus</i> catalase replacement
Glucose conversion (%)	72.0±	80.5±0.8

Example 9: *Talaromyces stipitatus* genomic DNA extraction

Talaromyces stipitatus strain NN70 was grown in PDA agar plate at 45°C for 3 days. Mycelia were collected directly from the agar plate into a sterilized mortar and frozen under liquid nitrogen. Frozen mycelia were ground, by mortar and pestle, to a fine powder, and genomic DNA was isolated using a DNeasy® Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA).

Example 10: Cloning of the *Talaromyces stipitatus* catalase gene from genomic DNA
Based on the DNA information of European Molecular Biology Laboratory (EMBL):EQ962660 (*i.e.*, SEQ ID NO: 3) and protein sequence SWISSPROT:B8MT74 (*i.e.*, SEQ ID NO: 4), oligonucleotide primers, shown in below, were designed to amplify the catalase gene from the genomic DNA of *Talaromyces stipitatus* NN70. Primers were fabricated by Invitrogen (Invitrogen, Beijing, China).

Forward primer: 5' ACACAACTGGGGATCC ACC atgcgaggggcatactctctc 3' (SEQ ID NO: 38)

Reverse primer: 5' GTCACCCTCTAGATCT aacaagtactcgtgttaatcgtggaa 3' (SEQ ID NO: 39)

Lowercase characters represent the sequences of the gene, while capitalized parts were homologous to the insertion sites of pPFJO355 vector which has been described in US2010306879.

The expression vector pPFJO355 contains the TAKA-amylase promoter derived from *Aspergillus oryzae* and the *Aspergillus niger* glucoamylase terminator elements. Furthermore pPFJO355 has pUC18 derived sequences for selection and propagation in *E. coli*, and a *pyrG* gene, which encodes an orotidine decarboxylase derived from *Aspergillus nidulans* for selection of a transformant of a *pyrG* mutant *Aspergillus* strain.

Twenty picomoles of primer pair (forward and reverse) were used in a PCR reaction composed of 2 µl of *Talaromyces stipitatus* NN70 genomic DNA, 10 µl of 5X GC Buffer, 1.5 µl of DMSO, 2.5 mM each of dATP, dTTP, dGTP, and dCTP, and 0.6 unit of PHUSION™ High-Fidelity DNA Polymerase (Finnzymes Oy, Espoo, Finland) in a final volume of 50 µl. The amplification was performed using a Peltier Thermal Cycler (MJ Research Inc., South San Francisco, CA, USA) programmed for denaturing at 98°C for 40 seconds; 8 cycles of denaturing at 98°C for 15 seconds, annealing at 70°C for 30 seconds, with 1 °C decrease per cycle and elongation at 72°C for 80 seconds; and another 23 cycles each at 98°C for 15 seconds, 62°C for 30 seconds and 72°C for 80 seconds; final extension at 72°C for 5 minutes. The heat block then went to a 4°C soak cycle.

The PCR reaction products were isolated by 1.0% agarose gel electrophoresis using 90mM Tris-borate and 1 mM EDTA (TBE) buffer where a single product band of the expected size, approximate 2.4 kb, was visualized under UV light, and then purified from solution using an ILLUSTRATE™ GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, Buckinghamshire, UK) according to the manufacturer's instructions.

Plasmid pPFJO355 was digested with *Bam* HI and *Bgl* II, isolated by 1.0% agarose gel electrophoresis using TBE buffer, and purified using an ILLUSTRATE™ GFX™ PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

An IN-FUSION™ CF Dry-down Cloning Kit (Clontech Laboratories, Inc., Mountain View, CA, USA) was used to clone the fragment directly into the expression vector pPFJO355, without the need for restriction digestion and ligation.

The PCR reaction product and the digested vector were ligated together using an IN-FUSION™ CF Dry-down PCR Cloning resulting in plasmid pTs in which the transcription of *Talaromyces stipitatus* catalase gene was under the control of a promoter from the gene for *Aspergillus oryzae* alpha-amylase. The cloning operation was conducted according to the manufacturer's instruction. In brief, 30 ng of pPFJO355 digested with *Bam* HI and *Bgl* II, and 60 ng of the purified *Talaromyces stipitatus* catalase PCR reaction products were added to the reaction vial and resuspended the powder in a final volume of 10 µl with addition of deionized water. The reaction was incubated at 37°C for 15 minutes and then 50°C for 15 minutes. Three µl of the reaction products were used to transform *E. coli* TOP10 competent cells (TIANGEN Biotech (Beijing) Co. Ltd., Beijing, China). *E. coli* transformants containing expression constructs were detected by colony PCR which is a method for quick screening of plasmid inserts directly from *E.*

coli colonies. Briefly, in the premixed PCR solution aliquot in each PCR tube, including PCR buffer, MgCl₂, dNTP and primer pairs for which the PCR fragment generated, a single colony was added by picking up with a sterile tip and twirling the tip in the reaction solution. Normaly 7-10 colonies were screened. After the PCR program, reactions were checked on agarose gel. The colony giving the amplification of expected size was possibly to contain the correct insert. The plasmid DNA was prepared using a QIAprep Spin Miniprep Kit (QIAGEN Inc., Valencia, CA, USA). The *Talaromyces stipitatus* catalase gene inserted in plasmid pTs was confirmed by DNA sequencing using a 3730XL DNA Analyzers (Applied Biosystems Inc, Foster City, CA, USA).

Example 11: Expression of *Talaromyces stipitatus* catalase gene in *Aspergillus oryzae*

Aspergillus oryzae HowB101 (described in patent WO9535385 example 1) protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422. Three µg of plasmid pTs were used to transform *Aspergillus oryzae* HowB101.

The transformation of *Aspergillus oryzae* HowB101 with plasmid pTs yielded about 50 transformants for each transformation. Eight transformants were isolated to individual Minimal medium plates.

Four transformants from each transformation were inoculated separately into 3 ml of YPM in 24-well plate and incubated at 30°C, 150 rpm. After 3 days incubation, 20 µl of supernatant from each culture were analyzed on NuPAGE Novex 4-12% Bis-Tris Gel with 2-(N-morpholino) ethanesulfonic acid (MES) (Invitrogen Corporation, Carlsbad, CA, USA) according to the manufacturer's instructions. The resulting gel was stained with INSTANT BLUE™ (Expedeon Ltd., Babraham Cambridge, UK). SDS-PAGE profiles of the cultures showed expression with protein bands detected. The size of major band of the gene was around 92KD. The expression strain was designated as EXP84.

Example 12: Fermentation of the expression strain EXP84

A slant of the expression strain, EXP84, was washed with 10 ml of YPM and inoculated into eight 2-liter flasks containing 400 ml of YPM medium to generate broth for characterization of the enzyme. The culture was harvested on day 3 and filtered using a 0.45 µm DURAPORE Membrane (Millipore, Bedford, MA, USA).

Example 13: Purification of recombinant *Talaromyces stipitatus* catalase from *Aspergillus oryzae* EXP84

3200 ml supernatant of the recombinant strain EXP84 was precipitated with ammonium sulfate (80% saturation) and re-dissolved in 50 ml 20mM Tris-HCl buffer, pH7.5, then dialyzed

against the same buffer and filtered through a 0.45 mm filter, the final volume was 100 ml. The solution was applied to a 40 ml Q SEPHAROSE® Fast Flow column (GE Healthcare, Buckinghamshire, UK) equilibrated in 20mM Tris-HCl buffer, pH7.5. Fractions eluted with 0.08-0.2M NaCl were collected and further purified on a 40 ml Q SEPHAROSE® Fast Flow column (GE Healthcare, Buckinghamshire, UK) with a linear NaCl gradient (0.14 – 0.2M). Fractions were evaluated by SDS-PAGE (NP0336BOX, NUPAGE 4-12% BT GEL 1.5MM15W). Fractions containing a band of approximately 92 kDa were pooled. Then the pooled solution was concentrated by ultrafiltration.

Example 14: *Humicola insolens* genomic DNA extraction

Humicola insolens strain NN38 was inoculated onto a PDA plate and incubated for 3 days at 45°C in the darkness. Several mycelia-PDA plugs were inoculated into 500 ml shake flasks containing 100 ml of YPG medium. The flasks were incubated for 3 days at 45°C with shaking at 160 rpm. The mycelia were collected by filtration through MIRACLOTH® (Calbiochem, La Jolla, CA, USA) and frozen in liquid nitrogen. Frozen mycelia were ground, by a mortar and a pestle, to a fine powder, and genomic DNA was isolated using DNeasy® Plant Maxi Kit (QIAGEN Inc., Valencia, CA, USA) following the manufacturer's instruction.

Example 15: Genome sequencing, assembly and annotation of *Humicola insolens* strain NN38

The extracted genomic DNA samples were delivered to Beijing Genome Institute (BGI, Shenzhen, China) for genome sequencing using ILLUMINA® GA2 System (Illumina, Inc., San Diego, CA, USA). The raw reads were assembled at BGI using program SOAPdenovo (Li *et al.*, 2010, *Genome Research* 20(2): 265-72). The assembled sequences were analyzed using standard bioinformatics methods for gene finding and functional prediction. Briefly, geneID (Parra *et al.*, 2000, *Genome Research* 10(4):511-515) was used for gene prediction. Blastall version 2.2.10 ((Altschul *et al.*, 1990, *J. Mol. Biol.* 215 (3): 403–410; National Center for Biotechnology Information (NCBI), Bethesda, MD, USA) and HMMER version 2.1.1 (National Center for Biotechnology Information (NCBI), Bethesda, MD, USA) were used to predict function based on structural homology. The catalase gene, (SEQ ID NO: 5 for DNA sequence, SEQ ID NO: 6 for protein sequence), was identified directly by analysis of the Blast results. The Agene program (Munch and Krogh, 2006, *BMC Bioinformatics* 7:263) and SignalP program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6) were used to identify starting codons. SignalP was further used to predict the signal peptide. Pepstats (European Bioinformatics Institute, Hinxton, Cambridge CB10 1SD, UK) was used to estimate isoelectric point of proteins, and molecular weight.

Example 16: Cloning of the *Humicola insolens* catalase gene from genomic DNA

Based on the DNA information of the *Humicola insolens* catalase, oligonucleotide primers, shown in below, were designed to amplify the catalase gene from the genomic DNA of *Humicola insolens* NN38. Primers were fabricated by Invitrogen (Invitrogen, Beijing, China).

5 Forward primer: 5' ACACAACTGGGGATCC ACC atgaacagagtcacgaatctcctcg 3'
(SEQ ID NO: 40)

Reverse primer: 5' GTCACCCTCTAGATCT ggtacaactcccaccctattccttctc 3' (SEQ ID
NO: 41)

10 Lowercase characters represent the sequences of the gene in the forward primer and the
flanking region of the 3' end of the gene in the reverse primer, while capitalized parts were
homologous to the insertion sites of pPFJO355 vector which has been described in
US2010306879.

The expression vector pPFJO355 contains the TAKA-amylase promoter derived from
Aspergillus oryzae and the *Aspergillus niger* glucoamylase terminator elements. Furthermore
15 pPFJO355 has pUC18 derived sequences for selection and propagation in *E. coli*, and a *pyrG*
gene, which encodes an orotidine decarboxylase derived from *Aspergillus nidulans* for selection of
a transformant of a *pyrG* mutant *Aspergillus* strain.

Twenty picomoles of primer pair (forward and reverse) were used in a PCR reaction
composed of 2µl of *Humicola insolens* NN38 genomic DNA, 10 µl of 5X GC Buffer, 1.5µl of DMSO,
20 2.5 mM each of dATP, dTTP, dGTP, and dCTP, and 0.6 unit of PHUSION™ High-Fidelity DNA
Polymerase (Finnzymes Oy, Espoo, Finland) in a final volume of 50 µl. The amplification was
performed using a Peltier Thermal Cycler (MJ Research Inc., South San Francisco, CA, USA)
programmed for denaturing at 98°C for 1 minute; 6 cycles of denaturing at 98°C for 15 seconds,
annealing at 63°C for 30 seconds, with 1 °C decrease per cycle and elongation at 72°C for 3
25 minutes; and another 22 cycles each at 98°C for 15 seconds, 62°C for 30 seconds and 72°C for 3
minutes; final extension at 72°C for 7 minutes. The heat block then went to a 4°C soak cycle.

The PCR reaction products were isolated by 1.0% agarose gel electrophoresis using 90mM
Tris-borate and 1 mM EDTA (TBE) buffer where a single product band of the expected size,
approximate 3.1 kb, was visualized under UV light and then purified from solution using an
30 ILLUSTRATE™ GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, Buckinghamshire,
UK) according to the manufacturer's instructions.

Plasmid pPFJO355 was digested with *Bam* HI and *Bgl* II, isolated by 1.0% agarose gel
electrophoresis using TBE buffer, and purified using an ILLUSTRATE™ GFX™ PCR DNA and Gel
Band Purification Kit according to the manufacturer's instructions.

35 An IN-FUSION™ CF Dry-down Cloning Kit (Clontech Laboratories, Inc., Mountain View, CA,

USA) was used to clone the fragment directly into the expression vector pPFJO355, without the need for restriction digestion and ligation.

The PCR reaction product and the digested vector were ligated together using an IN-FUSION™ CF Dry-down PCR Cloning resulting in plasmid pHi in which the transcription of *Humicola insolens* catalase gene was under the control of a promoter from the gene for *Aspergillus oryzae* alpha-amylase. The cloning operation was conducted according to the manufacturer's instruction. In brief, 30 ng of pPFJO355 digested with *Bam* HI and *Bgl* II, and 60 ng of the purified *Humicola insolens* catalase PCR product were added to the reaction vial and resuspended the powder in a final volume of 10µl with addition of deionized water. The reaction was incubated at 37°C for 15 minutes and then 50°C for 15 minutes. Three µl of the reaction products were used to transform *E. coli* TOP10 competent cells (TIANGEN Biotech (Beijing) Co. Ltd., Beijing, China). *E. coli* transformants containing expression constructs were detected by colony PCR which is a method for quick screening of plasmid inserts directly from *E. coli* colonies. Briefly, in the premixed PCR solution aliquot in each PCR tube, including PCR buffer, MgCl₂, dNTP and primer pairs for which the PCR fragment generated, a single colony was added by picking up with a sterile tip and twirling the tip in the reaction solution. Normally 7-10 colonies were screened. After the PCR program, reactions were checked on agarose gel. The colony giving the amplification of expected size was possibly to contain the correct insert. The plasmid DNA was prepared using a QIAprep Spin Miniprep Kit (QIAGEN Inc., Valencia, CA, USA). The *Humicola insolens* catalase gene inserted in plasmid pHi was confirmed by DNA sequencing using a 3730XL DNA Analyzers (Applied Biosystems Inc, Foster City, CA, USA).

Example 17: Expression of *Humicola insolens* catalase gene in *Aspergillus oryzae*

Aspergillus oryzae HowB101 (described in patent WO9535385 example 1) protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422. Three µg of plasmid pHi were used to transform *Aspergillus oryzae* HowB101.

The transformation of *Aspergillus oryzae* HowB101 with plasmid pHi yielded about 50 transformants for each transformation. Eight transformants were isolated to individual Minimal medium plates.

Four transformants from each transformation were inoculated separately into 3 ml of YPM medium in 24-well plate and incubated at 30°C, 150 rpm. After 3 days incubation, 20 µl of supernatant from each culture were analyzed on NuPAGE Novex 4-12% Bis-Tris Gel w/MES (Invitrogen Corporation, Carlsbad, CA, USA) according to the manufacturer's instructions. The resulting gel was stained with INSTANT BLUE™ (Expedeon Ltd., Babraham Cambridge, UK). SDS-PAGE profiles of the cultures showed expression with protein bands detected. The size of

major band of the gene was around 80KD. The expression strain was designated as O5.

Example 18: Fermentation of expression strain O5

A slant of the expression strain, O5, was washed with 10 ml of YPM and inoculated into
5 twelve 2-liter flasks each containing 400 ml of YPM medium to generate broth for characterization
of the enzyme. The culture was harvested on day 3 and filtered using a 0.45 μ m DURAPORE
Membrane (Millipore, Bedford, MA, USA).

Example 19: Purification of recombinant *Humicola insolens* catalase from
10 *Aspergillus oryzae* O5

4000ml supernatant of the recombinant strain O5 was precipitated with ammonium sulfate
(80% saturation) and re-dissolved in 50 ml 20mM Bis-Tris buffer, pH6.0, then dialyzed against the
same buffer and filtered through a 0.45 μ m filter, the final volume was 140 ml. The solution was
applied to a 40 ml Q SEPHAROSE® Fast Flow column (GE Healthcare, Buckinghamshire, UK)
15 equilibrated in 20 mM Bis-Tris buffer, pH6.0, and the proteins was eluted with a linear NaCl
gradient (0–0.25M). Fractions eluted with 0.2-0.5M NaCl were collected and further purified on a
40ml Q SEPHAROSE® Fast Flow column (GE Healthcare, Buckinghamshire, UK) equilibrated in
20 mM Bis-Tris buffer, pH6.0, and the proteins was eluted with a linear NaCl gradient (0.2–0.5M).
Fractions were evaluated by SDS-PAGE (NP0336BOX, NUPAGE 4-12% BT GEL 1.5MM15W).
20 Fractions containing a band of approximately 80 kDa were pooled. Then the pooled solution was
concentrated by ultrafiltration.

The mature polypeptide of the *Humicola insolens* catalase shares 99.25% identity with the
mature polypeptide of *Humicola grisea* thermotolerant catalase protein (WO2009104622-A1).

25 Example 20: Catalase activity assay

The purified *Humicola insolens* catalase was checked for catalase activity by using the
following protocol.

The substrate was prepared by 1000 times dilution of 30% H₂O₂ (from Xilong Chemical,
Guangdong, China) with double distilled H₂O (ddH₂O), the final concentration was 10.3 mM. The
30 reaction was started by adding 1 μ l of purified *Humicola insolens* catalase sample into 1000 μ l of
substrate. The optical density (OD) at 240 nm was read by Ultrospec 3300 (GE Healthcare,
Buckinghamshire, UK) at second of 0 and 16 respectively, and the decrease of the OD (from 0.400
to 0.102) showed the relative activity of the *Humicola insolens* catalase.

35 Example 21: *Penicillium emersonii* Genomic DNA extraction

Penicillium emersonii strain NN051602 was inoculated onto a PDA plate and incubated for 3 days at 45°C in the darkness. Several mycelia-PDA plugs were inoculated into 500 ml shake flasks containing 100 ml of YPG medium. The flasks were incubated for 3 days at 45°C with shaking at 160 rpm. The mycelia were collected by filtration through MIRACLOTH® (Calbiochem, La Jolla, CA, USA) and frozen in liquid nitrogen. Frozen mycelia were ground, by a mortar and a pestle, to a fine powder, and genomic DNA was isolated using a Large-Scale Column Fungal DNAout (Baoman Biotechnology, Shanghai, China) according to the manufacturer's instructions.

Example 22: Genome sequencing, assembly and annotation

The extracted genomic DNA samples were delivered to Beijing Genome Institute (BGI, Shenzhen, China) for genome sequencing using ILLUMINA® GA2 System (Illumina, Inc., San Diego, CA, USA). The raw reads were assembled at BGI using program SOAPdenovo (Li *et al.*, 2010, *Genome Res* 20: 265-72). The assembled sequences were analyzed using standard bioinformatics methods for gene identification and functional prediction. Briefly, geneID (Parra *et al.*, 2000, *Genome Research* 10(4):511-515) was used for gene prediction. Blastall version 2.2.10 (Altschul *et al.*, 1990, *J. Mol. Biol.* 215 (3): 403-410, <http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and HMMER version 2.1.1 (National Center for Biotechnology Information (NCBI), Bethesda, MD, USA, <http://hmmer.janelia.org>) were used to predict function based on structural homology. The catalase was identified by analysis of the Blast results. The Agene program (Munch and Krogh, 2006, *BMC Bioinformatics* 7:263) and SignalP program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6) were used to identify start codons. SignalP was further used to predict the signal peptide. Pepstats (Rice *et al.*, 2000, *Trends Genet.* 16(6): 276-277) was used to estimate isoelectric point of proteins, and molecular weight.

Example 23: Cloning of the *Penicillium emersonii* catalase from genomic DNA

One catalase gene, PE04230007241 (SEQ ID NO: 7), was selected for expression cloning.

Based on the gene information obtained by genome sequencing, oligonucleotide primers as shown below, were designed to amplify the catalase gene, PE04230007241, from the genomic DNA of *Penicillium emersonii*. Primers were fabricated by Invitrogen (Invitrogen, Beijing, China).

Forward primer	5' ACACAACTGGGGATCC ACC atgcgcgcagtcagct 3'	SEQ ID NO: 42
Reverse primer	5' GTCACCCTCTAGATCT gtcgactattccaaccttctatatggacac 3'	SEQ ID NO: 43

Lowercase characters of the forward primer represent the coding regions of the gene and lowercase characters of the reverse primer represent the flanking region of the gene, while capitalized parts were homologous to the insertion sites of pPFJO355 vector which has been described in US2010306879.

An IN-FUSION™ CF Dry-down Cloning Kit (Clontech Laboratories, Inc., Mountain View, CA, USA) was used to clone the fragment directly into the expression vector pPFJO355 which has been described in US2010306879, without the need for restriction digestion and ligation.

5 The expression vector pPFJO355 contains the TAKA-amylase promoter derived from *Aspergillus oryzae* and the *Aspergillus niger* glucoamylase terminator elements. Furthermore pPFJO355 has pUC18 derived sequences for selection and propagation in *E. coli*, and a pyrG gene, which encodes an orotidine decarboxylase derived from *Aspergillus nidulans* for selection of a transformant of a pyrG mutant *Aspergillus* strain.

10 Twenty picomoles of each of the primers above were used in a PCR reaction composed of 2µl of *Penicillium emersonii* genomic DNA, 10 µl of 5X GC Buffer, 1.5µl of DMSO, 2.5 mM each of dATP, dTTP, dGTP, and dCTP, and 0.6 unit of PHUSION™ High-Fidelity DNA Polymerase (Finnzymes Oy, Espoo, Finland) in a final volume of 50 µl. The amplification was performed using a Peltier Thermal Cycler (MJ Research Inc., South San Francisco, CA, USA) programmed for denaturing at 98°C for 1 minutes; 8 cycles of denaturing at 98°C for 15 seconds, annealing at 65°C
15 for 30 seconds, with 1 °C decrease per cycle and elongation at 72°C for 3 minute 15 second; and another 22 cycles each at 98°C for 15 seconds, 58C for 30 seconds and 72°C for 3 minute 15 second; final extension at 72°C for 10 minutes. The heat block then went to a 4°C soak cycle.

The reaction product was isolated by 1.0% agarose gel electrophoresis using 90mM Tris-borate and 1 mM EDTA (TBE) buffer where an approximate 2.5kb product band was excised from
20 the gel, and purified using an ILLUSTRA™ GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, Buckinghamshire, UK) according to the manufacturer's instructions.

Plasmid pPFJO355 was digested with *Bam* HI and *Bgl* II, isolated by 1.0% agarose gel electrophoresis using TBE buffer, and purified using an ILLUSTRA™ GFX™ PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

25 The PCR product and the digested vector were ligated together using an IN-FUSION™ CF Dry-down PCR Cloning resulting in pCat_PE04230007241 in which the transcription of the *Penicillium emersonii* catalase gene was under the control of a promoter from the gene for *Aspergillus oryzae* alpha-amylase. The cloning operation was conducted according to the manufacturer's instruction. In brief, 30 ng of pPFJO355 digested with *Bam* HI and *Bgl* II, and 60 ng
30 of the purified *Penicillium emersonii* catalase gene PCR product were added to the reaction vial and resuspended the powder in a final volume of 10µl with addition of deionized water. The reaction was incubated at 37°C for 15 minutes and then 50°C for 15 minutes. Three µl of the reaction products were used to transform *E. coli* TOP10 competent cells (TIANGEN Biotech (Beijing) Co. Ltd., Beijing, China). An *E. coli* transformant containing pCat_PE04230007241 was
35 detected by colony PCR. Colony PCR is a method for quick screening of plasmid inserts directly from *E. coli* colonies. Briefly, in the premixed PCR solution aliquot in each PCR tube, including

PCR buffer, MgCl₂, dNTPs, and primer pairs from which the PCR fragment was generated, a single colony was added by picking with a sterile tip and twirling the tip in the reaction solution. Normally 7-10 colonies were screened. After the PCR, reactions were analyzed by 1.0% agarose gel electrophoresis using TBE buffer. The plasmid DNA was prepared using a QIAprep Spin Miniprep Kit (QIAGEN Inc., Valencia, CA, USA). The *Penicillium emersonii* catalase gene inserted in pCat_PE04230007241 was confirmed by DNA sequencing using a 3730XL DNA Analyzer (Applied Biosystems Inc, Foster City, CA, USA).

Example 24: Expression of *Penicillium emersonii* catalase gene in *Aspergillus oryzae*

Aspergillus oryzae HowB101 (described in patent WO9535385 example 1) protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422. Three µg of pCat_PE04230007241 were used to transform *Aspergillus oryzae* HowB101.

The transformation of *Aspergillus oryzae* HowB101 with pCat_PE04230007241 yielded about 50 transformants. Four transformants were isolated to individual Minimal medium plates.

Four transformants were inoculated separately into 3 ml of YPM medium in 24-well plate and incubated at 30°C, 150 rpm. After 3 days incubation, 20 µl of supernatant from each culture were analyzed on NuPAGE Novex 4-12% Bis-Tris Gel with 2-(N-morpholino)ethanesulfonic acid (MES) (Invitrogen Corporation, Carlsbad, CA, USA) according to the manufacturer's instructions. The resulting gel was stained with INSTANT BLUE™ (Expedeon Ltd., Babraham Cambridge, UK). SDS-PAGE profiles of the cultures showed that all transformants had a band of approximately 80 kDa. The expression strain was designated as O6YTS.

Example 25: Fermentation of *Aspergillus oryzae* expression strain O6YTS

A slant of the expression strain, O6YTS, was washed with 10 ml of YPM and inoculated into 7 2-liter flasks containing 400 ml of YPM medium to generate broth for characterization of the enzyme. The culture was harvested on day 3 and filtered using a 0.45 µm DURAPORE Membrane (Millipore, Bedford, MA, USA).

Example 26: Purification of recombinant *Penicillium emersonii* catalase from *Aspergillus oryzae* O6YTS

2800 ml supernatant of the recombinant strain O6YTS was precipitated with ammonium sulfate (80% saturation) and re-dissolved in 50 ml 20mM Tris-HCl buffer, pH8.0, then dialyzed against the same buffer and filtered through a 0.45 µm filter, the final volume was 80 ml. The solution was applied to a 40 ml Q SEPHAROSE® Fast Flow column (GE Healthcare, Buckinghamshire, UK) equilibrated in 20mM Tris-HCl buffer, pH8.0. Fractions eluted with 0.18-

0.25M NaCl were evaluated by SDS-PAGE (NP0336BOX, NUPAGE 4-12% BT GEL 1.5MM15W). Fractions containing a band of approximately 80 kDa were pooled. Then the pooled solution was concentrated by ultrafiltration.

5 Example 27: Catalase activity assay

The purified *Penicillium emersonii* catalase was checked for catalase activity by using the following protocol.

10 The substrate was prepared by 1000 times dilution of 30% H₂O₂ (from Xilong Chemical, Guangdong, China) with double distilled H₂O (ddH₂O), the final concentration was 10.3 mM. The reaction was started by adding 1μl of purified *Penicillium emersonii* catalase sample into 1000μl of substrate. The optical density (OD) at 240 nm was read by Ultrospec 3300 (GE Healthcare, Buckinghamshire, UK) at second of 0 and 16 respectively, and the decrease of the OD (from 0.505 to 0.284) showed the relative activity of the *Penicillium emersonii* catalase.

15

The present invention is further described by the following numbered paragraphs:

[1] A method for degrading or converting a cellulosic material, comprising: treating the cellulosic material with an enzyme composition in the presence of a polypeptide having catalase activity.

20 [2] The method of paragraph 1, wherein the enzyme composition comprises one or more (*e.g.*, several) enzymes selected from the group consisting of a cellulase, a GH61 polypeptide having cellulolytic enhancing activity, a hemicellulase, an esterase, an expansin, a laccase, a ligninolytic enzyme, a pectinase, a peroxidase, a protease, and a swollenin.

25 [3] The method of paragraph 2, wherein the cellulase is one or more (*e.g.*, several) enzymes selected from the group consisting of an endoglucanase, a cellobiohydrolase, and a beta-glucosidase.

[4] The method of paragraph 2, wherein the hemicellulase is one or more enzymes selected from the group consisting of a xylanase, an acetylxylan esterase, a feruloyl esterase, an arabinofuranosidase, a xylosidase, and a glucuronidase.

30 [5] The method of any of paragraphs 1-4, wherein the cellulosic material is selected from the group consisting of agricultural residue, herbaceous material, municipal solid waste, pulp and paper mill residue, waste paper, and wood; preferably, arundo, bagasse, bamboo, corn cob, corn fiber, corn stover, miscanthus, orange peel, rice straw, switchgrass, wheat straw, eucalyptus, fir, pine, poplar, spruce, willow, algal cellulose, bacterial cellulose, cotton linter, filter paper, 35 microcrystalline cellulose, or phosphoric-acid treated cellulose.

[6] The method of any of paragraphs 1-5, wherein the cellulosic material is pretreated, especially by chemical pretreatment, physical pretreatment, or biochemical pretreatment.

[7] The method of any of paragraphs 1-6, further comprising recovering the degraded cellulosic material.

5 [8] The method of paragraph 7, wherein the degraded cellulosic material is a sugar.

[9] The method of paragraph 8, wherein the sugar is selected from the group consisting of glucose, xylose, mannose, galactose, and arabinose.

[10] The method of any of paragraphs 1-9, wherein the presence of the polypeptide having catalase activity increases the hydrolysis of the cellulosic material compared to the absence of the
10 polypeptide having catalase activity.

[11]. The method of any of paragraphs 1-10, wherein the polypeptide having catalase activity is selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to the mature polypeptide of SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6,
15 or the mature polypeptide of SEQ ID NO: 8;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low, medium, medium-high, high, or very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 7, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii);

20 (c) a polypeptide encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 1, the mature polypeptide coding sequence of SEQ ID NO: 3, the mature polypeptide coding sequence of SEQ ID NO: 5, or the mature polypeptide coding sequence of SEQ ID NO: 7; or the cDNA sequence thereof;

(d) a variant of the mature polypeptide of SEQ ID NO: 2, a variant of the mature polypeptide of SEQ ID NO: 4, a variant of the mature polypeptide of SEQ ID NO: 6, or a variant of
25 the mature polypeptide of SEQ ID NO: 8, comprising a substitution, deletion, and/or insertion at one or more (e.g., several) positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has catalase activity.

[12] The method of any of paragraphs 1-11, wherein the polypeptide having catalase
30 activity is a catalase from *Thermoascus*, *Talaromyces*, *Humicola*, or *Penicillium*.

[13] The method of paragraph 12, wherein the polypeptide having catalase activity is a catalase from *Thermoascus aurantiacus*, *Talaromyces stipitatus*, *Humicola insolens*, or *Penicillium emersonii*.

[14] A method for producing a fermentation product, comprising:

35 (a) saccharifying a cellulosic material with an enzyme composition in the presence of a polypeptide having catalase activity;

(b) fermenting the saccharified cellulosic material with one or more (e.g., several) fermenting microorganisms to produce the fermentation product; and

(c) recovering the fermentation product from the fermentation.

5 [15] The method of paragraph 14, wherein the enzyme composition comprises one or more (e.g., several) enzymes selected from the group consisting of a cellulase, a GH61 polypeptide having cellulolytic enhancing activity, a hemicellulase, an esterase, an expansin, a laccase, a ligninolytic enzyme, a pectinase, a peroxidase, a protease, and a swollenin.

10 [16] The method of paragraph 15, wherein the cellulase is one or more (e.g., several) enzymes selected from the group consisting of an endoglucanase, a cellobiohydrolase, and a beta-glucosidase.

[17] The method of paragraph 15, wherein the hemicellulase is one or more enzymes selected from the group consisting of a xylanase, an acetylxylan esterase, a feruloyl esterase, an arabinofuranosidase, a xylosidase, and a glucuronidase.

15 [18] The method of any of paragraphs 14-17, wherein the cellulosic material is selected from the group consisting of agricultural residue, herbaceous material, municipal solid waste, pulp and paper mill residue, waste paper, and wood; preferably, arundo, bagasse, bamboo, corn cob, corn fiber, corn stover, miscanthus, orange peel, rice straw, switchgrass, wheat straw, eucalyptus, fir, pine, poplar, spruce, willow, algal cellulose, bacterial cellulose, cotton linter, filter paper, microcrystalline cellulose, or phosphoric-acid treated cellulose.

20 [19] The method of any of paragraphs 14-18, wherein the cellulosic material is pretreated, especially by chemical pretreatment, physical pretreatment, or biochemical pretreatment; or wherein steps (a) and (b) are performed simultaneously in a simultaneous saccharification and fermentation.

25 [20] The method of any of paragraphs 14-19, wherein the fermentation product is an alcohol, an alkane, a cycloalkane, an alkene, an amino acid, a gas, isoprene, a ketone, an organic acid, or polyketide.

[21] The method of any of paragraphs 14-20, wherein the presence of the polypeptide having catalase activity increases the hydrolysis of the cellulosic material compared to the absence of the polypeptide having catalase activity.

30 [22]. The method of any of paragraphs 14-21, wherein the polypeptide having catalase activity is selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to the mature polypeptide of SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6, or the mature polypeptide of SEQ ID NO: 8;

35 (b) a polypeptide encoded by a polynucleotide that hybridizes under low, medium, medium-high, high, or very high stringency conditions with (i) the mature polypeptide coding

sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 7, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii);

(c) a polypeptide encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 1, the mature polypeptide coding sequence of SEQ ID NO: 3, the mature polypeptide coding sequence of SEQ ID NO: 5, or the mature polypeptide coding sequence of SEQ ID NO: 7; or the cDNA sequence thereof;

(d) a variant of the mature polypeptide of SEQ ID NO: 2, a variant of the mature polypeptide of SEQ ID NO: 4, a variant of the mature polypeptide of SEQ ID NO: 6, or a variant of the mature polypeptide of SEQ ID NO: 8, comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has catalase activity.

[23] The method of any of paragraphs 14-22, wherein the polypeptide having catalase activity is a catalase from *Thermoascus*, *Talaromyces*, *Humicola*, or *Penicillium*.

[24] The method of paragraph 23, wherein the polypeptide having catalase activity is a catalase from *Thermoascus aurantiacus*, *Talaromyces stipitatus*, *Humicola insolens*, or *Penicillium emersonii*.

[25] A method of fermenting a cellulosic material, comprising: fermenting the cellulosic material with one or more (*e.g.*, several) fermenting microorganisms, wherein the cellulosic material is hydrolyzed with an enzyme composition in the presence of a polypeptide having catalase activity.

[26] The method of paragraph 25, wherein the enzyme composition comprises one or more (*e.g.*, several) enzymes selected from the group consisting of a cellulase, a GH61 polypeptide having cellulolytic enhancing activity, a hemicellulase, an esterase, an expansin, a laccase, a ligninolytic enzyme, a pectinase, a peroxidase, a protease, and a swollenin.

[27] The method of paragraph 26, wherein the cellulase is one or more (*e.g.*, several) enzymes selected from the group consisting of an endoglucanase, a cellobiohydrolase, and a beta-glucosidase.

[28] The method of paragraph 26, wherein the hemicellulase is one or more enzymes selected from the group consisting of a xylanase, an acetylxytan esterase, a feruloyl esterase, an arabinofuranosidase, a xylosidase, and a glucuronidase.

[29] The method of any of paragraphs 25-28, wherein the cellulosic material is selected from the group consisting of agricultural residue, herbaceous material, municipal solid waste, pulp and paper mill residue, waste paper, and wood; preferably, arundo, bagasse, bamboo, corn cob, corn fiber, corn stover, miscanthus, orange peel, rice straw, switchgrass, wheat straw, eucalyptus, fir, pine, poplar, spruce, willow, algal cellulose, bacterial cellulose, cotton linter, filter paper, microcrystalline cellulose, or phosphoric-acid treated cellulose.

[30] The method of any of paragraphs 25-29, wherein the cellulosic material is pretreated, especially by chemical pretreatment, physical pretreatment, or biochemical pretreatment.

[31] The method of any of paragraphs 25-30, wherein the fermentation produces a fermentation product.

5 [32] The method of paragraph 31, further comprising recovering the fermentation product.

[33] The method of paragraph 32, wherein the fermentation product is an alcohol, an alkane, a cycloalkane, an alkene, an amino acid, a gas, isoprene, a ketone, an organic acid, or polyketide.

10 [34] The method of any of paragraphs 25-33, wherein the presence of the polypeptide having catalase activity increases the hydrolysis of the cellulosic material compared to the absence of the polypeptide having catalase activity.

[35] The method of any of paragraphs 25-34, wherein the polypeptide having catalase activity is selected from the group consisting of:

15 (a) a polypeptide having at least 60% sequence identity to the mature polypeptide of SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6, or the mature polypeptide of SEQ ID NO: 8;

20 (b) a polypeptide encoded by a polynucleotide that hybridizes under low, medium, medium-high, high, or very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 7, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii);

(c) a polypeptide encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 1, the mature polypeptide coding sequence of SEQ ID NO: 3, the mature polypeptide coding sequence of SEQ ID NO: 5, or the mature polypeptide coding sequence of SEQ ID NO: 7; or the cDNA sequence thereof;

25 (d) a variant of the mature polypeptide of SEQ ID NO: 2, a variant of the mature polypeptide of SEQ ID NO: 4, a variant of the mature polypeptide of SEQ ID NO: 6, or a variant of the mature polypeptide of SEQ ID NO: 8, comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has catalase activity.

30 [36] The method of any of paragraphs 25-35, wherein the polypeptide having catalase activity is a catalase from *Thermoascus*, *Talaromyces*, *Humicola*, or *Penicillium*.

[37] The method of paragraph 36, wherein the polypeptide having catalase activity is a catalase from *Thermoascus aurantiacus*, *Talaromyces stipitatus*, *Humicola insolens*, or *Penicillium emersonii*.

35 [38] An enzyme composition for degrading or converting a cellulosic material comprising one or more (*e.g.*, several) enzymes having cellulolytic and/or hemicellulolytic activity and a

polypeptide having catalase activity.

[39] The enzyme composition of paragraph 38, further comprising one or more (e.g., several) enzymes selected from the group consisting of a GH61 polypeptide having cellulolytic enhancing activity, an esterase, an expansin, a laccase, a ligninolytic enzyme, a pectinase, a peroxidase, a protease, and a swollenin.

[40] The enzyme composition of paragraph 38 or 39, wherein the enzymes having cellulolytic activity are selected from the group consisting of an endoglucanase, a cellobiohydrolase, and a beta-glucosidase.

[41] The enzyme composition of any of paragraphs 38-40, wherein the enzymes having hemicellulolytic activity are selected from the group consisting of a xylanase, an acetylxylan esterase, a feruloyl esterase, an arabinofuranosidase, a xylosidase, and a glucuronidase.

[42] The enzyme composition of any of paragraphs 38-41, wherein the polypeptide having catalase activity is selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to the mature polypeptide of SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6, or the mature polypeptide of SEQ ID NO: 8;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low, medium, medium-high, high, or very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 7, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii);

(c) a polypeptide encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 1, the mature polypeptide coding sequence of SEQ ID NO: 3, the mature polypeptide coding sequence of SEQ ID NO: 5, or the mature polypeptide coding sequence of SEQ ID NO: 7; or the cDNA sequence thereof;

(d) a variant of the mature polypeptide of SEQ ID NO: 2, a variant of the mature polypeptide of SEQ ID NO: 4, a variant of the mature polypeptide of SEQ ID NO: 6, or a variant of the mature polypeptide of SEQ ID NO: 8, comprising a substitution, deletion, and/or insertion at one or more (e.g., several) positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has catalase activity.

[43] The enzyme composition of any of paragraphs 38-42, wherein the polypeptide having catalase activity is a catalase from *Thermoascus*, *Talaromyces*, *Humicola*, or *Penicillium*.

[44] The enzyme composition of paragraph 43, wherein the polypeptide having catalase activity is a catalase from *Thermoascus aurantiacus*, *Talaromyces stipitatus*, *Humicola insolens*, or *Penicillium emersonii*.

[45] Use of the enzyme composition of any of paragraphs 38-44 in degrading or converting a cellulosic material.

[46] The use of paragraph 45, wherein the cellulosic material is selected from the group consisting of agricultural residue, herbaceous material, municipal solid waste, pulp and paper mill residue, waste paper, and wood; preferably, arundo, bagasse, bamboo, corn cob, corn fiber, corn stover, miscanthus, orange peel, rice straw, switchgrass, wheat straw, eucalyptus, fir, pine, poplar, spruce, willow, algal cellulose, bacterial cellulose, cotton linter, filter paper, microcrystalline cellulose, or phosphoric-acid treated cellulose.

[47] The use of paragraph 45 or 46, wherein the cellulosic material is pretreated, especially by chemical pretreatment, physical pretreatment, or biochemical pretreatment.

[48] A whole broth formulation or cell culture composition comprising one or more (e.g., several) enzymes having cellulolytic and/or hemicellulolytic activity and a polypeptide having catalase activity.

The invention described and claimed herein is not to be limited in scope by the specific aspects herein disclosed, since these aspects are intended as illustrations of several aspects of the invention. Any equivalent aspects are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. In the case of conflict, the present disclosure including definitions will control.

CLAIMS

What is claimed is:

- 5 1. A method for degrading or converting a cellulosic material, comprising: treating the cellulosic material with an enzyme composition in the presence of a polypeptide having catalase activity.
2. The method of claim 1, wherein the enzyme composition comprises one or more (*e.g.*, several) enzymes selected from the group consisting of a cellulase, a GH61 polypeptide having cellulolytic enhancing activity, a hemicellulase, an esterase, an expansin, a laccase, a ligninolytic enzyme, a pectinase, a peroxidase, a protease, and a swollenin.
- 10 3. The method of claim 1 or 2, wherein the cellulosic material is selected from the group consisting of agricultural residue, herbaceous material, municipal solid waste, pulp and paper mill residue, waste paper, and wood; preferably, arundo, bagasse, bamboo, corn cob, corn fiber, corn stover, miscanthus, orange peel, rice straw, switchgrass, wheat straw, eucalyptus, fir, pine, poplar, spruce, willow, algal cellulose, bacterial cellulose, cotton linter, filter paper, microcrystalline cellulose, or phosphoric-acid treated cellulose.
- 15 4. The method of any of claims 1-3, wherein the cellulosic material is pretreated, especially by chemical pretreatment, physical pretreatment, or biochemical pretreatment.
- 20 5. The method of any of claims 1-4, wherein the polypeptide having catalase activity is selected from the group consisting of:
- (a) a polypeptide having at least 60% sequence identity to the mature polypeptide of SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6, or the mature polypeptide of SEQ ID NO: 8;
- 25 (b) a polypeptide encoded by a polynucleotide that hybridizes under low, medium, medium-high, high, or very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 7, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii);
- (c) a polypeptide encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 1, the mature polypeptide coding sequence of SEQ ID NO: 3, the mature polypeptide coding sequence of SEQ ID NO: 5, or the mature polypeptide coding sequence of SEQ ID NO: 7; or the cDNA sequence thereof;
- 30

(d) a variant of the mature polypeptide of SEQ ID NO: 2, a variant of the mature polypeptide of SEQ ID NO: 4, a variant of the mature polypeptide of SEQ ID NO: 6, or a variant of the mature polypeptide of SEQ ID NO: 8, comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions; and

5 (e) a fragment of the polypeptide of (a), (b), (c), or (d) that has catalase activity.

6. The method of any of claims 1-5, wherein the polypeptide having catalase activity is a catalase from *Thermoascus*, *Talaromyces*, *Humicola*, or *Penicillium*.

7. A method for producing a fermentation product, comprising:

10 (a) saccharifying a cellulosic material with an enzyme composition in the presence of a polypeptide having catalase activity;

(b) fermenting the saccharified cellulosic material with one or more (*e.g.*, several) fermenting microorganisms to produce the fermentation product; and

(c) recovering the fermentation product from the fermentation.

15 8. The method of claim 7, wherein the enzyme composition comprises one or more (*e.g.*, several) enzymes selected from the group consisting of a cellulase, a GH61 polypeptide having cellulolytic enhancing activity, a hemicellulase, an esterase, an expansin, a laccase, a ligninolytic enzyme, a pectinase, a peroxidase, a protease, and a swollenin.

20 9. The method of claim 7 or 8, wherein the cellulosic material is selected from the group consisting of agricultural residue, herbaceous material, municipal solid waste, pulp and paper mill residue, waste paper, and wood; preferably, arundo, bagasse, bamboo, corn cob, corn fiber, corn stover, miscanthus, orange peel, rice straw, switchgrass, wheat straw, eucalyptus, fir, pine, poplar, spruce, willow, algal cellulose, bacterial cellulose, cotton linter, filter paper, microcrystalline cellulose, or phosphoric-acid treated cellulose.

25 10. The method of any of claims 7-9, wherein the fermentation product is an alcohol, an alkane, a cycloalkane, an alkene, an amino acid, a gas, isoprene, a ketone, an organic acid, or polyketide.

11. The method of any of claims 7-10, wherein the presence of the polypeptide having catalase activity increases the hydrolysis of the cellulosic material compared to the absence of the polypeptide having catalase activity.

12. The method of any of claims 7-11, wherein the polypeptide having catalase activity is selected

from the group consisting of:

- (a) a polypeptide having at least 60% sequence identity to the mature polypeptide of SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6, or the mature polypeptide of SEQ ID NO: 8;
- 5 (b) a polypeptide encoded by a polynucleotide that hybridizes under low, medium, medium-high, high, or very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 7, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii);
- (c) a polypeptide encoded by a polynucleotide having at least 60% sequence identity to
10 the mature polypeptide coding sequence of SEQ ID NO: 1, the mature polypeptide coding sequence of SEQ ID NO: 3, the mature polypeptide coding sequence of SEQ ID NO: 5, or the mature polypeptide coding sequence of SEQ ID NO: 7; or the cDNA sequence thereof;
- (d) a variant of the mature polypeptide of SEQ ID NO: 2, a variant of the mature polypeptide of SEQ ID NO: 4, a variant of the mature polypeptide of SEQ ID NO: 6, or a variant of
15 the mature polypeptide of SEQ ID NO: 8, comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions; and
- (e) a fragment of the polypeptide of (a), (b), (c), or (d) that has catalase activity.

13. The method of any of claims 7-12, wherein the polypeptide having catalase activity is a catalase from *Thermoascus*, *Talaromyces*, *Humicola*, or *Penicillium*.

20 14. A method of fermenting a cellulosic material, comprising: fermenting the cellulosic material with one or more (*e.g.*, several) fermenting microorganisms, wherein the cellulosic material is hydrolyzed with an enzyme composition in the presence of a polypeptide having catalase activity.

15. The method of claim 14, wherein the enzyme composition comprises one or more (*e.g.*, several) enzymes selected from the group consisting of a cellulase, a GH61 polypeptide having
25 cellulolytic enhancing activity, a hemicellulase, an esterase, an expansin, a laccase, a ligninolytic enzyme, a pectinase, a peroxidase, a protease, and a swollenin.

16. The method of claim 14 or 15, wherein the cellulosic material is selected from the group consisting of agricultural residue, herbaceous material, municipal solid waste, pulp and paper mill residue, waste paper, and wood; preferably, arundo, bagasse, bamboo, corn cob, corn fiber, corn
30 stover, miscanthus, orange peel, rice straw, switchgrass, wheat straw, eucalyptus, fir, pine, poplar, spruce, willow, algal cellulose, bacterial cellulose, cotton linter, filter paper, microcrystalline cellulose, or phosphoric-acid treated cellulose.

17. The method of any of claims 14-16, wherein the polypeptide having catalase activity is selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to the mature polypeptide of SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6, or the mature polypeptide of SEQ ID NO: 8;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low, medium, medium-high, high, or very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 7, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii);

(c) a polypeptide encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 1, the mature polypeptide coding sequence of SEQ ID NO: 3, the mature polypeptide coding sequence of SEQ ID NO: 5, or the mature polypeptide coding sequence of SEQ ID NO: 7; or the cDNA sequence thereof;

(d) a variant of the mature polypeptide of SEQ ID NO: 2, a variant of the mature polypeptide of SEQ ID NO: 4, a variant of the mature polypeptide of SEQ ID NO: 6, or a variant of the mature polypeptide of SEQ ID NO: 8, comprising a substitution, deletion, and/or insertion at one or more (e.g., several) positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has catalase activity.

18. The method of any of claims 14-17, wherein the polypeptide having catalase activity is a catalase from *Thermoascus*, *Talaromyces*, *Humicola*, or *Penicillium*.

19. An enzyme composition for degrading or converting a cellulosic material comprising one or more (e.g., several) enzymes having cellulolytic and/or hemicellulolytic activity and a polypeptide having catalase activity.

20. The enzyme composition of claim 19, further comprising one or more (e.g., several) enzymes selected from the group consisting of a GH61 polypeptide having cellulolytic enhancing activity, an esterase, an expansin, a laccase, a ligninolytic enzyme, a pectinase, a peroxidase, a protease, and a swollenin.

21. The enzyme composition of claim 19 or 20, wherein the polypeptide having catalase activity is selected from the group consisting of:

(a) a polypeptide having at least 60% sequence identity to the mature polypeptide of SEQ ID NO: 2, the mature polypeptide of SEQ ID NO: 4, the mature polypeptide of SEQ ID NO: 6, or the mature polypeptide of SEQ ID NO: 8;

(b) a polypeptide encoded by a polynucleotide that hybridizes under low, medium, medium-high, high, or very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 7, (ii) the cDNA sequence thereof, or (iii) the full-length complement of (i) or (ii);

5 (c) a polypeptide encoded by a polynucleotide having at least 60% sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 1, the mature polypeptide coding sequence of SEQ ID NO: 3, the mature polypeptide coding sequence of SEQ ID NO: 5, or the mature polypeptide coding sequence of SEQ ID NO: 7; or the cDNA sequence thereof;

10 (d) a variant of the mature polypeptide of SEQ ID NO: 2, a variant of the mature polypeptide of SEQ ID NO: 4, a variant of the mature polypeptide of SEQ ID NO: 6, or a variant of the mature polypeptide of SEQ ID NO: 8, comprising a substitution, deletion, and/or insertion at one or more (e.g., several) positions; and

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has catalase activity.

15 22. The enzyme composition of any of claims 19-21, wherein the polypeptide having catalase activity is a catalase from *Thermoascus*, *Talaromyces*, *Humicola*, or *Penicillium*.

23. Use of the enzyme composition of any of claims 19-22 in degrading or converting a cellulosic material.

20 24. The use of claim 23, wherein the cellulosic material is selected from the group consisting of agricultural residue, herbaceous material, municipal solid waste, pulp and paper mill residue, waste paper, and wood; preferably, arundo, bagasse, bamboo, corn cob, corn fiber, corn stover, miscanthus, orange peel, rice straw, switchgrass, wheat straw, eucalyptus, fir, pine, poplar, spruce, willow, algal cellulose, bacterial cellulose, cotton linter, filter paper, microcrystalline cellulose, or phosphoric-acid treated cellulose.

25 25. A whole broth formulation or cell culture composition comprising one or more (e.g., several) enzymes having cellulolytic and/or hemicellulolytic activity and a polypeptide having catalase activity.

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      M R G A Y S L G A F A S L I A V A S A A
1  atgcgaggggcatactctctcggcgccctttgccagttctcatcgcggtagcttcgggtgcc
      C P M L T G E I P A G S I A N P H H L G
61  tgcccaatgctgactggcgaaatcccagcaggcagcattgcaaaccctcatcaccttgga
      S R A D S N A S D E T E A F L S E F Y L
121  agccgcgctgactcgaatgcttcggacgaaacagaagcctttctgtccgaattctacctt
      N D N N S F L T T D V G G P I E D Q N S
181  aatgacaacaacagcttcctcactaccgatgtggcgcccgatagaagacaaaaacagt
      L K A G I R G S T L L E D F I F R Q K I
241  ctcaaggccggcattcgcggatcaacgctcttgaggatttcatctttcgccagaagatt
      Q R F D H E R
301  cagcgctttgatcacgagcgtGTAAGTTCTTGAAATCATATGACTACTTCGATGTGTACT
      V P E R A V H A R G A G A H G V
361  TACGACTTCTAGgtgccgaacgcgctgtgcatgctcgaggtgctggtgctcatggtgta
      F T S Y A D W S N I T A A S F L G A A G
421  ttcacatcgatgctgattggtccaacatcacgcgctgcttcattcctaggagctgccgga
      K E T P T F V R F S T V A G S R G S A D
481  aaggaacgcccacctttgtacgcttctcgaactgttgccggcagtcgtggtagtgccgat
      T A R D V H G F A T R F Y T D E G N Y
541  accgctcgtgatgttcacggctttgctacccgcttctatactgacgaaggcaactacgGT
      D I V G
601  AAGATCTATCCATGGTCATAGCAGCCTATACATTTGCTAACTCACAGCAGatctcgttgg
      N N I P V F F I Q D A I Q F P D L I H A
661  aaacaacattcccgctcttctcatccaagacgtattcagttccctgacctcattcatgc
      V K P Q P A S E I P Q A A T A H D T A Y
721  agtcaagccacagccagccagtgaaatcccacaggccgctactgcccacgacactgctta
      D F F G Q Q P S T L H T L F W A M A G H
781  tgattttcttcggccagcagcctagtaccttgcataccctcttctgggcaatggcagggtca
      G I P R S F R H V D G F G V H A Y R F V
841  tggtatcccgcggtctttccgcatgttgacggatttcggcggttcacgcttaccgatttgt
      T D D G S S K L V K F H W K S L Q G R A
901  gactgacgacggctcttcaaagctagtcaaattccactggaagtccttacagggtcgtgc
      S L V W E E A Q A T A G K N A D F M R Q
961  aagcttagtctggaagaagctcaagccactgctggcaagaatgctgatttcatgaggca
      D L F D N I A A G R Y P E W E
1021  agatctgtttgacaatatattgcagctggccggtaccagaatgggagGTGAGTATAAGTTG
      L G V Q L I E E P
1081  CCTGTCCTCCGAAGAATTTCTACTAACATGAATAGctcggcggtgcaacttatcgaggaacc
      D Q L S Y G F D L L D P T K I L P V E Q
1141  agaccagctcagctacggattttgatctgcttgatcccaccaagatactcccagttgaaca
      V P I T P L G K M Q L N R N P L N Y F A
1201  agttccaatcaccccgctcggaataatgcaactcaaccgtaaccgctaaactactttgc
      E T E Q V M
1261  tgagaccgagcaagtaatgGTACGTTAACTTCTCTTCTCCCCCTCCCCCAACCAACTCC
      F Q P G H I V R G I D
1321  GGGTACAGCTCATGCTGATCATTTTAGttccaacctgggtcacattgttcgtggcattgat
      F T E D P L L Q G R L F S Y L D T Q L N
1381  ttcacagaggacctcttctccaaggccgctctattttcttacctcgacactcagcttaat
      R N G G P N F E Q I P I N R P R V P I H
1441  cgcaacgggtggcccaactttgagcagattccgatcaaccgctcctcgtgttcctatccac
      N N N R D G F G Q M F I P L N D A A Y S
1501  aataacaaccgagacgggttttgccagatgtttattccactcaacgatgcgggcatactcg

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Fig. 1

P N T L S D G N P K Q A N E T V G N G F
1561 ccaaaccaccctaagcgatggcaaccctaagcaggcaaagagactggtggaaatggtttc
F T T P G R T A N G N L V R A K S P T F
1621 ttactactccaggacgcactgccaatggaaacctcgctccgcgccccaaagcccaacgttt
A D V W S Q P G L F Y N S L T A T E Q Q
1681 gcggatgtgtggtcccaacctggcctcttttacaactccttgacagccaccgaacaacag
F V I N A L R F E L A N V A S E T V K N
1741 tttgtcatcaatgctctgcggttcgagctagccaatgtagcaagtgagactgtgaagaat
N F I T Q I N R V N N T L A T L V A T A
1801 aacttcatacaccagatcaatcgcgtaaacacacacttggaacactttagccactgca
I G V N A P E P D P T Y Y H H N K T S D
1861 attggtgtcaatgctcctgaacccgacccgacttactaccaccacaacaagacgtctgat
V G T F G T P L K K I D G L K V G V L A
1921 gtgggaacattcggtactcctctgaagaagattgatggtctcaaggtcggagtccttgct
S V N D E N S I S E G Q S L A R S L A D
1981 tctgtcaacgatgaaaacagtatcttcgagggacagtcctctagcacgaagcttgccggtat
L N V D V V I V A E R L A G N V S A T Y
2041 ttgaatgtggacgtcggttattgtcgctgaacgacttgctggtaatgtctcagctacatac
S A S D A I N F D A V I V T S G A K G L
2101 tccgcatctgacgctatcaacttcgatgctgttattgtcacttcaggggctaagggtctc
F G P Q T F T A V S N T T L Y P V G R P
2161 ttggacctcaaaccttcaccgcgctatccaacaccactctttatcccggtgggcccgtccc
T Q I L V D A F R Y G K P V G A V G S A
2221 acgcagatcttggcgacgctttccgctacggcaagccggttgagcagtggttagtgca
S E A L T V S D I D T D R S G V I T G D
2281 agcgaagcgctgactgtttcggacattgatactgaccgcagtggtgtgattactggtgat
L N D E F V K Q L S E D L A T F K F L D
2341 ttgaacgacgagtttgtgaagcaactgtcggaggaccttgcaacattcaagttccttgac
R F T V D E *
2401 cgcttcaccgtcgacgagtag

Fig. 1 continued

M N R V T N L L A W A G A I G L A Q A T
 1 atgaacagagtcacgaatctcctcgccctggcgccggcgatagggctcgcccaagcaaca
 C P F A D P A A L Y R R Q D T T S G Q S
 61 tgccccctttgcggaacctgcccgtctgtataggcgtcaagatactaccagcgccagtcg
 P L A A Y E V D D S T G Y L T S D V G G
 121 ccacttgacagcagaggtggatgacagcaccggatacctgacctccgatgttgccggg
 P I Q D Q T S L K A G I R G P T L L E D
 181 cccattcaggaccagaccagcctcaaggcaggcatccgggggtccgaccttcttgaggac
 F M F R Q K I Q H F D H E R
 241 tttatgttccgccagaagatccagcacttcgacctgaacggGTAAGGACATAATGCTCA
 301 CACGAGCGGCTGCGTACCTATTTATTTCCGAGACATTGGGCTGGCTGGCTGGCTGTGACT
 V P
 361 GCCTGAGTTTGGGGACATACGGAGTACCTTACTGACGCGCTGATCCACTCCAGgttcccg
 E R A V H A R G A G A H G T F T S Y A D
 421 aaaggcggtccatgctcgaggcgctggagcacacgggaccttcacgagttacgcccgaact
 W S N I T A A S F L N A T G K Q T P V F
 481 ggagtaacatcacagcggtcctttctgaacgccacaggaaagcagacgcccgtgtttg
 V R F S T V A G S R G S A D T A R D V H
 541 tccggttctcgacctgtgctgggtctcgaggagcgcagacacggcgagagacgttcatg
 G F A T R F
 601 gtttcgcgacgcggttGTAAGTTTTGTTGTGTTTCATTTCGTTCCGGTCTGTAGAGGAGGG
 Y T D E
 661 TTAGGATATGAGCTAATGTGTGTGTGTGTGTGTGTGTGTGAAGttacactgatgaa
 G N F
 721 ggcaacttttgGTACGTCCCATGCATGGTCTCAATTCTCTTATCTGGCAGCGATGTGGTC
 D I V G N N I P V F F I
 781 ATTGTCGACGTTGCTAAGTTGCGTAGatatacgtcgaaacaacatcccggtattcttcat
 Q D A I Q F P D L I H S V K P R P D N E
 841 tcaagatgcaatccagttccctgaccttatccactcggtcaagccgctcccgacaacga
 I P Q A A T A H D S A W D F F S Q Q P S
 901 gattccccaagcgcgacgggtcatgattcagcttgggacttcttcagccagcagccaag
 T M
 961 caccatgGTAAGCAATGGACCAAGGAGCCGCACCTGGGGTGACATGCCAGGGAGTACAG
 1021 GAGCGTTCCGATGACTCTCGTGTGACCAAGGCAGTACAACACTCCACGAGGACTCGAAG
 H T L F W A M
 1081 AGATTCGGAATATGGAACACAGAACTGACAGGATGGTAGcacacgttgttctgggccat
 S G H G I P R S Y R H M
 1141 gtctggccacggaatccctcgagctatcgccatatgGTACGTTTGCCTGGCTGAGATGA
 D G F G V H T F R F
 1201 CCGTGAATCCATTTCTAACCTCAAGTCCAGgatggcttcggcggtccacacgttccggttt
 V K D D G S S K L I K W H F K S R Q G K
 1261 gtcaaagatgacggctcgtccaagttgatcaagtggcatttcaagtcacgccagggaag
 A S L V W E E A Q V L S G K N A D F H R
 1321 gcgagtctagtctgggaagaggcgaggttctgtctggcaagaatgccgacttccaccgt
 Q D L W D A I E S G N G P E W D V C V Q
 1381 caggacctctgggatgctattgagtcgggaacggaccagaatgggatgtctgcgtccag
 I V D E S Q A Q A F G F D L L D P T K I
 1441 attgtcgatgagtcacggcgcaagcctttggcttcgacttgctggacccgacaaagatc
 I P E E Y A P L T K L G L L K L D R N P
 1501 atccccgaggagtacgcccccttgacgaagctgggcctcttgaaactggatcgcaatccg

Fig. 2

T N Y F A E T E Q V M F Q P G H I V R G
 1561 accaactacttcgccgagacggagcaggtcatgttccaaccgggtcatatcgtccgcggc
 I D F T E D P L L Q G R L F S Y L D T Q
 1621 atcgacttcacggaggatccccctgctacagggaagctctcttctcgtaccttgacacgcag
 L N R N G G P N F E Q L P I N M P R V P
 1681 ctgaaccggaatggcggggcccaacttttgagcagctgccccatcaacatgccgcggtgccg
 I H N N N R D G A G Q M F I H R N K Y P
 1741 attcacaacaataatcgcgacggcgccggccagatgttcatccacaggaacaagtatcct
 Y T
 1801 tGTAAGTACCTCTTTTGCCCTCGATCGTTGTGGTGCCGGCTTGCTGACAGACGCAGacact
 P N T L N S G Y P R Q A N Q N A G R G F
 1861 cccaacaccctgaacagtggttatccgcggaagccaacaaaatgccggacgcggattc
 F T A P G R T V S G A L V R E V S P T F
 1921 ttcacagcgctggccgtaccgtcagcggtgccctcgtccgtgaggtgtcgccaacattc
 N D H W S Q P R L F F N S L T P V E Q Q
 1981 aacgaccactggtcgcagccccgtctcttcttcaactccctcactcccgctcgaacagcag
 F L V N A M R F E I S L V K S E E V R K
 2041 ttctcgtcaacgccatgcgcttcgaaatcagccttgtagagtcggaagaagtcaggaag
 N V L T Q L N R V S H D V A G R V A A A
 2101 aacgtgctcaccagctcaaccgcgtcagccatgatgtggcggggcgcggtggccgcccgt
 I G L A A P D A D D T Y Y H N N K T A G
 2161 atcgccctcgccgcgcccgcgacgcggacgacacatactaccacaacaacaagacgggtggc
 V S I L G S G P L P T I K T L R V G I L
 2221 gtctcgatccttggaagcggggcccttgccatcaagactctccgctcggtcggtatcctg
 A T T S E S S A L D Q A A Q L R T R L E
 2281 gctaccacgagcagtcgagcgcgctggatcaggcagcccagctccgcacccgtctggaa
 K D G L V V T V V A E T L R E G V D Q T
 2341 aaggacgggcttggtgcaggttggtggtgaaacgctgcgcgaggggtagaccagaca
 Y S T A D A T G F D G V V V V D G A A A
 2401 tactcgacggcgatgccacgggtttcgacggcggttggtgttggtggacggggcgcgcg
 L F A S T A S S P L F P T G R P L Q I F
 2461 ctgtttgccagcaccgcgtcgtcgccgttggtcccgacgggcaggccgttgacagatcttt
 V D A Y R W G K P V G V C G G K S S E V
 2521 gtggacgcgtatcggtggggaaagccggtcggtgtgtgtggtgggaagtcgagcaggtg
 L D A A D V P E N G D G V Y S E E S V D
 2581 ttggatgcgccggtgttccggaaaatggggacggggtgtattcggaggagtcggtggac
 K F V E E F E K G L A T F R
 2641 aagtttggtgaggagtttgagaaggggttggtactttcaggGTGAGTCTTGGTGCCTTT
 2701 GTTTTTTGTGATGTTATTGTTTTGTTTCGTCTCGGACTTTGTGAAAGAATGACGGACTGA
 2761 CGTCTTTGGTATCTAGTTTACCGATCGGTTTGCTCTCGACTCTTAGGAGGACGAATGGAC
 E S E L
 2821 AGAAAGTGAGACCGAGAGTGACTCAGAGACTGAGTTGGAGTCGGAATAGgaatcggaatt
 E S E S G S E S G S E N E F C I Q E V D
 2881 ggaatcgagtcagggtcggagtcaggatcagagaatgaattttgtatccaggaagtcga
 G V Y Q I C N H Q E D E D S E D S T S E
 2941 tggggtgtatcagatttgtaatcaccaggaggatgaggattctgaggatagcacttcgga
 E E G D E V E P V I S W G *
 3001 agaggagggagatgaagttgagcctgtcatttcatggggatga

Fig. 2 continued

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      M R A V Q L L P S L A G L I G A A S A V
1  atgcgcgcagtgacagcttctgcccagcctcgccggcctgattggcgctgcctctgccgtt
   G C P Y L T G Q L D A R D V H N P H E F
61 ggatgtccgtatctgacgggccagctcgatgccagagacgtgcacaatccgcacgagttc
   Q R R Q D P G D A A A S T E Q F L S Q F
121 cagcgctcgacaggatcccggagatgcccgtcgctccacagagcagttcctgtcccagttc
   Y L N D S N S Y M T T D V G G P I S D Q
181 tatctcaatgacagcaacagctacatgaccactgatgtcgggggcccatctcggatcag
   N S L K A G E R G P T L L E D F I F R Q
241 aacagtttgaaggccggagagcgggtccaaccctgttgaggacttcatcttccgtcag
   K I Q H F D H E R
301 aagatccagcactttgatcacgagcggGTAGGTTGTACCATCCATGCGAGAGAGATCGAT
      V P E R A V H A R G A G A
361 CGATGTTGACGTGGTGGCAGgtcccagaacgcgcagtcctatgctcgaggagccggcgccc
   H G T F T S Y G N W S N I T A A S F L S
421 acggaacgttccacttccctacgaaactgggtccaacatcactgcccctccttccctgagcg
   A E G K E T P V F V R F S T V A G S R G
481 ctgaagggaaggagacccccgtgtttgtgcgttctccaccgtggccggaagtgcaggca
   S A D T A R D V H G F A T R F Y T D E G
541 gtgcggacacggcgccgcatgtgcattggcttggccaccaggttctacactgacgaggca
   N F D I
601 actttgGTACGTCGTCTCACAATCCTCTCGACTGGCATCGTCTGACCGCTGAGCAGatatt
   V G N N I P V F F I Q D A I L F P D L I
661 cgtcggcaacaacattccagttcttcttccatccaggacgccattctcttccctgatctgat
   H A V K P S P D N E I P Q A A T A H D T
721 ccatgctgtcaagcccagccccgacaacgagatccccaggtgctgcgactgctcatgacac
   A W D F F S Q Q P S A L H T L F W A M S
781 ggctggggaacttcttccagccagcagcccagtgcggttgacacgctcttctgggctatgtc
   G H G I P R S F R H M D G F G V H T F R
841 cggccatggaatccctcgctcttttccgacatggacggctttggcgctccacactttccg
   F V T D D G A S K L V K F H W T S L Q G
901 attcgtgactgacgacggcgccctccaagctgggtcaaattccactggacctgctcaggg
   R A S L V W E E A Q A A A G K N L D Y M
961 ccgggcccagcctggtctgggaggaggcgcaagcggcgagcgggaaagaacctggactatat
   R Q D L Y D N I E A G R Y P E W E
1021 gcgccaggacctctatgacaacatcgaagccgggtcgatatcctgaatgggagGTAGGTGG
      L G I Q I V
1081 CCGCATTTTCTCGGCATATATATGTCCATGCTGACGTTCTAGctgggcattcaaactcgt
   D E E D Q L K F G F D L L D P T K I I P
1141 cgacgaggaggatcagctcaagtttggatttgatctgctggatccaaccaagatcattcc
   V E Y V P I T P L G K L Q L N R N P L N
1201 tgttgaatatgtccccatcacgccgttgggaagctgcagctcaaccggaatccgctcaa
   Y F A E T E Q I M
1261 ctatttgcgcgagacggagcagataatGTATGTAAACAGTTTGTGTTGATTCTTTGC
      F Q P G H I V R G I D F T
1321 AGTAGACTGACGATACATAGttccaaccggccatattgtgcgcggaattgactttaccg
   E D P L L Q G R L F S Y L D T Q L N R N
1381 aagaccccccttctccagggaaggctcttctcctatctcgacacgcagttgaatcggaatg
   G G P N F E Q L P I N R P R V P W H N N
1441 gaggccccaatttgcgacgacttcccatcaatcgctcctaggggtgccatggcataacaaca
   N R D G F
1501 accgtgatggattcaGTAAGTTTACCCCCCTGCGCTGACTCTCTGCATGCTAACTCCACC

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Fig. 3

S Q A F I P L N K A A Y S P N T L N N G
1561 AGGccaagcggtttatccccctgaacaaggcggcctacagcccgaacacgctcaacaatgg
N P K Q A N Q T V G D G F F T T P G R T
1621 caaccccaagcaggcgaaccagactgtggcgatggattcttcaccactcccggacgtac
T S G R L M R T V S S T F S D V W S Q P
1681 gaccagtggccggctcatgcgacccgtcagttcgaccttctccgacgtctggctgcagcc
R L F Y N S L V P A E Q Q F L V N A I R
1741 tcggctgttctacaactcgctgggtgccggccgagcagcagttcctcgtaacgccatccg
F E N S N V K S E V V R N N V I I Q L N
1801 tttcgagaactccaacgtcaagagcggaagtgggtccggaacaatgtcatcatccagctcaa
R V D N D L A R R V A R V I G V A E P E
1861 ccgctcgataacgacctcgcccgccgggtgtcgggtcattggcgttgcagaacccga
P D P T Y Y H N N K T A N V G T F G T P
1921 gcccgatccaacctattatcacacaacaagacggccaacgtgggtacgtttggcacgcc
L K R I D G L K V G V L A T V G D P D S
1981 gctcaagcggatcgacgggtctcaaagtcggtgtgcttgccacagttggcgacccagacag
I S Q G Q S L S D A L S D S K V D V T V
2041 tatcagtcagggccagagcctcagtgacgcgtctcggactccaaggtcgatgtcactgt
V A E S F T D G V D A L Y T N S D A T G
2101 cgttgtgagtcctttcacggacgggtcgatgcgctctacaccaactcggacgcgaccgg
F D A V I V A D G A E G L F T P S S F T
2161 cttcgacgcggttatcggtgggtgatggcgccgaagggtttttaccccgagtagcttcac
A K P T N S F S T T T L Y P A G R P L Q
2221 agccaaaccgacgaactcattctcgacgacaacgctttatccggccgggtcggtccgctgoa
I L V D A F R F G K P V G A L G S G A K
2281 gatcctgggtcgacgccttccggttcggcaagcccgtcggcgctctgggcagcggagctaa
A L D A A G I S T S R P G V Y V A N S T
2341 ggcgcttgatgcggcaggtatctcgactagccggcctgggtgtgtacgtcgccaactcgac
S E A F T D D I E D G L R T F K F L D R
2401 cagcgaggcggttcacggacgatatcgaggatggtttgcgaacgttcaagttcctcgaccg
F A L D E *
2461 gtttgcgctggatgagtga

Fig. 3 continued

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2012/073040

A. CLASSIFICATION OF SUBJECT MATTER

See extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: C12N, C12S

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DATABASES: CPRSABS, CNABS, CJFD, CSCD, SIPONPL, CNKI, DWPI, SIPOABS, CPEA, ISI WEB OF KNOWLEDGE, CA, BA, MEDLINE, PUBMED

SEARCH TERMS: SEQUENCE SEARCH ON THE SEQUENCES OF SEQ ID NO: 1-8, CATALASE?, EC 1.11.1.6, CELLULOSE, DEGRADATION, CONVERSION, FERMENT, CELLULOLYTIC, SACCHARIFY, SACCHARIFICATION, LIQUEFY, LIQUEFACTION

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010080408 A2 (NOVOZYMES INC) 15 Jul. 2010 (15.07.2010), see the whole document, especially the claims 1-25 and Examples 1-7	1-25
A	GenBank accession No.: ABH85895.1 11 Aug. 2006 (11.08.2006) , searched on 12 Jun. 2012 (12.06.2012) from GenBank database, see the sequence	1-25

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:	“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
“A” document defining the general state of the art which is not considered to be of particular relevance	“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
“E” earlier application or patent but published on or after the international filing date	“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
“L” document which may throw doubts on priority claim (S) or which is cited to establish the publication date of another citation or other special reason (as specified)	“&” document member of the same patent family
“O” document referring to an oral disclosure, use, exhibition or other means	
“P” document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 12 June 2012 (12.06.2012)	Date of mailing of the international search report 19 Jul. 2012 (19.07.2012)
Name and mailing address of the ISA/CN The State Intellectual Property Office, the P.R.China 6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China 100088 Facsimile No. 86-10-62019451	Authorized officer LIAO, Yajing Telephone No. (86-10)62411074

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/CN2012/073040

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GenBank accession No.: XP_002487825.1 02 Jul. 2009 (02.07.2009) , searched on 12 Jun. 2012 (12.06.2012) from GenBank database, see the sequence	1-25
A	GenBank accession No.: XP_AAB76420.1 08 Oct. 1997 (08.10.1997) , searched on 12 Jun. 2012 (12.06.2012) from GenBank database, see the sequence	1-25
A	GenBank accession No.: XP_748550.1 27 Feb. 2008 (27.02.2008) , searched on 12 Jun. 2012 (12.06.2012) from GenBank database, see the sequence	1-25

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2012/073040

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Claims 1-25 relate to 4 groups of inventions based on different catalases which consist of SEQ ID NO:2, 4, 6 and 8 respectively. These catalases do not process a common structure except for the known domains of catalase. Therefore, there are no common or corresponding technical features among the 4 inventions relating to different catalases, obviously, there are no common or corresponding special technical features within the meaning of Rule 13.2 among the 4 inventions. The application, hence does not meet the requirements of unity of invention as defined in Rule 13.1.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

International application No.
PCT/CN2012/073040

Form PCT/ISA /210 (patent family annex) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2012/073040

A. CLASSIFICATION OF SUBJECT MATTER

C12N 9/08(2006.01)

C12N 9/42(2006.01)i

C12N15/53 (2006.01)i

C12N15/56 (2006.01)i

C12S 3/04 (2006.01)i