Abstract:

Title: COMPOSITIONS COMPRISING A POLYPEPTIDE HAVING CELLULOLYTIC ENHANCING ACTIVITY AND A BICYClic COMPOUND AND USES THEREOF

(57) Abstract: The present invention relates to compositions comprising: a polypeptide having cellulolytic enhancing activity and a bicyclic compound. The present invention also relates to methods of using the compositions.
COMPOSITIONS COMPRISING A POLYPEPTIDE HAVING CELLULOLYTIC
ENHANCING ACTIVITY AND A BICYCLIC COMPOUND AND USES THEREOF

Statement as to Rights to Inventions Made Under
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This invention was made with Government support under Cooperative Agreement DE-FC36-08GO18080 awarded by the Department of Energy. The government has certain rights in this invention.

Cross-Reference to Related Applications
This application claims the benefit of U.S. Provisional Application Serial No. 61/373,124, filed August 12, 2010, U.S. Provisional Application Serial No. 61/373,128, filed August 12, 2010, U.S. Provisional Application Serial No. 61/373,145, filed August 12, 2010, U.S. Provisional Application Serial No. 61/373,150, filed August 12, 2010, U.S. Provisional Application Serial No. 61/373,157, filed August 12, 2010, U.S. Provisional Application Serial No. 61/373,166, filed August 12, 2010, U.S. Provisional Application Serial No. 61/373,170, filed August 12, 2010, and U.S. Provisional Application Serial No. 61/373,210, filed August 12, 2010, which applications are incorporated herein by reference.

Reference to a Sequence Listing
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
The present invention relates to compositions comprising a polypeptide having cellulolytic enhancing activity and a bicyclic compound, and to methods of using the compositions.

Description of the Related Art
Cellulose is a polymer of the simple sugar glucose 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.

The conversion of lignocellulosic feedstocks into ethanol has the advantages of the ready availability of large amounts of feedstock, the desirability of avoiding burning or land filling the materials, and the cleanliness of the ethanol fuel. Wood, agricultural residues, herbaceous crops, and municipal solid wastes have been considered as feedstocks for ethanol production. These materials primarily consist of cellulose, hemicellulose, and lignin. Once the lignocellulose is converted to fermentable sugars, e.g., glucose, the fermentable sugars are easily fermented by yeast into ethanol.


It would be advantageous in the art to improve the ability of polypeptides having cellulolytic enhancing activity to enhance enzymatic hydrolysis of lignocellulosic feedstocks.

The present invention relates to compositions comprising a polypeptide having cellulolytic enhancing activity and a bicyclic compound, and to methods of using the compositions.
Summary of the Invention

The present invention relates to compositions comprising: (a) a polypeptide having cellulolytic enhancing activity; and (b) a bicyclic compound, wherein the combination of the polypeptide having cellulolytic enhancing activity and the bicyclic compound enhances hydrolysis of a cellulosic material by a cellulolytic enzyme.

The present invention also 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 cellulolytic enhancing activity and a bicyclic compound, wherein the combination of the polypeptide having cellulolytic enhancing activity and the bicyclic compound enhances hydrolysis of the cellulosic material by the enzyme composition.

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 cellulolytic enhancing activity and a bicyclic compound, wherein the combination of the polypeptide having cellulolytic enhancing activity and the bicyclic compound enhances hydrolysis of the cellulosic material by the enzyme composition;

(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 also 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 saccharified with an enzyme composition in the presence of a polypeptide having cellulolytic enhancing activity and a bicyclic compound, wherein the combination of the polypeptide having cellulolytic enhancing activity and the bicyclic compound enhances hydrolysis of the cellulosic material by the enzyme composition.

In one aspect, the bicyclic compound is a compound of formula (I) or (II):

wherein the bond indicated with a dashed line can be single or double;
R₁ and R₂ are independently hydrogen, -C(0)R₅, -C(0)OR₆, -C(0)NHR₇, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, alkenyl, heteroaryl, and heteroaralkyl;

R₃, R₄a, R₄b, and R₄c are independently hydrogen, halogen, -OH, -OR₈, -CN, -N₉O₂, -N(R₉)₈(R₉)₈, -C(0)R₅, -C(0)OR₆, -C(0)NHR₇, -OC(0)R₁₁, -NHC(0)OR₁₃, -NHC(0)OR₁₄, -OC(0)NHR₁₅, -NHC(0)NHR₁₆, -SO₂R₁₇, -SO₂N(R₁₈)(R₁₉), -SR₂₀, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, alkenyl, heteroaryl, and heteroaralkyl; and

wherein two of R₄a, R₄b, and R₄c may together form an optionally substituted fused ring;

R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₁, R₁₂, R₁₃, R₁₄, R₁₅, R₁₆, R₁₇, and R₂₀ are independently hydrogen, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, alkenyl, heteroaryl, and heteroaralkyl; and

R₁₇ is an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, alkenyl, heteroaryl, and heteroaralkyl;

or a salt or solvate thereof.

**Brief Description of the Figures**

Figure 1 shows the effect of the *Thermoascus aurantiacus* GH61A polypeptide on the hydrolysis of AVICEL® by a *Trichoderma reesei* cellulase composition in the presence of the various bicyclic compounds. White bars: 3-day saccharification; black bars: 7-day saccharification.

Figures 2A and 2B show the effect of the 7. *aurantiacus* GH61A polypeptide concentration on percent conversion of AVICEL® by a 7. *reesei* cellulase composition at various concentrations of quercitin. In each panel, the results of a 7. *aurantiacus* GH61 titration are plotted. Panel A: percent conversion - day 3; panel B: percent conversion - day 7.

Figure 3 shows the effect of quercitin concentration on the GH61 effect of the 7. *aurantiacus* GH61A polypeptide during hydrolysis of AVICEL® by a 7. *reesei* cellulase composition. Solid line, open circles: 4 mg/g 7. *reesei* cellulase composition; dashed line, open squares: 2 mg/g 7. *reesei* cellulase composition.

Figures 4A (epicatechin), 4B (isorhamnatin), 4C (taxifolin), 4D (keracyanin), 4E (kaempferol), 4F (apigenin), and 4G (naringenin) show (1) the effect of a bicyclic compound on hydrolysis of AVICEL® by the 7. *reesei* cellulase composition in the absence of a GH61 polypeptide (white bars), (2) the effect of a bicyclic compound on hydrolysis of AVICEL® by the 7. *reesei* cellulase composition in the presence of a GH61 polypeptide (grey bars), and...
(3) the effect of a GH61 polypeptide on hydrolysis of AVICEL® by the 7 reesei cellulase composition in the presence of a bicyclic compound (black bars) at 3 and 7 days of hydrolysis.

Figure 5 shows (A) the fractional hydrolysis of AVICEL® by the 7 reesei cellulase composition with various GH61 polypeptides as indicated, and combinations of compounds as indicated; and (B) the GH61 effect for mixtures of compounds at 1 mM and 3 mM concentration for various GH61 polypeptides as indicated. White bars: 3-days of hydrolysis; black bars: 7-days of hydrolysis. DHA: dehydroascorbate; pyro: pyrogallol; quer: quercitin hydrate; 2AP: 2-aminophenol; naph: 2-hydroxy-1,4-naphthoquinone; morin: morin hydrate; naring: naringenin; Theau: Thermoascus aurantiacus GH61A polypeptide; Aspfu: Aspergillus fumigatus GH61B polypeptide and 15B show the fractional hydrolysis of AVICEL® by the 7 reesei cellulase composition with various GH61 polypeptides as indicated, and combinations of compounds.

Definitions

Acetylxylan esterase: The term "acetylxylan esterase" means a carboxylesterase (EC 3.1.1.72) that catalyzes the hydrolysis of acetyl groups from polymeric xylan, acetylated xylose, acetylated glucose, alpha-napthyl acetate, and p-nitrophenyl acetate. For purposes of the present invention, acetylxylan 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 acetylxylan esterase is defined as the amount of enzyme capable of releasing 1 µmol of p-nitrophenolate anion per minute at pH 5, 25°C.

Alkenyl: The term "alkenyl" refers to unsaturated aliphatic groups including straight-chain (linear; unbranched), branched-chain groups, and combinations thereof, having the number of carbon atoms specified, if designated, which contain at least one double bond (-C=C-). All double bonds may be independently either (E) or (Z) geometry, as well as mixtures thereof. Examples of alkenyl groups include, but are not limited to, -CH2-CH=CH-CH3, -CH=CH-CH=CH2 and -CH2-CH=CH-CH(CH3)-CH2-CH3. If no size is designated, the alkenyl groups mentioned herein contain 2-20 carbon atoms, typically 2-10 carbon atoms, or 2-8 carbon atoms, or 2-6 carbon atoms, or 2-4 carbon atoms. The term "alkenylene" is by itself or in combination with other terms, represents a divalent radical derived from an alkenyl, as exemplified, but not limited, by -CH2CHCHCH2-.

Alkyl: The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a fully saturated straight-chain (linear; unbranched) or branched chain, or combination thereof, having the number of carbon atoms specified, if designated (i.e., CrC10 means one to ten carbons). Examples include, but are not limited to, groups such as methyl,
ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. If no size is designated, the alkyl groups mentioned herein contain 1-20 carbon atoms, typically 1-10 carbon atoms, or 1-8 carbon atoms, or 1-6 carbon atoms, or 1-4 carbon atoms. The term "alkylene" is by itself or in combination with other terms, represents a divalent radical derived from an alkyl, as exemplified, but not limited, by \(-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\) - .

**Alkynyl:** The term "alkynyl" refers to unsaturated aliphatic groups including straight-chain (linear; unbranched), branched-chain groups, and combinations thereof, having the number of carbon atoms specified, if designated, which contain at least one carbon-carbon triple bond (-C≡C-). Examples of alkynyl groups include, but are not limited to, \(-\text{CH}_2\text{C}≡\text{C}-\text{CH}_3\); \(-\text{C}≡\text{C}≡\text{CH}\) and \(-\text{CH}_2\text{C}≡\text{C}≡\text{CH(\text{CH}_3)}\)- \(-\text{CH}_2\text{CH}_3\) . If no size is designated, the alkynyl groups mentioned herein contain 2-20 carbon atoms, typically 2-10 carbon atoms, or 2-8 carbon atoms, or 2-6 carbon atoms, or 2-4 carbon atoms. The term "alkynylene" is by itself or in combination with other terms, represents a divalent radical derived from an alkynyl, as exemplified, but not limited, by \(-\text{CH}_2\text{CCCH}_2\) - .

**Allelic variant:** The term "allelic variant" means any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequences. An allelic variant of a polypeptide is a polypeptide encoded by an allelic variant of a gene.

**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 µl 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 μmol of glucuronic or 4-O-methylglucuronic acid per minute at pH 5, 40°C.

Aryl: The term "aryl" means, unless otherwise stated, a polyunsaturated, aromatic, hydrocarbon substituent. Aryl may contain additional fused rings (e.g., from 1 to 3 rings), including additionally fused aryl, heteroaryl, cycloalkyl, and/or heterocycloalkyl rings. Examples of aryl groups include, but are not limited to, phenyl, 1-naphthyl, 2-naphthyl, and 4-biphenyl.

Arylene/heteroarylene: The term "arylene" and "heteroarylene" means a divalent radical derived from an aryl and heteroaryl, respectively. Each of the two valencies of arylene and heteroarylene may be located at any suitable portion of the ring (e.g.,

![Arylene/heteroarylene structure]

) and may be fused to another ring, as appropriate. Non-limiting examples of arylene include phenylene, biphenylene, naphthylene, and the like. Examples of heteroarylene groups include, but are not limited to, pyridinylene, oxazolylene, thioazolylene, pyrazolylene, pyranylene, and furanylene.

Aralkyl: The term "aralkyl" designates an alkyl-substituted aryl group, where the alkyl portion is attached to the parent structure. Examples are benzyl, phenethyl, and the like. "Heteroaralkyl" designates a heteroaryl moiety attached to the parent structure via an alkyl residue. Examples include furanylmethyl, pyridinylmethyl, pyrimidinylethyl, and the like. Aralkyl and heteroaralkyl also include substituents in which at least one carbon atom of the alkyl group is present in the alkyl group and wherein another carbon of the alkyl group has been replaced by, for example, an oxygen, nitrogen or sulfur atom (e.g., phenoxymethyl, 2-pyridylmethoxy, 3-(1-naphthoxy)propyl, and the like).

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 ei 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 μmol 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 µmol 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.

**Carbohydrate moiety:** The term "carbohydrate moiety" as used herein refers to an optionally substituted 5 or 6-membered cyclic monosaccharide radical comprising an oxygen bridge between two carbon atoms. Non-limiting examples include an optionally substituted radical of glucose, sucrose, and fructose. In some aspects, the carbohydrate moiety is optionally substituted with a second carbohydrate moiety (to generate a disaccharide moiety, e.g., a lactose moiety). In some aspects, the carbohydrate moiety contains only one cyclic monosaccharide.

**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 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) that catalyzes the hydrolysis of 1,4-beta-D-glucosidic linkages in cellulose, cellobiohexaose, 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). For purposes of the present invention, 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 Claeyssens, 1985, *FEBS Letters*, 187: 283-288; and Tomme et al., 1988, *Eur. J. Biochem.* 170: 575-581. In the present invention, the Lever et al. method can be employed to assess hydrolysis of cellulose in corn stover, while the methods of van Tilbeurgh et al. and Tomme et al. can be used to determine the cellobiohydrolase activity on a fluorescent disaccharide derivative, 4-methylumbelliferyl -beta-D-lactoside.

**Cellulolytic enhancing activity:** The term "cellulolytic enhancing activity" means a biological activity catalyzed by a GH61 polypeptide that enhances the hydrolysis of a cellulosic material by enzyme having cellulolytic activity. For purposes of the 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 at 50°C compared to a control hydrolysis without addition of cellulolytic enzyme protein (of cellulose in PCS). In a preferred aspect, a mixture of CELLUCLAST® 1.5L (Novozymes A/S, Bagsvaerd, Denmark) in the presence of 2-3% of total protein weight Aspergillus oryzae beta-glucosidase (recombinantly produced in Aspergillus oryzae according to WO 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, more preferably at least 1.05-fold, more preferably at least 1.10-fold, more preferably at least 1.25-fold, more preferably at least 1.5-fold, more preferably at least 2-fold, more preferably at least 3-fold, more preferably at least 4-fold, and most preferably at least 5-fold, even more preferably at least 10-fold.

**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 N°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 N°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: 1-20 mg of cellulolytic enzyme protein/g of cellulose in PCS for 3-7 days at 50°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, 72 hours, sugar analysis by AMINEX® HPX-87H column (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

**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, arabinoylans, 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, Wiselogel 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. Schepet, 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 corn stover.
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.

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 usually begins with the ATG start codon or alternative start codons such as GTG and TTG and ends with a stop codon such as TAA, TAG, and TGA. The coding sequence may be a DNA, cDNA, synthetic, or recombinant polynucleotide.

Control sequences: The term "control sequences" means all components necessary for the expression of a polynucleotide encoding a polypeptide. Each control sequence may be native or foreign 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 transcriptional 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.

Cycloalkyl: The term "cycloalkyl" by itself or in combination with other terms, represents, unless otherwise stated, a saturated or unsaturated cyclic non-aromatic hydrocarbon radical (e.g., cyclic versions of alkyl, alkenyl, or alkynyl, or mixtures thereof).
Cydoalkyi may contain additional fused rings (e.g., from 1 to 3 rings), including additionally fused cydoalkyi and/or heterocydoalkyi rings, but excludes additionally fused aryl and/or heteroaryl groups. Examples of cydoalkyi include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, norbornyl, and the like. If no size is designated, the alkylnyl groups mentioned herein contain 3-9 carbon atoms, typically 3-7 carbon atoms. The term "cycloalkylene" by itself or as part of another substituent means a divalent radical derived from a cydoalkyi, as exemplified, but not limited, by -cyclohexyl-.

**Cycloalkyl-alkyl/heterocycloalkyl-alkyl:** The terms "cycloalkyl-alkyl" and "heterocycloalkyl-alkyl" designate an alkyl/substituted cydoalkyi group and alkyl-substituted heterocycloalkyi, respectively, where the alkyl moiety is attached to the parent structure. Non-limiting examples include cyclopropylethyl, cyclobutyl-propyl, cyclopentyl-hexyl, cyclohexyl-isopropyl, 1-cyclohexenyl-propyl, 3-cyclohexenyl-t-butyl, cycloheptyl-heptyl, norbornyl-methyl, 1-piperidinyl-ethyl, 4-morpholinyl-propyl, 3-morpholinyl-t-butyl, tetrahydrofuran-2-yl-hexyl, tetrahydrofuran-3-ylisopropyl, and the like. Cycloalkyl-alkyl and heterocycloalkyl-alkyl also include substituents in which at least one carbon atom is present in the alkyl group and wherein another carbon atom of the alkyl group has been replaced by, for example, an oxygen, nitrogen or sulfur atom (e.g., cyclopropoxymethy1, 2-piperidinylxylo-t-butyl, and the like).

**Endoglucanase:** The term "endoglucanase" means an endo-1,4-(1,3;1,4)-beta-D-glucan 4-glucanohydrolase (E.C. 3.2.1.4), which 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 the 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 additional nucleotides that provide for its expression.

**Family 61 glycoside hydrolase:** The term "Family 61 glycoside hydrolase" or

**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 µmol of p-nitrophenolate anion per minute at pH 5, 25°C.

**Halogen:** The terms "halo" or "halogen," by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom.

**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, Shalom, 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 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. 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 marked by numbers. Some families, with 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 on the Carbohydrate-Active Enzymes (CAZy) database. Hemicellulolytic enzyme activities can be measured according to

Heteroaryl: The term "heteroaryl" refers to aryl groups (or rings) that contain from one to four annular heteroatoms selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule at an annular carbon or annular heteroatom. Heteroaryl may contain additional fused rings (e.g., from 1 to 3 rings), including additionally fused aryl, heteroaryl, cycloalkyi, and/or heterocycloalkyi rings. Non-limiting examples of heteroaryl groups are 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalinyl, 5-quinoxalinyl, 3-quinolyl, and 6-quinolyl.

Heterocycloalkyi: The term "heterocycloalkyi," by itself or in combination with other terms, represents a saturated or unsaturated cyclic non-aromatic hydrocarbon radical containing of at least one carbon atom and at least one annular heteroatom selected from the group consisting of O, N, P, Si and S, and wherein the nitrogen and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N, P, S and Si may be placed at any interior position of the heterocycloalkyi group or at the position at which the heterocycloalkyi group is attached to the remainder of the molecule. Heterocycloalkyi may contain additional fused rings (e.g., from 1 to 3 rings), including additionally fused cycloalkyi and/or heterocycloalkyi rings, but excludes additionally fused aryl and/or heteroaryl groups. Examples of heterocycloalkyi include, but are not limited to, thiazolidinonyl, 1H-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like. The term "heterocycloalkylene" by itself or as part of another substituent means a divalent radical derived from a heterocycloalkyi, as exemplified, but not limited, by

Host cell: The term "host cell" means any cell type that is susceptible to transformation, transfection, transduction, and the like with a nucleic acid construct or expression vector comprising a polynucleotide 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.

Isolated or Purified: The term "isolated" or "purified" means a polypeptide or polynucleotide that is removed from at least one component with which it is naturally
associated. For example, a polypeptide may be at least 1% pure, e.g., at least 5% pure, at least 10% pure, at least 20% pure, at least 40% pure, at least 60% pure, at least 80% pure, at least 90% pure, or at least 95% pure, as determined by SDS-PAGE, and a polynucleotide may be at least 1% pure, e.g., at least 5% pure, at least 10% pure, at least 20% pure, at least 40% pure, at least 60% pure, at least 80% pure, at least 90% pure, or at least 95% pure, as determined by agarose electrophoresis.

**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. 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. The mature polypeptide can be predicted using the SignalP program (Nielsen et al., 1997, *Protein Engineering* 10: 1-6).

**Mature polypeptide coding sequence:** The term "mature polypeptide coding sequence" is defined herein as a nucleotide sequence that encodes a mature polypeptide having biological activity. The mature polypeptide coding sequence can be predicted using the SignalP program (Nielsen et al., 1997, *supra*).

**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. The term nucleic acid construct is synonymous with the term "expression cassette" when the nucleic acid construct contains the control sequences required for expression of a coding sequence of the present invention.

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

**Polypeptide fragment:** The term "fragment" means a polypeptide having one or more (e.g., several) amino acids deleted from the amino and/or carboxyl terminus of a mature polypeptide; wherein the fragment has biological activity.

**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 degree of 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 3.0.0, 5.0.0, or later. The
optional 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:

\[
\text{Identity} = \frac{\text{Identical Residues} \times 100}{\text{Length of Alignment} - \text{Total Number of Gaps in Alignment}}
\]

For purposes of the present invention, the degree of 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 3.0.0 or later. The optional 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:

\[
\text{Identity} = \frac{\text{Identical Deoxyribonucleotides} \times 100}{\text{Length of Alignment} - \text{Total Number of Gaps in Alignment}}
\]

**Subsequence:** The term "subsequence" means a polynucleotide having one or more
(e.g., several) nucleotides deleted from the 5' and/or 3' end of a mature polypeptide coding
sequence; wherein the subsequence encodes a fragment having biological activity.

**Substituted:** The term "substituted" refers to the replacement of one or more (e.g.,
several) hydrogen atoms of a moiety with a monovalent or divalent radical. "Optionally
substituted" indicates that the moiety may be substituted or unsubstituted. A moiety lacking
the terms "optionally substituted" and "substituted" is intended an unsubstituted moiety (e.g.,
"phenyl" is intended an unsubstituted phenyl unless indicated as a substituted phenyl or an
optionally substituted phenyl). Suitable substituent groups for indicated optionally substituted
moieties include, for example, hydroxyl, nitro, amino (e.g., -NH₂ or dialkyl amino), imino,
cyano, halo (such as F, Cl, Br, I), halo alkyl (such as -CCl₃ or -CF₃), thio, sulfonyl, thioamido,
amidino, imidino, oxo, oxamidino, methoxamidino, imidino, guanidino, sulfonamido, carboxyl,
formyl, alkyl, alkoxy, alkoxy-alkyl, alkylcarbonyl, alkylcarbonyloxy (-OCOR), aminocarbonyl,
arylcarbonyl, aralkylcarbonyl, carbamolino, heteroarylcarbonyl, heteroaralkyl-carbonyl,
alkylthio, amino alkyl, cyanoalkyl, carbamoyl (-NHCOOR- or -OCONHR-), urea (-
NHCONHR-), aryl and the like, where R is any suitable group, e.g., alkyl or alkyne. In
some embodiments, the optionally substituted moiety is optionally substituted only with
select radicals, as described. In some embodiments, the above groups (e.g., alkyl groups)
are optionally substituted with, for example, alkyl (e.g., methyl or ethyl), halo alkyl (e.g., -
CCI₃, -CH₂CHCl₂ or -CF₃), cycloalkyl (e.g., -C₃H₅, -C₄H₇, -C₅H₉), amino (e.g., -NH₂ or dialkyl amino), alkoxy (e.g., methoxy), heterocycloalkyl (e.g., as morpholine, piperazine, piperidine, azetidine), hydroxyl, and/or heteroaryl (e.g., oxazolyl). Other suitable substituent groups for indicated optionally substituted moieties are described herein. In some embodiments, a substituent group is itself optionally substituted. In some embodiments, a substituent group is not itself substituted. The group substituted onto the substitution group can be, for example, carboxyl, halo, nitro, amino, cyano, hydroxyl, alkyl, alkenyl, alkynyl, alkoxy, aminocarbonyl, -SR, thioamido, -SO₃H, -SO₂R or cycloalkyl, where R is any suitable group, e.g., a hydrogen or alkyl.

When the substituted substituent includes a straight chain group, the substituent can occur either within the chain (e.g., 2-hydroxypropyl, 2-aminobutyl, and the like) or at the chain terminus (e.g., 2-hydroxyethyl, 3-cyanopropyl, and the like). Substituted substituents can be straight chain, branched or cyclic arrangements of covalently bonded carbon or heteroatoms (N, O or S).

**Variant:** The term "variant" means a polypeptide having cellulolytic enhancing activity comprising an alteration, i.e., a substitution, insertion, and/or deletion of one or more (e.g., several) amino acid residues at one or more (e.g., several) positions. A substitution means a replacement of an amino acid occupying a position with a different amino acid; a deletion means removal of an amino acid occupying a position; and an insertion means adding one or more (e.g., several) amino acids, e.g., 1-5 amino acids, adjacent to an amino acid occupying a position.

**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, (arabinoglu)arabinoxylans, (glucoarabin)arabinoxylans, and complex heteroxylans. See, for example, Ebringerova et al., 2005, *Adv. Polym. Sci.* 186: 1-67.

In the methods 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.,

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 dried 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 μmol 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 μmol 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.

As used herein and in the appended claims, the singular forms "a," "or," and "the"
include plural referents unless the context clearly dictates otherwise. It is understood that the aspects of the invention described herein include “consisting” and/or “consisting essentially of” aspects.

**Detailed Description of the Invention**

The present invention relates to compositions comprising: (a) a polypeptide having cellulolytic enhancing activity; and (b) a bicyclic compound, wherein the combination of the polypeptide having cellulolytic enhancing activity and the bicyclic compound enhances hydrolysis of a cellulosic material by a cellulolytic enzyme. In one aspect, the compositions further comprise (c) one or more (e.g., several) enzymes selected from the group consisting of a cellulase, a hemicellulase, an esterase, an expansin, a laccase, a ligninolytic enzyme, a pectinase, a peroxidase, a protease, and a swollenin.

The present invention also 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 cellulolytic enhancing activity and a bicyclic compound, wherein the combination of the polypeptide having cellulolytic enhancing activity and the bicyclic compound enhances hydrolysis of the cellulosic material by the enzyme composition. In one aspect, the method above further comprises recovering the degraded or converted cellulosic material. Soluble products of degradation or conversion of the cellulosic material can be separated from the insoluble cellulosic material using technology well known in the art such as, for example, centrifugation, filtration, and gravity settling.

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 cellulolytic enhancing activity and a bicyclic compound, wherein the combination of the polypeptide having cellulolytic enhancing activity and the bicyclic compound enhances hydrolysis of the cellulosic material by the enzyme composition;

(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 also 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 saccharified with an enzyme composition in the presence of a polypeptide having cellulolytic enhancing activity and a bicyclic compound, wherein the combination of the polypeptide having cellulolytic enhancing activity and the bicyclic compound enhances hydrolysis of the cellulosic material by the enzyme...
composition. 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.

5 Bicyclic Compounds

The bicyclic compounds used the methods and compositions of the present invention include any substituted fused ring system as described herein. The compounds may comprise one or more (e.g., several) additional rings, and are not limited to a specific number of rings unless otherwise stated.

In one aspect of the methods and compositions of the present invention, 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 flavylium ion, such as an optionally substituted anthocyanidin or optionally substituted anthocyanin, or derivative thereof.

In another aspect, the bicyclic compound is of formula (I), (I’), (II), or (II’):

wherein the bond indicated with a dashed line can be single or double;

R\(^1\) and R\(^2\) are independently hydrogen, -C(0)R\(^5\), -C(0)OR\(^6\), -C(0)NHR\(^7\), or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, ary, aralkyl, heteroaryl, and heteroaralkyl:

R\(^3\), R\(^{4a}\), R\(^{4b}\), and R\(^{4c}\) are independently hydrogen, halogen, -OH, -OR\(^8\), -CN, -N\(^0\)\(2\), -N(R\(^9\))(R\(^{10}\)), -C(0)R\(^5\), -C(0)OR\(^6\), -C(0)NHR\(^7\), -OC(0)R\(^{11}\), -NH(0)R\(^{12}\), -OC(0)OR\(^{13}\), -NHC(0)OR\(^{14}\), -OC(0)NHR\(^{15}\), -NHC(0)NHR\(^{16}\), -S0\(_2\)R\(^{17}\), -S0\(_2\)N(R\(^{18}\))(R\(^{19}\)), -SR\(^{20}\), or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, ary, aralkyl, heteroaryl, and heteroaralkyl; and
wherein two of $R^{4a}$, $R^{4b}$, and $R^{4c}$ may together form an optionally substituted fused ring:

$$R^5, R^6, R^7, R^8, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, \text{ and } R^{20} \text{ are independently hydrogen, or an optionally substituted moiety selected from alkyl, alkenyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl; and}$$

$$R^{17} \text{ is an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl; and}$$

or a salt or solvate thereof.

In another aspect the bicyclic compound is of formula (I). In another aspect the bicyclic compound is of formula ('I'). In any of these aspects, the bond indicated with a dashed line is single bond. In other aspects, the bond indicated with a dashed line is a double bond.

In another aspect, the bicyclic compound is of formula (II). In another aspect, the bicyclic compound is of formula ('II').

In another aspect, the bicyclic compound of formula (I) has the formula (I-C), (I-D), (I-E), or (I-F):

$$\text{(I-C)} \quad \text{(I-D)} \quad \text{(I-E)} \quad \text{(I-F)}$$

wherein $R^1, R^2, R^3, R^{4a}, R^{4b}, \text{ and } R^{4c}$ are as defined above.

In another aspect the bicyclic compound is of formula (I-C). In another aspect the bicyclic compound is of formula (I-D). In another aspect the bicyclic compound is of formula (I-E). In another aspect the bicyclic compound is of formula (I-F).

In some aspects of formula (I), (I), (I-C), (I-D), (I-E), and (I-F), $R^1$ and $R^2$ are independently hydrogen, or an optionally substituted moiety selected from alkyl, alkenyl,
alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl; heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl. In other aspects, R and R are independently hydrogen, an optionally substituted carbohydrate moiety, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, or cycloalkyl-alkyl. In some aspects, R and R are independently hydrogen, an optionally substituted carbohydrate moiety, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, and alkyl. In some aspects, R and R are independently hydrogen, an optionally substituted carbohydrate moiety, or an optionally substituted alkyl. In some aspects, R and R are independently hydrogen, or an optionally substituted carbohydrate moiety. In some aspects, at least one of R and R is hydrogen. In other aspects, both R and R are hydrogen. In some aspects, at least one of R and R is other than hydrogen. In some aspects, R is hydrogen. In some aspects, R is other than hydrogen. In some aspects, R is -OH. In some aspects, R is independently hydrogen, -OH, -OR, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl; and wherein two of R, R, and R may together form an optionally substituted fused ring (e.g., an optionally substituted cycloalkylene, arylene, heteroarylene, or heterocycloalkylene, such as an optionally substituted cyclic diether). In some aspects, R, R, R, and R are independently hydrogen, -OH, -OR, an optionally substituted carbohydrate moiety, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, or cycloalkyl-alkyl. In some aspects, R, R, R, and R are independently hydrogen, -OH, -OR, an optionally substituted carbohydrate moiety, or an optionally substituted moiety selected from alkyl, alkenyl, and alkynyl. In some aspects, R, R, R, and R are independently hydrogen, -OH, -OR, or an optionally substituted carbohydrate moiety. In some aspects, at least one of R, R, and R is hydrogen. In some aspects, at least two of R, R, and R are hydrogen. In some aspects, each of R, R, and R are hydrogen. In some aspects, at least one of R, R, and R is -OH. In some aspects, at least two of R, R, and R is -OH. In some aspects, one of R, R, and R is hydrogen and the other two of R, R, and R are each -OH. In some aspects, at least one of R, R, and R is other than hydrogen. In some aspects, at least two of R, R, and R are other than hydrogen. In some aspects, R and at least one of R, R, and R are other than hydrogen. In some aspects, R and at least two of R, R, and R are other than hydrogen. In some aspects, at least one of R, R, R, and R is an optionally substituted carbohydrate moiety. In some aspects, two of R, R, R, and R are optionally substituted carbohydrate moieties. In some aspects, R is hydrogen, -OH, or an optionally substituted carbohydrate moiety. In some aspects, R is hydrogen. In some aspects, R is other than hydrogen. In some aspects, R is -OH. In some
aspects, $R^3$ is an optionally substituted carbohydrate moiety.

In another aspect, the bicyclic compound of formula (I) has the formula (I-A) or (II-A):

$$\text{(I-A)}$$

$$\text{(II-A)}$$

wherein $R^1$, $R^2$, $R^8$, and $R^{4a}$ are as defined above.

In another aspect, the bicyclic compound has the formula (I-B) or (II-B):

$$\text{(I-B)}$$

$$\text{(II-B)}$$

wherein $R^1$, $R^2$, and $R^{4a}$ are as defined above.

In some aspects of formula (I-A), (II-A), (I-B), and (II-B), $R^1$ and $R^2$ are independently hydrogen, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl. In other aspects, $R^1$ and $R^2$ are independently hydrogen, an optionally substituted carbohydrate moiety, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, or cycloalkyl-alkyl. In some aspects, $R^1$ and $R^2$ are independently hydrogen, an optionally substituted carbohydrate moiety, or an optionally substituted moiety selected from alkyl, alkenyl, and alkynyl. In some aspects, $R^1$ and $R^2$ are independently hydrogen, an optionally substituted carbohydrate moiety, or an optionally substituted alkyl. In some aspects, $R^1$ and $R^2$ are independently hydrogen, or an optionally substituted carbohydrate moiety. In some aspects, at least one of $R^1$ and $R^2$ is hydrogen. In other aspects, both $R^1$ and $R^2$ are hydrogen. In some aspects, at least one of $R^1$ and $R^2$ is other than hydrogen. In some aspects, at least one of $R^1$ and $R^2$ is an optionally substituted carbohydrate moiety. In some aspects, both $R^1$ and $R^2$ are other than hydrogen.

In some aspects of formula (I-A), (II-A), (I-B), and (II-B), $R^{4a}$ is independently hydrogen, halogen, -OH, -OR$^8$, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl. In some aspects, $R^{4a}$ is hydrogen, -OH, -OR$^8$, an
optionally substituted carbohydrate moiety, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, or cycloalkyl-alkyl. In some aspects, R⁴a is hydrogen, -OH, -OR⁸, an optionally substituted carbohydrate moiety, or an optionally substituted moiety selected from alkyl, alkenyl, and alkynyl. In some aspects, R⁴a is hydrogen, -OH, -OR⁸, or an optionally substituted carbohydrate moiety. In some aspects, R⁴a is hydrogen. In some aspects, R⁴a is -OH. In some aspects, R⁴a is other than hydrogen. In some aspects, R⁴a is an optionally substituted carbohydrate moiety.

In some of these aspects of the bicyclic compounds (e.g., a bicyclic compound of formula I, I’, II, II’, I-A, II-A, I-B, II-B, I-C, I-D, I-E, or I-F), each optionally substituted moiety is the indicated moiety optionally substituted with one or more (e.g., several) groups selected from hydroxyl, nitro, amino, imino, cyano, halo, haloalkyl, thiol, thioalkyl, sulfonyl, thioamido, amidino, oxo, oxamidino, methoxamidino, imidino, guanidino, sulfonamido, carboxyl, formyl, alkyl, cycloalkyl, alkoxy, alkoxy-alkyl, alkylcarbonyl, alkylcarbonyl-alkyl, alkylcarbonyloxoy, aminocarbonyl, aryl, heteroaryl, aryalkylcarbonyl, aralkylcarbonyl, carbonylamino, heteroarylcarbonyl, heteroaralkylcarbonyl, alkythio, amino alkyl, cyanocarbonyl, carbamoyl, and urea. In some embodiments, each optionally substituted moiety is the indicated moiety optionally substituted with one or more (e.g., several) groups selected from hydroxyl, nitro, amino, cyano, halo, haloalkyl, thiol, thioalkyl, sulfonyl, thioamido, amidino, carboxyl, formyl, alkyl, cycloalkyl, alkoxy, and alkoxyalkyl.

All aspects described for formulas I, I-A, I-B, I-C, I-D, I-E, and I-F, are also contemplated for the corresponding isomer of formula I’. Likewise, all aspects described for formulas II, II-A, and II-B, are also contemplated for the corresponding isomer of formula I’.

In another aspect, the bicyclic compound is selected from the group consisting of:

(I-1): Epicatechin;

(1-2): Quercetin;
(1-3): Myricetin;

(1-4): Taxifolin;

(1-5): Kaempferol;

(1-6): Morin;

(1-7): Acacetin;

(1-8): Naringenin;
(I-9): Isorhamnetin;

(II-1): Cyanidin;

(II-2): Cyanin;

(II-3): Kuromanin;
(11-4): Keracyanin;
or a salt or solvate thereof.

In some aspects, the bicyclic compound described herein (e.g., a compound of formula I, I', II, II', I-A, II-A, I-B, II-B, I-C, I-D, I-E, or I-F) is in substantially pure form. With respect to the bicyclic compounds, unless otherwise stated, "substantially pure" intends a preparation of the bicyclic compound that contains no more than 15% impurity, wherein the impurity intends compounds other than the bicyclic compound, but does not include other forms of the bicyclic compound (e.g., different salt form or a different stereoisomer, conformer, rotamer, or tautomer of the analog depicted). In one variation, a preparation of substantially pure bicyclic compound is provided wherein the preparation contains no more than 25% impurity, or no more than 20% impurity, or no more than 10% impurity, or no more than 5% impurity, or no more than 3% impurity, or no more than 1% impurity, or no more than 0.5% impurity.

In some aspects the bicyclic compound described herein (e.g., a compound of formula I, II, III, or IV) is not in substantially pure form. For example, the bicyclic compound may be added or supplemented as part of an impure composition (e.g., unpurified biological material) wherein the composition is rich in the compound or one or more (e.g., several) chemical precursors thereof. In a one aspect, an impure composition (e.g., unpurified biological material) comprising one or more (e.g., several) bicyclic compounds is pretreated, e.g., as described herein for cellulosic material, and/or added to cellulosic material and/or combined with the cellulosic material prior to pretreatment of the cellulosic material. In another aspect, an impure composition (e.g., unpurified biological material) comprising one or more (e.g., several) bicyclic compounds is added to an enzyme composition involved in saccharification, enhancement of saccharification, liquefaction, etc. In another aspect, an impure composition (e.g., unpurified biological material) comprising one or more (e.g., several) bicyclic compounds is added to a fermentation or simultaneous saccharification-fermentation reaction. In any of these aspects, the impure composition comprising a bicyclic
compound (e.g., unpurified biological material) is a preparation that contains more than 0.5% impurity, or more than 1% impurity, or more than 3% impurity, or more than 5% impurity, or more than 10% impurity, or more than 20% impurity, or more than 30% impurity, or more than 40% impurity, or more than 50% impurity, or more than 60% impurity, or more than 70% impurity, or more than 80% impurity, or more than 90% impurity, or more than 95% impurity, or more than 97% impurity, or more than 98% impurity, or more than 99% impurity.

In another aspect the bicyclic compound described herein (e.g., a compound of formula I, I', II, II', I-A, II-A, I-B, II-B, I-C, I-D, I-E, or I-F) is not substantially pure, but rather is added or supplemented as a raw biological feedstock known to be rich in the bicyclic compound. In one preferred aspect, the biological feedstock is citrus rind or citrus fruit. In another preferred aspect, the biological feedstock is antioxidant fruit including, but not limited to blueberry, raspberry, blackberry, etc., in another preferred aspect, these biological feedstocks are pretreated as cellulose feedstocks or in combination with cellulosic feedstocks as described herein.

The bicyclic compounds described herein (e.g., any compound of formula I, I', II, II', I-A, II-A, I-B, II-B, I-C, I-D, I-E, or I-F) and methods of using the same, unless otherwise stated, include all solvate and/or hydrate forms. In some aspects, the bicyclic compounds described herein can exist in unsolvated forms as well as solvated forms (i.e., solvates). The bicyclic compounds may also include hydrated forms (i.e., hydrates).

The bicyclic compounds described herein (e.g., any compound of formula I, I', II, II', I-A, II-A, I-B, II-B, I-C, I-D, I-E, or I-F), as well as methods of using such compounds, unless otherwise stated, include all salt forms of the compounds. The compounds also include all non-salt forms of any salt of a bicyclic compound described herein, as well as other salts of any salt of a bicyclic compound described herein. The desired salt of a basic functional group of a bicyclic compound may be prepared by methods known to those of skill in the art by treating the compound with an acid. The desired salt of an acidic functional group of a bicyclic compound can be prepared by methods known to those of skill in the art by treating the compound with a base. Examples of inorganic salts of acid compounds include, but are not limited to, alkali metal and alkaline earth salts, such as sodium salts, potassium salts, magnesium salts, bismuth salts, and calcium salts; ammonium salts; and aluminum salts. Examples of organic salts of acid compounds include, but are not limited to, procaine, dibenzylamine, N-ethylpiperidine, N,N'-dibenzylethlenediamine, trimethylamine, and triethylamine salts. Examples of inorganic salts of base compounds include, but are not limited to, hydrochloride and hydrobromide salts. Examples of organic salts of base compounds include, but are not limited to, tartrate, citrate, maleate, fumarate, and succinate.

Unless stereochemistry is explicitly indicated in a chemical structure or chemical name, the chemical structure or chemical name is intended to embrace all possible
stereoisomers, conformers, rotamers, and tautomers of the bicyclic compounds depicted. For example, a bicyclic compound containing a chiral carbon atom is intended to embrace both the \((R)\) enantiomer and the \((S)\) enantiomer, as well as mixtures of enantiomers, including racemic mixtures; and a bicyclic compound containing two chiral carbons is intended to embrace all enantiomers and diastereomers (including \((R,R)\), \((S,S)\), \((R,S)\), and \((R,S)\) isomers). In some aspects, a bicyclic compound described herein (e.g., any compound of formula I, \(I'\), II, \(I''\), I-A, I-B, I-B, I-C, I-D, I-E, or I-F) is in the form of the \((R)\) enantiomer. In some aspects, a bicyclic compound described herein (e.g., any compound of formula I, \(I'\), II, \(I''\), I-A, I-B, I-B, I-C, I-D, I-E, or I-F) is in the form of the \((S)\) enantiomer. Included in all uses of the bicyclic compounds disclosed herein, is any or all of the stereochemical, enantiomeric, diastereomeric, conformational, rotomeric, tautomeric, solvate, hydrate, and salt forms of the compounds as described.

The effective amount of the bicyclic compound can depend on one or more (e.g., several) factors including, but not limited to, the mixture of component cellulolytic enzymes, the cellulosic substrate, the concentration of cellulosic substrate, the pretreatment(s) of the cellulosic substrate, non-cellulosic components (e.g., native or degraded lignin or hemicellulose), non-cellulase components, temperature, and reaction time.

The bicyclic compound is preferably present in an amount that is not limiting with regard to the GH61 polypeptide having cellulolytic enhancing activity, cellulolytic enzyme(s), and cellulose. In one aspect, the compound is present in an amount that is not limiting with regard to the GH61 polypeptide having cellulolytic enhancing activity. In another aspect, the compound is present in an amount that is not limiting with regard to the cellulolytic enzyme(s). In another aspect, the compound is present in an amount that is not limiting with regard to the cellulose. In another aspect, the compound is present in an amount that is not limiting with regard to the GH61 polypeptide having cellulolytic enhancing activity and the cellulolytic enzyme(s). In another aspect, the compound is present in an amount that is not limiting with regard to the GH61 polypeptide having cellulolytic enhancing activity and the cellulose. In another aspect, the compound is present in an amount that is not limiting with regard to the cellulolytic enzyme(s) and the cellulose. In another aspect, the compound is present in an amount that is not limiting with regard to the GH61 polypeptide having cellulolytic enhancing activity, the cellulolytic enzyme(s), and the cellulose.

In one aspect, an effective amount of the bicyclic compound 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 \(10^{-6}\) to about 1, about \(10^{-6}\) to about 10, about \(10^{-6}\) to about 10, or about \(10^{-6}\) to about \(10^{-2}\). In another aspect, an effective amount of the bicyclic compound to cellulosic material as a molar ratio to glucosyl units of cellulose is about \(10^{-6}\) to about 10. In another
aspect, an effective amount of the bicyclic compound to cellulosic material as a molar ratio to
5 glucosyl units of cellulose is about $10^{-6}$ to about 7.5. In another aspect, an effective amount
of the bicyclic compound to cellulosic material as a molar ratio to glucosyl units of cellulose
is about $10^4$ to about 5. In another aspect, an effective amount of the bicyclic compound to
10 cellulosic material as a molar ratio to glucosyl units of cellulose is about $10^6$ to about 2.5. In
another aspect, an effective amount of the bicyclic compound to cellulosic material as a
molar ratio to glucosyl units of cellulose is about $10^6$ to about 1. In another aspect, an
effective amount of the bicyclic compound to cellulosic material as a molar ratio to glucosyl
units of cellulose is about $10^6$ to about 1. In another aspect, an effective amount of the
bicyclic compound to cellulosic material as a molar ratio to glucosyl units of cellulose is
about $10^5$ to about $10^{-1}$. In another aspect, an effective amount of the bicyclic compound to
15 cellulosic material as a molar ratio to glucosyl units of cellulose is about $10^3$ to about $10^6$. In another aspect, an
effective amount of the bicyclic compound to cellulosic material as a molar ratio to glucosyl
units of cellulose is about $10^3$ to about $10^6$.

In another aspect, an effective amount of the bicyclic compound to cellulose is about
20 $10^6$ to about 10 g per g of cellulose, e.g., about $10^4$ to about 7.5, about $10^6$ to about 5,
about $10^6$ to about 2.5, about $10^6$ to about 1, about $10^6$ to about 1, about $10^5$ to about $10^3$,
about $10^4$ to about $10^1$, about $10^3$ to about $10^1$, or about $10^2$ g per g of
25 cellulose. In another aspect, an effective amount of the bicyclic compound to cellulose is
about $10^3$ to about 10 g per g of cellulose. In another aspect, an effective amount of the
bicyclic compound to cellulose is about $10^6$ to about 5 g per g of cellulose. In another aspect,
an effective amount of the bicyclic compound to cellulose is about $10^6$ to about 2.5 g per g of
30 cellulose. In another aspect, an effective amount of the bicyclic compound to cellulose is
about $10^4$ to about 1 g per g of cellulose. In another aspect, an effective amount of the
bicyclic compound to cellulose is about $10^4$ to about $10^3$ g per g of cellulose. In another aspect,
an effective amount of the bicyclic compound to cellulose is about $10^3$ to about $10^4$ g per g of
35 cellulose.

In another aspect, an effective amount of the bicyclic compound is about 0.1 µM to
about 1 M, e.g., about 0.5 µM to about 0.75 M, about 0.75 µM to about 0.5 M, about 1 µM to about 0.25 M, about 1 µM to about 0.1 M, about 5 µM to about 50 mM, about 10 µM to about 25 mM, about 50 µM to about 25 mM, about 10 µM to about 10 mM, about 5 µM to about 5 mM, or about 0.1 mM to about 1 mM. In another aspect, an effective amount of the bicyclic compound is about 0.1 µM to about 1 M. In another aspect, an effective amount of the bicyclic compound is about 0.5 µM to about 0.75 M. In another aspect, an effective amount of the bicyclic compound is about 0.75 µM to about 0.5 M. In another aspect, an effective amount of the bicyclic compound is about 1 µM to about 0.25 M. In another aspect, an effective amount of the bicyclic compound is about 1 µM to about 0.1 M. In another aspect, an effective amount of the bicyclic compound is about 5 µM to about 50 mM. In another aspect, an effective amount of the bicyclic compound is about 10 µM to about 25 mM. In another aspect, an effective amount of the bicyclic compound is about 50 µM to about 25 mM. In another aspect, an effective amount of the bicyclic compound is about 10 µM to about 10 mM. In another aspect, an effective amount of the bicyclic compound is about 5 µM to about 5 mM. In another aspect, an effective amount of the bicyclic compound is about 0.1 mM to about 1 mM.

In another aspect, one or more (e.g., several) bicyclic compounds are used in any of the methods of the present invention.

In another aspect of the present invention, the bicyclic compound(s) may be recycled from a completed saccharification or completed saccharification and fermentation to a new saccharification. The bicyclic compound(s) can be recovered using standard methods in the art, e.g., filtration/centrifugation pre- or post-distillation, to remove residual solids, cellular debris, etc. and then recirculated to the new saccharification.

Polypeptides Having Cellulolytic Enhancing Activity and Polynucleotides Thereof

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

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

\[[ILMV]-P-X(4,5)-G-X-Y-[ILMV]-X-R-X-[EQ]-X(4)-[HNQ] \quad (\text{SEQ ID NO: 125 or SEQ ID NO: 126}) \text{ and } [FW]-[TF]-K-[AIV],\]

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

\[H-X(1,2)-G-P-X(3)-[YW]-[AILMV] \quad \text{(SEQ ID NO: 127 or SEQ ID NO: 128)},\]
\[[EQ]-X-Y-X(2)-C-X-[EHQN]-[FILV]-X-[ILV] \quad \text{(SEQ ID NO: 129)}, \text{ or}\]
\[H-X(1,2)-G-P-X(3)-[YW]-[AILMV] \quad \text{(SEQ ID NO: 130 or SEQ ID NO: 131)} \text{ and } [EQ]-X-\]
Y-X(2)-C-X-[EHQN]-[FILV]-X-[ILV] (SEQ ID NO: 132),
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 contiguous
positions. In the above motifs, the accepted IUPAC single letter amino acid abbreviation is
employed.

In a preferred embodiment, the isolated GH61 polypeptide having cellulolytic
enhancing activity further comprises H-X(1,2)-G-P-X(3)-[YW]-[AILMV] (SEQ ID NO: 133 or
SEQ ID NO: 134). In another preferred embodiment, the isolated GH61 polypeptide having
cellulolytic enhancing activity further comprises [EQ]-X-Y-X(2)-C-X-[EHQN]-[FILV]-X-[ILV]
(SEQ ID NO: 135). In another preferred embodiment, the isolated GH61 polypeptide having
cellulolytic enhancing activity further comprises H-X(1,2)-G-P-X(3)-[YW]-[AILMV] (SEQ ID
NO: 136 or SEQ ID NO: 137) and [EQ]-X-Y-X(2)-C-X-[EHQN]-[FILV]-X-[ILV] (SEQ ID NO:
138).

In a second aspect, isolated polypeptides having cellulolytic enhancing activity,
comprise the following motif:

[ILMV]-P-X(4,5)-G-X-Y-[ILMV]-X-R-X-[EQ]-X(3)-A-[HNQ] (SEQ ID NO: 139 or SEQ
ID NO: 140),
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.

In a third aspect, the polypeptide having cellulolytic enhancing activity comprises an
amino acid sequence that has a degree of identity to the mature polypeptide of SEQ ID NO:
2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID
NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24,
SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID
NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46,
SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID
NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 142, SEQ ID NO:
144, SEQ ID NO: 146, SEQ ID NO: 148, SEQ ID NO: 150, SEQ ID NO: 152, SEQ ID NO:
154, SEQ ID NO: 156, SEQ ID NO: 158, SEQ ID NO: 160, SEQ ID NO: 162, or SEQ ID NO:
164 of preferably at least 60%, more preferably at least 65%, more preferably at least 70%,
more preferably at least 75%, more preferably at least 80%, more preferably at least 85%,
even more preferably at least 90%, most preferably at least 91%, at least 92%, at least 93%,
at least 94%, or at least 95%, or at least 100% and even most preferably at least 96%, at
least 97%, at least 98%, at least 99%, or at least 100%.

In a preferred aspect, the mature polypeptide is amino acids 20 to 326 of SEQ ID
NO: 2, amino acids 18 to 239 of SEQ ID NO: 4, amino acids 20 to 258 of SEQ ID NO: 6,
amino acids 19 to 226 of SEQ ID NO: 8, amino acids 20 to 304 of SEQ ID NO: 10, amino acids 23 to 250 of SEQ ID NO: 12, amino acids 22 to 249 of SEQ ID NO: 14, amino acids 20 to 249 of SEQ ID NO: 16, amino acids 18 to 232 of SEQ ID NO: 18, amino acids 16 to 235 of SEQ ID NO: 20, amino acids 19 to 323 of SEQ ID NO: 22, amino acids 16 to 310 of SEQ ID NO: 24, amino acids 20 to 246 of SEQ ID NO: 26, amino acids 22 to 354 of SEQ ID NO: 28, amino acids 22 to 250 of SEQ ID NO: 30, or amino acids 22 to 322 of SEQ ID NO: 32, amino acids 24 to 444 of SEQ ID NO: 34, amino acids 26 to 253 of SEQ ID NO: 36, amino acids 20 to 223 of SEQ ID NO: 38, amino acids 18 to 246 of SEQ ID NO: 40, amino acids 20 to 334 of SEQ ID NO: 42, amino acids 18 to 227 of SEQ ID NO: 44, amino acids 22 to 368 of SEQ ID NO: 46, amino acids 25 to 330 of SEQ ID NO: 48, amino acids 17 to 236 of SEQ ID NO: 50, amino acids 17 to 250 of SEQ ID NO: 52, amino acids 23 to 478 of SEQ ID NO: 54, amino acids 17 to 230 of SEQ ID NO: 56, amino acids 20 to 257 of SEQ ID NO: 58, amino acids 23 to 251 of SEQ ID NO: 60, amino acids 19 to 349 of SEQ ID NO: 62, amino acids 24 to 436 of SEQ ID NO: 64, amino acids 21 to 344 of SEQ ID NO: 142, amino acids 21 to 389 of SEQ ID NO: 144, amino acids 22 to 406 of SEQ ID NO: 146, amino acids 20 to 427 of SEQ ID NO: 148, amino acids 18 to 267 of SEQ ID NO: 150, amino acids 21 to 273 of SEQ ID NO: 152, amino acids 21 to 322 of SEQ ID NO: 154, amino acids 18 to 234 of SEQ ID NO: 156, amino acids 24 to 233 of SEQ ID NO: 158, amino acids 17 to 237 of SEQ ID NO: 160, amino acids 20 to 484 of SEQ ID NO: 162, or amino acids 22 to 320 of SEQ ID NO: 164.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 2 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 2. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 2. In another preferred aspect, the polypeptide comprises or consists of amino acids 20 to 236 of SEQ ID NO: 2, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 20 to 326 of SEQ ID NO: 2.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 4 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 4. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 4. In another preferred aspect, the polypeptide comprises or consists of amino acids 18 to 239 of SEQ ID NO: 4, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 18 to 239 of SEQ ID NO: 4.
A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 6 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 6. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 6. In another preferred aspect, the polypeptide comprises or consists of amino acids 20 to 258 of SEQ ID NO: 6, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 20 to 258 of SEQ ID NO: 6.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 8 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 8. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 8. In another preferred aspect, the polypeptide comprises or consists of amino acids 19 to 226 of SEQ ID NO: 8, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 19 to 226 of SEQ ID NO: 8.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 10 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 10. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 10. In another preferred aspect, the polypeptide comprises or consists of amino acids 20 to 304 of SEQ ID NO: 10, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 20 to 304 of SEQ ID NO: 10.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 12 or an allelic variant thereof; or a fragment thereof having cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 12. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 12. In another preferred aspect, the polypeptide comprises or consists of amino acids 16 to 317 of SEQ ID NO: 12, or an allelic variant thereof; or a fragment thereof having cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 16 to 317 of SEQ ID NO: 12.
of the amino acid sequence of SEQ ID NO: 14 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 14. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 14.

In another preferred aspect, the polypeptide comprises or consists of amino acids 23 to 250 of SEQ ID NO: 14, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 23 to 250 of SEQ ID NO: 14.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 16 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 16. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 16. In another preferred aspect, the polypeptide comprises or consists of amino acids 20 to 249 of SEQ ID NO: 16, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 20 to 249 of SEQ ID NO: 16.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 18 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 18. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 18. In another preferred aspect, the polypeptide comprises or consists of amino acids 18 to 232 of SEQ ID NO: 18, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 18 to 232 of SEQ ID NO: 18.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 20 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 20. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 20. In another preferred aspect, the polypeptide comprises or consists of amino acids 16 to 235 of SEQ ID NO: 20, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 16 to 235 of SEQ ID NO: 20.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 22 or an allelic variant thereof; or a fragment
thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 22. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 22. In another preferred aspect, the polypeptide comprises or consists of amino acids 19 to 323 of SEQ ID NO: 22, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 19 to 323 of SEQ ID NO: 22.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 24 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 24. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 24. In another preferred aspect, the polypeptide comprises or consists of amino acids 16 to 310 of SEQ ID NO: 24, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 16 to 310 of SEQ ID NO: 24.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 26 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 26. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 26. In another preferred aspect, the polypeptide comprises or consists of amino acids 20 to 246 of SEQ ID NO: 26, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 20 to 246 of SEQ ID NO: 26.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 28 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 28. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 28. In another preferred aspect, the polypeptide comprises or consists of amino acids 22 to 354 of SEQ ID NO: 28, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 22 to 354 of SEQ ID NO: 28.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 30 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide
comprises or consists of the amino acid sequence of SEQ ID NO: 30. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 30. In another preferred aspect, the polypeptide comprises or consists of amino acids 22 to 250 of SEQ ID NO: 30, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 22 to 250 of SEQ ID NO: 30.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 32 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 32. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 32. In another preferred aspect, the polypeptide comprises or consists of amino acids 22 to 322 of SEQ ID NO: 32, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 22 to 322 of SEQ ID NO: 32.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 34 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 34. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 34. In another preferred aspect, the polypeptide comprises or consists of amino acids 24 to 444 of SEQ ID NO: 34, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 24 to 444 of SEQ ID NO: 34.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 36 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 36. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 36. In another preferred aspect, the polypeptide comprises or consists of amino acids 26 to 253 of SEQ ID NO: 36, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 26 to 253 of SEQ ID NO: 36.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 38 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 38. In another preferred
aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 38. In another preferred aspect, the polypeptide comprises or consists of amino acids 20 to 223 of SEQ ID NO: 38, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 20 to 223 of SEQ ID NO: 38.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 40 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 40. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 40.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 42 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 42. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 42.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 44 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 44. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 44.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 46 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 46. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 46.
In another preferred aspect, the polypeptide comprises or consists of amino acids 22 to 368 of SEQ ID NO: 46, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 22 to 368 of SEQ ID NO: 46.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 48 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 48. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 48.

In another preferred aspect, the polypeptide comprises or consists of amino acids 25 to 330 of SEQ ID NO: 48, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 25 to 330 of SEQ ID NO: 48.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 50 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 50. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 50. In another preferred aspect, the polypeptide comprises or consists of amino acids 17 to 236 of SEQ ID NO: 50, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 17 to 236 of SEQ ID NO: 50.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 52 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 52. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 52. In another preferred aspect, the polypeptide comprises or consists of amino acids 19 to 250 of SEQ ID NO: 52, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 19 to 250 of SEQ ID NO: 52.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 54 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 54. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 54. In another preferred aspect, the polypeptide comprises or consists of amino acids 23 to 478.
of SEQ ID NO: 54, or an allelic variant thereof; or a fragment thereof that has cellulolytic
enhancing activity. In another preferred aspect, the polypeptide comprises or consists of
amino acids 23 to 478 of SEQ ID NO: 54.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists
of the amino acid sequence of SEQ ID NO: 56 or an allelic variant thereof; or a fragment
thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide
comprises or consists of the amino acid sequence of SEQ ID NO: 56. In another preferred
aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 56.
In another preferred aspect, the polypeptide comprises or consists of amino acids 17 to 230
of SEQ ID NO: 56, or an allelic variant thereof; or a fragment thereof that has cellulolytic
enhancing activity. In another preferred aspect, the polypeptide comprises or consists of
amino acids 17 to 230 of SEQ ID NO: 56.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists
of the amino acid sequence of SEQ ID NO: 58 or an allelic variant thereof; or a fragment
thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide
comprises or consists of the amino acid sequence of SEQ ID NO: 58. In another preferred
aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 58.
In another preferred aspect, the polypeptide comprises or consists of amino acids 20 to 257
of SEQ ID NO: 58, or an allelic variant thereof; or a fragment thereof that has cellulolytic
enhancing activity. In another preferred aspect, the polypeptide comprises or consists of
amino acids 20 to 257 of SEQ ID NO: 58.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists
of the amino acid sequence of SEQ ID NO: 60 or an allelic variant thereof; or a fragment
thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide
comprises or consists of the amino acid sequence of SEQ ID NO: 60. In another preferred
aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 60.
In another preferred aspect, the polypeptide comprises or consists of amino acids 23 to 251
of SEQ ID NO: 60, or an allelic variant thereof; or a fragment thereof that has cellulolytic
enhancing activity. In another preferred aspect, the polypeptide comprises or consists of
amino acids 23 to 251 of SEQ ID NO: 60.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists
of the amino acid sequence of SEQ ID NO: 62 or an allelic variant thereof; or a fragment
thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide
comprises or consists of the amino acid sequence of SEQ ID NO: 62. In another preferred
aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 62.
In another preferred aspect, the polypeptide comprises or consists of amino acids 19 to 349
of SEQ ID NO: 62, or an allelic variant thereof; or a fragment thereof that has cellulolytic
enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 19 to 349 of SEQ ID NO: 62.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 64 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 64. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 64. In another preferred aspect, the polypeptide comprises or consists of amino acids 24 to 436 of SEQ ID NO: 64, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 24 to 436 of SEQ ID NO: 64.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 142 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 142. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 142. In another preferred aspect, the polypeptide comprises or consists of amino acids 21 to 344 of SEQ ID NO: 142, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 21 to 344 of SEQ ID NO: 142.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 144 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 144. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 144. In another preferred aspect, the polypeptide comprises or consists of amino acids 21 to 389 of SEQ ID NO: 144, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 21 to 389 of SEQ ID NO: 144.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 146 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 146. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 146. In another preferred aspect, the polypeptide comprises or consists of amino acids 22 to 406 of SEQ ID NO: 146, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of
amino acids 22 to 406 of SEQ ID NO: 146.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 148 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 148. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 148. In another preferred aspect, the polypeptide comprises or consists of amino acids 20 to 427 of SEQ ID NO: 148, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 20 to 427 of SEQ ID NO: 148.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 150 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 150. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 150. In another preferred aspect, the polypeptide comprises or consists of amino acids 18 to 267 of SEQ ID NO: 150, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 18 to 267 of SEQ ID NO: 150.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 152 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 152. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 152. In another preferred aspect, the polypeptide comprises or consists of amino acids 21 to 273 of SEQ ID NO: 152, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 21 to 273 of SEQ ID NO: 152.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 154 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 154. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 154. In another preferred aspect, the polypeptide comprises or consists of amino acids 21 to 322 of SEQ ID NO: 154, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 21 to 322 of SEQ ID NO: 154.
A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 156 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 156. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 156.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 158 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 158. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 158.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 160 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 160. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 160.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of the amino acid sequence of SEQ ID NO: 162 or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 162. In another preferred aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 162.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of amino acids 18 to 234 of SEQ ID NO: 156, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 18 to 234 of SEQ ID NO: 156.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of amino acids 18 to 234 of SEQ ID NO: 158, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 24 to 233 of SEQ ID NO: 158.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of amino acids 18 to 234 of SEQ ID NO: 158, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 24 to 233 of SEQ ID NO: 158.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of amino acids 18 to 234 of SEQ ID NO: 158, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 24 to 233 of SEQ ID NO: 158.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of amino acids 18 to 234 of SEQ ID NO: 158, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 24 to 233 of SEQ ID NO: 158.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of amino acids 18 to 234 of SEQ ID NO: 158, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 24 to 233 of SEQ ID NO: 158.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of amino acids 18 to 234 of SEQ ID NO: 158, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 24 to 233 of SEQ ID NO: 158.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of amino acids 18 to 234 of SEQ ID NO: 158, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 24 to 233 of SEQ ID NO: 158.

A polypeptide having cellulolytic enhancing activity preferably comprises or consists of amino acids 18 to 234 of SEQ ID NO: 158, or an allelic variant thereof; or a fragment thereof that has cellulolytic enhancing activity. In another preferred aspect, the polypeptide comprises or consists of amino acids 24 to 233 of SEQ ID NO: 158.
of the amino acid sequence of SEQ ID NO: 164 or an allelic variant thereof; or a fragment
thereof that has cellulolytic enhancing activity. In a preferred aspect, the polypeptide
comprises or consists of the amino acid sequence of SEQ ID NO: 164. In another preferred
aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 164.

In another preferred aspect, the polypeptide comprises or consists of amino acids 22 to 320
of SEQ ID NO: 164, or an allelic variant thereof; or a fragment thereof that has cellulolytic
enhancing activity. In another preferred aspect, the polypeptide comprises or consists of
amino acids 22 to 320 of SEQ ID NO: 164.

Preferably, a fragment of the mature polypeptide of SEQ ID NO: 2 contains at least
277 amino acid residues, more preferably at least 287 amino acid residues, and most
preferably at least 297 amino acid residues. Preferably, a fragment of the mature
polypeptide of SEQ ID NO: 4 contains at least 185 amino acid residues, more preferably at
least 195 amino acid residues, and most preferably at least 205 amino acid residues.

Preferably, a fragment of the mature polypeptide of SEQ ID NO: 6 contains at least 200
amino acid residues, more preferably at least 212 amino acid residues, and most
preferably at least 224 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ
ID NO: 8 contains at least 175 amino acid residues, more preferably at least 185 amino acid
residues, and most preferably at least 195 amino acid residues. Preferably, a fragment of the
mature polypeptide of SEQ ID NO: 10 contains at least 240 amino acid residues, more
preferably at least 255 amino acid residues, and most preferably at least 270 amino acid
residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 12 contains at
least 255 amino acid residues, more preferably at least 270 amino acid residues, and most
preferably at least 285 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ
ID NO: 14 contains at least 175 amino acid residues, more preferably at least 190 amino acid
residues, and most preferably at least 205 amino acid residues.

Preferably, a fragment of the mature polypeptide of SEQ ID NO: 16 contains at least 200
amino acid residues, more preferably at least 210 amino acid residues, and most
preferably at least 220 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ
ID NO: 18 contains at least 185 amino acid residues, more preferably at least 195 amino
acid residues, and most preferably at least 205 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 20 contains at least 190 amino acid residues, more
preferably at least 200 amino acid residues, and most preferably at least 210 amino acid
residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 22 contains at
least 260 amino acid residues, more preferably at least 275 amino acid residues, and most
preferably at least 290 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 24 contains at least 250 amino acid residues, more preferably at
least 265 amino acid residues, and most preferably at least 280 amino acid residues.
Preferably, a fragment of the mature polypeptide of SEQ ID NO: 26 contains at least 195 amino acid residues, more preferably at least 205 amino acid residues, and most preferably at least 214 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 28 contains at least 285 amino acid residues, more preferably at least 300 amino acid residues, and most preferably at least 315 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 30 contains at least 200 amino acid residues, more preferably at least 210 amino acid residues, and most preferably at least 220 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 32 contains at least 255 amino acid residues, more preferably at least 270 amino acid residues, and most preferably at least 285 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 34 contains at least 360 amino acid residues, more preferably at least 380 amino acid residues, and most preferably at least 400 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 36 contains at least 200 amino acid residues, more preferably at least 210 amino acid residues, and most preferably at least 220 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 38 contains at least 170 amino acid residues, more preferably at least 180 amino acid residues, and most preferably at least 190 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 40 contains at least 190 amino acid residues, more preferably at least 200 amino acid residues, and most preferably at least 210 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 42 contains at least 265 amino acid residues, more preferably at least 280 amino acid residues, and most preferably at least 295 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 44 contains at least 180 amino acid residues, more preferably at least 190 amino acid residues, and most preferably at least 200 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 46 contains at least 320 amino acid residues, more preferably at least 335 amino acid residues, and most preferably at least 350 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 48 contains at least 255 amino acid residues, more preferably at least 270 amino acid residues, and most preferably at least 285 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 50 contains at least 190 amino acid residues, more preferably at least 200 amino acid residues, and most preferably at least 210 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 52 contains at least 200 amino acid residues, more preferably at least 210 amino acid residues, and most preferably at least 220 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 54 contains at least 380 amino acid residues, more preferably at least 400 amino acid residues, and most preferably at least 420 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 56 contains at least 180
amino acid residues, more preferably at least 190 amino acid residues, and most preferably at least 200 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 58 contains at least 210 amino acid residues, more preferably at least 220 amino acid residues, and most preferably at least 230 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 60 contains at least 190 amino acid residues, more preferably at least 200 amino acid residues, and most preferably at least 210 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 62 contains at least 270 amino acid residues, more preferably at least 290 amino acid residues, and most preferably at least 310 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 64 contains at least 340 amino acid residues, more preferably at least 360 amino acid residues, and most preferably at least 380 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 142 contains at least 280 amino acid residues, more preferably at least 295 amino acid residues, and most preferably at least 310 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 144 contains at least 310 amino acid residues, more preferably at least 330 amino acid residues, and most preferably at least 350 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 146 contains at least 320 amino acid residues, more preferably at least 340 amino acid residues, and most preferably at least 360 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 148 contains at least 350 amino acid residues, more preferably at least 370 amino acid residues, and most preferably at least 390 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 150 contains at least 220 amino acid residues, more preferably at least 230 amino acid residues, and most preferably at least 240 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 152 contains at least 220 amino acid residues, more preferably at least 230 amino acid residues, and most preferably at least 240 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 154 contains at least 255 amino acid residues, more preferably at least 270 amino acid residues, and most preferably at least 285 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 156 contains at least 185 amino acid residues, more preferably at least 195 amino acid residues, and most preferably at least 205 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 158 contains at least 180 amino acid residues, more preferably at least 190 amino acid residues, and most preferably at least 200 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 160 contains at least 190 amino acid residues, more preferably at least 200 amino acid residues, and most preferably at least 210 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ ID NO: 162 contains at least 385 amino acid residues, more preferably at least 410 amino acid residues, and most preferably
at least 435 amino acid residues. Preferably, a fragment of the mature polypeptide of SEQ
ID NO: 164 contains at least 255 amino acid residues, more preferably at least 270 amino
acid residues, and most preferably at least 285 amino acid residues.

Preferably, a subsequence of the mature polypeptide coding sequence of SEQ ID
NO: 1 contains at least 831 nucleotides, more preferably at least 861 nucleotides, and most
preferably at least 891 nucleotides. Preferably, a subsequence of the mature polypeptide
coding sequence of SEQ ID NO: 3 contains at least 555 nucleotides, more preferably at
least 585 nucleotides, and most preferably at least 615 nucleotides. Preferably, a
subsequence of the mature polypeptide coding sequence of SEQ ID NO: 5 contains at least
600 nucleotides, more preferably at least 636 nucleotides, and most preferably at least 672
nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of SEQ
ID NO: 7 contains at least 525 nucleotides, more preferably at least 555 nucleotides, and
most preferably at least 585 nucleotides. Preferably, a subsequence of the mature polypeptide
coding sequence of SEQ ID NO: 9 contains at least 720 nucleotides, more preferably
at least 765 nucleotides, and most preferably at least 810 nucleotides. Preferably, a
subsequence of the mature polypeptide coding sequence of SEQ ID NO: 11 contains at
least 765 nucleotides, more preferably at least 810 nucleotides, and most preferably at least
855 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of
nucleotides 67 to 796 of SEQ ID NO: 13 contains at least 525 nucleotides, more preferably
at least 570 nucleotides, and most preferably at least 615 nucleotides. Preferably, a
subsequence of the mature polypeptide coding sequence of SEQ ID NO: 15 contains at
least 600 nucleotides, more preferably at least 630 nucleotides, and most preferably at least
660 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of
SEQ ID NO: 17 contains at least 555 nucleotides, more preferably at least 585 nucleotides,
and most preferably at least 615 nucleotides. Preferably, a subsequence of the mature polypeptide
coding sequence of SEQ ID NO: 19 contains at least 570 nucleotides, more preferably at least 600 nucleotides, and most preferably at least 630 nucleotides. Preferably, a
subsequence of the mature polypeptide coding sequence of SEQ ID NO: 21 contains at
least 780 nucleotides, more preferably at least 825 nucleotides, and most preferably at least
870 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of
SEQ ID NO: 23 contains at least 750 nucleotides, more preferably at least 795 nucleotides,
and most preferably at least 840 nucleotides. Preferably, a subsequence of the mature polypeptide
coding sequence of SEQ ID NO: 25 contains at least 585 nucleotides, more preferably at least 615 nucleotides, and most preferably at least 645 nucleotides. Preferably, a
subsequence of the mature polypeptide coding sequence of SEQ ID NO: 27 contains at
least 855 nucleotides, more preferably at least 900 nucleotides, and most preferably at least
945 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of
SEQ ID NO: 29 contains at least 600 nucleotides, more preferably at least 630 nucleotides, and most preferably at least 660 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of SEQ ID NO: 31 contains at least 765 nucleotides, more preferably at least 810 nucleotides, and most preferably at least 855 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of SEQ ID NO: 33 contains at least 1180 nucleotides, more preferably at least 1140 nucleotides, and most preferably at least 1200 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of SEQ ID NO: 35 contains at least 600 nucleotides, more preferably at least 630 nucleotides, and most preferably at least 660 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of SEQ ID NO: 37 contains at least 170 amino acid residues, more preferably at least 180 amino acid residues, and most preferably at least 190 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of SEQ ID NO: 39 contains at least 570 nucleotides, more preferably at least 600 nucleotides, and most preferably at least 630 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of SEQ ID NO: 41 contains at least 795 nucleotides, more preferably at least 840 nucleotides, and most preferably at least 885 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of SEQ ID NO: 43 contains at least 540 nucleotides, more preferably at least 570 nucleotides, and most preferably at least 600 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of SEQ ID NO: 45 contains at least 960 nucleotides, more preferably at least 1005 nucleotides, and most preferably at least 1050 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of SEQ ID NO: 47 contains at least 765 nucleotides, more preferably at least 810 nucleotides, and most preferably at least 855 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of SEQ ID NO: 49 contains at least 570 nucleotides, more preferably at least 600 nucleotides, and most preferably at least 630 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of SEQ ID NO: 51 contains at least 600 nucleotides, more preferably at least 630 nucleotides, and most preferably at least 660 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of SEQ ID NO: 53 contains at least 1140 nucleotides, more preferably at least 1200 nucleotides, and most preferably at least 1260 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of SEQ ID NO: 55 contains at least 540 nucleotides, more preferably at least 570 nucleotides, and most preferably at least 600 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of SEQ ID NO: 57 contains at least 630 nucleotides, more preferably at least 690 nucleotides, and most preferably at least 720 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of SEQ ID NO: 59 contains at least 570 nucleotides, more preferably at least 600 nucleotides, and most preferably at least
630 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of
SEQ ID NO: 61 contains at least 810 nucleotides, more preferably at least 870 nucleotides,
and most preferably at least 930 nucleotides. Preferably, a subsequence of the mature
polypeptide coding sequence of SEQ ID NO: 63 contains at least 1020 nucleotides, more
preferably at least 1080 nucleotides, and most preferably at least 1140 nucleotides.
Preferably, a subsequence of the mature polypeptide coding sequence of SEQ ID NO: 141
contains at least 840 nucleotides, more preferably at least 885 nucleotides, and most
preferably at least 930 nucleotides. Preferably, a subsequence of the mature polypeptide
coding sequence of SEQ ID NO: 143 contains at least 930 nucleotides, more preferably at
least 960 nucleotides, and most preferably at least 1050 nucleotides. Preferably, a
subsequence of the mature polypeptide coding sequence of SEQ ID NO: 145 contains at
least 960 nucleotides, more preferably at least 1020 nucleotides, and most preferably at
least 1080 nucleotides. Preferably, a subsequence of the mature polypeptide coding
sequence of SEQ ID NO: 147 contains at least 1050 nucleotides, more preferably at least
1110 nucleotides, and most preferably at least 1170 nucleotides. Preferably, a subsequence
of the mature polypeptide coding sequence of SEQ ID NO: 149 contains at least 660
nucleotides, more preferably at least 690 nucleotides, and most preferably at least 720
nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of SEQ
ID NO: 151 contains at least 660 nucleotides, more preferably at least 690 nucleotides, and
most preferably at least 720 nucleotides. Preferably, a subsequence of the mature polypeptide
coding sequence of SEQ ID NO: 153 contains at least 765 nucleotides, more preferably at
least 810 nucleotides, and most preferably at least 855 nucleotides. Preferably, a
subsequence of the mature polypeptide coding sequence of SEQ ID NO: 155 contains at
least 555 nucleotides, more preferably at least 585 nucleotides, and most preferably at least
615 nucleotides. Preferably, a subsequence of the mature polypeptide coding sequence of
SEQ ID NO: 157 contains at least 540 nucleotides, more preferably at least 570 nucleotides,
and most preferably at least 600 nucleotides. Preferably, a subsequence of the mature
polypeptide coding sequence of SEQ ID NO: 159 contains at least 570 nucleotides, more
preferably at least 600 nucleotides, and most preferably at least 630 nucleotides. Preferably,
a subsequence of the mature polypeptide coding sequence of SEQ ID NO: 161 contains at
least 1155 nucleotides, more preferably at least 1230 nucleotides, and most preferably at
least 1305 nucleotides. Preferably, a subsequence of the mature polypeptide coding
sequence of SEQ ID NO: 163 contains at least 765 nucleotides, more preferably at least 810
nucleotides, and most preferably at least 855 nucleotides.

In a fourth aspect, the polypeptide having cellulolytic enhancing activity is encoded
by a polynucleotide that hybridizes under at least very low stringency conditions, preferably
at least low stringency conditions, more preferably at least medium stringency conditions,
more preferably at least medium-high stringency conditions, even more preferably at least high stringency conditions, and most preferably at least very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 141, SEQ ID NO: 143, SEQ ID NO: 145, SEQ ID NO: 151, SEQ ID NO: 153, SEQ ID NO: 15, SEQ ID NO: 161, or SEQ ID NO: 163, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 157, or SEQ ID NO: 159, or the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 141, SEQ ID NO: 143, SEQ ID NO: 145, SEQ ID NO: 147, SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 153, SEQ ID NO: 161, or SEQ ID NO: 163, (iii) a subsequence of (i) or (ii), or (iv) a full-length complementary strand of (i), (ii), or (iii) (J. Sambrook, E.F. Fritsch, and T. Maniatis, 1989, supra). A subsequence of the mature polypeptide coding sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 141, SEQ ID NO: 143, SEQ ID NO: 145, SEQ ID NO: 147, SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 153, SEQ ID NO: 155, SEQ ID NO: 157, SEQ ID NO: 159, or the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 141, SEQ ID NO: 143, SEQ ID NO: 145, SEQ ID NO: 147, SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 153, SEQ ID NO: 155, SEQ ID NO: 157, SEQ ID NO: 159, SEQ ID NO: 161, or SEQ ID NO: 163 contains at least 100 contiguous nucleotides or preferably at least 200 contiguous nucleotides. Moreover, the subsequence may encode a polypeptide fragment that has cellulolytic enhancing activity. In a preferred aspect, the mature polypeptide coding sequence is nucleotides 388 to 1332 of SEQ ID NO: 1, nucleotides 98 to 821 of SEQ ID NO: 3, nucleotides 126 to 978 of SEQ ID NO: 5, nucleotides 55 to 678 of SEQ ID NO: 7, nucleotides 58 to 912 of SEQ ID NO: 9, nucleotides 46 to 951 of SEQ ID NO: 11,
nucleotides 67 to 796 of SEQ ID NO: 13, nucleotides 77 to 766 of SEQ ID NO: 15,
nucleotides 52 to 921 of SEQ ID NO: 17, nucleotides 46 to 851 of SEQ ID NO: 19,
nucleotides 55 to 1239 of SEQ ID NO: 21, nucleotides 46 to 1250 of SEQ ID NO: 23,
nucleotides 58 to 811 of SEQ ID NO: 25, nucleotides 64 to 1112 of SEQ ID NO: 27,
nucleotides 64 to 859 of SEQ ID NO: 29, nucleotides 64 to 1018 of SEQ ID NO: 31,
nucleotides 70 to 1483 of SEQ ID NO: 33, nucleotides 76 to 832 of SEQ ID NO: 35,
nucleotides 58 to 974 of SEQ ID NO: 37, nucleotides 52 to 875 of SEQ ID NO: 39,
nucleotides 58 to 1250 of SEQ ID NO: 41, nucleotides 52 to 795 of SEQ ID NO: 43,
nucleotides 64 to 1104 of SEQ ID NO: 45, nucleotides 73 to 990 of SEQ ID NO: 47,
nucleotides 49 to 1218 of SEQ ID NO: 49, nucleotides 55 to 930 of SEQ ID NO: 51,
nucleotides 67 to 1581 of SEQ ID NO: 53, nucleotides 49 to 865 of SEQ ID NO: 55,
nucleotides 58 to 1065 of SEQ ID NO: 57, nucleotides 67 to 868 of SEQ ID NO: 59,
nucleotides 55 to 1099 of SEQ ID NO: 61, nucleotides 70 to 1483 of SEQ ID NO: 63,
nucleotides 61 to 1032 of SEQ ID NO: 141, nucleotides 61 to 1167 of SEQ ID NO: 143,
nucleotides 64 to 1218 of SEQ ID NO: 145, nucleotides 58 to 1281 of SEQ ID NO: 147,
nucleotides 52 to 801 of SEQ ID NO: 149, nucleotides 61 to 819 of SEQ ID NO: 151,
nucleotides 61 to 966 of SEQ ID NO: 153, nucleotides 52 to 702 of SEQ ID NO: 155,
nucleotides 70 to 699 of SEQ ID NO: 157, nucleotides 49 to 711 of SEQ ID NO: 159,
nucleotides 76 to 1452 of SEQ ID NO: 161, or nucleotides 64 to 1018 of SEQ ID NO: 163.

The nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID
NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17,
SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID
NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39,
SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID
NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61,
SEQ ID NO: 63, SEQ ID NO: 141, SEQ ID NO: 143, SEQ ID NO: 145, SEQ ID NO: 147,
SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 153, SEQ ID NO: 155, SEQ ID NO: 157,
SEQ ID NO: 159, SEQ ID NO: 161, or SEQ ID NO: 163, or a subsequence thereof; as well
as the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8,
SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID
NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30,
SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID
NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52,
SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID
NO: 64, SEQ ID NO: 142, SEQ ID NO: 144, SEQ ID NO: 146, SEQ ID NO: 148, SEQ ID NO:
150, SEQ ID NO: 152, SEQ ID NO: 154, SEQ ID NO: 156, SEQ ID NO: 158, SEQ ID NO:
160, SEQ ID NO: 162, or SEQ ID NO: 164, or a fragment thereof, may be used to design a
nucleic acid probe to identify and clone DNA encoding polypeptides having cellulolytic
enhancing activity from strains of different genera or species according to methods well
known in the art. In particular, such probes can be used for hybridization with the genomic
DNA or cDNA of the genus or species of interest, following standard Southern blotting
procedures, in order to identify and isolate the corresponding gene therein. Such probes can
be considerably shorter than the entire sequence, but should be at least 14, preferably at
least 25, more preferably at least 35, and most preferably at least 70 nucleotides in length. It
is, however, preferred that the nucleic acid probe is at least 100 nucleotides in length. For
example, the nucleic acid probe may be at least 200 nucleotides, preferably at least 300
nucleotides, more preferably at least 400 nucleotides, or most preferably at least 500
nucleotides in length. Even longer probes may be used, e.g., nucleic acid probes that are
preferably at least 600 nucleotides, more preferably at least 700 nucleotides, even more
preferably at least 800 nucleotides, or most preferably at least 900 nucleotides in length.
Both DNA and RNA probes can be used. The probes are typically labeled for detecting the
polypeptide corresponding gene (for example, with $^{32}$P, $^{3}$H, $^{35}$S, biotin, or avidin). Such probes are
encompassed by the present invention.

A genomic DNA or cDNA library prepared from such other strains may, therefore, be
screened for DNA that hybridizes with the probes described above and encodes a
polypeptide having cellulolytic enhancing activity. Genomic or other DNA from such other
strains may be separated by agarose or polyacrylamide gel electrophoresis, or other
separation techniques. DNA from the libraries or the separated DNA may be transferred to
and immobilized on nitrocellulose or other suitable carrier material. In order to identify a
clone or DNA that is homologous with SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID
NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17,
SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID
NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39,
SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID
NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61,
SEQ ID NO: 63, SEQ ID NO: 141, SEQ ID NO: 143, SEQ ID NO: 145, SEQ ID NO: 147,
SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 153, SEQ ID NO: 155, SEQ ID NO: 157,
SEQ ID NO: 159, SEQ ID NO: 161, or SEQ ID NO: 163, or a subsequence thereof, the
carrier material is preferably used in a Southern blot.

For purposes of the present invention, hybridization indicates that the nucleotide
sequence hybridizes to a labeled nucleic acid probe corresponding to the mature
polypeptide coding sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID
NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID
NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29,
SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 141, SEQ ID NO: 143, SEQ ID NO: 145, SEQ ID NO: 147, SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 153, SEQ ID NO: 155, SEQ ID NO: 157, SEQ ID NO: 159, SEQ ID NO: 161, or SEQ ID NO: 163; the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 155, SEQ ID NO: 157, or SEQ ID NO: 159, or the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 141, SEQ ID NO: 143, SEQ ID NO: 145, SEQ ID NO: 147, SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 153, SEQ ID NO: 161, or SEQ ID NO: 163; the full-length complementary strand thereof; or a subsequence thereof, under very low to very high stringency conditions, as described supra.

In a preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 1. In another preferred aspect, the nucleic acid probe is nucleotides 388 to 1332 of SEQ ID NO: 1. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 2, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 1. In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pEJG120 which is contained in E. coli NRRL B-30699, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pEJG120 which is contained in E. coli NRRL B-30699.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 3. In another preferred aspect, the nucleic acid probe is nucleotides 98 to 821 of SEQ ID NO: 3. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 4, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 3. In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pTter61C which is contained in E. coli NRRL B-30813, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pTter61C which is contained in E. coli NRRL B-30813.
In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 5. In another preferred aspect, the nucleic acid probe is nucleotides 126 to 978 of SEQ ID NO: 5. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 6, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 5.

In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pTter61D which is contained in E. coli NRRL B-30812, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pTter61D which is contained in E. coli NRRL B-30812.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 7. In another preferred aspect, the nucleic acid probe is nucleotides 55 to 678 of SEQ ID NO: 7. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 8, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 7.

In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pTter61E which is contained in E. coli NRRL B-30814, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pTter61E which is contained in E. coli NRRL B-30814.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 9. In another preferred aspect, the nucleic acid probe is nucleotides 58 to 912 of SEQ ID NO: 9. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 10, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 9.

In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pTter61G which is contained in E. coli NRRL B-3081 1, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pTter61G which is contained in E. coli NRRL B-3081 1.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 11. In another preferred aspect, the nucleic acid probe is nucleotides 46 to 951 of SEQ ID NO: 11. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 12, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 11.

In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pTter61 F which is contained in E. coli NRRL B-50044, wherein the polynucleotide
sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding region contained in plasmid pTTer61 F which is contained in *E. coli* NRRL B-50044.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 13. In another preferred aspect, the nucleic acid probe is nucleotides 67 to 796 of SEQ ID NO: 13. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 14, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 13. In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pDZA2-7 which is contained in *E. coli* NRRL B-30704, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pDZA2-7 which is contained in *E. coli* NRRL B-30704.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 15. In another preferred aspect, the nucleic acid probe is nucleotides 77 to 766 of SEQ ID NO: 15. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 16, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 15. In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pTr3337 which is contained in *E. coli* NRRL B-30878, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pTr3337 which is contained in *E. coli* NRRL B-30878.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 17. In another preferred aspect, the nucleic acid probe is nucleotides 52 to 921 of SEQ ID NO: 17. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 18, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 17. In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pSMai190 which is contained in *E. coli* NRRL B-50084, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pSMai190 which is contained in *E. coli* NRRL B-50084.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 19. In another preferred aspect, the nucleic acid probe is nucleotides 46 to 851 of SEQ ID NO: 19. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 20, or a
subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 19.
In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained
in plasmid pSMai192 which is contained in E. coli NRRL B-50086, wherein the
polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing
activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding
sequence contained in plasmid pSMai192 which is contained in E. coli NRRL B-50086.
In another preferred aspect, the nucleic acid probe is the mature polypeptide coding
sequence of SEQ ID NO: 21. In another preferred aspect, the nucleic acid probe is
nucleotides 55 to 1239 of SEQ ID NO: 21. In another preferred aspect, the nucleic acid
probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 22, or a
subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 21.
In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained
in plasmid pSMai191 which is contained in E. coli NRRL B-50085, wherein the
del polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing
activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding
sequence contained in plasmid pSMai191 which is contained in E. coli NRRL B-50085.
In another preferred aspect, the nucleic acid probe is the mature polypeptide coding
sequence contained in plasmid pSMai191 which is contained in E. coli NRRL B-50085.
In another preferred aspect, the nucleic acid probe is the mature polypeptide coding
sequence of SEQ ID NO: 23. In another preferred aspect, the nucleic acid probe is
nucleotides 46 to 1250 of SEQ ID NO: 23. In another preferred aspect, the nucleic acid
probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 24, or a
subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 23.
In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained
in plasmid pSMai193 which is contained in E. coli NRRL B-50087, wherein the
del polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing
activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding
sequence contained in plasmid pSMai193 which is contained in E. coli NRRL B-50087.
In another preferred aspect, the nucleic acid probe is the mature polypeptide coding
sequence of SEQ ID NO: 25. In another preferred aspect, the nucleic acid probe is
nucleotides 58 to 811 of SEQ ID NO: 25. In another preferred aspect, the nucleic acid probe
is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 26, or a
subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 25.
In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained
in plasmid pSMai187 which is contained in E. coli NRRL B-50083, wherein the
del polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing
activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding
sequence contained in plasmid pSMai187 which is contained in E. coli NRRL B-50083.
In another preferred aspect, the nucleic acid probe is the mature polypeptide coding
sequence of SEQ ID NO: 27. In another preferred aspect, the nucleic acid probe is nucleotides 64 to 1112 of SEQ ID NO: 27. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 28, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 27.

In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pXYZ1473 which is contained in *E. coli* DSM 22075, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pXYZ1473 which is contained in *E. coli* DSM 22075.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 29. In another preferred aspect, the nucleic acid probe is nucleotides 64 to 859 of SEQ ID NO: 29. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 30, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 29.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 31. In another preferred aspect, the nucleic acid probe is nucleotides 64 to 1018 of SEQ ID NO: 31. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 32, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 31.

In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pGEM-T-Ppin7 which is contained in *E. coli* DSM 22711, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pGEM-T-Ppin7 which is contained in *E. coli* DSM 22711.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 33. In another preferred aspect, the nucleic acid probe is nucleotides 70 to 1483 of SEQ ID NO: 33. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 34, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 33.

In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pXYZ1483 which is contained in *E. coli* DSM 22600, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pXYZ1483 which is contained in *E. coli* DSM 22600.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 35. In another preferred aspect, the nucleic acid probe is nucleotides 76 to 832 of SEQ ID NO: 35. In another preferred aspect, the nucleic acid probe...
is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 36, or a
subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 35.
In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained
in plasmid pGEM-T-GH61D23Y4 which is contained in *E. coli* DSM 22882, wherein the
polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing
activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding
sequence contained in plasmid pGEM-T-GH61D23Y4 which is contained in *E. coli* DSM
22882.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding
sequence of SEQ ID NO: 37. In another preferred aspect, the nucleic acid probe is
nucleotides 58 to 974 of SEQ ID NO: 37. In another preferred aspect, the nucleic acid probe
is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 38, or a
subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 37.
In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained
in plasmid pSMai213 which is contained in *E. coli* NRRL B-50300, wherein the
polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing
activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding
sequence contained in plasmid pSMai213 which is contained in *E. coli* NRRL B-50300.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding
sequence of SEQ ID NO: 39. In another preferred aspect, the nucleic acid probe is
nucleotides 52 to 875 of SEQ ID NO: 39. In another preferred aspect, the nucleic acid probe
is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 40, or a
subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 39.
In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained
in plasmid pSMai216 which is contained in *E. coli* NRRL B-50301, wherein the
polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing
activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding
sequence contained in plasmid pSMai216 which is contained in *E. coli* NRRL B-50301.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding
sequence of SEQ ID NO: 41. In another preferred aspect, the nucleic acid probe is
nucleotides 58 to 1250 of SEQ ID NO: 41. In another preferred aspect, the nucleic acid
probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 42, or a
subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 41.
In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained
in plasmid *p* pSMai217 which is contained in *E. coli* NRRL B-50302, wherein the
polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing
activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding
sequence contained in plasmid pSMai217 which is contained in *E. coli* NRRL B-50302.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 43. In another preferred aspect, the nucleic acid probe is nucleotides 52 to 795 of SEQ ID NO: 43. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 44, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 43. In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pSMai218 which is contained in *E. coli* NRRL B-50303, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pSMai218 which is contained in *E. coli* NRRL B-50303.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 45. In another preferred aspect, the nucleic acid probe is nucleotides 64 to 1104 of SEQ ID NO: 45. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 46, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 45. In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pAG68 which is contained in *E. coli* NRRL B-50320, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pAG68 which is contained in *E. coli* NRRL B-50320.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 47. In another preferred aspect, the nucleic acid probe is nucleotides 73 to 990 of SEQ ID NO: 47. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 48, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 47. In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pAG69 which is contained in *E. coli* NRRL B-50321, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pAG69 which is contained in *E. coli* NRRL B-50321.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 49. In another preferred aspect, the nucleic acid probe is nucleotides 49 to 1218 of SEQ ID NO: 49. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 50, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 49. In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained
in plasmid pAG75 which is contained in *E. coli* NRRL B-50322, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pAG75 which is contained in *E. coli* NRRL B-50322.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 51. In another preferred aspect, the nucleic acid probe is nucleotides 55 to 930 of SEQ ID NO: 51. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 52, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 51.

In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pAG76 which is contained in *E. coli* NRRL B-50323, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pAG76 which is contained in *E. coli* NRRL B-50323.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 53. In another preferred aspect, the nucleic acid probe is nucleotides 67 to 1581 of SEQ ID NO: 53. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 54, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 53.

In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pAG77 which is contained in *E. coli* NRRL B-50324, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pAG77 which is contained in *E. coli* NRRL B-50324.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 55. In another preferred aspect, the nucleic acid probe is nucleotides 49 to 865 of SEQ ID NO: 55. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 56, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 55.

In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pAG78 which is contained in *E. coli* NRRL B-50325, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pAG78 which is contained in *E. coli* NRRL B-50325.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 57. In another preferred aspect, the nucleic acid probe is nucleotides 58 to 1065 of SEQ ID NO: 57. In another preferred aspect, the nucleic acid
probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 58, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 57. In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pAG79 which is contained in *E. coli* NRRL B-50326, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pAG79 which is contained in *E. coli* NRRL B-50326.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 59. In another preferred aspect, the nucleic acid probe is nucleotides 67 to 868 of SEQ ID NO: 59. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 60, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 59. In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid plasmid pGEM-T-GH61a51486 which is contained in *E. coli* DSM 22656, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pGEM-T-GH61a51486 which is contained in *E. coli* DSM 22656.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 61. In another preferred aspect, the nucleic acid probe is nucleotides 55 to 1099 of SEQ ID NO: 61. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 62, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 61. In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pGEM-T-GH61DYF which is contained in *E. coli* DSM 22654, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pGEM-T-GH61DYF which is contained in *E. coli* DSM 22654.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 63. In another preferred aspect, the nucleic acid probe is nucleotides 70 to 1483 of SEQ ID NO: 63. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 64, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 63. In another preferred aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pGEM-T-GH61D14YH which is contained in *E. coli* DSM 22657, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing
activity. In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence contained in plasmid pGEM-T-GH61D14YH which is contained in E. coli DSM 22657.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 141. In another preferred aspect, the nucleic acid probe is nucleotides 61 to 1032 of SEQ ID NO: 141. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 141, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 141.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 143. In another preferred aspect, the nucleic acid probe is nucleotides 61 to 1167 of SEQ ID NO: 143. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 143, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 143.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 145. In another preferred aspect, the nucleic acid probe is nucleotides 64 to 1218 of SEQ ID NO: 145. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 145, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 145.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 147. In another preferred aspect, the nucleic acid probe is nucleotides 58 to 1281 of SEQ ID NO: 147. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 147, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 147.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 149. In another preferred aspect, the nucleic acid probe is nucleotides 52 to 801 of SEQ ID NO: 149. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 149, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 149.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 151. In another preferred aspect, the nucleic acid probe is nucleotides 61 to 819 of SEQ ID NO: 151. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 151, or a
In another preferred aspect, the nucleic acid probe is SEQ ID NO: 151.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 153. In another preferred aspect, the nucleic acid probe is nucleotides 61 to 966 of SEQ ID NO: 153. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 153, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 153.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 155. In another preferred aspect, the nucleic acid probe is nucleotides 52 to 702 of SEQ ID NO: 155. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 155, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 155.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 157. In another preferred aspect, the nucleic acid probe is nucleotides 70 to 699 of SEQ ID NO: 157. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 157, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 157.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 159. In another preferred aspect, the nucleic acid probe is nucleotides 49 to 711 of SEQ ID NO: 159. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 159, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 159.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 161. In another preferred aspect, the nucleic acid probe is nucleotides 76 to 1452 of SEQ ID NO: 161. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 161, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO: 161.

In another preferred aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 163. In another preferred aspect, the nucleic acid probe is nucleotides 64 to 1018 of SEQ ID NO: 163. In another preferred aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 163, or a subsequence thereof. In another preferred aspect, the nucleic acid probe is SEQ ID NO:
For long probes of at least 100 nucleotides in length, very low to very high stringency conditions are defined as prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and either 25% formamide for very low and low stringencies, 35% formamide for medium and medium-high stringencies, or 50% formamide for high and very high stringencies, following standard Southern blotting procedures for 12 to 24 hours optimally. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 45°C (very low stringency), at 50°C (low stringency), at 55°C (medium stringency), at 60°C (medium-high stringency), at 65°C (high stringency), and at 70°C (very high stringency).

For short probes of about 15 nucleotides to about 70 nucleotides in length, stringency conditions are defined as prehybridization and hybridization at about 5°C to about 10°C below the calculated Tm using the calculation according to Bolton and McCarthy (1962, Proc. Natl. Acad. Sci. USA 48:1390) in 0.9 M NaCl, 0.09 M Tris-HCl pH 7.6, 6 mM EDTA, 0.5% NP-40, 1X Denhardt’s solution, 1 mM sodium pyrophosphate, 1 mM sodium monobasic phosphate, 0.1 mM ATP, and 0.2 mg of yeast RNA per ml following standard Southern blotting procedures for 12 to 24 hours optimally. The carrier material is finally washed once in 6X SSC plus 0.1% SDS for 15 minutes and twice each for 15 minutes using 6X SSC at 5°C to 10°C below the calculated Tm.

In a fifth aspect, the polypeptide having cellulolytic enhancing activity is encoded by a polynucleotide comprising or consisting of a nucleotide sequence that has a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 141, SEQ ID NO: 143, SEQ ID NO: 145, SEQ ID NO: 147, SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 153, SEQ ID NO: 155, SEQ ID NO: 157, SEQ ID NO: 159, SEQ ID NO: 161, or SEQ ID NO: 163 of preferably at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 91%, at least 92%, at least 93%, at least 94%, or at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, at least 99%, or at least 100%.

In a sixth aspect, the polypeptide having cellulolytic enhancing activity is an artificial variant comprising a substitution, deletion, and/or insertion of one or more (e.g., several)
amino acids of the mature polypeptide of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, or SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 142, SEQ ID NO: 144, SEQ ID NO: 146, SEQ ID NO: 148, SEQ ID NO: 150, SEQ ID NO: 152, SEQ ID NO: 154, SEQ ID NO: 156, SEQ ID NO: 158, SEQ ID NO: 160, SEQ ID NO: 162, or SEQ ID NO: 164; or a homologous sequence thereof. Preferably, amino acid changes are 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 one to about 30 amino acids; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue; a small linker peptide of up to about 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 group 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. The most commonly occurring exchanges 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/AI, 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 parent 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 cellulolytic enhancing 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; Wodaver et al., 1992, FEBS Lett. 309: 59-64. The identities of essential amino acids can also be inferred from analysis of identities with polypeptides that are related to the parent 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 total number of amino acid substitutions, deletions and/or insertions of the mature polypeptide of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, or SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 142, SEQ ID NO: 144, SEQ ID NO: 146, SEQ ID NO: 148, SEQ ID NO: 150, SEQ ID NO: 152, SEQ ID NO: 154, SEQ ID NO: 156, SEQ ID NO: 158, SEQ ID NO: 160, SEQ ID NO: 162, or SEQ ID NO: 164, is not more than 10, e.g., 1, 2, 3, 4, 5, 6, 7, 8 or 9.

A polypeptide having cellulolytic enhancing activity may be obtained from microorganisms of any genus. For purposes of the present invention, the term "obtained from" as used herein in connection with a given source shall mean that the polypeptide encoded by a polynucleotide is produced by the source or by a strain in which the polynucleotide from the source has been inserted. In one aspect, the polypeptide obtained from a given source is secreted extracellularly.
A polypeptide having cellulolytic enhancing 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, or Oceanobacillus polypeptide having cellulolytic enhancing activity, or a Gram negative bacterial polypeptide such as an E. coli, Pseudomonas, Salmonella, Campylobacter, Helicobacter, Flavobacterium, Fusobacterium, Ilyobacter, Neisseria, or Ureaplasma polypeptide having cellulolytic enhancing 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 cellulolytic enhancing activity.

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

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

The polypeptide having cellulolytic enhancing 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 cellulolytic enhancing 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, Penicillum, Phanerochaete, Piromyces, Poitrasia, Pseudoplectania, Pseudotrichonympha, Rhizomucor, Schizopyllum, Scytalidium, Talaromyces, Thermoascus, Thielavia, Tolypocladium, Trichoderma, Trichophyta, Verticillium, Volvariella, or Xylaria polypeptide having cellulolytic enhancing activity.

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

In another aspect, the polypeptide is an Acremonium cellulolyticus, Aspergillus aculeatus, Aspergillus awamori, Aspergillus fumigatus, Aspergillus foetidus, Aspergillus

It will be understood that for the aforementioned species the invention encompasses both the perfect and imperfect states, and other taxonomic equivalents, e.g., anamorphs, regardless of the species name by which they are known. Those skilled in the art will readily recognize the identity of appropriate equivalents.

Strains of these species are readily accessible to the public in a number of culture collections, such as the American Type Culture Collection (ATCC), Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM), Centraalbureau Voor Schimmelcultures (CBS), and Agricultural Research Service Patent Culture Collection, Northern Regional Research Center (NRRL).

Furthermore, polypeptides having cellulolytic enhancing activity may be identified and obtained from other sources including microorganisms isolated from nature (e.g., soil, composts, water, etc.) using the above-mentioned probes. Techniques for isolating microorganisms from natural habitats are well known in the art. The polynucleotide may then be obtained by similarly screening a genomic DNA or cDNA library of such a microorganism. Once a polynucleotide encoding a polypeptide has been detected with the probe(s), the polynucleotide can be isolated or cloned by utilizing techniques that are well known to those of ordinary skill in the art (see, e.g., Sambrook et al., 1989, supra).

Polynucleotides comprising nucleotide sequences that encode polypeptide having cellulolytic enhancing activity can be isolated and utilized to express the polypeptide having cellulolytic enhancing activity for evaluation in the methods of the present invention.
The techniques used to isolate or clone a polynucleotide encoding a polypeptide are known in the art and include isolation from genomic DNA, preparation from cDNA, or a combination thereof. The cloning of the polynucleotides from such genomic DNA can be effected, e.g., by using the well known polymerase chain reaction (PCR) or antibody screening of expression libraries to detect cloned DNA fragments with shared structural features. See, e.g., Innis et al., 1990, PCR: A Guide to Methods and Application, Academic Press, New York. Other nucleic acid amplification procedures such as ligase chain reaction (LCR), ligation activated transcription (LAT) and polynucleotide-based amplification (NASBA) may be used. The polynucleotides may be cloned from a strain or a related organism and thus, for example, may be an allelic or species variant of the polypeptide encoding region of the polynucleotide.

The polynucleotides comprise nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 141, SEQ ID NO: 143, SEQ ID NO: 145, SEQ ID NO: 147, SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 153, SEQ ID NO: 155, SEQ ID NO: 157, SEQ ID NO: 159, SEQ ID NO: 161, or SEQ ID NO: 163 of preferably at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 91%, at least 92%, at least 93%, at least 94%, or at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellulolytic enhancing activity.

The polynucleotide may also be a polynucleotide encoding a polypeptide having cellulolytic enhancing activity that hybridizes under at least very low stringency conditions, preferably at least low stringency conditions, more preferably at least medium stringency conditions, more preferably at least medium-high stringency conditions, even more preferably at least high stringency conditions, and most preferably at least very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO:
57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 141, SEQ ID NO: 143,
SEQ ID NO: 145, SEQ ID NO: 147, SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 153,
SEQ ID NO: 155, SEQ ID NO: 157, SEQ ID NO: 159, SEQ ID NO: 161, or SEQ ID NO: 163,
(ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO:
7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 155, SEQ ID NO: 157, or
SEQ ID NO: 159 or the cDNA sequence of the mature polypeptide coding sequence of SEQ
ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 19,
SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID
NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41,
SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID
NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63,
SEQ ID NO: 141, SEQ ID NO: 143, SEQ ID NO: 145, SEQ ID NO: 147, SEQ ID NO: 149,
SEQ ID NO: 151, SEQ ID NO: 153, SEQ ID NO: 161, or SEQ ID NO: 163, or (iii) a full-length
complementary strand of (i) or (ii); or allelic variants and subsequences thereof (Sambrook
et al., 1989, supra), as defined herein.

As described earlier, the techniques used to isolate or clone a polynucleotide
encoding a polypeptide are known in the art and include isolation from genomic DNA,
preparation from cDNA, or a combination thereof.

20 Enzyme Compositions

The enzyme compositions can comprise any protein that is useful in degrading or
converting a cellulosic material.

In one aspect, the enzyme composition comprises or further comprises one or more
(e.g., several) proteins selected from the group consisting of a cellulase, 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.

In another aspect, 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., 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 an endoglucanase and a cellobiohydrolase. In another aspect, the enzyme 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 beta-glucosidase.

In another aspect, 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, 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 aspect, 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 $\text{H}_2\text{O}_2$-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 fermentation, e.g., during saccharification or during or after propagation of the fermenting microorganism(s).

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 crude fermentation broth with or without cells removed, 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 enzymes can be derived or obtained from any suitable origin, including, bacterial, fungal, yeast, plant, or mammalian origin. The term “obtained” means herein that the enzyme may have been isolated from an organism that naturally produces the enzyme as a native enzyme. 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.

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

In another preferred 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*, *Melnocarpus*, *Meripilus*, *Mucor*, *Myceliophthora*, *Neoallomastix*, *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 a preferred aspect, the polypeptide is a *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae*, *Saccharomyces diastaticus*, *Saccharomyces douglasii*, *Saccharomyces kluyveri*, *Saccharomyces norbensis*, or *Saccharomyces oviformis* polypeptide having enzyme activity.

fimeti, Thielavia microspora, Thielavia ovispora, Thielavia peruviana, Thielavia spedeonium, Thielavia setosa, Thielavia subthermophila, Thielavia terrestris, Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Trichoderma reesei, Trichoderma viride, or Trichophaga saccata polypeptide having enzyme activity.

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

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 enzymes may also be prepared by purifying such a protein from 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 (Novozymes A/S), CELLIC™ CTec2 (Novozymes A/S), CELLUCLAST™ (Novozymes A/S), NOVOZYM™ 188 (Novozymes A/S), CELLUZYM™ (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 (Rohm 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, more preferably from about 0.025 to about 4.0 wt % of solids, and most preferably from about 0.005 to about 2.0 wt % of solids. The cellulase enzymes are added in amounts effective from about 0.001 to about 5.0 wt % of solids, more preferably from about 0.025 to about 4.0 wt % of solids, and most preferably from about 0.005 to about 2.0 wt % of solids.

Examples of bacterial endoglucanases that can be used in the methods 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. - 74 -
Trichoderma reesei endoglucanase (Saloheimo, et al., 1988, Gene 63:1 1-22; 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; SEQ ID NO: 70); Trichoderma reesei endoglucanase V (Saloheimo et al., 1994, Molecular Microbiology 13: 219-228; GENBANK™ accession no. Z33381; SEQ ID NO: 72); 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 carotovara 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 (SEQ ID NO: 74); Myceliophthora thermophila CBS 117.65 endoglucanase (SEQ ID NO: 76); basidiomycete CBS 495.95 endoglucanase (SEQ ID NO: 78); basidiomycete CBS 494.95 endoglucanase (SEQ ID NO: 80); Thielavia terrestris NRRL 8126 CEL6B endoglucanase (SEQ ID NO: 82); Thielavia terrestris NRRL 8126 CEL6C endoglucanase (SEQ ID NO: 84); Thielavia terrestris NRRL 8126 CEL7C endoglucanase (SEQ ID NO: 86); Thielavia terrestris NRRL 8126 CEL7E endoglucanase (SEQ ID NO: 88); Thielavia terrestris NRRL 8126 CEL7F endoglucanase (SEQ ID NO: 90); Cladophrina foecundissimum ATCC 62373 CEL7A endoglucanase (SEQ ID NO: 92); and Trichoderma reesei strain No. VTT-D-80133 endoglucanase (SEQ ID NO: 94; GENBANK™ accession no. M15665). The endoglucanases of SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, and SEQ ID NO: 94 described above are encoded by the mature polypeptide coding sequence of SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, and SEQ ID NO: 93, respectively.

Examples of cellobiohydrolases useful in the present invention include, but are not limited to, Trichoderma reesei cellobiohydrolase I (SEQ ID NO: 96); Trichoderma reesei cellobiohydrolase II (SEQ ID NO: 98); Humicola insolens cellobiohydrolase I (SEQ ID NO: 100); Myceliophthora thermophila cellobiohydrolase II (SEQ ID NO: 102 and SEQ ID NO: 104); Thielavia terrestris cellobiohydrolase II (CEL6A) (SEQ ID NO: 106); Chaetomium thermophilum cellobiohydrolase I (SEQ ID NO: 108); and Chaetomium thermophilum cellobiohydrolase II (SEQ ID NO: 110). The cellobiohydrolases of SEQ ID NO: 96, SEQ ID
Examples of beta-glucosidases useful in the present invention include, but are not limited to, *Aspergillus oryzae* beta-glucosidase (SEQ ID NO: 112); *Aspergillus fumigatus* beta-glucosidase (SEQ ID NO: 114); *Penicillium brasiliannum* IBT 20888 beta-glucosidase (SEQ ID NO: 116); *Aspergillus niger* beta-glucosidase (SEQ ID NO: 118); and *Aspergillus aculeatus* beta-glucosidase (SEQ ID NO: 120). The beta-glucosidases of SEQ ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 116, SEQ ID NO: 118, and SEQ ID NO: 120 described above are encoded by the mature polypeptide coding sequence of SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 115, SEQ ID NO: 117, and SEQ ID NO: 119, respectively.

Examples of other beta-glucosidases useful in the present invention include a *Aspergillus oryzae* beta-glucosidase variant fusion protein of SEQ ID NO: 122 or the *Aspergillus oryzae* beta-glucosidase fusion protein of SEQ ID NO: 124. The beta-glucosidase fusion proteins of SEQ ID NO: 122 and SEQ ID NO: 124 are encoded by SEQ ID NO: 121 and SEQ ID NO: 123, respectively.


In one aspect, 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), and Thielavia terrestris NRRL 8126 xylanases (WO 2009/07921 0).

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, Hypocrea jecorina acetylxylan esterase (WO 2005/001036), Neurospora crassa acetylxylan esterase (UniProt accession number Q7s259), Thielavia terrestris NRRL 8126 acetylxylan esterase (WO 2009/042846), Chaetomium globosum acetylxylan esterase (Uniprot accession number Q2GWX4), Chaetomium gracile acetylxylan esterase (GeneSeqP accession number AAB82124), Phaeosphaeria nodorum acetylxylan esterase (Uniprot accession number Q4UHJ1), and Humicola insolens DSM 1800 acetylxylan esterase (WO 2009/073709).

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, *Humicola insolens* DSM 1800 arabinofuranosidase (WO 2009/073383) and *Aspergillus niger* arabinofuranosidase (GeneSeqP accession number AAR94170).

Examples of alpha-glucuronidases useful in the methods of the present invention include, but are not limited to, *Aspergillus clavatus* alpha-glucuronidase (UniProt accession number alcc12), *Trichoderma reesei* alpha-glucuronidase (UniProt accession number Q99024), *Talaromyces emersonii* alpha-glucuronidase (UniProt accession number Q8X211), *Aspergillus niger* alpha-glucuronidase (UniProt accession number Q96WX9), *Aspergillus terreus* alpha-glucuronidase (SwissProt accession number Q0CJP9), and *Aspergillus fumigatus* alpha-glucuronidase (SwissProt accession number Q4WW45).

The enzymes and proteins 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. 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.

**Nucleic Acid Constructs**

An isolated polynucleotide encoding a polypeptide, e.g., a polypeptide having cellulolytic enhancing activity, a cellulolytic enzyme, a hemicellulolytic enzyme, 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. Manipulation of the polynucleotide's sequence 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 sequence, a polynucleotide that is recognized by a host cell for expression of a polynucleotide encoding a polypeptide. The promoter sequence 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 of choice 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 the transcription of the nucleic acid constructs in the present invention in a bacterial host cell are the promoters obtained from the Bacillus amylo liquefaciens 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, E. coli lac operon, 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 lac 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 suitable promoters for directing the transcription of the nucleic acid constructs in 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 (glA), Aspergillus oryzae TAKA amylase, Aspergillus oryzae alkaline protease, Aspergillus oryzae triose phosphate isomerase, Fusarium oxysporum trypsin-like protease (WO 96/00787), Fusarium venenatum amylloglucosidase (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 V, Trichoderma reesei xylanase I, Trichoderma reesei xylanase II, Trichoderma reesei beta-xilosidas, as well as the NA2-tpi promoter (a modified promoter from a gene encoding a neutral alpha-amylase in Aspergillus in which the untranslated leader has been replaced by an untranslated leader from a gene encoding triose phosphate isomerase in Aspergillus; non-limiting examples include modified promoters from the gene encoding neutral alpha-amylase in Aspergillus niger in which the untranslated leader has
been replaced by an untranslated leader from the gene encoding triose phosphate isomerase in *Aspergillus nidulans* or *Aspergillus oryzae*; 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 suitable transcription terminator sequence, which is recognized by a host cell to terminate transcription. The terminator sequence is operably linked to the 3'-terminus of the polynucleotide encoding the polypeptide. Any terminator that is functional in the host cell of choice may be used in the present invention.

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

Preferred polyadenylation sequences for filamentous fungal host cells are obtained


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. The foreign signal peptide coding sequence may be required where the coding sequence does not naturally contain a signal peptide coding sequence. Alternatively, the 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 of choice 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.


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 at the N-terminus of a polypeptide, 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 allow the regulation of the expression of the polypeptide relative to the growth of the host cell. Examples of regulatory systems are those that cause the 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 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 polypeptide having cellulolytic enhancing activity, a cellulolytic enzyme, a hemicellulolytic enzyme, etc., at such sites. Alternatively, the polynucleotide may be expressed by inserting the polynucleotide or a nucleic acid construct comprising the sequence 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 the *dal* genes from *Bacillus subtilis* or *Bacillus licheniformis*, or markers that confer antibiotic resistance such as ampicillin, chloramphenicol, kanamycin, or tetracycline resistance. Suitable markers for yeast host cells are 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* (oroticine-5'-phosphate decarboxylase), *sC* (sulfate adenyltransferase), and *trpC* (anthranilate synthase), as well as equivalents thereof. Preferred for use in an *Aspergillus* cell are the *amdS* and *pyrG* genes of *Aspergillus nidulans* or *Aspergillus oryzae* and the *bar* gene of *Streptomyces hygroscopicus*.

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's sequence 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 pUB1 10, pE194, pTA1060, and pAMβI 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 ANSI (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 polypeptide having cellulolytic enhancing activity, a cellulolytic enzyme, a hemicellulolytic enzyme, etc., can be advantageously used in the recombinant production of the polypeptide. A construct or vector comprising such a polynucleotide is introduced into a host cell so that the vector is maintained as a chromosomal integrant or as a self-replicating extrachromosomal 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 amylo liquefaciens*, *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.

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. *Zooplasma* cells.

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

189-207), by electroporation (see, e.g., Buckley et al., 1999, Appl. Environ. Microbiol. 65: 3800-3804) or by 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.

The host cell may also be a eukaryote, such as a mammalian, insect, plant, or fungal cell.

The host cell may be a fungal cell. "Fungi" as used herein includes the phyla Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota (as defined by Hawksworth et al., In, Ainsworth and Bisby's Dictionary of The Fungi, 8th edition, 1995, CAB International, University Press, Cambridge, UK) as well as the Oomycota (as cited in Hawksworth et al., 1995, supra, page 171) and all mitosporic fungi (Hawksworth et al., 1995, supra).

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, Schizosaccharomyces, or Yarrowia cell such as a Kluyveromyces lactis, Saccharomyces carlsbergensis, Saccharomyces cerevisiae, Saccharomyces diastaticus, Saccharomyces douglasi, Saccharomyces kluyveri, Saccharomyces norbensis, Saccharomyces oviformis, or Yarrowia lipolytica cell.

The fungal host cell may be a filamentous fungal cell. "Filamentous fungi" include all 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 catabolism may be fermentative.

The filamentous fungal host cell may be an Acremonium, Aspergillus, Aureobasidium, Bjerkandera, Ceriporiopsis, Chrysosporium, Coprinus, Coriolus, Cryptococcus, Filobasidium, Fusarium, Humicola, Magnaporthe, Mucor, Myceliophthora, Neocallimastix, Neurospora, Paecilomyces, Penicillium, Phanerochaete, Phlebia, Piromyces, Pleurotus, Schizophyllum, 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,


Methods of Production

Methods for producing a polypeptide, e.g., a polypeptide having cellulolytic enhancing activity, a cellulolytic enzyme, a hemicellulolytic enzyme, etc., comprise (a) cultivating a cell, which in its wild-type form is capable of producing the polypeptide, under conditions conducive for production of the polypeptide; and (b) recovering the polypeptide.

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

In the production methods, the cells are cultivated in a nutrient medium suitable for...
production of the polypeptide using methods well known in the art. For example, the cell may be cultivated by shake flask cultivation, and small-scale 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 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 may include 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.

The resulting broth may be used as is or 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, centrifugation, filtration, extraction, spray-drying, evaporation, or precipitation.

The polypeptides 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, J.-C. Janson and Lars Ryden, editors, VCH Publishers, New York, 1989) to obtain substantially pure polypeptides.

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.

Methods for Processing Cellulosic Material

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

The present invention also 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 cellulolytic enhancing activity and a bicyclic compound. In one aspect, the method above further comprises recovering the degraded or converted cellulosic material. Soluble products of degradation or conversion of the cellulosic material can be separated from the insoluble cellulosic material using technology well known in the art such as, for example, centrifugation, filtration, and gravity settling.

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 cellulolytic enhancing activity and a bicyclic compound; (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 also 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 saccharified with an enzyme composition in the presence of a polypeptide having cellulolytic enhancing activity and a bicyclic compound. 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 one aspect, the bicyclic compound is recovered following saccharification or fermentation and recycled back to a new saccharification reaction. Recycling of the bicyclic compound can be accomplished using processes conventional in the art.

The processing of 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 cofermentation (SSCF); hybrid hydrolysis and fermentation (HFF); 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 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 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 cofermentation 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.


The cellulosic material can also be subjected to particle size reduction, pre-soaking, wetting, washing, 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, 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 1-15 minutes, 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 H$_2$SO$_4$ or S0$_2$ (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), and organosolv pretreatments.

In dilute acid pretreatment, cellulosic material is mixed with dilute acid, typically H$_2$SO$_4$, 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 2006/1 10891, WO 2006/1 1899, WO 2006/1 1900, and WO 2006/1 10901 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: 1133-141; 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.


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.

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.


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 fermentable sugars, such as glucose, cellobiose, xylose, xylulose, arabinose, mannose, galactose, and/or soluble oligosaccharides. The hydrolysis is performed enzymatically by an enzyme composition in the presence of a polypeptide having cellulolytic enhancing activity and a bicyclic compound. The enzyme and protein components of the compositions can be added 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 a preferred 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 pretreated cellulosic material (substrate) 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 96 hours, more preferably about 16 to about 72 hours, and most preferably about 24 to about 48 hours. The temperature is in the range of preferably about 25°C to about 70°C, more preferably about 30°C to about 65°C, and more preferably about 40°C to 60°C, in particular about 50°C. The pH is in the range of preferably about 3 to about 8, more preferably about 3.5 to about 7, and most preferably about 4 to about 6, in particular about pH 5. The dry solids content is in the range of preferably about 5 to about 50 wt %, more preferably about 10 to about 40 wt %, and most preferably about 20 to about 30 wt %.

The optimum amounts of the enzymes and polypeptides having cellulolytic enhancing activity depend on several factors including, but not limited to, the mixture of component cellulolytic enzymes, the cellulosic substrate, the concentration of cellulosic substrate, the pretreatment(s) of the cellulosic substrate, temperature, time, pH, and inclusion of fermenting organism (e.g., yeast for Simultaneous Saccharification and Fermentation).

In one aspect, an effective amount of cellulolytic or hemicellulolytic enzyme protein to cellulosic material is about 0.5 to about 50 mg, preferably at about 0.5 to about 40 mg, more preferably at about 0.5 to about 25 mg, more preferably at about 0.75 to about 20 mg, more preferably at about 0.75 to about 15 mg, even more preferably at about 0.5 to about 10 mg, and most preferably at about 2.5 to about 10 mg per g of cellulosic material.

In another aspect, an effective amount of a 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 at about 0.025 to about 1.5 mg, more preferably at about 0.05 to about 1.25
mg, more preferably at about 0.075 to about 1.25 mg, more preferably at about 0.1 to about
1.25 mg, even more preferably at about 0.15 to about 1.25 mg, and most preferably at about
0.25 to about 1.0 mg per g of cellulosic material.

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

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 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 C₆ and/or C₅ fermenting organisms, or
a combination thereof. Both C₆ and C₅ fermenting organisms are well known in the art. Suitable
fermenting microorganisms are able to ferment, i.e., convert, sugars, such as glucose,
xyllose, xylulose, arabinose, maltose, mannose, galactose, or oligosaccharides, directly or
indirectly into the desired fermentation product.


Examples of fermenting microorganisms that can ferment C₆ sugars include bacterial and fungal organisms, such as yeast. Preferred yeast includes strains of the *Saccharomyces* spp., preferably *Saccharomyces cerevisiae*.

Examples of fermenting organisms that can ferment C₅ sugars include bacterial and fungal organisms, such as some yeast. Preferred C₅ fermenting yeast include strains of *Pichia*, preferably *Pichia stipitis*, such as *Pichia stipitis* CBS 5773; strains of *Candida*, preferably *Candida boidinii*, *Candida brassicae*, *Candida sheatae*, *Candida diddensii*, *Candida pseudotropicalis*, or *Candida utilis*.

Other fermenting organisms include strains of *Zymomonas*, such as *Zymomonas mobilis*; *Hansenula*, such as *Hansenula anomala*; *Kluvyeromyces*, such as *K. fragilis*; *Schizosaccharomyces*, such as *S. pombe*; *E. coli*, especially *E. coli* strains that have been genetically modified to improve the yield of ethanol; *Clostridium*, such as *Clostridium acetobutylicum*, *Clostridium thermocellum*, and *Clostridium phytofermentans*; *Geobacillus* *sp.*; *Thermoanaerobacter*, such as *Thermoanaerobacter saccharolyticum*; and *Bacillus*, such as *Bacillus coagulans*.

In a preferred aspect, the yeast is a *Saccharomyces* spp. In a 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*. In another preferred aspect, the yeast is a *Kluvyeromyces*. In another more preferred aspect, the yeast is *Klyveromyces marxianus*. In another more preferred aspect, the yeast is *Klyveromyces fragilis*. In another preferred aspect, the yeast is a *Candida*. In another more preferred aspect, the yeast is *Candida boidinii*. 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 pseudotropicalis*. In another more preferred aspect, the yeast is *Candida utilis*. In 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 *Pachysolen*. In another more preferred aspect, the yeast is *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 *Bretannomyces*. In another more preferred aspect, the yeast is *Bretannomyces clausenii* (Philippidis, G. P., 1996, Cellulose bioconversion technology, in *Handbook on Bioethanol: Production and Utilization*, Wyman, C. E., ed., Taylor & Francis, Washington, DC, 179-212).

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

In a preferred aspect, the bacterium is a *Zymomonas*. In a more preferred aspect, the bacterium is *Zymomonas mobilis*. In another preferred aspect, the bacterium is a *Clostridium*. In another more preferred aspect, the bacterium is *Clostridium thermocellum*.

Commercially available yeast suitable for ethanol production includes, e.g., ETHANOL RED™ yeast (available from Fermentis/Lesaffre, USA), FALI™ (available from Fleischmann’s Yeast, USA), SUPERSTART™ and THERMOSACC™ fresh yeast (available from Ethanol Technology, WI, USA), BIOFERM™ AFT and XR (available from NABC - North American Bioproducts Corporation, GA, USA), GERT STRAND™ (available from Gert Strand AB, Sweden), and FERMIOL™ (available from DSM Specialties).

In a preferred aspect, the fermenting microorganism has been genetically modified to provide the ability to ferment pentose sugars, such as xylose utilizing, arabinose utilizing, and xylose and arabinose co-utilizing microorganisms.


In a preferred aspect, the genetically modified fermenting microorganism is *Saccharomyces cerevisiae*. In another preferred aspect, the genetically modified fermenting microorganism is *Zymomonas mobilis*. 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* sp.

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 lignocellulose or hydrolysate and the fermentation is performed for about 8 to about 96 hours, such as about 24 to about 60 hours. The temperature is typically between about 26°C to about 60°C, in particular about 32°C or 50°C, and at about pH 3 to about pH 8, such as around pH 4-5, 6, or 7.

In a preferred aspect, the yeast and/or another microorganism is 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 a preferred aspect, the temperature is preferably between about 20°C to about 60°C, more preferably about 25°C to about 50°C, and most preferably about 32°C to about 50°C, in particular about 32°C or 50°C, and the pH is generally from about pH 3 to about pH 7, preferably around pH 4-7. However, some fermenting organisms, e.g., bacteria, have higher fermentation temperature optima. Yeast or another microorganism is preferably applied in amounts of approximately 10⁵ to 10¹⁰, preferably from approximately 10⁷ to 10⁹, especially approximately 2 x 10⁸ 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. 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 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 glycol], butanediol, glyc erin, 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, acetonic acid, adipic acid, ascorbic acid, citric acid, 2,5-diketo-D-gluconic 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.

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 (e.g., several) 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 another more preferred aspect, the alcohol is butanediol. 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, Song, C. S., Cao, N. J., Du, J., and Tsao, G. T., 1999, Ethanol production from renewable resources, in Advances in Biochemical Engineering/Biotechnology, Schöter, 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,
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. Kiriyama, 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*,
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
(e.g., several) ketone moieties. In another more preferred aspect, the ketone is acetone.
See, for example, Qureshi and Blaschek, 2003, *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 acetoic 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 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 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.

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 example, fuel ethanol, drinking ethanol, i.e., potable neutral spirits, or industrial ethanol.

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

Examples

Example 1: Methods of evaluating the effect of bicyclic compounds on GH61 polypeptides having cellulosolytic enhancing activity

The effect of various bicyclic compounds on the cellulosolytic enhancing activity of GH61 polypeptides was evaluated according to the procedures described below.

Microcrystalline cellulose and milled washed pretreated corn stover (milled washed PCS) were used as sources of the cellulosic material. Microcrystalline cellulose (AVICEL® PH101) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Milled washed PCS was prepared according to the procedure described below.

Corn stover was pretreated at the U.S. Department of Energy National Renewable
Energy Laboratory (NREL) using 1.4% (w/v) sulfuric acid at 165°C and 107 psi for 8 minutes. The water-insoluble solids in the pretreated corn stover (PCS) contained 56.5% cellulose, 4.6% hemicelluloses, and 28.4% lignin. The cellulose and hemicellulose composition were determined by a two-stage sulfuric acid hydrolysis with subsequent analysis of sugars by high performance liquid chromatography using NREL Standard Analytical Procedure #002. Lignin was determined gravimetrically after hydrolyzing the cellulose and hemicellulose fractions with sulfuric acid using NREL Standard Analytical Procedure #003. Whole slurry PCS was prepared by adjusting the pH to 5.0 by addition of 10 M NaOH with extensive mixing, and then autoclaving for 20 minutes at 120°C. The dry weight of the whole slurry PCS was 29% TS (total solids). Milled washed PCS (dry weight 32.35%) was prepared by milling whole slurry PCS in a Cosmos ICMG 40 wet multi-utility grinder (EssEmm Corporation, Tamil Nadu, India), followed by washing with deionized water and decanting off the supernatant fraction repeatedly until the pH was greater than 4.

A *Trichoderma reesei* cellulase composition (CELLUCLAST® supplemented with *Aspergillus oryzae* beta-glucosidase, available from Novozymes A/S, Bagsvaerd, Denmark) was used as the cellulase preparation. The cellulase preparation is designated herein in the Examples as "*Trichoderma reesei* cellulase composition".

The hydrolysis reactions of AVICEL® or milled washed PCS were conducted using 2.0 ml deep-well plates (Axygen, Union City, CA, USA) in a total reaction volume of 1.0 ml. Each hydrolysis was performed with 29.5 mg of AVICEL® or 50 mg of PCS (total insoluble solids; 28.8 mg of cellulose) per ml of 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate and the *T. reesei* cellulase composition at 4 mg protein per gram of cellulose with or without a bicyclic compound and with or without the *T. aurantiacus* GH61A polypeptide having cellulolytic enhancing activity at 50% (w/w) of total protein, equivalent to 2 mg protein per gram of cellulose (unless otherwise specified). The bicyclic compounds tested were dissolved or suspended at 10 mM in either 20% (v/v) methanol in 50 mM sodium acetate pH 5.0 with 1 mM manganese sulfate or in 100% methanol and were added to saccharification reactions at a final concentration of 1 mM. Control saccharification reactions containing equivalent methanol-containing buffer concentrations, 2% and 10% (v/v) methanol, or no methanol, were performed on each 96-well plate. Plates were then sealed using an ALPS-3000™ plate heat sealer (Abgene, Epsom, United Kingdom), mixed thoroughly, and incubated at 50°C for 7 days in an IsoTemp Incubator (Fisher Scientific, Hampton, NH, USA). All experiments were performed in triplicate, unless otherwise indicated.

In some experiments, GH61 polypeptide having cellulolytic enhancing activity was titrated into the saccharification reactions. These hydrolysis reactions were performed as described above, with the following exceptions: the concentration of the *T. reesei* cellulase
composition was maintained fixed at 4 mg protein per gram of cellulose, while the GH61 polypeptide was varied between 0 and 50% (w/w) of total protein, as indicated, equivalent to 0 to 2 mg of GH61 polypeptide per gram of cellulose.

At various times during saccharification, typically 3 and 7 days, 100 µl aliquots were removed and filtered using a 0.45 µm MULTISCREEN® 96-well filter plate (Millipore, Bedford, MA, USA). The filtrates were analyzed for sugar content as described below. When not used immediately, filtered aliquots were frozen at -20°C. The sugar concentrations of samples, diluted 2-fold in 0.05% w/w benzoic acid in 0.005 M H$_2$SO$_4$, were measured using a 4.6 x 250 mm AMINEX® HPX-87H column (Bio-Rad Laboratories, Inc., Hercules, CA, USA) by elution with 0.05% w/w benzoic acid-0.005 M H$_2$SO$_4$ at 65°C at a flow rate of 0.6 ml per minute, and quantification by integration of the glucose signal from refractive index detection (CHEMSTATION®, AGILENT® 1100 HPLC, Agilent Technologies, Santa Clara, CA, USA) calibrated with pure sugar samples. The resulting glucose concentrations were adjusted for dilution and then used to calculate the percentage of cellulose conversion for each reaction. Data were processed using MICROSOFT EXCEL® software (Microsoft, Richland, WA, USA), or KALEIDAGRAPH® software (Synergy Software, Reading, PA, USA).

The bicyclic compounds evaluated include taxifolin, cyanin, cyanidin, keracyanin, naringenin, morin, kuromanin, quercitin, myricitin, kaempferol, acacetin, epicatechin, naringenin and isorhamnetin. The compounds were obtained from Sigma-Aldrich Co. (St. Louis, Missouri, USA).

The extent of cellulose conversion was calculated based on the mass ratio of glucose to the initial mass of insoluble cellulose. The extent of total cellulose conversion was calculated using the following equation:

$$\text{% conversion} = \frac{\text{[glucose]}_{\text{mg/ml}}}{\text{[cellulose]}_{\text{mg/ml}}} \times 100$$

(Equation 1)

where 1.111 represents the mass conversion factor accounting for the hydration of glucose produced from the cellulose polymer. The fractional hydrolysis is defined herein as the conversion expressed as a fraction rather than as a percent (i.e., % conversion / 100).

The effect of each bicyclic compound tested was quantified by calculating the ratio of percent conversion for the T. reesei cellulase composition with supplemented a GH61 polypeptide having cellulolytic enhancing activity in the presence of each compound to the percent conversion in the absence of each compound:

$$\text{bicyclic compound effect} = \frac{\text{% conversion}_{\text{+compound}}}{\text{% conversion}_{\text{-compound}}}$$

(Equation 2)

A bicyclic compound that enhances saccharification will yield a compound effect ($\text{+GH61} > 1$), a bicyclic compound that inhibits saccharification will yield a compound effect ($\text{+GH61} < 1$), and a bicyclic compound that does not affect saccharification will yield a compound effect ($\text{+GH61} = 1$).
Similarly, the effect of each bicyclic compound tested in the absence of GH61 polypeptide was quantified by calculating the ratio of percent conversion for the 7. reesei cellulase composition without supplemented GH61 polypeptide having cellulolytic enhancing activity in the presence of each compound to the percent conversion in the absence of each compound:

\[
\text{bicyclic compound effect}_{(\text{no GH61})} = \frac{\%_{\text{conversion}}_{\text{GH61}}}{\%_{\text{conversion}}_{\text{(no compound)}}} \quad (\text{Equation 3})
\]

A bicyclic compound that enhances saccharification will yield a bicyclic compound effect_{(\text{no GH61})} > 1, a bicyclic compound that inhibits saccharification will yield a compound effect_{(\text{no GH61})} < 1, and a bicyclic compound that does not affect saccharification will yield a compound effect_{(\text{no GH61})} = 1.

The effect of GH61 polypeptide in the presence of each bicyclic compound was quantified by calculating ratio of percent conversion for the 7. reesei cellulase composition in the presence of each compound with a GH61 polypeptide present to the percent conversion in the absence of GH61 polypeptide:

\[
\text{GH61 effect} = \frac{\%_{\text{conversion}}_{\text{GH61}}}{\%_{\text{conversion}}_{\text{GH61}}} \quad (\text{Equation 4})
\]

Saccharification conditions under which the GH61 polypeptide enhances saccharification will yield a GH61 effect > 1, conditions under which GH61 polypeptide inhibits saccharification will yield a GH61 effect < 1, and conditions under which the GH61 polypeptide does not affect saccharification will yield a GH61 effect = 1. Thus for saccharification reactions where only the presence of a bicyclic compound differs, a difference in GH61 effect can be attributed to the effect of the bicyclic compound on the cellulolytic enhancing effect of the GH61 polypeptide.

Example 2: Preparation of *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity

7. *aurantiacus* GH61A polypeptide having cellulolytic enhancing activity (SEQ ID NO: 13 [DNA sequence] and SEQ ID NO: 14 [deduced amino acid sequence]) was recombinantly prepared according to WO 2005/074656 using *Aspergillus oryzae* Jal_250 as a host. The recombinantly produced 7. *aurantiacus* GH61A polypeptide was first concentrated from 60 ml to 7 ml, by ultrafiltration using a 10 kDa membrane (VIVASPIN®, GE Healthcare, Piscataway, NJ, USA), buffer exchanged into 20 mM Tris-HCl plus 150 mM NaCl pH 8.0, and then purified using a 320 ml SUPERDEX® 75 column (GE Healthcare, Piscataway, NJ, USA) equilibrated with 20 mM Tris-HCl plus 150 mM NaCl pH 8.0 at a flow rate of 1 ml per minute. Fractions of 5 ml were collected and pooled based on SDS-PAGE. Protein
concentration was determined using a Microplate BCA™ Protein Assay Kit (Thermo Fisher Scientific Inc., Rockford, IL, USA) in which bovine serum albumin was used as a protein standard.

Example 3: Effect of Thermoascus aurantiacus GH61A polypeptide having cellulolytic enhancing activity on hydrolysis of microcrystalline cellulose or PCS by the Trichoderma reesei cellulase composition

The effect of the T. aurantiacus GH61A polypeptide on the hydrolysis of AVICEL® or milled washed PCS by the 7. reesei cellulase composition was determined using the experimental conditions and procedures described in Example 1 in the absence of a bicyclic compound.

The percent conversion in the absence of the T. aurantiacus GH61A polypeptide (Equation 1) averaged over all plates was 26.8±1.27% after 3 days of hydrolysis and 42.0±1.33% after 7 days of hydrolysis. The percent conversion in the presence of 50% (w/w) T. aurantiacus GH61A polypeptide was 26.2±1.17% after 3 days of hydrolysis and 41.4±1.67% after 7 days of hydrolysis. This yielded a GH61 effect of 0.98+0.064 at 3 days, and 0.99+0.051 at 7 days of saccharification (Equation 4). The standard deviations reflect plate-to-plate differences, and within a specific plate, the average standard deviation for conversion was 0.52%.

The percent conversion in the absence of the T. aurantiacus GH61A polypeptide (Equation 1) and the presence of 10% methanol was 11.8+0.702 after 3 days of hydrolysis and 14.7+1.01 after 7 days, whereas in the presence of 50% (w/w) T. aurantiacus GH61A polypeptide, the percent conversions were 11.8+0.379 and 15.1+0.388 at the same time points. These values gave GH61 effects of 1.00+0.068 and 1.02+0.075 (Equation 4). The percent conversion in the absence of the T. aurantiacus GH61A polypeptide and the presence of 2% methanol was 24.9+1.39 and 37.9+1.79 at 3 and 7 days, respectively, of hydrolysis, whereas in the presence of the T. aurantiacus GH61A polypeptide these values were 24.5+1.36 and 38.2+1.54, respectively. This yielded GH61 effects of 0.98+0.078 at 3 days and 1.01+0.06 at 7 days of hydrolysis. The bicyclic compound effect (no GH61) for 2% methanol was 0.93 at 3 days and 0.90 at 7 days (Equation 2). The bicyclic compound effect (no GH61) of 10% methanol was 0.43 at 3 days and 0.35 at 7 days, values that are significantly less than 1. It can therefore be concluded that methanol inhibited saccharification by the T. reesei cellulase composition; however since the GH61 effect was 1, the solvent used for dissolution of a bicyclic compound did not alter the GH61 polypeptide enhancing effect.

Example 4: Effect of bicyclic compounds on Thermoascus aurantiacus GH61A
polypeptide during hydrolysis of microcrystalline cellulose by the *Trichoderma reesei* cellulase composition

The effect of various bicyclic compounds on the cellulolytic enhancing activity of the *T. aurantiacus* GH61A polypeptide during hydrolysis of AVICEL® by the *T. reesei* cellulase composition was determined using the experimental conditions and procedures described in Example 1. The concentration of each compound tested was 1 mM, with the following exceptions: cyanidin chloride (0.52 mM), cyanin chloride (0.26 mM), genistin (0.39 mM), kuromanin chloride (0.34 mM), keracyanin chloride (0.26 mM), and rhamnetin (0.53 mM).

The effect of each bicyclic compound on hydrolysis of a cellulosic material by the *T. reesei* cellulase composition in the absence of a GH61 polypeptide was quantified using the

bicyclic compound effect \( \rho_{\text{o, AVICEL}} \) and bicyclic compound effect \( \rho_{\text{GH61}} \) (Equations 2 and 3); determining the ratio of percent conversion of the cellulosic material in the presence of the bicyclic compound to the percent conversion of the cellulosic material in the absence of the bicyclic compound, but in the presence of the buffer used to dissolve the bicyclic under examination. In general, the bicyclic compound effect \( \rho_{\text{o, GH61}} \) was approximately 1, though the methanol solvent was inhibitory to cellulolysis (Example 3).

Figure 1 shows the effect of the *T. aurantiacus* GH61A polypeptide on the hydrolysis of AVICEL® by the *T. reesei* cellulase composition in the presence of the various bicyclic compounds. The GH61 effect (Equation 4) was plotted for the bicyclic compounds indicated at 3 days (white bars) and 7 days (black bars) of saccharification.

Hydrolysis of AVICEL® by the *T. reesei* cellulase composition was enhanced by the presence of the *T. aurantiacus* GH61A polypeptide and various bicyclic compounds (GH61 effect > 1). Since the *T. aurantiacus* GH61A polypeptide had no cellulolytic enhancing effect on microcrystalline cellulose in the absence of bicyclic compounds (Example 3), it can be concluded that the presence of both the bicyclic compound and the *T. aurantiacus* GH61A polypeptide were required for the observed enhancement of cellulolysis.

The observed enhancement of hydrolysis by GH61 polypeptide was particularly high in the presence of anthocyanins and anthocyanin-like compounds including cyanidin chloride, cyanin chloride, and kuromanin chloride, which had GH61 effects of 1.62 ± 0.136, 1.12 ± 0.0403 and 1.29 ± 0.0219 at 3 days of hydrolysis, and 1.86 ± 0.171, 1.11 ± 0.0197 and 1.41 ± 0.0184 at 7 days of hydrolysis respectively. The observed enhancement of hydrolysis by GH61 polypeptide was also high in the presence of flavonoids including acacetin, quercitin hydrate and morin hydrate, yielding GH61 effects of 0.990 ± 0.0395, 1.02 ± 0.0271 and 1.13 ± 0.0644 at 3 days of hydrolysis and 1.16 ± 0.0536, 1.11 ± 0.0382 and 1.17 ± 0.125 at 7 days of hydrolysis. The overall results demonstrated that cellulolytic enhancing activity of the GH61 polypeptide was apparent in the presence of a bicyclic
compound during hydrolysis of AVICEL® by the *T. reesei* cellulase composition. However, the *T. aurantiacus* GH61 A polypeptide had no detectable effect on hydrolysis of AVICEL® by the *T. reesei* cellulase composition in the absence of a bicyclic compound.

**Example 5: Effect of quercitin concentration on *Thermoascus aurantiacus* GH61 A polypeptide during hydrolysis of microcrystalline cellulose by the *Trichoderma reesei* cellulase composition**

The concentration-dependence of a representative bicyclic compound, quercitin, on the cellulolytic enhancing activity of the *T. aurantiacus* GH61 A polypeptide during hydrolysis of AVICEL® by the *T. reesei* cellulase composition was determined by filtrating the GH61 polypeptide, as described in Example 1, at various concentrations of quercitin between 2 µM and 10 mM.

Figure 2 shows the effect of the *T. aurantiacus* GH61 A polypeptide concentration on percent conversion (Equation 1) of AVICEL® by the *T. reesei* cellulase composition at various concentrations of quercitin between 0 and 10 mM. Panel A shows 3 days of saccharification; panel B shows 7 days of saccharification. Calculations were performed as described in Example 1. In the absence of quercitin, increasing GH61 polypeptide concentration did not enhance percent conversion (solid line, diamonds). For any concentration of quercitin, a net increase in percent conversion was indicated by a higher percent conversion than an equivalent concentration of GH61 polypeptide in the absence of quercitin, evident as a data point that lies above the solid line. The lowest concentrations of quercitin were very weakly enhancing, and increasing concentrations of quercitin were increasingly inhibitory. Increasing GH61 polypeptide concentrations in the presence of quercitin led to higher conversion, and the presence of both GH61 polypeptide and quercitin yielded a net enhancement of hydrolysis at quercitin concentrations of 1 mM or less. Furthermore, the magnitude of the increase in percent conversion with GH61 polypeptide concentration was largest at the highest quercitin concentrations tested.

Figure 3 shows the effect of quercitin concentration on *GH61 effect* (Equation 4) at concentrations of quercitin between 0 and 10 mM for two concentrations of the *T. reesei* cellulase compositions, 2 mg (dashed lines) and 4 mg (solid lines) protein per gram of cellulose, for 7 days of hydrolysis. For 4 mg of the *T. reesei* cellulase composition per g cellulose, the *GH61 effect* was greater than 1 at quercitin concentrations greater than 20 µM. For 2 mg of the *T. reesei* cellulase composition per g cellulose, the *GH61 effect* was greater than 1 at all non-zero quercitin concentrations tested. Increasing concentrations of quercitin increased the magnitude of the *GH61 effect* at both cellulase concentrations.

Since the *T. aurantiacus* GH61 A polypeptide did not enhance cellulolysis of microcrystalline cellulose in the absence of bicyclic compounds (the *GH61 effect* on
AVICEL® = 1, Example 3), it can be concluded that cellulolytic enhancing activity of the GH61 polypeptide was apparent in the presence of a bicyclic compound during hydrolysis of AVICEL® by the 7. reesei cellulase composition, and that increased concentrations of quercitin were required for increased GH61 polypeptide-dependent enhancement of cellulolysis by the 7. reesei cellulase composition.

Example 6: Effect of bicyclic compounds on Thermoascus aurantiacus aurantiacus GH61A polypeptide during hydrolysis of microcrystalline cellulose by the Trichoderma reesei cellulase composition

The effect of various bicyclic compounds on the cellulolytic enhancing activity of the 7. aurantiacus GH61A polypeptide during hydrolysis of AVICEL® by the 7. reesei cellulase composition was determined using the experimental conditions and procedures and quantified as described in Example 1. The bicyclic compounds examined include epicatechin, isorhamnitin, taxifolin, keracyanin, kaempferol, apigenin, and naringenin.

Figures 4A (epicatechin), 4B (isorhamnitin), 4C (taxifolin), 4D (keracyanin), 4E (kaempferol), 4F (apigenin), and 4G (naringenin) show (1) the effect of a bicyclic compound on hydrolysis of AVICEL® by the 7. reesei cellulase composition in the absence of a GH61 polypeptide (white bars), (2) the effect of a bicyclic compound on hydrolysis of AVICEL® by the 7. reesei cellulase composition in the presence of a GH61 polypeptide (grey bars), and (3) the effect of a GH61 polypeptide on hydrolysis of AVICEL® by the 7. reesei cellulase composition in the presence of a bicyclic compound (black bars) at 3 and 7 days of hydrolysis.

Figures 4A to 4G all have the following common characteristic. For all of the bicyclic compounds shown in these figures, the 7 day saccharification time points show an enhancement of cellulose conversion by the 7. reesei cellulase composition with 7. aurantiacus GH61A polypeptide in the presence of the bicyclic compound in comparison to the conversion by the 7. reesei cellulase composition in the absence of the 7. aurantiacus GH61A polypeptide. The bicyclic compounds showed greater or lesser degrees of inhibition of the cellulase composition in the absence of the GH61A polypeptide (Figures 4A to 4G, white bars), and greater or lesser inhibition of the cellulase composition in the presence of the 7. aurantiacus GH61A polypeptide (Figures 4A to 4G, gray bars). In each case, however, addition of the 7. aurantiacus GH61A polypeptide to hydrolysates in the presence of the bicyclic compound enhanced the hydrolysis, compared to hydrolysates performed in the presence of the bicyclic compound without the GH61 polypeptide as indicated by a bicyclic compound effect $\gamma_{(A,H61)}$ greater than the bicyclic compound effect $\gamma_{(A,H61)}$, and a GH61 effect greater than 1, as defined by Equations 2, 3, and 4.

In the presence of many of these bicyclic compounds, the hydrolysis of AVICEL® in
the presence of both the *7. aurantiacus* GH61 A polypeptide and the bicyclic compound was greater than the inhibition by the bicyclic compound, leading to an overall enhancement of cellulolysis. Examples of this include, but are not limited to, keracyanin (Figure 4D).

These results demonstrated that cellulolytic enhancing activity of the GH61 polypeptide was apparent in the presence of these bicyclic compounds during hydrolysis of AVICEL® by the *7. reesei* cellulase composition. However, the *7. aurantiacus* GH61 A polypeptide had no detectable effect on hydrolysis of AVICEL® by the *7. reesei* cellulase composition in the absence of a bicyclic compound (Example 2).

**Example 7: Enhancement of microcrystalline cellulose cellulolysis by the *T. reesei* cellulose composition using combinations of compounds and various GH61 polypeptides**

Combinations of compounds including: pyrogallol, 2-aminophenol, quercitin, 2-hydroxy-1,4-naphthoquinone, morin hydrate and naringenin (Sigma, St. Louis, MO) were tested in conjunction with various GH61 polypeptides for their combined ability to enhance cellulolysis by *7. reesei* cellulases. Saccharification reactions were performed as described (Example 8), using 29.5 mg per ml microcrystalline cellulose (AVICEL®) and 4 mg per g cellulose of *7. reesei* cellulase composition in 50 mM sodium acetate, 1 mM manganese sulfate at pH 5.0 at either a total compound concentration of 3 mM (1 mM of each compound) or a total concentration of 1 mM (0.33 mM of each compound) with GH61's including *Thermoascus aurantiacus* GH61 A polypeptide and *Aspergillus fumigatus* GH61 B polypeptide. Solutions of each compound were made in either 20% or 50% (v/v) methanol in 50 mM sodium acetate pH 5.0 with 1 mM manganese sulfate. These were were added to saccharification reactions at a final concentration of 1 mM or 3 mM as described above. As a control, methanol was added to saccharification reactions at equivalent final concentrations.

Figure 5A shows the fractional hydrolysis of AVICEL® by the *7. reesei* cellulase composition with various GH61 polypeptides as indicated, and combinations of compounds as indicated. Figure 5B shows the GH61 effect for each of these mixtures. The compound mixtures included: dehydroascorbate (DHA), pyrogallol (pyro) and quercitin (querc); pyrogallol, 2-aminophenol (2-AP), 2-hydroxy-1,4-naphthoquinone (naphtho); 2-aminophenol, quercitin, dehydroascorbate and 2-hydroxy-1,4-naphthoquinone, morin hydrate, naringenin. In each case the overall hydrolysis was enhanced by the combined presence of the compound mixtures and the GH61 polypeptides. In each case, the apparent fractional hydrolysis was higher at 1 mM concentration of compounds than either 3 mM compounds or control saccharifications. For most mixtures of compounds examined at 1 mM, *7. aurantiacus* GH61 A polypeptide gave the greatest overall conversion, whereas at 3 mM, *A. fumigatus* GH61 B generally gave the highest overall conversion.
The present invention is further described by the following numbered paragraphs:

[1] A composition comprising: (a) a polypeptide having cellulolytic enhancing activity and (b) a bicyclic compound, wherein the combination of the polypeptide having cellulolytic enhancing activity and the bicyclic compound enhances hydrolysis of a cellulosic material by a cellulolytic enzyme.

[2] The composition of paragraph 1, wherein the bicyclic compound is of formula (I) or (II):

wherein the bond indicated with a dashed line can be single or double;

R¹ and R² are independently hydrogen, -C(0)R ⁵, -C(0)OR ⁶, -C(0)NHR ⁷, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl;

R³, R⁴a, R⁴b, and R⁴c are independently hydrogen, halogen, -OH, -OR ⁸, -CN, -NHR ⁹, -N(R ⁹)(R ¹⁰), -C(0)R ⁵, -C(0)OR ⁶, -C(0)NHR ⁷, -OC(0)R ¹¹, -NHC(0)R ¹², -OC(0)OR ¹³, -NHC(0)OR ¹⁴, -OC(0)NHR ¹⁵, -NHC(0)NHR ¹⁶, -SO₂R ¹⁷, -SO₂N(R ¹⁸)(R ¹⁹), -SR ²⁰, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl; and

wherein two of R⁴a, R⁴b, and R⁴c may together form an optionally substituted fused ring;

R⁵, R⁶, R⁷, R⁸, R⁹, R¹⁰, R¹¹, R¹², R¹³, R¹⁴, R¹⁵, R¹⁶, R¹⁸, R¹⁹, and R²⁰ are independently hydrogen, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl; and

R¹⁷ is an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl;

or a salt or solvate thereof.

[3] The composition of paragraph 2, wherein the bond indicated with a dashed line is single bond.

[4] The composition of paragraph 2, wherein the bond indicated with a dashed line is
a double bond.

[5] The composition of any one of paragraphs 2-4, wherein R¹ and R² are independently hydrogen, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyi, cycloalkyl-alkyl, heterocycloalkyi, heterocycloalkyl-alkyl, aryl, aralkyi, heteroaryl, and heteroaralkyi.

[6] The composition of any one of paragraphs 2-4, wherein R¹ and R² are independently hydrogen, an optionally substituted carbohydrate moiety, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyi, or cycloalkyl-alkyl.

[7] The composition of any one of paragraphs 2-4, wherein R¹ and R² are independently hydrogen, an optionally substituted carbohydrate moiety, or an optionally substituted moiety selected from alkyl, alkenyl, and alkynyl.

[8] The composition of any one of paragraphs 2-4, wherein R¹ and R² are independently hydrogen, an optionally substituted carbohydrate moiety, or an optionally substituted alkyl.

[9] The composition of any one of paragraphs 2-4, wherein R¹ and R² are independently hydrogen, or an optionally substituted carbohydrate moiety.

[10] The composition of any one of paragraphs 2-4, wherein at least one of R¹ and R² is hydrogen.

[11] The composition of any one of paragraphs 2-4, wherein both R¹ and R² are hydrogen.

[12] The composition of any one of paragraphs 2-4, wherein at least one of R¹ and R² is other than hydrogen.

[13] The composition of any one of paragraphs 2-4, wherein at least one of R¹ and R² is an optionally substituted carbohydrate moiety.

[14] The composition of any one of paragraphs 2-4, wherein both R¹ and R² are other than hydrogen.

[15] The composition of any one of paragraphs 2-14, wherein R³, R⁴a, R⁴b, and R⁴c are independently hydrogen, halogen, -OH, -OR⁸, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyi, cycloalkyl-alkyl, heterocycloalkyi, heterocycloalkyl-alkyl, aryl, aralkyi, heteroaryl, and heteroaralkyi; and wherein two of R⁴a, R⁴b, and R⁴c may together form an optionally substituted cyclic diether.

[16] The composition of any one of paragraphs 2-14, wherein R³, R⁴a, R⁴b, and R⁴c are independently hydrogen, -OH, -OR⁸, an optionally substituted carbohydrate moiety, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyi, or cycloalkyl-alkyl.

[17] The composition of any one of paragraphs 2-14, wherein R³, R⁴a, R⁴b, and R⁴c are independently hydrogen, -OH, -OR⁸, an optionally substituted carbohydrate moiety, or an
optionally substituted moiety selected from alkyl, alkenyl, and alkynyl.

[18] The composition of any one of paragraphs 2-14, wherein \( R^3 \), \( R^{4a} \), \( R^{4b} \), and \( R^{4c} \) are independently hydrogen, -OH, -OR, or an optionally substituted carbohydrate moiety.

[19] The composition of any one of paragraphs 2-18, wherein at least one of \( R^{4a} \), \( R^{4b} \), and \( R^{4c} \) is hydrogen.

[20] The composition of any one of paragraphs 2-18, wherein at least two of \( R^{4a} \), \( R^{4b} \), and \( R^{4c} \) are hydrogen.

[21] The composition of any one of paragraphs 2-18, wherein each of \( R^{4a} \), \( R^{4b} \), and \( R^{4c} \) are hydrogen.

[22] The composition of any one of paragraphs 2-18, wherein at least one of \( R^{4a} \), \( R^{4b} \), and \( R^{4c} \) is -OH.

[23] The composition of any one of paragraphs 2-18, wherein at least two of \( R^{4a} \), \( R^{4b} \), and \( R^{4c} \) is -OH.

[24] The composition of any one of paragraphs 2-18, wherein one of \( R^{4a} \), \( R^{4b} \), and \( R^{4c} \) is hydrogen and the other two of \( R^{4a} \), \( R^{4b} \), and \( R^{4c} \) are each -OH.

[25] The composition of any one of paragraphs 2-18, wherein at least one of \( R^{4a} \), \( R^{4b} \), and \( R^{4c} \) is other than hydrogen.

[26] The composition of any one of paragraphs 2-18, wherein at least two of \( R^{4a} \), \( R^{4b} \), and \( R^{4c} \) are other than hydrogen.

[27] The composition of any one of paragraphs 2-18, wherein \( R^3 \) and at least one of \( R^{4a} \), \( R^{4b} \), and \( R^{4c} \) are other than hydrogen.

[28] The composition of any one of paragraphs 2-18, wherein \( R^3 \) and at least two of \( R^{4a} \), \( R^{4b} \), and \( R^{4c} \) are other than hydrogen.

[29] The composition of any one of paragraphs 2-18, wherein at least one of \( R^3 \), \( R^{4a} \), \( R^{4b} \), and \( R^{4c} \) is an optionally substituted carbohydrate moiety.

[30] The composition of any one of paragraphs 2-18, wherein two of \( R^3 \), \( R^{4a} \), \( R^{4b} \), and \( R^{4c} \) are optionally substituted carbohydrate moieties.

[31] The composition of any one of paragraphs 2-30, wherein \( R^3 \) is hydrogen, -OH, or an optionally substituted carbohydrate moiety.

[32] The composition of any one of paragraphs 2-30, wherein \( R^3 \) is hydrogen.

[33] The composition of any one of paragraphs 2-30, wherein \( R^3 \) is other than hydrogen.

[34] The composition of any one of paragraphs 2-30, wherein \( R^3 \) is -OH.

[35] The composition of any one of paragraphs 2-30, wherein \( R^3 \) is an optionally substituted carbohydrate moiety.

[36] The composition of paragraph 2, wherein the bicyclic compound is of the formula (I-A) or (II-A):
wherein \( R_1, R_2, R_4^a, \) and \( R_8 \) are as defined in any one of the preceding paragraphs; or a salt or solvate thereof.

[37] The composition of paragraph 2, wherein the bicyclic compound is of the formula (I-B) or (II-B):

wherein \( R_1, R_2, \) and \( R_4^a \) are as defined in any one of the preceding paragraphs; or a salt or solvate thereof.

[38] The composition of paragraph 2, wherein the bicyclic compound is of the formula (I-C), (I-D), (I-E), or (I-F):

wherein \( R_1, R_2, R_3, R_4^a, R_4^b, \) and \( R_4^c \) are as defined in any one of the preceding paragraphs.
paragraphs;
or a salt or solvate thereof.

[39] The composition of paragraph 2, wherein the bicyclic compound is selected from the group consisting of: (I-1): epicatechin; (I-2): quercetin; (I-3): myricetin; (I-4): taxifolin; (I-5): kaempferol; (I-6): morin; (I-7): acacetin; (I-8): naringenin; (I-9):isorhamnetin; (I-10): apigenin; (I-11): cyanidin; (II-2): cyanin; (II-3): turomanin; and (II-4): keracyanin; or a salt or solvate thereof.

[40] The composition of any one of paragraphs 1-39, which further comprises (c) one or more enzymes selected from the group consisting of a cellulase, a hemicellulase, an esterase, an expansin, a laccase, a ligninolytic enzyme, a pectinase, a peroxidase, a protease, and a swollenin.

[41] The composition of paragraph 40, wherein the cellulase is one or more enzymes selected from the group consisting of an endoglucanase, a cellobiohydrolase, and a beta-glucosidase.

[42] The composition of paragraph 40, 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.

[43] 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 cellulosolytic enhancing activity and a bicyclic compound, wherein the combination of the polypeptide having cellulosolytic enhancing activity and the bicyclic compound enhances hydrolysis of the cellulosic material by the enzyme composition.

[44] The method of paragraph 43, wherein the cellulosic material is pretreated.

[45] The method of paragraph 43 or 44, further comprising recovering the degraded cellulosic material.

[46] The method of any one of paragraphs 43-45, wherein the enzyme composition comprises one or more enzymes selected from the group consisting of a cellulase, a hemicellulase, an esterase, an expansin, a laccase, a ligninolytic enzyme, a pectinase, a peroxidase, a protease, and a swollenin.

[47] The method of paragraph 46, wherein the cellulase is one or more enzymes selected from the group consisting of an endoglucanase, a cellobiohydrolase, and a beta-glucosidase.

[48] The method of paragraph 46, 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.

[49] The method of any one of paragraphs 43-48, wherein the degraded cellulosic material is a sugar.
[50] The method of paragraph 49, wherein the sugar is selected from the group
consisting of glucose, xylose, mannose, galactose, and arabinose.

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

(a) saccharifying a cellulosic material with an enzyme composition in the
presence of a polypeptide having cellulolytic enhancing activity and a bicyclic compound,
wherein the combination of the polypeptide having cellulolytic enhancing activity and the
bicyclic compound enhances hydrolysis of the cellulosic material by the enzyme
composition;

(b) fermenting the saccharified cellulosic material with one or more fermenting
microorganisms to produce the fermentation product; and

(c) recovering the fermentation product from the fermentation.

[52] The method of paragraph 51, wherein the cellulosic material is pretreated.

[53] The method of paragraph 51 or 52, wherein the enzyme composition comprises
one or more enzymes selected from the group consisting of a cellulase, a hemicellulase, an
esterase, an expansin, a laccase, a ligninolytic enzyme, a pectinase, a peroxidase, a
protease, and a swollenin.

[54] The method of paragraph 53, wherein the cellulosic material is one or more enzymes
selected from the group consisting of an endoglucanase, a cellbiohydrolase, and a beta-
glucosidase.

[55] The method of paragraph 53, 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.

[56] The method of any one of paragraphs 51-55, wherein steps (a) and (b) are
performed simultaneously in a simultaneous saccharification and fermentation.

[57] The method of any one of paragraphs 51-56, 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.

[58] A method of fermenting a cellulosic material, comprising: fermenting the
cellulosic material with one or more fermenting microorganisms, wherein the cellulosic
material is saccharified with an enzyme composition in the presence of a polypeptide having
cellulolytic enhancing activity and a bicyclic compound, wherein the combination of the
polypeptide having cellulolytic enhancing activity and the bicyclic compound enhances
hydrolysis of the cellulosic material by the enzyme composition.

[59] The method of paragraph 58, wherein the cellulosic material is pretreated before
saccharification.

[60] The method of paragraph 58 or 59, wherein the enzyme composition comprises
one or more enzymes selected from the group consisting of a cellulase, a hemicellulase, an
esterase, an expansin, a laccase, a ligninolytic enzyme, a pectinase, a peroxidase, a protease, and a swollenin.

[61] The method of paragraph 60, wherein the cellulase is one or more enzymes selected from the group consisting of an endoglucanase, a cellobiohydrolase, and a beta-glucosidase.

[62] The method of paragraph 60, 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.

[63] The method of any one of paragraphs 58-62, wherein the fermenting of the cellulosic material produces a fermentation product.

[64] The method of paragraph 63, further comprising recovering the fermentation product from the fermentation.

[65] The method of paragraph 63 or 64, 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 a polyketide.

[66] The method of any one of paragraphs 43-65, wherein the bicyclic compound is of formula (I) or (II):

\[
\begin{align*}
\text{(I)} & \quad \text{or } \\
\text{(II)} & \\
\end{align*}
\]

wherein the bond indicated with a dashed line can be single or double;

\( R^1 \) and \( R^2 \) are independently hydrogen, -C(0)R, -C(0)OR, -C(0)NHR, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl;

\( R^3, R^{4a}, R^{4b}, \) and \( R^{4c} \) are independently hydrogen, halogen, -OH, -OR, -CN, -NO, -N(R)(R), -C(0)R, -C(0)OR, -C(0)NHR, -OC(0)R, -NH(0)R, -OC(0)OR, -NHC(0)OR, -OC(0)NHR, -S0(R)R, -S0(R)N(R)(R), -NR, -SR, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl; and wherein two of \( R^{4a}, R^{4b}, \) and \( R^{4c} \) may together form an optionally substituted fused ring;

\( R^{5}, R^{6}, R^{7}, R^{8}, R^{9}, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, \) and \( R^{20} \) are independently hydrogen, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and
heteroaralkyl; and

R\textsuperscript{17} is an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl;

5 or a salt or solvate thereof.

[67] The method of paragraph 66, wherein the bond indicated with a dashed line is a single bond.

[68] The method of paragraph 66, wherein the bond indicated with a dashed line is a double bond.

[69] The method of any one of paragraphs 66-68, wherein R\textsuperscript{1} and R\textsuperscript{2} are independently hydrogen, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl.

[70] The method of any one of paragraphs 66-68, wherein R\textsuperscript{1} and R\textsuperscript{2} are independently hydrogen, an optionally substituted carbohydrate moiety, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, or cycloalkyl-alkyl.

[71] The method of any one of paragraphs 66-68, wherein R\textsuperscript{1} and R\textsuperscript{2} are independently hydrogen, an optionally substituted carbohydrate moiety, or an optionally substituted moiety selected from alkyl, alkenyl, and alkynyl.

[72] The method of any one of paragraphs 66-68, wherein R\textsuperscript{1} and R\textsuperscript{2} are independently hydrogen, an optionally substituted carbohydrate moiety, or an optionally substituted alkyl.

[73] The method of any one of paragraphs 66-68, wherein R\textsuperscript{1} and R\textsuperscript{2} are independently hydrogen, or an optionally substituted carbohydrate moiety.

[74] The method of any one of paragraphs 66-68, wherein at least one of R\textsuperscript{1} and R\textsuperscript{2} is hydrogen.

[75] The method of any one of paragraphs 66-68, wherein both R\textsuperscript{1} and R\textsuperscript{2} are hydrogen.

[76] The method of any one of paragraphs 66-68, wherein at least one of R\textsuperscript{1} and R\textsuperscript{2} is other than hydrogen.

[77] The method of any one of paragraphs 66-68, wherein at least one of R\textsuperscript{1} and R\textsuperscript{2} is an optionally substituted carbohydrate moiety.

[78] The method of any one of paragraphs 66-68, wherein both R\textsuperscript{1} and R\textsuperscript{2} are other than hydrogen.

[79] The method of any one of paragraphs 66-78, wherein R\textsuperscript{3}, R\textsuperscript{4a}, R\textsuperscript{4b}, and R\textsuperscript{4c} are independently hydrogen, halogen, -OH, -OR\textsuperscript{8}, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-
alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl; and wherein two of $R^4_a$, $R^4_b$, and $R^4_c$ may
together form an optionally substituted cyclic diether.

[80] The method of any one of paragraphs 66-78, wherein $R^3$, $R^4_a$, $R^4_b$, and $R^4_c$ are
independently hydrogen, -OH, -OR$_8$, an optionally substituted carbohydrate moiety, or an
optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, or cycloalkyl-
alkyl.

[81] The method of any one of paragraphs 66-78, wherein $R^3$, $R^4_a$, $R^4_b$, and $R^4_c$ are
independently hydrogen, -OH, -OR$_8$, an optionally substituted carbohydrate moiety, or an
optionally substituted moiety selected from alkyl, alkenyl, and alkynyl.

[82] The method of any one of paragraphs 66-78, wherein $R^3$, $R^4_a$, $R^4_b$, and $R^4_c$ are
independently hydrogen, -OH, -OR$_8$, an optionally substituted carbohydrate moiety.

[83] The method of any one of paragraphs 66-82, wherein at least one of $R^4_a$, $R^4_b$, and $R^4_c$ is hydrogen.

[84] The method of any one of paragraphs 66-82, wherein at least two of $R^4_a$, $R^4_b$, and $R^4_c$ are hydrogen.

[85] The method of any one of paragraphs 66-82, wherein each of $R^4_a$, $R^4_b$, and $R^4_c$ are hydrogen.

[86] The method of any one of paragraphs 66-82, wherein at least one of $R^4_a$, $R^4_b$, and $R^4_c$ is -OH.

[87] The method of any one of paragraphs 66-82, wherein at least two of $R^4_a$, $R^4_b$, and $R^4_c$ is -OH.

[88] The method of any one of paragraphs 66-82, wherein one of $R^4_a$, $R^4_b$, and $R^4_c$ is hydrogen and the other two of $R^4_a$, $R^4_b$, and $R^4_c$ are each -OH.

[89] The method of any one of paragraphs 66-82, wherein at least one of $R^4_a$, $R^4_b$, and $R^4_c$ is other than hydrogen.

[90] The method of any one of paragraphs 66-82, wherein at least two of $R^4_a$, $R^4_b$, and $R^4_c$ are other than hydrogen.

[91] The method of any one of paragraphs 66-82, wherein $R^3$ and at least one of $R^4_a$, $R^4_b$, and $R^4_c$ are other than hydrogen.

[92] The method of any one of paragraphs 66-82, wherein $R^3$ and at least two of $R^4_a$, $R^4_b$, and $R^4_c$ are other than hydrogen.

[93] The method of any one of paragraphs 66-92, wherein at least one of $R^3$, $R^4_a$, $R^4_b$, and $R^4_c$ is an optionally substituted carbohydrate moiety.

[94] The method of any one of paragraphs 66-92, wherein two of $R^3$, $R^4_a$, $R^4_b$, and $R^4_c$ are optionally substituted carbohydrate moieties.

[95] The method of any one of paragraphs 66-94, wherein $R^3$ is hydrogen, -OH, or an optionally substituted carbohydrate moiety.
[96] The method of any one of paragraphs 66-94, wherein R³ is hydrogen.
[97] The method of any one of paragraphs 66-94, wherein R³ is other than hydrogen.
[98] The method of any one of paragraphs 66-94, wherein R³ is -OH.
[99] The method of any one of paragraphs 66-94, wherein R³ is an optionally substituted carbohydrate moiety.

[100] The method of any one of paragraphs 43-65, wherein the bicyclic compound is of the formula (I-A) or (II-A):

![Chemical Structure](image)

wherein R¹, R², R⁴a, and R⁸ are as defined in any one of the preceding paragraphs; or a salt or solvate thereof.

[101] The method of any one of paragraphs 43-65, wherein the bicyclic compound is of the formula (I-B) or (II-B):

![Chemical Structure](image)

wherein R¹, R², and R⁴a are as defined in any one of the preceding paragraphs; or a salt or solvate thereof.

[102] The method of any one of paragraphs 43-65, wherein the bicyclic compound is of the formula (I-C), (I-D), (I-E), or (I-F):

![Chemical Structure](image)
wherein \( R^1, R^2, R^3, R^{4a}, R^{4b}, \) and \( R^{4c} \) are as defined in any one of the preceding paragraphs; 
or a salt or solvate thereof.

[103] The method of any one of paragraphs 43-65, wherein the bicyclic compound is 
selected from the group consisting of: (I-1): epicatechin; (I-2): quercetin; (I-3): myricetin; (I-4): 
taxifolin; (I-5): kaempferol; (I-6): morin; (I-7): acacetin; (I-8): naringenin; (I-9): isorhamnetin; 
(1-10): apigenin; (11-1): cyanidin; (II-2): cyanin; (II-3): turomanin; and (II-4): keracyanin; or a 
salt or solvate thereof.

[104] The method of any of paragraphs 43-103, wherein an effective amount of the 
bicyclic compound to cellulosic material as a molar ratio to glucosyl units of cellulose is 
about \( 10^{-8} \) to about 10, e.g., about \( 10^{-6} \) to about 7.5, about \( 10^{-6} \) to about 5, about \( 10^{-5} \) to 
about 2.5, about \( 10^{-6} \) to about 1, about \( 10^{-5} \) to about 1, about \( 10^{-6} \) to about \( 10^{-4} \), about \( 10^{-3} \) to 
about \( 10^{-1} \), or about \( 10^{-2} \) to about \( 10^{0} \).

[105] The method of any of paragraphs 43-103, wherein an effective amount of the 
bicyclic compound to cellulose is about \( 10^{0} \) to about 10 per g of cellulose, e.g., about \( 10^{0} \) to 
about 7.5, about \( 10^{0} \) to about 5, about \( 10^{0} \) to about 2.5, about \( 10^{0} \) to about 1, about \( 10^{0} \) to 
about 1, about \( 10^{0} \) to about \( 10^{0} \), about \( 10^{0} \) to about \( 10^{1} \), or about \( 10^{0} \) to about \( 10^{2} \) per g of cellulose.

[106] The method of any of paragraphs 43-103, wherein an effective amount of the 
bicyclic compound is about 0.1 \( \mu \text{M} \) to about 1 \( \text{M} \), e.g., about 0.5 \( \mu \text{M} \) to about 0.75 \( \text{M} \), about 
0.75 \( \mu \text{M} \) to about 0.5 \( \text{M} \), about 1 \( \mu \text{M} \) to about 0.25 \( \text{M} \), about 1 \( \mu \text{M} \) to about 0.1 \( \text{M} \), about 5 
\( \mu \text{M} \) to about 50 \( \text{mM} \), about 10 \( \mu \text{M} \) to about 25 \( 
\text{mM} \), about 50 \( \mu \text{M} \) to about 25 \( \text{mM} \), about 10 \( \mu \text{M} \) to 
about 10 \( \text{mM} \), about 5 \( \mu \text{M} \) to about 5 \( \text{mM} \), or about 0.1 \( \text{mM} \) to about 1 \( \text{mM} \).

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:

1. A composition comprising: (a) a polypeptide having cellulolytic enhancing activity and (b) a bicyclic compound, wherein the combination of the polypeptide having cellulolytic enhancing activity and the bicyclic compound enhances hydrolysis of a cellulosic material by a cellulolytic enzyme.

2. The composition of claim 1, wherein the bicyclic compound is of formula (I) or (II):

   \[
   \begin{align*}
   &\text{(I)} \\
   &\text{(II)}
   \end{align*}
   \]

   wherein the bond indicated with a dashed line can be single or double;

   \( R^1 \) and \( R^2 \) are independently hydrogen, \(-\text{C}(0)R^5 \), \(-\text{C}(0)\text{OR}^6 \), \(-\text{C}(0)\text{NHR}^7 \), or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl;

   \( R^3 \), \( R^{4a} \), \( R^{4b} \), and \( R^{4c} \) are independently hydrogen, halogen, \(-\text{OH}\), \(-\text{OR}^8 \), \(-\text{CN}\), \(-\text{N}^2\text{O}_2 \), \(-\text{N}(R^9)(R^{10})\), \(-\text{C}(0)R^5 \), \(-\text{C}(0)\text{OR}^6 \), \(-\text{C}(0)\text{NHR}^7 \), \(-\text{OC}(0)R^{11} \), \(-\text{NHC}(0)R^{12} \), \(-\text{OC}(0)\text{OR}^{13} \), \(-\text{NHC}(0)\text{OR}^{14} \), \(-\text{OC}(0)\text{NHR}^{15} \), \(-\text{NHC}(0)\text{NHR}^{16} \), \(-\text{SO}_2\text{R}^{17} \), \(-\text{SO}_2\text{N}(R^{18})(R^{19}) \), \(-\text{SR}^{20} \), or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl; and

   wherein two of \( R^{4a} \), \( R^{4b} \), and \( R^{4c} \) may together form an optionally substituted fused ring;

   \( R^5 \), \( R^6 \), \( R^7 \), \( R^8 \), \( R^9 \), \( R^{10} \), \( R^{11} \), \( R^{12} \), \( R^{13} \), \( R^{14} \), \( R^{15} \), \( R^{16} \), \( R^{18} \), \( R^{19} \), and \( R^{20} \) are independently hydrogen, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl; and

   \( R^{17} \) is an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl;

   or a salt or solvate thereof.

3. The composition of claim 2, wherein the bicyclic compound is of the formula (I-A) or (II-A):
wherein \( R_1, R_2, R_4^a, \) and \( R_8 \) are as defined in any one of the preceding claims; or a salt or solvate thereof.

4. The composition of claim 2, wherein the bicyclic compound is of the formula (I-B) or (II-B):

wherein \( R_1, R_2, \) and \( R_4^a \) are as defined in any one of the preceding claims; or a salt or solvate thereof.

5. The composition of claim 2, wherein the bicyclic compound is of the formula (I-C), (I-D), (I-E), or (I-F):

wherein \( R_1, R_2, R_3, R_4^a, R_4^b, \) and \( R_4^c \) are as defined in any one of the preceding claims;
6. The composition of claim 2, wherein the bicyclic compound is selected from the group consisting of: (I-1): epicatechin; (I-2): quercetin; (I-3): myricetin; (I-4): taxifolin; (I-5): kaempferol; (I-6): morin; (I-7): acacetin; (I-8): naringenin; (I-9): isorhamnetin; (I-10): apigenin; (II-1): cyanidin; (II-2): cyanin; (II-3): turomanin; and (II-4): keracyanin; or a salt or solvate thereof.

7. The composition of any one of claims 1-6, which further comprises (c) one or more enzymes selected from the group consisting of a cellulase, a hemicellulase, an esterase, an expansin, a laccase, a ligninolytic enzyme, a pectinase, a peroxidase, a protease, and a swollenin.

8. 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 cellulolytic enhancing activity and a bicyclic compound, wherein the combination of the polypeptide having cellulolytic enhancing activity and the bicyclic compound enhances hydrolysis of the cellulosic material by the enzyme composition.

9. The method of claim 8, wherein the cellulosic material is pretreated.

10. The method of claim 8 or 9, further comprising recovering the degraded cellulosic material.

11. The method of any one of claims 8-10, wherein the enzyme composition comprises one or more enzymes selected from the group consisting of a cellulase, a hemicellulase, an esterase, an expansin, a laccase, a ligninolytic enzyme, a pectinase, a peroxidase, a protease, and a swollenin.

12. A method for producing a fermentation product, comprising:
   (a) saccharifying a cellulosic material with an enzyme composition in the presence of a polypeptide having cellulolytic enhancing activity and a bicyclic compound, wherein the combination of the polypeptide having cellulolytic enhancing activity and the bicyclic compound enhances hydrolysis of the cellulosic material by the enzyme composition;
   (b) fermenting the saccharified cellulosic material with one or more fermenting microorganisms to produce the fermentation product; and
   (c) recovering the fermentation product from the fermentation.
13. The method of claim 12, wherein the enzyme composition comprises one or more enzymes selected from the group consisting of a cellulase, a hemicellulase, an esterase, an expansin, a laccase, a ligninolytic enzyme, a pectinase, a peroxidase, a protease, and a swollenin.

14. A method of fermenting a cellulosic material, comprising: fermenting the cellulosic material with one or more fermenting microorganisms, wherein the cellulosic material is saccharified with an enzyme composition in the presence of a polypeptide having cellulolytic enhancing activity and a bicydic compound, wherein the combination of the polypeptide having cellulolytic enhancing activity and the bicydic compound enhances hydrolysis of the cellulosic material by the enzyme composition.

15. The method of claim 14, wherein the enzyme composition comprises one or more enzymes selected from the group consisting of a cellulase, 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 fermenting of the cellulosic material produces a fermentation product.

17. The method of claim 16, further comprising recovering the fermentation product from the fermentation.

18. The method of any of one claims 8-17, wherein the bicydic compound is of formula (I) or (II):

![Chemical Structures](image)

wherein the bond indicated with a dashed line can be single or double;
R¹ and R² are independently hydrogen, -C(0)R⁵, -C(0)OR⁶, -C(0)NHR⁷, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl;
R³, R⁴a, R⁴b, and R⁴c are independently hydrogen, halogen, -OH, -OR⁸, -CN, -N=O₂.
-N(R\(^8\))(R\(^{10}\)), -C(0)R\(^6\), -C(0)OR\(^6\), -C(0)NHR\(^7\), -OC(0)R\(^{11}\), -NHC(0)R\(^{12}\), -OC(0)OR\(^{13}\),
-NHC(0)OR\(^{14}\), -OC(0)NHR\(^{15}\), -NHC(0)NHR\(^{16}\), -S\(^2\)O\(^2\)R\(^{17}\), -S\(^2\)O\(^2\)N(R\(^{18}\))(R\(^{19}\)), -SR\(^{20}\), or an
optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyi, cycloalkyl-alkyl,
heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl; and
wherein two of R\(^{4a}\), R\(^{4b}\), and R\(^{4c}\) may together form an optionally substituted fused ring;

R\(^5\), R\(^6\), R\(^7\), R\(^8\), R\(^9\), R\(^{10}\), R\(^{11}\), R\(^{12}\), R\(^{13}\), R\(^{14}\), R\(^{15}\), R\(^{16}\), R\(^{18}\), R\(^{19}\), and R\(^{20}\) are independently
hydrogen, or an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyi,
cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and
heteroaralkyl; and
R\(^{17}\) is an optionally substituted moiety selected from alkyl, alkenyl, alkynyl, cycloalkyi,
cycloalkyl-alkyl, heterocycloalkyl, heterocycloalkyl-alkyl, aryl, aralkyl, heteroaryl, and
heteroaralkyl;
or a salt or solvate thereof.

19. The method of claim 18, wherein the bicyclic compound is of the formula (I-A) or (II-A):

![Chemical Structure](image)

wherein R\(^1\), R\(^2\), R\(^{4a}\), and R\(^8\) are as defined in any one of the preceding claims;
or a salt or solvate thereof.

20. The method of claim 18, wherein the bicyclic compound is of the formula (I-B) or (II-B):

![Chemical Structure](image)

wherein R\(^1\), R\(^2\), and R\(^{4a}\) are as defined in any one of the preceding claims;
or a salt or solvate thereof.
21. The method of claim 18, wherein the bicyclic compound is of the formula (I-C), (I-D), (I-E), or (I-F):

\[
\begin{align*}
&\text{(I-C)} & \text{(I-D)} \\
&\text{(I-E)} & \text{(I-F)}
\end{align*}
\]

wherein \(R^1, R^2, R^3, R^{4a}, R^{4b},\) and \(R^{4c}\) are as defined in any one of the preceding claims; or a salt or solvate thereof.

22. The method of claim 18, wherein the bicyclic compound is selected from the group consisting of: (I-1): epicatechin; (I-2): quercetin; (I-3): myricetin; (I-4): taxifolin; (I-5): kaempferol; (I-6): morin; (I-7): acacetin; (I-8): naringenin; (I-9): isorhamnetin; (I-10): apigenin; (II-1): cyanidin; (II-2): cyanin; (II-3): turomanin; and (II-4): keracyanin; or a salt or solvate thereof.
Fig. 1
Fig. 2B
Fig. 4A
Fig. 5A
Fig. 5B
A. CLASSIFICATION OF SUBJECT MATTER
INV. C12P7/10 C12P19/14 C12N9/42
ADD.

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)
C12P C12N

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 practical, search terms used)
EPO-Internal, BIOSIS, COMPENDEX, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>Wo 2005/074647 A2 (Novezymes Inc [US]) 18 August 2005 (2005-08-18) abstract; claims 48-74</td>
<td>1,7-17</td>
</tr>
<tr>
<td>A</td>
<td>Davin L B et al: &quot;Lignin primary structures and direct sites&quot;, CURRENT OPINION IN BIOTECHNOLOGY, LONDON, GB, vol. 16, no. 4, 1 August 2005 (2005-08-01), pages 407-415, XP027676725, ISSN: 0958-1669 [retrieved on 2005-08-01] page 409, left-hand column, paragraph 1 - figure 2, 4, 5</td>
<td>1-22</td>
</tr>
</tbody>
</table>

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

* Special categories of cited documents:
A: document defining the general state of the art which is not considered to be of particular relevance
E: earlier document but published on or after the international filing date
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)
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
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
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
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
A: document member of the same patent family

Date of the actual completion of the international search: 24 November 2011

Date of mailing of the international search report: 01/12/2011

Name and mailing address of the ISA:
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HJ Rijswijk
Tel. (+31-70) 340-2040, Fax. (+31-70) 340-3016

Authorized officer:
Schroder, Gunnar
<table>
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<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tr>
<td>A</td>
<td>ARANTES VALDEIR ET AL: &quot;Access to cellulose limits the efficiency of enzymatic hydrolysis: the role of amorphogenesis&quot;, BIOTECHNOLOGY FOR BIOFUELS, BIOMED CENTRAL LTD, LONDON UK, vol. 3, 4, 23 February 2010 (2010-02-23), pages 1-11, XP021070031, ISSN: 1754-6834 page 4, left-hand column, paragraph 2 - page 8, right-hand column, paragraph 1</td>
<td>1-22</td>
</tr>
<tr>
<td>Patent document cited in search report</td>
<td>Publication date</td>
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<tr>
<td>WO 2005074647 A2</td>
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<td>CN 1980953 A</td>
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<td>EP 1713825 A2</td>
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<td>EP 2305703 A1</td>
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<td>US 2005191736 A1</td>
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<td>US 2008206815 A1</td>
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<td>US 2010197556 A1</td>
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<td>WO 2005074647 A2</td>
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### INTERNATIONAL SEARCH REPORT

#### 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:

see additional sheet

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 fees.

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.
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-7

A composition for enhancing hydrolysis of a cellulose material by a cellulolytic enzyme comprising a polypeptide having cellulolytic enhancing activity and a bicyclic compound

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2. claims: 8-11 (completely) ; 18-22 (partially)

A method for degrading or converting a cellulose material, comprising treating the cellulose material with an enzyme composition comprising the above composition

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3. claims: 12, 13 (completely) ; 18-22 (partially)

A method for producing a fermentation product, comprising saccharifying a cellulose material with an enzyme composition comprising the above composition

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4. claims: 14-17 (completely) ; 18-22 (partially)

A method of fermenting a cellulose material, comprising saccharifying a cellulose material with an enzyme composition comprising the above composition

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